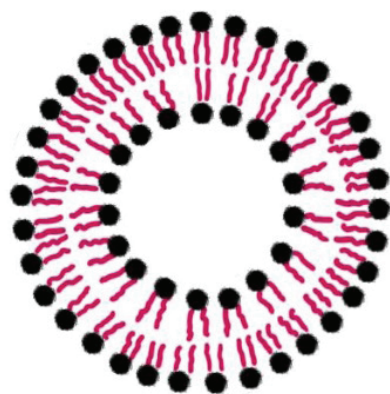
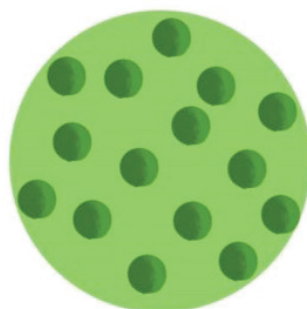


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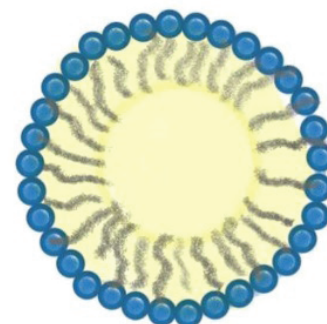
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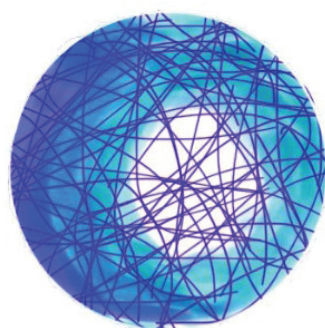
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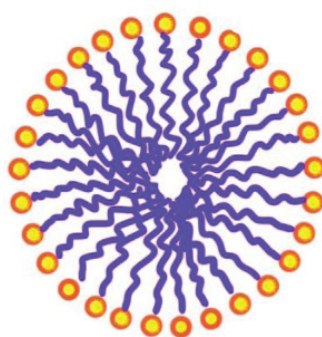
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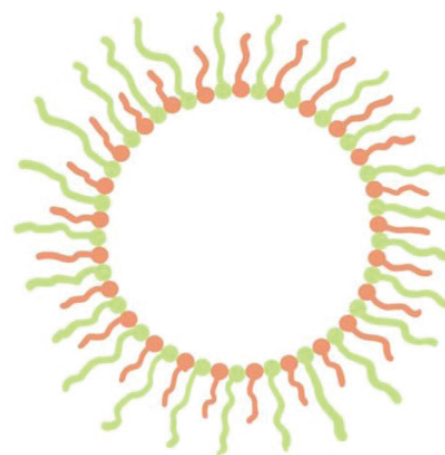
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Polymeric NPs



Micelles



Emulsifying system

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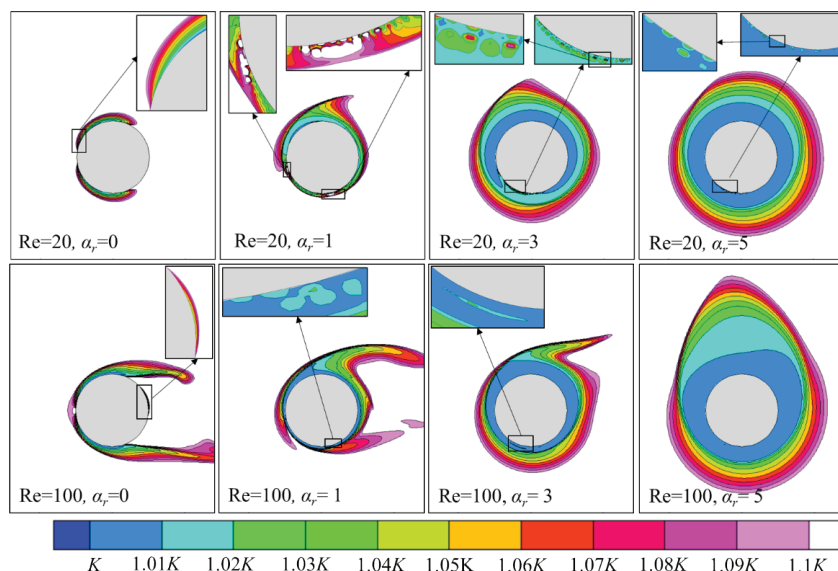
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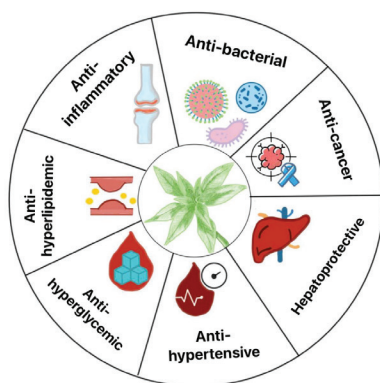
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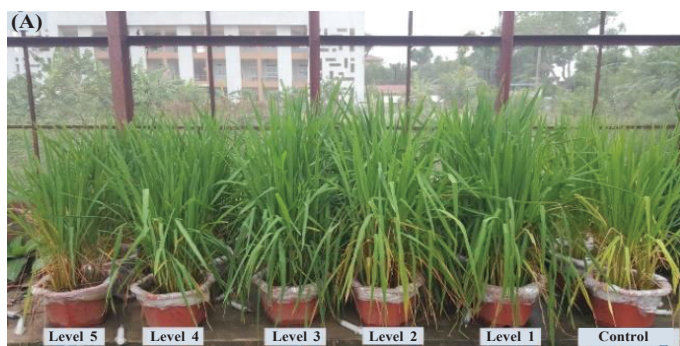
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The effects of pH on the precipitation of rice straw lignin from An Giang, Vietnam

Thuy-An Ngo*, Dao-Chi Vo Thi, Nhan-Tanh Nguyen Tran, Thien-Khanh Nguyen Tran,
Minh-Nguyet Doan Thi, Mai-Linh Duong, Lan-Tuyen Nguyen

An Giang University, Vietnam National University - Ho Chi Minh City, 18 Ung Van Khiem Street, Dong Xuyen Ward, Long Xuyen City, An Giang Province, Vietnam

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Abstract:

This research aimed to extract lignin from rice straw and reveal the pH value of diluted acid at which the most effective yield of lignin was precipitated. In this study, rice straw obtained from local fields in the An Giang province of Vietnam and sodium hydroxide 2 M was employed to extract lignin from rice straw. Hydrochloric acid was used to adjust the sample pH values to be in the range of 1.5-3.5. Fourier-transform infrared spectroscopy (FTIR) analysis was used to characterize the functional groups of the lignin materials. Thermogravimetric analysis (TGA) analysis was employed to supply information on the thermal decomposition of the lignin samples. Herein, the results showed that lignin precipitated at different pH values affected its thermal properties. At 700°C, the yield of the remaining lignin components were 56% at pH 2.0, but the weight loss of lignin samples precipitated at pH 3.5 dropped to 85% because many non-lignin substances existed in the samples. The yield of the crude lignin samples obtained were 16.51, 17.66, 15.27, 14.33 and 13.26% for pH of 1.5, 2.0, 2.5, 3.0 and 3.5 respectively. The crystallite regions played an important role in the lignin structures. The spectrum peaks at pH 1.5, 2.0 and 2.5 were broader than the peaks at pH 3.0 and 3.5. The results demonstrated the highest percentage of lignin precipitate was collected at pH 2.0.

Keywords: agglomerate, crystallinity region, decomposition, lignin, precipitate, redissolve, rice straw, thermal.

Classification number: 2.2

1. Introduction

Lignin is an aromatic organic polymer composed of three precursor aromatic alcohols, namely, p-coumaryl, syringyl, and guaiacyl [1, 2]. With an amorphous structure, lignin performs the function of plant cell binding and cell wall void filling along with cellulose, hemicellulose, and pectin [3]. Lignin does not exist as an independent polymer in plant cells but is always bound to carbohydrates (i.e., hemicellulose) to form lignin-carbohydrate complexes [2]. Due to stable binding with many functional groups, lignin is widely found in resins, emulsifiers, dyes, paints, asphalt, nutrients, and synthetic fuels [4, 5].

As a natural complex organic polymer with wide applications in many fields, lignin can be extracted by various methods depending on source material types (mainly herbaceous) and lignin-chemical structure [2]. The first lignin-carbohydrate complex was successfully extracted with hot water in 1953 [2, 6]. After that, organic solvents, alkaline solutions, acids, enzymes, microbiological methods, and ultrasonic treatments were tested toward the improvement

of lignin purity and recovery [2-5]. Among those methods, alkaline hydrolysis is proven to be a promising approach that is non-toxic to the environment and has a high lignin recovery rate [1, 4]. In this approach, α -ether bonds between lignin-hemicellulose and ester bonds (between lignin-hemicelluloses and hydroxycinnamic acids) are broken such that the lignin can be separated from the alkaline soluble complex mixture [1, 2]. More importantly, acids are also believed to have an essential influence on lignin precipitation from soluble mixtures with non-lignin components [2, 7]. However, when the concentration of H^+ is too high, the decomposition coefficient of lignin will increase, whereas, a low concentration of H^+ will affect the structure of the obtained lignin because the non-lignin components are not completely separated [7]. The differences in these structures of lignin can be examined by the following methods: FTIR spectroscopy, TGA, and X-ray diffraction analysis (XRD) [1, 4]. Therefore, to recover lignin from herbaceous plants, alkaline hydrolysis is considered efficient but depends upon acid concentration and lignin structure [1, 4, 7].

*Corresponding author: Email: ntan@agu.edu.vn

Raw materials for lignin extraction are diverse and include all woody plants of which the most important are herbaceous plants because of their abundance. Rice straw, an agricultural waste, is also considered an abundant lignin source due to it consisting of the three main carbon-rich components, namely, cellulose (32-47%), hemicellulose (19-27%) and lignin (5-24%) [8]. Approximately 370-520 million tons/year of this biomass source is generated globally of which Vietnam generates approximately 50 million annually [9, 10]. Furthermore, much of this biomass source is currently wasted due to policies allowing open burning, which causes air pollution [9].

In this study, various pH values were tested (using alkaline extraction) to determine which pH values would obtain a high yield of precipitated lignin. Then, the characteristics of the crude lignin were investigated using XRD, FTIR and TGA.

2. Materials and methods

2.1. Materials

Rice straw was obtained from local rice fields in An Giang Province, Vietnam. The rice straw was washed in deionized water to remove impurities [11] and troublesome elements like insect larvae, dust, soil, etc. After that, the rice straw was sun-dried to reduce its moisture content to 4-5.5% and was then chopped into lengths of 1-2 cm. The dry sample was ground to a fine powder using a commercial blender (DFY-2000, Vietnam), and then sieved to the size of 0.08 mm to achieve the so-called dried rice straw (DRS). The DRS was stored in sealed polyethylene bags at ambient temperature for future use. Then, 150 g of cleaned rice straw/DRS was mixed with 3600 ml of acetone 5% to remove oils, pigments, and wax [12, 13] to obtain dewaxed DRS.

The chemicals used for lignin extraction consisted of perchloric acid, hydrochloric acid, toluene, ethanol, and sodium hydroxide, which were obtained from Merck, Germany.

2.2. Methods

In this study, a chemical method was used to extract lignin from rice straw. Fifty grams of the dewaxed DRS was added to 1000 ml of NaOH 2 M [5, 14]. This mixture was sonicated in a S100-Elmasonic (Germany, 37 kHz) ultrasonic bath for 30 min at 90°C. Then, it was refluxed at 90°C for 90 min. After that, it was cooled to 40°C and filtered to remove residual biomass [15]. Hydrochloric acid 6 M was added into the filtrate until the pH reached 4.0, then it was stored at 4°C for 24 h. Next, three volumes of ethanol 95% were added to the liquid and kept at 4°C for 6 h for hemicellulose coagulation. Hemicellulose precipitates and the filtrate were obtained by vacuum suction. Ethanol was

recovered and the liquid containing lignin was obtained. According to the research of M.A. Hubbe, et al. (2019) [16], the hydroxycarboxylic acids in lignin molecules can become less soluble forms when pH is decreased to below 3.5. Therefore, to optimize lignin precipitates from the filtrate, the liquid's pH was adjusted from 3.5 to 1.5 and the pH values were measured by a pH meter (Extech 407228, USA). The diluted solution of hydrochloric acid 6 M was added to five samples containing 100 ml of the liquid to reach a target pH ranging from 3.5 to 1.5 [17]. All the samples were left for 24 h for lignin precipitation [15]. Each settled sediment was collected by a filter and then dried at 80°C until constant mass [4]. The yield of collected lignin was determined from the difference between the initial weight of the rice straw sample used for lignin extraction and the dried weight of lignin collected.

2.3. Characterisations

FTIR, TGA, and XRD were used to evaluate the fundamental properties of the lignin products. FTIR analysis was conducted with an Alpha-Bruker FTIR spectrometer using KBr powder to determine the absorbance of the functional groups of lignin, and the measurements were performed in the range of 500-4000 cm^{-1} with a resolution of 4 cm^{-1} [18].

TGA was accomplished on a Q500-TA instrument operating from temperature room (24-28°C) to 700°C with a heating rate of 20°C/min under a nitrogen atmosphere. The TGA presented the change in weight of the lignin samples as a function of temperature. The TGA curves indicated the rate of mass loss versus temperature, which was used to recognize the thermal stability of lignin [15].

The evaluation of lignin crystallinity was carried out using XRD on an Aeris Panalytical Diffractometer with Cu K α Ni-filtered radiation of $\lambda=1.543 \text{ \AA}$ with a working voltage of 45 kV. The diffraction patterns in the 2 θ mode between 10-50° were recorded with a step size of 0.019° and a scan time of 43.00 s/step [15, 19].

2.4. Statistical analysis

All experiments were carried out in triplicate. The paired t-test was performed to determine the statistically significant effect of pH values on lignin yields by using the SPSS package ver. 11-2018 (USA).

3. Results

3.1. The yields of raw lignin at different pH values

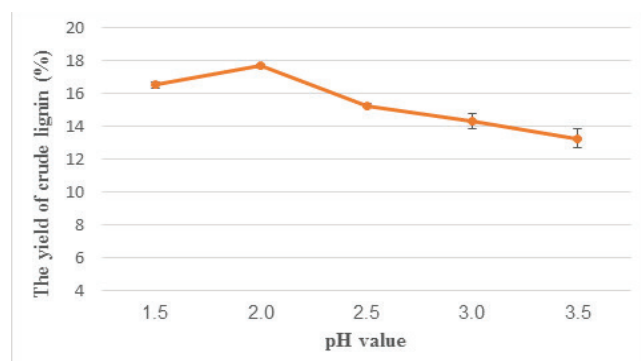
Table 1 shows the paired t-test results of the raw lignin yields that were precipitated with hydrochloric acid at different pH values in the range of 3.5-1.5.

Table 1. The Paired T-test result of raw lignin yields by pH.

Paired samples test	Mean	N	Standard deviation	Significant (2-tailed)
Pair 1	pH1.5	16.51	3	0.16
	pH2.0	17.66	3	0.13
Pair 2	pH1.5	16.51	3	0.16
	pH2.5	15.27	3	0.15
Pair 3	pH1.5	16.51	3	0.16
	pH3.0	14.33	3	0.47
Pair 4	pH1.5	16.51	3	0.16
	pH3.5	13.26	3	0.60
Pair 5	pH2.0	17.66	3	0.13
	pH2.5	15.27	3	0.15
Pair 6	pH2.0	17.66	3	0.13
	pH3.0	14.33	3	0.47
Pair 7	pH2.0	17.66	3	0.13
	pH3.5	13.26	3	0.60
Pair 8	pH2.5	15.27	3	0.15
	pH3.0	14.33	3	0.47
Pair 9	pH2.5	15.27	3	0.15
	pH3.5	13.26	3	0.60
Pair 10	pH3.0	14.33	3	0.47
	pH3.5	13.26	3	0.60

The paired sample t-test was used to assess the statistical difference between the pH values on the mean yields of lignin. The results showed that the pair of pH 2.5-3.0 (pair 8) and the pair of pH 3.0-3.5 (pair 10) were not significantly different ($\text{sig.} > 0.05$) within each pair at a confidence level of 95%. There were significant differences within each pair ($\text{sig.} < 0.05$) at a 95% confidence level. The mean yield obtained varied from 13.26 to 17.66 g.

Figure 1 indicates that the decrease of pH from 3.5 to 1.5 increased the yield. The highest percentage of lignin precipitate was above 17% at pH 2.0. These observations agree with previous studies [15, 19]. The curve was divided into two stages: firstly, when pH was reduced from 3.5 to 2,

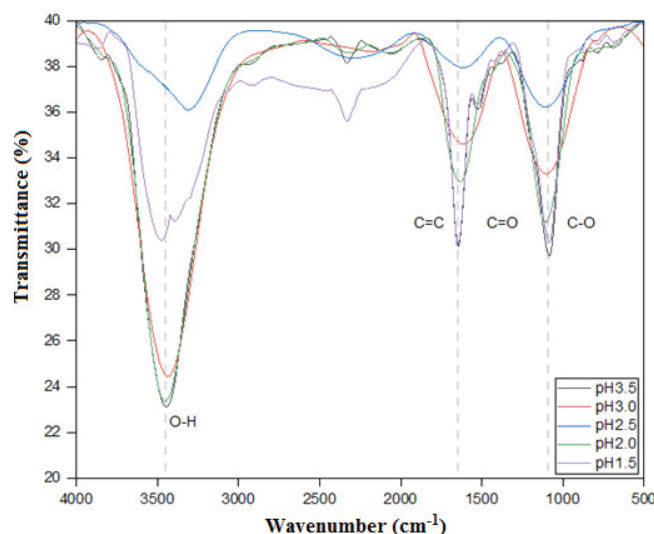
**Fig. 1. The change in yield at different pH values.**

more lignin started to precipitate because the hydrogen ions in the hydrochloric acid solution interacted with the negatively charged lignin molecules. The repulsive forces between suspended particles were reduced and the coagulation of lignin occurred. Thus, a higher concentration of hydrogen ions increased the precipitation of lignin. At pH 2.0, the highest yield was obtained because of the lower amount of soluble carboxyl acids. In the second stage, the yield of lignin precipitation decreased when more hydrochloric acid was added to the mixture because the precipitates became unstable and re-dissolved. According to S. Priyanto, et al. (2019) [7], the higher the concentration of acidity, the easier the lignin coagulation formed. However, if the mixture was too acidic, the lignin yield was potentially damaged. Therefore, the target pH to precipitate lignin was pH 2.0 [15].

4. Characterisations

4.1. FTIR analysis

The spectra of the lignin samples obtained at pH values from 3.5 to 1.5 are analysed using FTIR in the range of 500-4000 cm^{-1} and are shown in Fig. 2. All the spectra show variations in band intensities that are related to the functional groups of lignin. From 3000-3450 cm^{-1} , the broad vibration was assigned to hydroxyl groups and did not correlate with the pH changes of the solutions. The carbonyl groups corresponded to the absorbance at 1650 cm^{-1} , however, there was a significant drop in peaks when the pH values were higher (pH 3.5, pH 3.0). At 1592 cm^{-1} , which are the bands of aromatic skeletal vibrations, there were changes in the peak intensities with a decrease in pH. The other bands related to methoxyl groups were around 1454-1421 cm^{-1} . The absorbance signals between 1305-1090 cm^{-1} show the C=C, C=O, and C-O groups [4, 15].

**Fig. 2. FTIR spectra of crude lignin at different pH.**

4.2. TGA analysis

Figure 3 indicates weight loss occurring as the TGA curves decrease. The degradation temperature of lignin occurred over a wide temperature range, such as between 100 and 700°C, due to lignin being a natural polyphenolic polymer containing various branching [20]. The degradation of rice straw lignin was seen over three stages. In the first stage, weight loss at 30-100°C occurred due to water evaporation in the lignin samples. For the second stage, the degradation of carbohydrate components occurred and released carbon greenhouse gases such as CO, CO₂ and CH₄ at 180-370°C. In this stage, there was a significant change in the slope of the thermal decomposition. The final stage of lignin degradation started above 450°C, but mass loss rates were much smaller than in the second stage [21]. Also, in the final stage, the breakdown of lignin molecules only occurred when the biomass reached the target thermal energy. From pH 3.5 to 1.5, about 44-15% of all crude lignin samples remained at 700°C [15, 20] because there were trends to form chars from the highly condensed aromatic structure of lignin [22]. However, over 75 and 85% of the weight loss were decomposed at pH 3.5 and pH 3.0, respectively and implied the evidence for higher impurities of lignin precipitates.

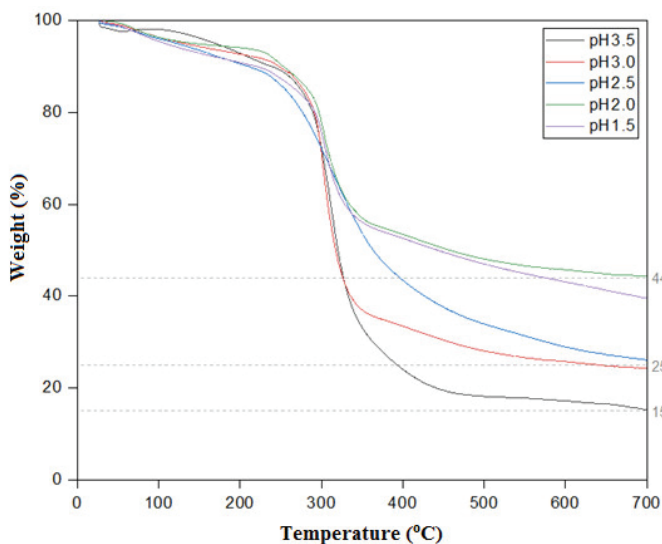


Fig. 3. TGA spectra of crude lignin at different pH values.

4.3. XRD analysis

The XRD analysis in Fig. 4 indicates changes in the crystallinity of lignin samples with pH. At pH values of 3.5 and 3.0, a wide peak at 2θ values ranging from 17 to 32°C is seen, but the lignin peaks obtained at pH 2.5, 1.5, and 2.0 were wider at 2θ from 12 to 38°C. This indicates that the broad peaks are related to the amorphous regions

of the lignin substances. At pH 1.5, the amorphous degree was narrower because of the non-lignin materials in the samples collected [15].

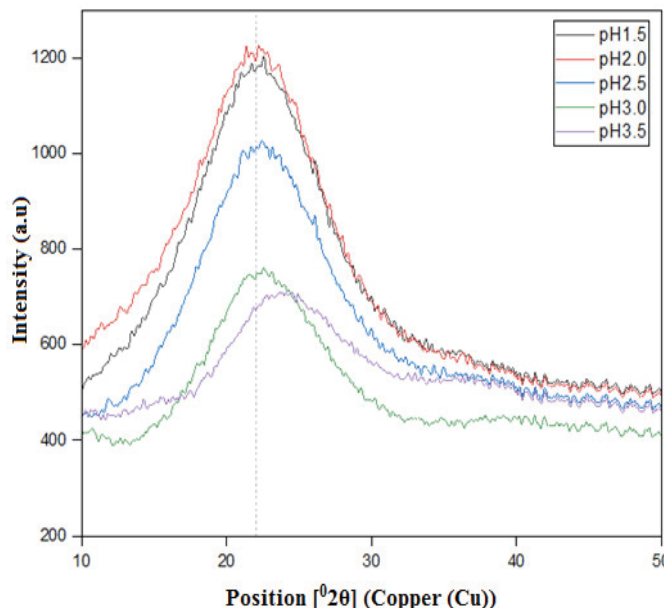


Fig. 4. XRD spectra of crude lignin obtained at different pH values.

5. Conclusions

The study results demonstrated that varying pH values of diluted hydrochloric acid affected lignin yields. The lignin precipitates were 16.51, 17.66, 15.27, 14.33 and 13.26% at pH 1.5, 2.0, 2.5, 3.0, 3.5, respectively. A pH of 2.0 was found to be the most effective to recover crude lignin from the solution. The FTIR spectra showed the most functional groups, such as carbonyl, aromatic rings, and methoxyl. The TGA results indicated that the thermal characteristics of lignin precipitates were related to the change in pH. About 75 and 85% of lignin materials precipitated at pH 3.0 and pH 3.5, respectively, which were thermally decomposed at 700°C. In contrast, 56% of samples at pH 2.0 remained at 700°C because of the condensed aromatic structures of the lignin substances. The XRD analysis indicated that different pH values also significantly affected the crystalline regions of lignin materials. At pH 2.0 and 2.5, the broadened peaks occurred at 2θ from 12 to 38°C, but those became narrower when the value of pH was 1.5.

This paper provides useful information on the efficiency of lignin precipitates from rice straw with respect to pH values. Therefore, it is noted that varying the pH in the lignin coagulation process should be considered and evaluated to obtain the desired lignin mass.

CRediT author statement

Thuy-An Ngo: Conceptualisation, Methodology, Supervision; Dao-Chi Vo Thi, Nhan-Tanh Nguyen Tran, Thien-Khanh Nguyen Tran: Data curation, Software, Visualisation; Minh-Nguyet Doan Thi, Mai-Linh Duong, Lan-Tuyen Nguyen: Investigation, Writing - original draft preparation.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] B. Xiao, X.F. Sun, R.C. Sun (2001), "Chemical, structural, and thermal characterizations of alkali-soluble lignins and hemicelluloses, and cellulose from maize stems, rye straw, and rice straw", *Polymer Degradation and Stability*, **74**(2), pp.307-319, DOI: 10.1016/S0141-3910(01)00163-X.
- [2] A.U. Buranov, G. Mazza (2008), "Lignin in straw of herbaceous crops", *Industrial Crops and Products*, **28**(3), pp.237-259, DOI: 10.1016/j.indcrop.2008.03.008.
- [3] Z. Borcsok, Z. Pasztory (2020), "The role of lignin in wood working processes using elevated temperatures: An abbreviated literature survey", *European Journal of Wood and Wood Products*, **79**, pp.511-526, DOI: 10.1007/s00107-020-01637-3.
- [4] S. De, S. Mishra, E. Poonguzhali, et al. (2020), "Fractionation and characterisation of lignin from waste rice straw: Biomass surface chemical composition analysis", *International Journal of Biological Macromolecules*, **145**, pp.795-803, DOI: 10.1016/j.ijbiomac.2019.10.068.
- [5] D.V. Ngo, T.H. Tran, D.N. Bui, et al. (2017), "Lignin and cellulose extraction from Vietnam's rice straw using ultrasound-assisted alkaline treatment method", *International Journal of Polymer Science*, **2**, pp.1-8, DOI: 10.1155/2017/1063695.
- [6] P. Traynard, A.A. Ayroud, A. Eymery (1953), "Existence d'une liaison lignine-hydrates de carbone dans le bois", *Association Technique Industrie Papetiere Bulletin*, **2**, pp.45-52.
- [7] S. Priyanto, A.P. Intan, B. Rianto, et al. (2019), "The effect of acid concentration (H_2SO_4) on the yield and functional group during lignin isolation of biomass waste pulp and paper industry", *Reaktor*, **19**(4), pp.162-167, DOI: 10.14710/reaktor.19.4.162-167.
- [8] A. Guzman, S. Delvasto, E. Sanchez (2015), "Valorisation of rice straw waste: An alternative ceramic raw material", *Ceramica*, **61**, pp.126-136, DOI: 10.1590/0366-69132015613571888.
- [9] V.H. Nguyen, C.M.D. Monet, M.V Migo, et al. (2020), "Rice straw overview: Availability, properties, and management practices", *Sustainable Rice Straw Management*, Springer, pp.1-14, DOI: 10.1007/978-3-030-32373-8_1.
- [10] N.Q. Diep, K. Sakanishi, N. Nakagoshi, et al. (2015), "Potential for rice straw ethanol production in the Mekong Delta, Vietnam", *Renewable Energy*, **74**, pp.456-463, DOI: 10.1016/j.renene.2014.08.051.
- [11] K. Luo, Y. Wang, H. Xiao, et al. (2019), "Preparation of convertible cellulose from rice straw using combined organosolv fractionation and alkaline bleaching", *Earth and Environmental Science*, **237**, pp.1-6, DOI: 10.1088/1755-1315/237/5/052053.
- [12] X.N. Nie, J. Liu, D. She, et al. (2013), "Physicochemical and structural characterization of hemicelluloses isolated by different alcohols from rice straw", *Bioresources*, **3**(8), pp.3817-3832, DOI: 10.15376/biores.8.3.3817-3832.
- [13] A.M. Mansora, J.S. Lima, F.N. Anib, et al. (2019), "Characteristics of cellulose, hemicellulose and lignin of MD2 pineapple biomass", *Chemical Engineering Transactions*, **72**, pp.79-84, DOI: 10.3303/CET1972014.
- [14] N. Nadiha, B. Jamilah (2020), "Hemicellulose extraction and characterization of rice straw and *leucaena Leucocephala*", *Knowledge E. Social Sciences*, pp.46-54, DOI: 10.18502/kss.v4i9.7314.
- [15] N.H. Do, H.H. Pham, T.M. Le, et al. (2020), "The novel method to reduce the silica content in lignin recovered from black liquor originating from rice straw", *Scientific Reports*, **10**, DOI: 41598.020-77868-5.
- [16] M.A. Hubbe, R. Alen, M. Paleologou, et al. (2019), "Lignin recovery from spent alkaline pulping liquors using acidification, membrane separation, and related processing steps: A review", *BioResources*, **14**(1), pp.2300-2351, DOI: 10.15376/biores.14.1.2300-2351.
- [17] M.G. Alriols, A. Tejado, M. Blanco, et al. (2009), "Agricultural palm oil tree residues as raw material for cellulose, lignin and hemicelluloses production by ethylene glycol pulping process", *Chemical Engineering Journal*, **148**(1), pp.106-114, DOI: 10.1016/j.cej.2008.08.008.
- [18] C.W. Dence (1992), "The determination of lignin", *Methods in Lignin Chemistry*, Springer, pp.33-61, DOI: 10.1007/978-3-642-74065-7-3.
- [19] P.S.B. Santosa, R. Pradoo, P.H.G. Cademartori, et al. (2013), "Effect of precipitation pH on kraft lignin composition", *4th International Conference on Biorefinery - Towards Bioenergy ICBB*, China.
- [20] D. Watkins, M.D. Nuruddin, M. Hosur, et al. (2015), "Extraction and characterization of lignin from different biomass resources", *Journal of Materials Research and Technology*, **4**(1), pp.26-32, DOI: 10.1016/j.jmrt.2014.10.009.
- [21] A. Tejado, C. Pena, J. Labidi, et al. (2007), "Physico-chemical characterization of lignins from different sources for use in phenol-formaldehyde resin synthesis", *Bioresource Technology*, **98**(8), pp.1655-1663, DOI: 10.1016/j.biortech.2006.05.042.
- [22] M.W.I. Schmidt, A. Noack (2000), "Black carbon in soils and sediments: Analysis, distribution, implications, and current challenges", *Global Biogeochem Cycles*, **14**(3), pp.777-793, DOI: 10.1029/1999GB001208.

Degradation of propanil by *Acinetobacter baumannii* DT immobilised in alginate

Danh Duc Ha*, Thi Oanh Nguyen

Dong Thap University, 783 Pham Huu Lau Street, Ward 6, Cao Lanh City, Dong Thap Province, Vietnam

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Abstract:

The herbicide propanil has been widely applied in Vietnam and around the world to control weeds. In this research, the bacterium *Acinetobacter baumannii* DT, which was isolated from soil, was utilized to investigate the degradation of propanil. Degradation experiments were carried out with condensed cells at 10^9 CFU/ml with both free and immobilized forms of the bacteria. Propanil degradation rates by bacteria immobilized in alginate were higher than those of free cells at the same concentrations. The degradation curve, which is a function of concentration, fit well to the degradation kinetics described by the Edwards model with a maximum degradation of 0.034 ± 0.003 mM/h for free cells and 0.053 ± 0.005 mM/h for immobilized cells. In addition, the immobilized bacteria could tolerate higher propanil concentrations and degrade propanil in a well-known herbicide more effectively compared to the freely suspended bacteria. These result of this manuscripts demonstrate that the bacterium *A. baumannii* DT immobilized in alginate is suitable for degradation of propanil in herbicides.

Keywords: *Acinetobacter baumannii* DT, degradation, immobilized cells, kinetics, propanil.

Classification number: 2.2

1. Introduction

The proper use of herbicides in agricultural production saves money, time and labor, especially considering they have become indispensable in today's agricultural sector. Propanil is a contact herbicide [1], which is used globally. This herbicide inhibits the photosynthesis of broadleaf weeds that leads to leaf chlorosis and the subsequent necrosis of leaves and other organs [2]. The herbicide is mainly applied to rice fields, which results in surface water, groundwater, and soil contamination [3-5]. Propanil has been detected at concentrations up to 3.6 mg/l in water [5], while the acceptable level of propanil in drinking water is 0.1 µg/l [6].

Propanil is usually mixed with butachlor to increase the efficiency of weed eradication [7]. The negative effects of butachlor on soil microorganisms have also been studied [8-10]. Therefore, the biodegradation of propanil may be influenced by the presence of butachlor. However, few reports describing the effects of butachlor on the degradation of propanil have been published [11].

Alginate is a natural polymer universally employed to immobilize cells in biodegradation experiments because alginate is relatively mild, chemically inert, inexpensive, and non-toxic [12]. Several bacteria and fungi such as

Fusarium oxysporum [13], *Paracoccus* sp. FLN-7 [14], *Ochrobactrum* sp. PP-2 [15], and *Spirosoma sordidissoli* TY50^T [16] have been isolated. However, to our knowledge, no publication has yet described the degradation of propanil by microorganisms immobilized in alginate.

In our previous report, propanil degradation by *A. baumannii* DT during the exponential growth phase with low cell density was determined [11, 17]. In this study, propanil degradation by *A. baumannii* DT immobilized in alginate and condensed counterparts is compared.

2. Materials and methods

2.1. Cultivation media

The mineral medium (MM) used in this study was prepared according to H.D. Duc (2017) [18]. The MM contained (in grams per liter) Na_2HPO_4 , 2.79; KH_2PO_4 , 1.00; $(\text{NH}_4)_2\text{SO}_4$, 1.00; $\text{MgSO}_4 \cdot \text{H}_2\text{O}$, 0.20; and 1.00 ml of trace mineral solution. The trace mineral solution consisted of (in grams per liter) H_3BO_3 , 0.30; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.20; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.10; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.03; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.03; $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 0.02; and $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 0.01. The pH of the MM was adjusted to 7.0 ± 0.1 . Ammonium sulfate (0.1%, w/v) and succinate (0.1%, w/v) were supplemented as a

*Corresponding author: Email: hadanhduc@gmail.com

nitrogen and carbon source, respectively. The medium was sterilized for 15 min at 121°C and stored at 4°C until further use. Pure propanil (99.6%) and herbicide with the trade name Cantanil 550EC (Thanhson Agrochem Company, Vietnam) were used. Cantanil 550EC contained 275 mg/l of propanil, 275 mg/l of butachlor, and adjuvant was used to determine the biodegradation of propanil.

2.2. Resting cells and immobilisation method

To prepare for immobilization, *A. baumannii* DT was cultured in MM amended with ammonium sulfate and succinate medium for 12 h. Bacteria were collected by centrifugation at 10,000 rpm for 5 min. Cell pellets were washed twice with sterile MM. The mixture was then re-suspended in MM used for degradation by free cells (resting cells) while 2×MM was used for immobilization.

The immobilization process was carried out according to a previous report [19] with modifications. The concentrated bacterial solution was mixed with a sterilized solution of Ca-alginate and glycerol to give final cell numbers of approximately 10^9 CFUs/ml, 3% alginate, and 10% glycerol. The solution was carefully blended and dripped into a solution containing 3% CaCl_2 (w/v) using a syringe. The beads formed in the solution were stirred for 1 h using a magnetic bar and then stored for 24 h at 4°C in this solution. The beads were collected and washed twice with MM before being used in experiments.

2.3. Propanil degradation by resting cells and immobilised cells

The degradation processes of propanil were carried out in MM with 10^9 CFUs/ml of both freely suspended and immobilized bacteria. Propanil was dissolved in absolute ethanol at 0.1 M and used as a stock solution. The degradation was carried out at propanil concentrations ranging from 0.1 to 0.7 mM. The degradation rates at various propanil concentrations were determined and expressed in mM/h. Nonlinear regression analysis was used to fit the trends of the degradation process. The obtained data were best fit by kinetic models that incorporate the equation for logarithmic degradation such as the V.H. Edwards model (1970) [20] given by $V = V_{\max} [e^{(-S/K_i)} - e^{(-S/K_i)}]$ or the Haldane equation [21] $V = (V_{\max} S) / (K_s + S + S^2/K_i)$ where V is the degradation velocity, $V_{\max}$ is the maximum degradation rate, S is the propanil concentration, K_i is the inhibition coefficient, and K_s is the half-saturation coefficient. The kinetic parameters were then calculated based on linear regression fitting of the H. Lineweaver, et al. (1934) [22] plot or double reciprocal plot. The Dixon plot was used to determine the inhibition constant in which the reciprocal of the velocity ($1/V$) was plotted against the inhibitor concentrations [i].

For the degradation of propanil in herbicide, Cantanil 550EC was added to 0.1 mM of propanil. The effects of butachlor on propanil degradation were also carried out. Pure butachlor or butachlor in the herbicide Cantanil 550EC were used at the same quantity (weight) as propanil. The incubation processes were conducted at room temperature (from 28 to 31°C) with a shaking speed of 150 rpm.

2.4. Long-term storage condition

For long-term storage, resting and entrapped bacteria were stored in Corning™ polyethylene terephthalate centrifuge tubes in the dark at 4°C. After 2 months of storage, both free and immobilized bacteria were kept at room temperature for 2 h before determining cell survival and biodegradation. The results were compared with bacteria numbers and degradation rates of fresh ones.

The number of viable bacterial cells in an alginate bead has been described by M. Schoebitz, et al. (2012) [23] with some modification. The beads (1.0 g) were transferred to 10 ml of sterile sodium citrate (6%, w/v) to dissolve at 30°C on a rotary shaker for 30 min. Then, the solution was serially diluted with MM and spread on an agar plate containing MM supplemented with ammonium sulfate and succinate. For the enumeration of non-immobilized cells, the bacteria solution was serially diluted and also spread on the plates. The number of survival bacteria was determined based on colonies emerging after being incubated for 24 h at 30°C in an incubator.

2.5. Analytical method

Propanil concentrations were measured using reverse phase high performance liquid chromatography (HPLC) (LC-10AD, Shimadzu, Japan) with a C18 column (5 μm , 250×4.6 mm; Hyperclone, Phenomenex, USA) at an absorbance of 240 nm. A mixture of acetonitrile and ultrapure water (7:3, v/v) served as a mobile phase at a flow rate of 1 ml/min.

2.6. Statistical analysis

The obtained data are shown as the mean $\pm$ standard deviation (SD). Duncan's multiple range test in the SPSS program (version 22.0) were used to determine differences among the treatments ($p < 0.05$).

3. Results and discussion

3.1. Propanil degradation by *A. baumannii* DT

A. baumannii DT isolated from soil can degrade propanil and 3,4-dichloroaniline [11]. *A. baumannii* DT was shown to utilize propanil as a sole carbon and nitrogen source in a previous report [11]. However, supplementation with succinate and ammonium sulfate increased the degradation

rates [11]. So, all experiments in this work were carried out with the amendment of these co-substrates.

The degradation by free bacteria and their immobilized counterparts were compared. The results showed that the degradation rates of propanil by immobilized cells were significantly higher than those of freely suspended cells at the same concentrations. At 0.5 mM, the complete degradation of propanil by immobilized bacteria was accomplished after 20 h while propanil degradation by free cells was not complete until after 24 h (Fig. 1). No propanil was lost in the control without alginate beads and bacteria, while the beads absorbed about 12% of the substrate after 24 h.

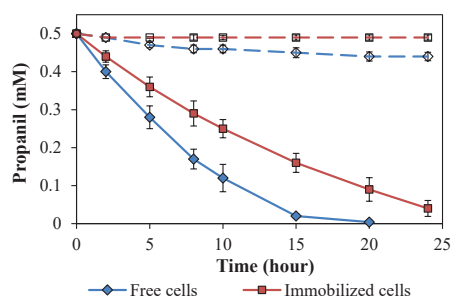


Fig. 1. Propanil degradation by free and immobilized cells (solid lines). The controls (dashed lines) were also run in parallel.

3.2. Degradation kinetics of freely suspended and immobilised cells.

The degradation rates at various concentrations showed that propanil degradation by freely suspended resting cells and immobilized cells in alginate beads both followed the Edwards model, in which degradation rates increase at low concentrations but are inhibited at high concentrations (Fig. 2). The degradation rates caused by the resting cells and their immobilized counterparts increased by 0.3 and 0.4 mM, respectively.

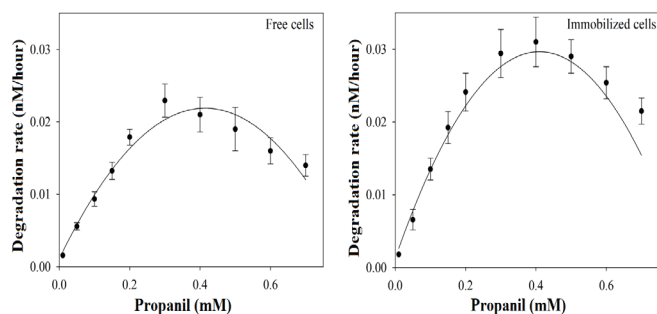


Fig. 2. Relation between propanil concentrations and degradation rates of free resting and immobilised cells.

The parameters of degradation kinetics were extracted from the Edwards equation and are shown in Table 1. The maximum degradation rate of immobilized bacteria

was statistically higher than that of the free counterparts. The inhibition coefficient of degradation, which is the concentration required to produce half maximum inhibition, by free cells was also significantly lower compared to the immobilized cells. However, the half-saturation coefficients, which are the reaction rates at half-maximum, of free cells and immobilized bacteria were not statistically different. These results showed that immobilized bacteria were more tolerant to the toxicity of propanil. Similarly, the degradation of toluene and chlorotoluene by *Comamonas testosteroni* KT5 immobilized in alginate was higher than that of freely suspended cells [24]. Propanil is a toxic compound, so the substrate inhibition occurred. The enhanced degradation of immobilized cells was because bacteria were protected by alginate matrix. In alginate beads, substrate has to diffuse through the immobilization barrier, and will then be available for the cells to utilize, which reduced the toxicity to bacteria.

Table 1. Degradation parameters of free suspended cells and immobilized cells.

Parameters	Free suspended cells	Immobilized cells
Maximum degradation rate (mM/hour)	0.034±0.003 ^a	0.053±0.005 ^b
Half-saturation coefficient (mM)	0.249±0.022 ^a	0.296±0.030 ^a
Inhibition coefficient (mM)	3.50±0.24 ^a	4.32±0.41 ^b

Data are shown as mean ±SD and different superscript letters (a and b) denote a significant difference ($p < 0.05$) between treatments in a line based on Duncan's test, whereas the same letter indicates no significant difference.

In our previous report, V_{max} , K_s and K_i of propanil utilization by freely suspended bacteria in the exponential growth phase were 0.027 ± 0.003 mM/h, 0.16 ± 0.02 mM and 0.33 ± 0.03 mM, respectively [11]. In this study, the V_{max} and K_s of concentrated bacteria were comparable higher than those of low bacteria density as described in the previous report [11]. The degradation and growth rates of bacteria in the exponential growth phase showed the activities of *A. baumannii* DT to propanil, while the degradation by concentrated bacteria provided a potential application to remediate the herbicide. Propanil detected in contaminated sites by E.G. Primel, et al. (2007) [5], of course, was mostly lower concentrations in this study. *A. baumannii* DT was investigated for degradation of various propanil concentrations, which showed the application ability to remediate in contaminated sites.

3.3. Effects of butachlor on degradation of pure propanil and degradation of propanil in herbicide Cantanil 550EC

Figure 3 shows that the percentage of propanil degradation for immobilized bacteria were significantly higher compared to those for free cells under the same conditions. The addition of pure butachlor and Cantanil 550EC decreased

the degradation rates. Similarly, the effects of butachlor on propanil degradation and growth of *A. baumannii* DT have been described in previous studies [11, 17]. A number of trace herbicides contain both butachlor and propanil, but both substrates inhibited each other in biodegradation [17]. The degradation was the lowest with the amendment of the herbicide Cantanil 550EC. These results can be explained by the negative effects from the adjuvants in the herbicide on the degradation propanil, which was also described by H.D. Duc, et al. (2020) [17].

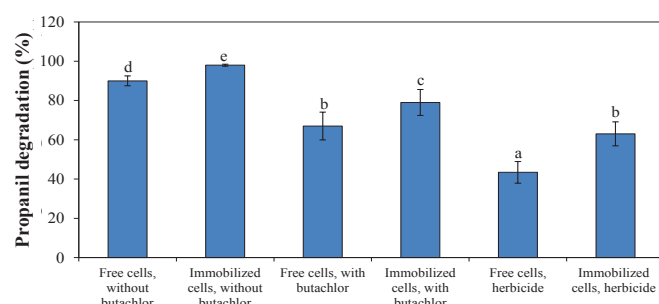


Fig. 3. Effects of butachlor on degradation of pure propanil and degradation of propanil in herbicide Cantanil 550EC. The degradation processes were carried out for 12 h at 0.1 mM propanil. Different letters (a, b, c, d and e) above the columns denote a significant difference ($p < 0.05$) among treatments.

3.4. Bacteria survival and propanil degradation for long-term storage

The number of live bacteria decreased after two months of storage. The survival percentages of bacteria amended with glycerol were higher than those of treatments without the cryoprotectant (Fig. 4). These results indicated that using glycerol as a cryoprotectant agent reduced the adverse effects of the bacteria. The survival of free and entrapped bacteria was not statistically different under the same conditions.

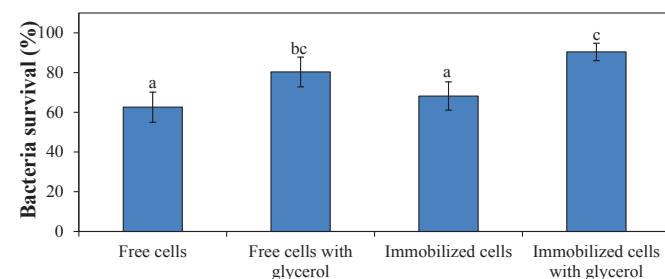


Fig. 4. Survival of *A. baumannii* DT after two months' storage at 4°C. Different letters (a, b, and c) above the columns denote a significant difference ($p < 0.05$) among treatments.

Table 2 shows that the degradation by immobilized bacteria was significantly higher than that of the resting cells, which was similar to the experiments described above. The addition of glycerol reduced propanil degradation from

about 8.2 to 15.6% at the beginning. However, the presence of glycerol resulted in a non-statistically significant reduction of degradation after two months of storage. The degradation rates of the treatments with glycerol decreased from only 6.1 to 12%, while data of treatments without glycerol were reduced from 27.9 to 36.4%.

Table 2. Propanil degradation by free cells and immobilized cells. The degradation processes were carried out for 10 h at 0.1 mM propanil.

		Propanil degradation (%)			
		Free suspended cells		Immobilized cells	
		At the beginning	After two months	At the beginning	After two months
Pure propanil	Without glycerol	92.1±4.2 ^{Dc}	55.5±8.1 ^{Ba}	98.4±1.5 ^{Cc}	68.4±7.9 ^{Bb}
	With glycerol	80.6±6.3 ^{Cab}	72.8±7.0 ^{Ca}	90.2±4.2 ^{Cb}	78.2±7.3 ^{Bab}
Herbicide	Without glycerol	43.4±5.5 ^{Bc}	15.5±4.3 ^{Aa}	63.4±6.1 ^{Bd}	30.4±5.2 ^{Ab}
	With glycerol	30.2±5.6 ^{Aa}	22.6±5.6 ^{Aa}	47.8±6.5 ^{Ab}	41.7±6.2 ^{Ab}

The lowercase superscript letters show statistically significant differences in the same row, while capitalized superscript letters indicate statistically significant differences among treatments within a column ($p < 0.05$). Data are means of the results from at least three individual experiments, and mean values and standard deviations are shown.

The results show that the addition of glycerol reduced the adverse effects of bacteria. Previous reports also presented that the survival of entrapped microorganisms is enhanced by the addition of glycerol [25, 26]. Glycerol is used as a cryoprotectant that can prevent ice crystal formation after penetration into the cells [27]. Another report shows that the addition of glycerol protects the microorganisms, increases pore size in beads, and controls the structure of dried macrocapsules [26].

4. Conclusions

A. baumannii DT immobilised in alginate showed more effective propanil degradation than free cells. It was confirmed that the chemical degradation over a wide range of concentrations by condensed free cells and immobilized cells followed the Edwards model with higher degradation rates and inhibition coefficients for immobilized bacteria. Besides, immobilized bacteria degraded propanil in an herbicide with higher rates than its free counterparts. Moreover, bacteria entrapped in alginate beads amended with glycerol reduced the adverse effects of long-term storage. These results indicate that *A. baumannii* DT immobilized in alginate can be applied to remediate propanil.

CRedit author statement

Danh Duc Ha, Thi Oanh Nguyen: Data curation, Software, Visualisation, Investigation, Writing original draft preparation, Conceptualisation, Methodology, Supervision.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] R.J. Smith (1965), "Propanil and mixtures with propanil for weed control in rice", *Weeds*, **13**, pp.236-238, DOI: 10.2307/4041035.
- [2] C. Tomlin (2009), *The Pesticide Manual*, 15th ed., BCPC Publications, pp.944-945.
- [3] J.M. Dabrowski, S.K. Peall, A.V. Niekerk, et al. (2002), "Predicting runoff-induced pesticide input in agricultural sub-catchment surface waters: Linking catchment variable sand contamination", *Water Research*, **36**, pp.4975-4984, DOI: 10.1016/S0043-1354(02)00234-8.
- [4] E. Silva, S. Batista, P. Viana, et al. (2006), "Pesticides and nitrates in groundwater from oriziculture areas of the 'Baixo Sado' region (Portugal)", *International Journal of Environmental Analytical Chemistry*, **86**, pp.955-972, DOI: 10.1080/03067310600833336.
- [5] E.G. Primel, R. Zanella, M.H.S. Kruz, et al. (2007), "Risk assessment of surface water contamination by herbicide residues: Monitoring of propanil degradation in irrigated rice field waters using HPLC-UV and confirmation by GC-MS", *Journal of The Brazilian Chemical Society*, **18(3)**, pp.585-589, DOI: 10.1590/S0103-50532007000300014.
- [6] T. Dolan, P. Howsam, D.J. Parsons, et al. (2013), "Is the EU drinking water directive standard for pesticides in drinking water consistent with the precautionary principle?", *Environmental Science & Technology*, **47(10)**, pp.4999-5006, DOI: 10.1021/es304955g.
- [7] P.O. Imeokparia, S.T.O. Lagoke, B.A. Olunuga (1992), "Evaluation of postemergence herbicides for broad-spectrum weed control in three cultivars of flooded rice in Nigeria", *Crop Protection*, **11(2)**, pp.165-173, DOI: 10.1016/0261-2194(92)90101-A.
- [8] H. Min, Y.F. Ye, Z.Y. Chen, et al. (2001), "Effects of butachlor on microbial populations and enzyme activities in paddy soil", *Journal of Environmental Science and Health B*, **36(5)**, pp.581-595, DOI: 10.1081/PFC-100106187.
- [9] J.H. Wang, Y.T. Lu, G.Q. Shen (2007), "Combined effects of cadmium and butachlor on soil enzyme activities and microbial community structure", *Environmental Geology*, **51**, pp.1221-1228, DOI: 10.1007/s00254-006-0414-y.
- [10] H. Fang, Y.L. Yu, X.G. Wang, et al. (2009), "Persistence of the herbicide butachlor in soil after repeated applications and its effects on soil microbial functional diversity", *Journal of Environmental Science and Health B*, **44**, pp.123-129, DOI: 10.1080/10934520802539657.
- [11] N.T. Oanh, H.D. Duc, D.T.H. Ngoc, et al. (2020), "Biodegradation of propanil by *Acinetobacter baumannii* DT in a biofilm-batch reactor and effects of butachlor on the degradation process", *FEMS Microbiology Letters*, **367**, DOI: 10.1093/femsle/fnaa005.
- [12] A. Partovinia, B. Rasekh (2018), "Review of the immobilized microbial cell systems for bioremediation of petroleum hydrocarbons polluted environments", *Critical Reviews in Environmental Science and Technology*, **48(1)**, pp.1-38, DOI: 10.1080/10643389.2018.1439652.
- [13] H. Reichel, H. Sisler, D. Kaufman (1991), "Inducers, substrates, and inhibitors of a propanil-degrading amidase of *Fusarium oxysporum*", *Pesticide Biochemistry and Physiology*, **39(3)**, pp.240-250, DOI: 10.1016/0048-3575(91)90119-7.
- [14] J. Zhang, J.G. Yin, B.J. Hang, et al. (2012), "Cloning of a novel arylamidase gene from *Paracoccus* sp. strain FLN-7 that hydrolyzes amide pesticides", *Applied and Environmental Microbiology*, **78**, pp.4848-4855, DOI: 10.1128/AEM.00320-12.
- [15] L. Zhang, Q. Hu, P. Hang, et al. (2019a), "Characterisation of an arylamidase from a newly isolated propaniltransforming strain of *Ochrobactrum* sp. PP-2", *Ecotoxicology and Environmental Safety*, **167**, pp.122-129, DOI: 10.1016/j.ecoenv.2018.09.127.
- [16] L. Zhang, X.Y. Zhou, X.J. Su, et al. (2019b), "*Spirosoma sordidoli* sp. nov., a propanil-degrading bacterium isolated from a herbicide-contaminated soil", *Antonie Van Leeuwenhoek*, **112**, pp.1523-1532, DOI: 10.1007/s10482-019-01278-4.
- [17] H.D. Duc, N.T.D. Thuy, H.T.T. Truc, et al. (2020), "Degradation of butachlor and propanil by *Pseudomonas* sp. strain But2 and *Acinetobacter baumannii* strain DT", *FEMS Microbiology Letters*, **367**, DOI: 10.1093/femsle/fnaa151.
- [18] H.D. Duc (2017), "Degradation of chlorotoluenes by *Comamonas testosteroni* KT5", *Applied Biological Chemistry*, **60**, pp.457-465, DOI: 10.1007/s13765-017-0299-3.
- [19] X. Bai, Z. Ye, Y. Li, et al. (2010), "Preparation and characterization of a novel macroporous immobilized micro-organism carrier", *Biochemical Engineering Journal*, **49(2)**, pp.264-270, DOI: 10.1016/j.bej.2010.01.004.
- [20] V.H. Edwards (1970), "The influence of high substrate concentration on microbial kinetics", *Biotechnology and Bioengineering*, **12**, pp.679-712, DOI: 10.1002/bit.260120504.
- [21] J.K. Versyck, E.J. Claes, F.J.V. Impe (1997), "Practical identification of unstructured growth kinetics by application of optimal experimental design", *Biotechnology Progress*, **13(5)**, pp.524-531, DOI: 10.1021/bp970080j.
- [22] H. Lineweaver, D. Burk (1934), "The determination of enzyme dissociation constants", *Journal of the American Chemical Society*, **56(3)**, pp.658-666, DOI: 10.1021/ja01318a036.
- [23] M. Schoebitz, H. Simonin, D. Poncelet (2012), "Starch filler and osmoprotectants improve the survival of rhizobacteria in dried alginate beads", *Journal of Microencapsulation*, **29(6)**, pp.532-538, DOI: 10.3109/02652048.2012.665090.
- [24] H.D. Duc (2020), "Determination of degradation of toluene and chlorotoluenes by *Comamonas testosteroni* KT5 immobilized in alginate", *Vietnam Journal of Science, Technology and Engineering B*, **62(6)**, pp.1-6 (in Vietnamese).
- [25] L. Kearney, M. Upton, A. McLoughlin (1990), "Enhancing the viability of *Lactobacillus plantarum* inoculum by immobilizing the cells in calcium-alginate beads incorporating cryoprotectants", *Applied and Environmental Microbiology*, **56**, pp.3112-3116, DOI: 10.1128/aem.56.10.3112-3116.1990.
- [26] C.Z. Perez, E. Ritte, L. Chernin, et al. (2002), "Preservation of chitinolytic pantoae agglomerans in a viable form by cellular dried alginate-based carriers", *Biotechnology Progress*, **18(6)**, pp.1133-1140, DOI: 10.1021/bp025532t.
- [27] M.T. Madigan, J.M. Martinko (1996), *Brock Biology of Microorganisms*, Prentice Hall, College Div., 1036pp.

Synthesis and characterisation of carboxymethyl cellulose with high degree substitution from Vietnamese pineapple leaf waste

Thi Dieu Phuong Nguyen, Nhu Thi Le, Tien Manh Vu, Truong Sinh Pham, Thi Dao Phan, Ngoc Lan Pham, Thi Tuyet Mai Phan*

Faculty of Chemistry, University of Science, Vietnam National University - Hanoi, 334, Nguyen Trai Street, Thanh Xuan District, Hanoi, Vietnam

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Abstract:

One of the most popular derivatives produced by carboxymethylating cellulose's hydroxyl groups is CMC. The use of CMC as a binder, thickening additive, film maker, suspending aid, and biodegradable substance has a lot of promise. Cellulose was first swelled in a NaOH solution to produce CMC, and then it was treated with monochloroacetic acid in alcohol. In this work, cellulose was successfully extracted from pineapple leaf waste by 0.75 M NaOH at 90°C and 5 M HNO₃ at 70°C for 1.5 h and 5 h, respectively. The obtained cellulose fibres, with average diameters of 150-300 nm, were converted to carboxymethyl cellulose (CMC) by esterification. The pure cellulose was soaked in a solution mixture of isopropanol and NaOH for 2 h. It was then reacted with chloroacetic acid (MCA) at 60°C for 1.5 h. The optimum conditions for carboxymethylation were found to be 5 g cellulose, 1.5 g MCA, and 15 ml 16% w/v NaOH. The obtained CMC had a high degree of substitution (DS) of 2.3. The properties of CMC were determined.

Keywords: carboxymethyl cellulose, cellulose degree of substitution, Vietnamese pineapple leaf waste.

Classification number: 2.2

1. Introduction

CMC is one of the most common derivatives obtained by the carboxymethylation of the hydroxyl groups of cellulose. CMC exhibits a great potential as thickening additives, film former, binder, suspending aid, and biodegradable materials [1-4]. In order to obtain CMC, first, cellulose was swollen in a NaOH solution, and then reacted with monochloroacetic acid in alcohol [5]. In this reaction, the sodium carboxymethyl groups substitutes the hydroxyl groups in C-2, C-3, and C-6 of the anhydro-glucose unit. It seems that substitution in the C2 position is slightly more dominant [6]. The solubility of CMC in water is a key parameter in their applications and a higher DS will normally improve the solubility of the CMC. Theoretically, the maximum DS is 3. CMC is soluble in water when DS is higher than 0.4. Most research [5-7] has achieved a DS ranging from 0.5 to 2.0. The DS of commercially available CMC is in the range of 0.4-1.4. Recently, many researchers are trying to find a way to achieve CMC with higher DS in order to improve commercial products. It has been shown that cellulose sources have a very important role since the crystalline content and the size of cellulose are the most crucial parameters for attaining CMC with a high DS [5]. Finding raw materials based on agricultural by-products to produce CMC has been obtaining more and more interest from researchers. For example, the use of cellulosic sources as an alternative to virgin softwood pulp to synthesize

CMC has been reported [4-10]. N. Haleem, et al. (2014) [7] obtained cellulose fibre with sizes of 15-20 µm from cotton waste by acid hydrolysis with 10 M H₂SO₄ at 70-80°C for 1 h. Generally, cellulose extraction is a complicated process, and several steps have to be performed to gain a high degree of substitution. Thus, finding new, available, and cheap cellulose sources for CMC preparation is of great significance.

Pineapple is one of the most popular tropical fruits in Vietnam. During harvesting, pineapple leaves are discarded. Their release into the environment, in turn, leads to pollution of our living environmental system [11, 12]. However, pineapple leaves are an abundantly available and potential source of cellulose. These leaves contain about 65-70% dry weight of cellulose [11, 13]. The process of extracting cellulose from pineapple leaf is simple [14-18], and the extracted cellulose has relatively low crystal content as compared to that of cotton waste [7], paper sludge [8], rice straw [19, 20], and other sources [3, 4, 6, 19]. These two factors positively affect the possibility of synthesis of CMC with high DS.

The purpose of this work is to confirm the potential of Vietnamese pineapple leaf waste as a raw material for industrial production of CMC with high degree of substitution.

*Corresponding author: Email: maimophong@gmail.com

2. Materials and methods

2.1. Materials

The pineapple leaves were collected from the pineapple Dong Giao farm, Tam Diep city, Ninh Binh province, Vietnam. The pineapple leaves were cut into 5 mm using a grinding machine, then dried in an oven at 60°C for 24 h. The samples were kept in zipper polyethylene bags.

For this study, the following acids, such as nitric acid 65%, monochloroacetic acid (MCA) (UK) 99.7%, acetic acid 99.9% and sodium hydroxide 99.9% (Merck), as well as methanol 99.8% and ethanol 99.9% from Xilong Chemical, isopropanol 99.7% (Merck), and acetone 99.8% (Merck) were used. They were of high purity.

2.2. Methods

2.2.1. Cellulose extraction: The extraction process of cellulose from pineapple leaf waste is illustrated in Fig. 1.

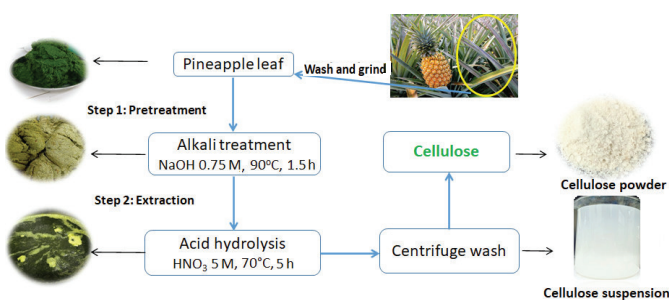


Fig. 1. Schematic illustration for the cellulose extraction process from pineapple leaf waste.

The dry pineapple leaf waste powder was treated with 0.75 M NaOH at 90°C and 5 M HNO₃ at 70°C for 1.5 and 5 h, respectively. This mixture was then centrifuged at 3000 rpm for 20 min to remove large particles and washed with warm distilled water until the indicator paper did not change colour. The residue was dried in an oven at 60°C overnight until the weight remained constant. Finally, the dried cellulose was ground and kept in a polyethylene bag for the next process modification.

The yield of the cellulose was gravimetrically determined and expressed as the weight of the extracted dried cellulose to 100 g of the dried pineapple leaf used for extraction. This was repeated 3 times for each extraction condition and the yield average and the standard deviation were calculated.

Equation (1) below was used for the determination of the yield of cellulose:

$$H(\%) = \frac{m}{m_0} \times 100 \quad (1)$$

where m_0 is the weight of initial dried pineapple leaf powder, m is the weight of obtained cellulose, and H is the yield of cellulose (named as HC).

2.2.2. Synthesis of CMC: Five grams of extracted cellulose from Vietnam's pineapple leaf powder was added to 150 ml of isopropanol under continuous stirring for 60 min. Then, 15 ml of 16% NaOH solution was dripped into the mixture and further stirred for 1 h at room temperature. The carboxymethylation was started when y grams of MCA ($y=0.5, 1.0, 1.5$, and 2.0 g) were added under continuous stirring for another 90 min at 60°C. The solid part was neutralized with acetic acid to pH=7.0 and washed two times by soaking in 20 ml of ethanol to remove undesirable by-products. The obtained CMC was filtered and dried at 60°C until it reached constant weight, and it was then kept in the polyethylene bag. Equation (1) above is also used to determine the yield of the CMC (HCMC) where m is the weight of the obtained CMC, and m_0 is the weight of the cellulose used for the CMC synthesis.

2.2.3. Infrared spectroscopy: FTIR analysis of the obtained cellulose and CMC were performed by a FT/IR-6300 spectrometer using KBr pellet methods. The spectral resolution was 4 cm⁻¹ and the absorption region was 600-4000 cm⁻¹.

2.3.4. X-ray diffraction: The crystallinity index (CrI) of the obtained cellulose and CMC were analysed by Shimadzu XRD-6100 diffractometer. The diffraction angle ranged from 5 to 80° (0.05°/min). The measurement was carried out at 30 kV and 15 mA under Cu K_α radiation. The CrI of the samples was calculated by Eq. (2):

$$CrI(\%) = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (2)$$

where I_{002} : ($2\theta=22.8^\circ$) and I_{am} : ($2\theta=18^\circ$) correspond to the crystalline and amorphous regions, respectively [21].

2.3.5. Particle size measurement: The particle size of the obtained cellulose was measured by a Shimadzu Sald-2001 Analyser. First, the cellulose suspension was diluted to 0.05-0.2 wt% concentration. Then, it was measured in a container.

2.3.6. Scanning electron microscopy (SEM): The surface of the separated cellulose is observed by the SEM images. The SEM images were done on a Hitachi S4800-NHE scanning electron microscope (Hitachi Co., Ltd., Japan).

2.3.7. Determination of Degree of Substitution (DS): Degree of Substitution of CMC is determined according to ASTM 1994 [22].

2.3.8. Sample preparation: 350 ml of ethanol was added to a 500 ml conical flask containing 5 g of CMC to the nearest 0.1 mg. The suspension in the flask was shaken for 30 min, then filtered through a porous funnel. The solvent was removed by heating at 100°C for 60 min. The sample was dried in an oven at 110°C until a constant weight was reached.

2.3.9. Procedure: 2 g of the dried obtained substance to the nearest 0.1 mg was put to a tared porcelain crucible. The crucible was carefully charred with a small flame, then with a large flame for 10 min. The cooled residue was moistened with 3-5 ml of concentrated sulfuric acid. Next, the crucible was cautiously heated until the fuming was finished. Then, the crucible was cooled to room temperature. About 1 g of ammonium carbonate was added. The powder was distributed over the content of the entire crucible. It was heated again with a small flame until the fuming stopped, and then was maintained at a dull red heat for 10 min. The treatment procedure was repeated with sulfuric acid and ammonium carbonate if the residual sodium sulphate still contained some carbon. The crucible was cooled in a desiccator and weighed. The sodium content, A, was calculated by Eq. (3):

$$A (\%) = \frac{a \times 32.28}{b} \quad (3)$$

where a is the weight of the sodium sulphate residue and b is the weight of the dry sample.

The degree of substitution was calculated by Eq. (4):

$$DS = \frac{162 \times A}{2300 - 80 \times A} \quad (4)$$

where 162 is the molecular weight of the glucose unit and 80 is the net increment in the anhydrous glucose unit for every substituted carboxymethyl group.

3. Results and discussion

3.1. Extraction of cellulose from Vietnam's pineapple leaf waste

The extracted cellulose yield was 55±1.75 wt.%. This yield value is much higher than that of cellulose extracted from other agricultural biomasses such as 37.67 wt.% from the Baobab fruit shell [19] and 32 wt.% from rice straw [20]. The high cellulose content would guarantee a lower price for cellulose derivatives.

The morphology of the obtained cellulose is shown in Fig. 2.

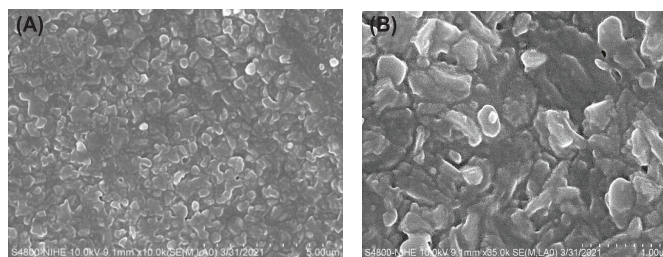


Fig. 2. SEM images of pineapple leaf cellulose at (A) 10,000 x magnification (5 µm size bar) and (B) 35,000 magnification (1 µm size bar).

As can be seen in from the SEM images, the obtained cellulose showed uniform size with average diameters of 150-300 nm, which was similar to that of another reported work [23]. It is worth mentioning that the separation of cellulose in this work is easier and the cellulose obtained had a significantly higher yield compared to that of previous reports [7, 14, 15, 16, 17]. Of course, this comparison is only relative because cellulose yield depends on the method and conditions of separation. The FTIR spectroscopy of obtained cellulose is displayed in Fig. 3.

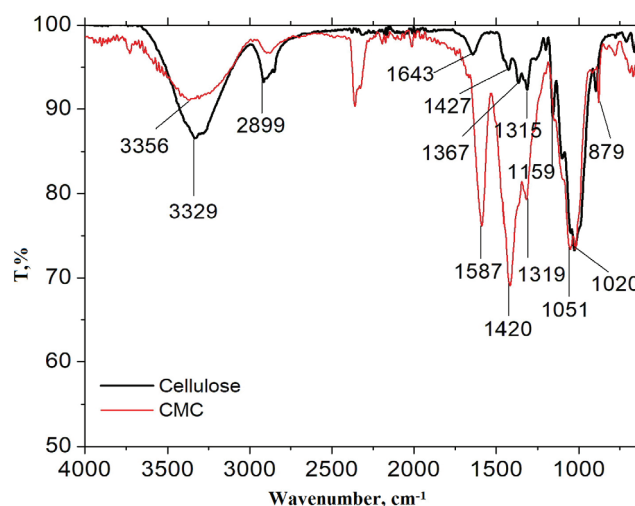


Fig. 3. FTIR spectroscopy of extracted cellulose and CMC from pineapple leaf waste.

As for cellulose, as shown in Fig. 3, there is a large band at 3329 cm⁻¹ corresponding to the OH group. The peak at 2899 cm⁻¹ represents the C-H stretching vibrations. The peak at 1159 cm⁻¹ can be assigned to C-O-C stretching of the β(1,4)-glycosidic linkage. Besides, the peaks at 1367 and 1427 cm⁻¹ are attributed to the -C-H and -C-O bending vibrations, respectively, in the polysaccharide rings. The vibration of the -C-O group of secondary alcohols in the cellulose chain backbone appears at 1105 cm⁻¹. The absorption band range of 879-1051 cm⁻¹ is assigned to the

β -(4,1)-glycosidic linkages between the glucose units of cellulose [4-10]. In this study, the crystalline nature of the obtained cellulose was investigated by use of XRD [13, 14, 21]. The XRD diffractogram of pineapple leaf cellulose (PLC) is shown in Fig. 4.

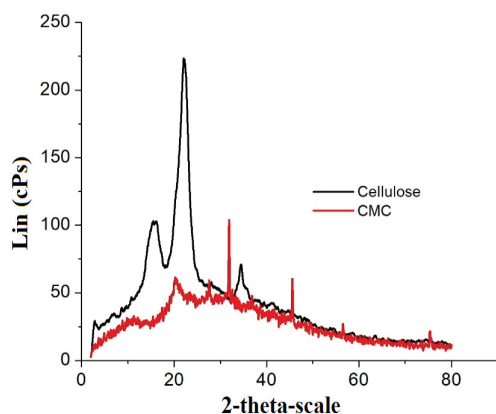


Fig. 4. XRD diffractogram of isolated cellulose and synthesized CMC from pineapple leaf waste.

As can be seen, the XRD diagrams of PLC showed peaks at $2\theta=16.6^\circ$, 22.8° , and 35.4° , which are attributed to the characteristic peaks of cellulose. The crystallinity index (CrI) of PLC is 68.7 and this CrI value is significantly lower than 82.7, which was reported by M. Mahardika, et al. (2008) [24]. As we all know, the crystallinity of cellulose depends on the method of separation and treatment. Thus, the separation method used in this study gives cellulose with relatively low crystallinity.

3.2. Synthesis of CMC from Vietnam's pineapple leaf cellulose

3.2.1. Distribution size of cellulose in suspension: Cellulose size plays an important role in gaining higher yields and degrees of substitution of CMC. In the carboxymethylation process, cellulose is often dispersed in the suspension of the solvent. The solvent increases the accessibility of the etherizing reagent to the cellulose chains [4, 7, 8, 22]. To date, many researchers have focused on the effect of cellulose size on the DS of CMC in the solid state. However, to our knowledge, there is no publication reporting on this effect on the DS of CMC in suspension, as well as on the efficiency of the denaturation reaction. This study is dealing with the effect of solvent on the cellulose fibre size in suspension. The pineapple leaf cellulose, with an average size of 150-300 nm, was ultrasonicated and dispersed in water, ethanol, and isopropanol. Spectra of cellulose size distributions are shown in Fig. 5.

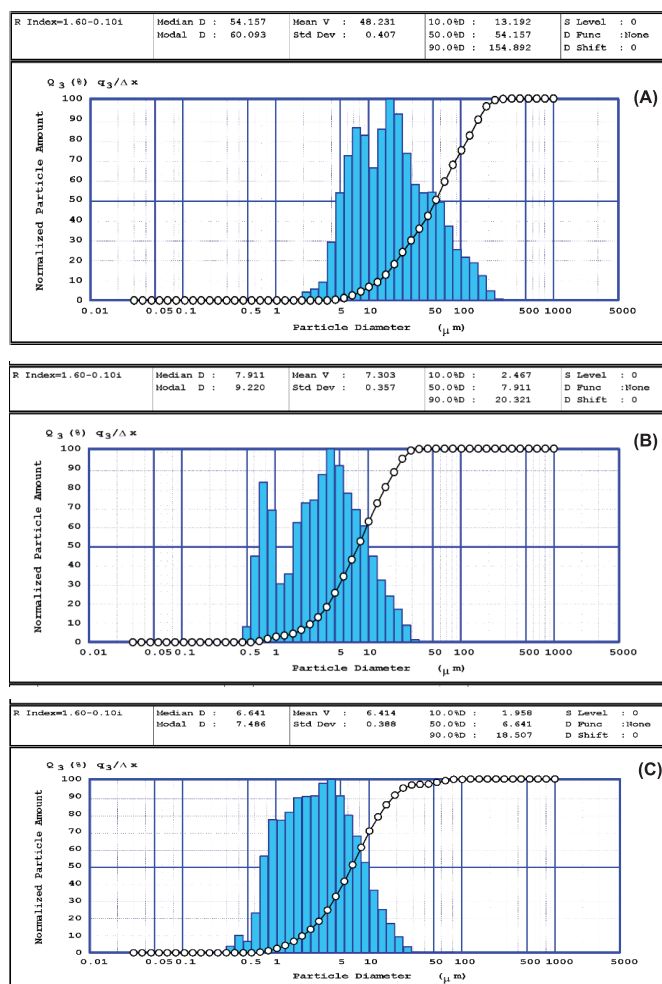


Fig. 5. Particle size distribution spectrum of cellulose in different solvents: (A) in water, (B) in ethanol, and (C) in isopropanol.

As can be seen, the average diameter of cellulose in water, ethanol, and isopropanol were 54.157, 7.911, and 6.641 μm , respectively. The cellulose size distribution is relatively narrow for isopropanol. Thus, isopropanol appears to be the best solvent to disperse cellulose. The differences in the particle sizes of cellulose can be due to the difference in the polarities and stereochemistry of the three solvents. The polarity index value of isopropanol, ethanol, and water are 5.0, 6.6, and 9.0, respectively. This implies that the lower the polarity of the solvent, the higher its dispersion for cellulose. These results are similar to those of other studies [4, 7, 8, 23] and serve as additional evidence of the successful synthesis of CMC in isopropanol [6, 25].

3.2.2. Effect of cellulose size on DS and yield of CMC: The reactant's accessibility and the presence of the activated hydroxyl groups are very important for the carboxymethylation reaction. As the particle size decreases, surface area and the free -OH groups for substitution increase, which leads to the reaction yield increasing.

Moreover, reduced cellulose particle size has larger specific surface areas meaning more cellulose accessibility for the reactants, and the reaction occurs at a faster rate [26-28]. In this work, the influence of the cellulose size in a suspension of isopropanol on the DS and yield of carboxymethylation reaction was studied. Cellulose was isolated from pineapple leaf waste at different concentration of HNO_3 (3, 4, 5 M) while other conditions were kept unchanged. The average sizes of the obtained cellulose in isopropanol were 42.421, 19.189, and 6.641 μm respectively. The DS and yield of CMC are shown in Table 1.

Table 1. The yield and DS of CMC synthesized with different sizes of cellulose in isopropanol.

Average diameter of cellulose, μm	6.641	19.189	42.421
H_{CMC} , %	136.6	121.2	115.1
DS	2.3	2.0	1.9

It is seen that the DS of CMC depends greatly on the size of cellulose in suspension. DS decreases with the increasing size of cellulose and reached 2.3 for cellulose with an average size of 6.641 μm , while cellulose with an average size of 42.421 μm produced a DS of only 1.9.

The yield of CMC greatly depends on the amount of monochloroacetic acid (MCA) used. The weight ratio of MCA to cellulose changed from 0.1 to 0.4. The yields of CMC and its dependence on MCA/cellulose ratios are shown in Table 2.

Table 2. The yield and DS of CMC synthesized with various amount of MCA.

Ratio of $m_{\text{MCA}}/m_{\text{cellulose}}$	0.1	0.2	0.3	0.4
H_{CMC} (%)	112.7	122.8	136.6	113.5

It can be seen from Table 2 that a maximum yield of 136.6% was obtained with an $m_{\text{MCA}}/m_{\text{cellulose}}$ ratio of 0.3. There was an increase in the yield of CMC with an increase of $m_{\text{MCA}}/m_{\text{cellulose}}$ ratio up to 0.3. The increase of CMC yield could be related to the greater availability of the acetate ions at higher concentrations. Nevertheless, as shown, further increase in $m_{\text{MCA}}/m_{\text{cellulose}}$ ratio leads to the CMC yield slightly decreasing. This could be due to the occurrence of undesired side reactions at high MCA amounts.

3.2.3. Structural characterisation of CMC: The CMC structure was characterized by FTIR spectroscopy, and the spectrum (see in Fig. 3).

From the IR spectra of CMC, a broad absorption band at 3356 cm^{-1} was found, which indicated the presence of O-H groups. The band at 2898 cm^{-1} is attributed to the C-H stretching vibration. The spectra shows peaks at 1319 and 1159 cm^{-1} , which are assigned to the C-O-C stretch vibrations

in the β (1,4)-glycosidic linkage. The absorption band at 1105 cm^{-1} is related to the C-O group of secondary alcohols and ethers in the cellulose molecules. The vibrations at 1051 and 1020 cm^{-1} are typical for the β -(1,4)-glycosidic linkages [8, 20, 29]. Besides, a new strong peak appears at 1587 cm^{-1} , corresponding to the COO- stretching vibrations, and also at 1420 cm^{-1} representing the salts of carboxyl groups. These two peaks are absent in the FTIR spectrum of cellulose (Fig. 3). A similar result was also shown by other researchers. For example, A.A.Y. Ahmed [19] for Baobab fruit shell and S. Sophonputtanaphoca [21] for pineapple leaves.

Figure 4 presents the XRD diffractogram of CMC from pineapple leaf waste. It can be seen that the cellulose has greater crystallinity as compared to CMC. Besides, fewer peaks were found for CMC in comparison with cellulose. It is notable that the characteristic peaks at $2\theta=16.6^\circ$, 22.8° , and 35.4° for CMC are broader and the intensity was significantly reduced. This means that this CMC represents a more amorphous structure than cellulose. Note that the typical peaks at $2\theta=16.6^\circ$ and 35.4° for cellulose are not present in the CMC curve. This shows that the formation of CMC - a product of carboxymethylation - has reduced the crystallinity of the reaction system. Indeed, the estimated crystallinity index was 68.7 for cellulose and 26.7 for CMC. The CMC being more amorphous than cellulose proves a more disordered molecular arrangement of CMC as compared to isolated holocellulose. This disordered molecular arrangement may be related to the cleavage of hydrogen bonds in cellulose by carboxymethyl substitution.

4. Conclusions

The cellulose extraction from Vietnamese pineapple leaf waste was successfully performed. The maximum extraction yield was $55\pm 1.75\text{ wt.}\%$ by using 0.75 M NaOH at 90°C for 1.5 h, and by 5 M HNO_3 at 70°C for 5 h. The average diameter of extracted cellulose was in the range of 150-300 nm. Pure cellulose was converted to CMC by esterification. The results showed that cellulose size and its distribution have a strong influence on the effectiveness of the carboxymethylation reaction. The DS and yield of CMC increases with decreasing the size of cellulose in suspension. The obtained CMC had a degree of substitution (DS) of 2.3 and a yield of 136.6%.

The study shows the successful separation of cellulose from Vietnamese pineapple leaf waste and the high-efficiency conversion of it into CMC, which both have great significance in utilizing pineapple leaf waste to create high-value products that contribute to environmental protection.

CRediT author statement

Thi Dieu Phuong Nguyen, Nhu Thi Le: Conceptualisation, Methodology, Software, Resources, Writing - Review and Editing; Tien Manh Vu, Truong Sinh Pham, Thi Dao Phan: Data curation, Software, Writing original draft preparation; Ngoc Lan Pham, Thi Tuyet Mai Phan: Supervision, Software, Validation.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] V. Stigsson, G. Kloow, U. Germgard (2001), "An historical overview of carboxymethyl cellulose (CMC) production on an industrial scale", *Paper Asia*, **17**(10), pp.16-21.
- [2] N.S.V. Capanema, A.A.P. Mansur, A.C.D. Jesus, et al. (2018), "Superabsorbent crosslinked carboxymethyl cellulose-PEG hydrogels for potential wound dressing applications", *Int. J. Bio. Macromol.*, **108**, pp.1218-1234, DOI: 10.1016/j.ijbiomac.2017.08.124.
- [3] M.S. Rahman, M.I.H. Mondal, M.S. Yeasmin, et al. (2020), "Conversion of lignocellulosic corn agro-waste into cellulose derivative and its potential application as pharmaceutical excipient", *Process.*, DOI: 10.3390/pr8060711.
- [4] I.H. Mondal, M.S. Yeasmin, M.S. Rahman (2015), "Preparation of food grade carboxymethyl cellulose from corn husk agrowaste", *Int. J. Biol. Macromol.*, **79**, pp.144-150, DOI: 10.1016/j.ijbiomac.2015.04.061.
- [5] M.S. Yeasmin, M.I.H. Mondal (2015), "Synthesis of highly substituted carboxymethyl cellulose depending on cellulose particle size", *Int. J. Biol. Macromol.*, **80**, pp.725-731, DOI: 10.1016/j.ijbiomac.2015.07.040.
- [6] V.L. Pushpamalar, S.J. Langford, M. Ahmad, et al. (2006), "Optimization of reaction conditions for preparing carboxymethyl cellulose from sago waste", *Carbohydr. Polym.*, **64**(2), pp.312-318, DOI: 10.1016/j.carbpol.2005.12.003.
- [7] N. Haleem, M. Arshad, M. Shahid, et al. (2014), "Synthesis of carboxymethyl cellulose from waste of cotton ginning industry", *Carbohydr. Polym.*, **113**, pp.249-255, DOI: 10.1016/j.carbpol.2014.07.023.
- [8] X. He, S. Wu, D. Fua, et al. (2009), "Preparation of sodium CMC from paper sludge", *J. Chem. Technol. Biotechnol.*, **84**(3), pp.427-434, DOI: 10.1002/jctb.2057.
- [9] C.H. Ünlü (2013), "Carboxymethylcellulose from recycled newspaper in aqueous medium", *Carbohydr. Polym.*, **97**(1), pp.159-164, DOI: 10.1016/j.carbpol.2013.04.039.
- [10] S.A. Asl, M. Mousavi, M. Labbafi (2017), "Synthesis and characterization of carboxymethyl cellulose from sugarcane bagasse", *J. F. Process. Technol.*, **8**(8), pp.1-6, DOI:10.4172/2157-7110.1000687.
- [11] M.E.R. Cassellis, M.E.S. Pardo, M.R. Lopez, et al. (2014), "Structural, physicochemical and functional properties of industrial residues of pineapple", *Cellul. Chem. Technol.*, **48**(7), pp.633-641.
- [12] T.T.M. Phan, T.H. Pham (2019), "Potential biogas production from wasted pineapple leaves", *Chem. J.*, **57**(6E1, 2), pp.235-239.
- [13] M.E.S. Pardo, M.E.R. Cassellis, R.M. Escobedo, et al. (2014), "Chemical characterisation of the industrial residues of the pineapple", *J. Agri. Chem. Environ.*, **3**(2), pp.53-56, DOI: 10.4236/jacen.2014.32B009.
- [14] G.I.B. Lopez, R.E.R. Alcudia, L. Veleza, et al. (2016), "Extraction and characterization of cellulose from agro-industrial waste of pineapple (*Ananas comosus* L. Merrill) crowns", *Chem. Sci. Rev. Lett.*, **5**(17), pp.198-204.
- [15] I.M. Fareez, N.A. Ibrahim, W.M.H.W. Yaacob, et al. (2018), "Characteristics of cellulose extracted from Jospine pineapple leaf fibre after alkali treatment followed by extensive bleaching", *Cellul.*, **25**(8), pp.4407-4421, DOI: 10.1007/s10570-018-1878-0.
- [16] N.A. Kassim, A.Z. Mohamed, E.S. Zainudin, et al. (2019), "Isolation and characterization of macerated cellulose from pineapple leaf", *Bioresour.*, **14**(1), pp.1198-1209, DOI:10.15376/biores.14.1.1198-1209.
- [17] S.B. Suhaimi, I. Patthare, S. Mooktida, et al. (2017), "Synthesis of methyl cellulose from nang lae pineapple leaves and production of methyl cellulose film", *Current Appl. Sci. Technol.*, **17**(2), pp.233-244.
- [18] M.T.T. Phan, L.N. Pham, L.H. Nguyen, et al. (2021), "Investigation on synthesis of hydrogel starting from Vietnamese pineapple leaf waste-derived carboxymethylcellulose", *J. Analyti. Methods in Chem*, DOI: 10.1155/2021/6639964.
- [19] A.A.Y. Ahmed, H.M. Taisser (2018), "Synthesis and characteristic of carboxymethyl cellulose from baobab (*Adansonia digitata* L.) fruit shell", *Inter. J. Eng. Appl. Sci.*, **5**(12), pp.1-10, DOI: 10.31873/IJEAS.5.12.02.
- [20] M.T.T. Phan, T.T. La, T.H.A. Ngo (2020), "Study on extracting hemicellulose, cellulose and carboxymethyl cellulose from Vietnamese rice straw waste", *Vietnam J. Sci. Technol. Engin.*, **63**(1), pp.15-20, DOI: 10.31276/VJSTE.63(1).15-20.
- [21] S. Sophonputtanaphoca, P. Chutong, L. Cha-aim, et al. (2019), "Potential of Thai rice straw as a raw material for the synthesis of carboxymethylcellulose", *Inter. Food Research. J.*, **26**(3), pp.969-978.
- [22] L. Segal, J.J. Creely, J.A.E. Martin, et al. (1959), "An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer", *Text. Res. J.*, **29**(10), pp.786-794, DOI: 10.1177/004051755902901003.
- [23] ASTM (1994), *Standard Test Methods for Sodium Carboxymethyl cellulose*, ASTM Committee on standards, Philadelphia, pp.291-298.
- [24] M. Mahardika, H. Abrial, A. Kasim, et al. (2018), "Production of nanocellulose from pineapple leaf fibers via high-shear homogenization and ultrasonication", *Fibers*, **6**(28), DOI: 10.3390/fib6020028.
- [25] S. Zhang, F. Li, J. Yu, et al. (2009), "Disolved state and viscosity properties of cellulose in a NaOH complex solvent", *Cellul. Chem. Technol.*, **43**(7-8), pp.241-249.
- [26] M.J. Nayef (2011), "Structure rheology of carboxymethyl cellulose (CMC) solutions", *B.Sc. in Chemical Engineering*, **1**, pp.1-103.
- [27] M.A. Millett, A.J. Baker, L.D. Scatter (1976), "Physical and chemical pretreatments for enhancing cellulose saccharification", *Biotechnol. Bioeng. Symp.*, **6**, pp.125-153.
- [28] L.T. Fan, Y. Lee, M.M. Gharpuray (1982), "The nature of lignocellulosics and their pretreatment for enzymatic hydrolysis", *Adv. Biochem. Eng.*, **23**, pp.157-187.
- [29] T.T.M. Phan, T.S. Ngo (2020), "Pectin and cellulose extraction from passion fruit peel waste", *Vietnam J. Sci. Technol. Eng.*, **62**(1), pp.32-37, DOI: 10.31276/VJSTE.62(1).32-37.

Curcuminoids from the Vietnamese *Curcuma longa*: Phytochemical analysis and biological activities

Thi Thu Ha Nguyen^{1,2}, Thi Tu Anh Le¹, Thanh Tra Nguyen^{1,2}, The Son Ninh^{1*}

¹Institute of Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Street, Nghia Do Ward, Cau Giay District, Hanoi, Vietnam

²Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Street, Nghia Do Ward, Cau Giay District, Hanoi, Vietnam

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Abstract:

A phytochemical investigation on the ethanolic (EtOH) extract of the Vietnamese *Curcuma longa* rhizomes has led to the isolation of four curcuminoids including curcumin (1), demethoxycurcumin (2), bisdemethoxycurcumin (3), and cyclocurcumin (4). The chemical structure of compounds 1-4 was elucidated by Nuclear magnetic resonance (NMR) and Mass spectrometry (MS) spectral data. Based on High-performance liquid chromatography (HPLC) quantitative analysis, the amounts of three major compounds 1-3 in the *C. longa* extract were calculated and reached 7.215±0.101, 3.927±0.031, and 2.255±0.049 mg/g, respectively. Curcuminoids 1-4 have induced IC₅₀ values of 9.23-14.6 µg/ml in a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay as compared with that of the positive control resveratrol (IC₅₀ 11.5 µg/ml). Compounds 1-4 with IC₅₀ values ranging between 8.7-15.54 µg/ml were better than the positive control acarbose (IC₅₀ 169.14 µg/ml) in α -glucosidase inhibitory examination. In addition, the EtOH extract and compounds 1-4 were also responsible for inhibitions against enzyme acetylcholinesterase and four cancer cell lines including epidermoid carcinoma (KB), hepatocellular carcinoma (HepG2), lung cancer (SK-LU-1), and breast cancer (MCF7).

Keywords: *Curcuma longa*, curcuminoids, cytotoxicity, DPPH radical scavenging, HPLC analysis, α -glucosidase inhibition.

Classification number: 2.2

1. Introduction

Curcuma longa L. (the Zingiberaceae family) is a perennial herb, which is distributed throughout the world and widely cultivated in Asian countries [1]. Its rhizomes have features like oblong, ovate, pyriform, and are often shortly branched [1, 2]. The powders derived from rhizomes have been in continuous use in food preparations [3]. Current users of traditional medicine claim the application of its rhizome powders has antioxidant, antibacterial, anticancer, anti-inflammatory, antimutagenic, antidiabetic, and hepatoprotective activities [4-6].

The extracts of the *C. longa* species react with alkalis to create red-brown salts. *C. longa* extracts are soluble in alkalis, ethanol, ketone, acetic acid, and chloroform [1]. This phenomenon is mainly due to the presence of curcumin and its derivatives [1]. Chromatographic HPLC is a powerful and robust technique for both qualitative and quantitative analysis of curcumin and its derivatives [7-10]. In the current paper, we report the phytochemical and HPLC quantitative procedures for identifying curcuminoids from the Vietnamese *C. longa* rhizomes together with their DPPH radical scavenging, α -glucosidase, acetylcholinesterase (AChE), and cytotoxic assays.

2. Materials and methods

2.1. General procedures

The Bruker Avance 500 MHz was used to measure 1D and 2D-NMR with TMS as an internal standard. A Thermo Scientific LTQ Orbitrap XL instrument was used to collect ESI-MS data. Silica gel (40-63 µm mesh, Sigma) and Sephadex LH-20 (75-150 µm, Bio-Science, Sweden) were used for column chromatography (CC). TLC examination was performed on plates that had been precoated with silica gel 60 F₂₅₄ (Merck, Germany). Compounds were seen using a UV lamp with wavelengths of 254 and 365 nm, as well as spraying with indicators (5% H₂SO₄ and vanillin).

Solvents for the HPLC analysis were purchased from Merck, Germany. HPLC-DAD data were obtained using a ZORBAX Eclipse XDB C18 column (150x4.6 mm, 5 µm) coupled with a ZORBAX Eclipse XDB guard C18 column (12.5x4.6 mm, 5 µm) on an Agilent Series 1260 (Agilent Technologies, USA) system, which included a vacuum degasser, a quaternary mixing pump, an auto-sampler, a column oven, and a diode-array detector (DAD) (Agilent Technologies, USA).

*Corresponding author: Email: yamantson@gmail.com

2.2. Plant material

The rhizomes of *C. longa* were collected in Bac Giang, Vietnam in December 2020 and were identified by the taxonomist Nguyen The Cuong of the Institute of Ecology and Biological Resources. A voucher specimen labelled CL-2020 was deposited in the Department of Applied Biochemistry, Institute of Chemistry, Vietnam Academy of Science and Technology.

2.3. Extraction and isolation

C. longa dried rhizomes (0.5 kg) were extracted with EtOH (4 l x 3 times) under reflux for 3.5 h. The crude EtOH extract (120 g) was obtained by evaporating the mixed extract under reduced pressure.

The EtOH extract was subjected to silica gel CC and eluted with *n*-hexane (1 l), CH₂Cl₂ (1.5 l), and EtOAc (1.2 l) to afford 7 fractions (CL1-CL7). Fraction CL2 (15.1 g) was chromatographed on silica gel CC [*n*-hexane-acetone (3:1, v/v)] to yield 7 fractions (CL21-CL27). The fraction CL22 (2.2 g) was subjected to sephadex LH-20 CC [MeOH/CH₂Cl₂ (9:1, v/v)] to give compound **2** (8.0 mg). The fraction CL3 (9.7 g) was separated on sephadex LH-20 column [MeOH (100%)] to yield 6 fractions (CL31-CL36). The fraction CL33 (1.2 g) was separated by sephadex LH-20 CC eluted with MeOH (100%) to provide 3 fractions (CL331-CL333). Compound **4** (1.5 mg) was isolated from the fraction CL332 (0.2 g) by using preparative TLC [CH₂Cl₂/EtOAc (6:1, v/v)]. Silica gel CC [CH₂Cl₂-EtOAc, 9:1 to 1:1, v/v] was utilized for fraction CL24 (8.9 g) to produce 8 fractions (CL241-CL248). Both compound **1** (9.1 mg) and compound **3** (12.1 mg) were derived from the fraction CL242 (1.5 g) by using silica gel CC [CH₂Cl₂/CH₃COCH₃ (3:1, v/v)].

Curcumin (1): Yellow powder; ESI-MS (+): *m/z* 369.1 [M+H]⁺ (calcd for C₂₁H₂₁O₆, 369.0); ¹H-NMR (500 MHz, CD₃OD, δ_H ppm): 7.60 (2H, d, 16.0 Hz, H-4), 7.23 (2H, d, 1.5 Hz, H-6), 7.13 (2H, dd, 1.5, 8.0 Hz, H-10), 6.85 (2H, d, 8.0 Hz, H-9), 6.62 (2H, d, 16.0 Hz, H-3), 5.99 (1H, s, H-1), 3.93 (6H, s, 7-OCH₃); ¹³C-NMR (125 MHz, CD₃OD, δ_C ppm): 184.8 (C-2), 150.5 (C-8), 149.5 (C-7), 142.1 (C-4), 128.6 (C-5), 124.1 (C-3), 122.3 (C-10), 116.6 (C-9), 111.8 (C-6), 101.4 (C-1), 56.5 (7-OCH₃).

Demethoxycurcumin (2): Yellow powder; ESI-MS (+): *m/z* 339.2 [M+H]⁺ (calcd for C₂₀H₁₉O₅, 339.0); ¹H-NMR (500 MHz, DMSO-*d*₆, δ_H ppm): 10.10 (1H, s, 8'-OH), 9.72 (1H, s, 8-OH), 7.61 (3H, m, H-4', H-6', H-10'), 7.57 (1H, d, 15.0 Hz, H-4), 7.20 (1H, d, 10.0 Hz, H-10), 7.32 (1H, s, H-6), 6.87 (3H, d, 10.0 Hz, H-9, H-7', H-9'), 6.79 (1H, d, 15.0 Hz, H-3), 6.74 (1H, d, 15.0 Hz, H-3'), 3.90 (3H, s, 7-OCH₃); ¹³C-NMR (125 MHz, DMSO-*d*₆, δ_C ppm): 184.1 (C-2'), 183.8 (C-2), 160.1 (C-8'), 148.7 (C-7), 148.5 (C-8), 141.5 (C-4), 141.0 (C-4'), 131.0 (C-7', C-9'), 126.8 (C-5), 126.1 (C-5'), 123.9 (C-3), 121.5 (C-3'), 115.2 (C-6', C-10'), 111.9 (C-6), 101.4 (C-1), 56.4 (7-OCH₃).

Bisdemethoxycurcumin (3): Yellow powder; ESI-MS (+): *m/z* 308.1 [M+H]⁺ (calcd for C₁₉H₁₇O₄, 308.0); ¹H-NMR (500 MHz, DMSO-*d*₆, δ_H ppm): 10.03 (2H, s, 8-OH), 7.55 (6H, m, H-4, H-6, H-10), 6.82 (4H, d, 8.5 Hz, H-7, H-9), 6.70 (2H, d, 15.5 Hz, H-3), 6.04 (1H, s, H-1); ¹³C-NMR (125 MHz, DMSO-*d*₆, δ_C ppm): 184.8 (C-2), 161.1 (C-8), 141.8 (C-4), 131.1 (C-7, C-9), 126.0 (C-5), 122.0 (C-3), 116.9 (C-6, C-10), 101.5 (C-1).

Cyclocurcumin (4): Yellow powder; ESI-MS (+): *m/z* 369.2 [M+H]⁺ (calcd for C₂₁H₂₁O₆, 369.0); ¹H-NMR (500 MHz, CDCl₃, δ_H ppm): 7.07 (1H, d, 10.0 Hz, H-9), 7.03 (4H, m, H-6, H-15, H-18, H-19), 6.95 (1H, d, 10.0 Hz, H-10), 6.50 (1H, d, 14.5 Hz, H-12), 7.33 (1H, d, 1.4.5 Hz, H-13), 5.60 (1H, s, H-3), 5.40 (1H, dd, 1.5, 14.5 Hz, H-4), 3.99 (3H, s, 7-OCH₃), 3.94 (3H, s, 16-OCH₃), 3.04 (1H, dd, 15.0, 20.0 Hz, H-2a), 2.90 (1H, d, 1.5, 14.5 Hz, H-2b); ¹³C-NMR (125 MHz, CDCl₃, δ_C ppm): 194.1 (C-1), 170.0 (C-11), 147.7 (C-17), 147.0 (C-16), 146.8 (C-7), 146.1 (C-8), 146.5 (C-7), 137.9 (C-13), 130.4 (C-5), 127.7 (C-14), 123.0 (C-19), 120.1 (C-10), 118.1 (C-12), 114.1 (C-18), 113.9 (C-9), 109.2 (C-6), 108.9 (C-15), 105.7 (C-3), 80.9 (C-4), 43.5 (C-2), 56.5 (16-OCH₃), 56.1 (16-OCH₃).

2.4. HPLC quantitative analysis

Quantitative analysis by HPLC has been applied to identify the amounts of curcuminoids in the EtOH extract of the Vietnamese *C. longa* rhizomes. Due to a material shortage of compound **4**, the analysis was focused on compounds **1-3**. The HPLC method has been carefully described in a previous publication [11]. Briefly, three isolated compounds were used as standard compounds in which their calibration solutions were prepared in a concentration range of 10-400 µg/ml by MeOH dilution. Linearity was evaluated in this range three times. The extract of *C. longa* was also diluted by MeOH and filtered before use. The mobile phase was made up of 0.1% acetic acid in water (mobile phase A) and ACN (mobile phase B). The HPLC running conditions were the same for standard compounds and extracts, which include sample injection (2 µl), flow rate (1.6 ml/min), column temperature (40°C), UV detector (λ=425 nm) for 0 min (75% A, 25% B), 0-20.0 min (30% A, 70% B), and 20-25 min (100% B).

The limit of detection (LOD) is the lowest concentration of the standard substance that can be detected. Similarly, the limit of quantitation (LOQ) shows the lowest concentration of analyte that can be quantified. Both LOD and LOQ were calculated using a calibration curve based on the standard deviation (SD or σ) of the data response and the slope of the calibration curve (*a*) using the following equations: LOD=3.3 σ/*a* and LOQ=10 σ/*a*.

2.5. Biological assays

Antioxidative assay: Our earlier reports have detailed descriptions of the DPPH antioxidant assay [12-14]. In brief, DPPH (0.1 mM) was diluted in MeOH. DPPH (200 µl) was

then continually added to 1.3 μl of various concentrations of samples in Dimethyl sulfoxide (DMSO) (128, 25.6, 5.12, and 1.024 $\mu\text{g/ml}$). A 96-well plate was used to mix the ingredients at 25°C for 30 min. The mixture was carried out by a 96-well plate at 25°C and 30 min. An Elisa reader was used to determine the absorbance at 517 nm.

The inhibitory rate (%) = $[(\text{OD}_0 - \text{OD}_1) / \text{OD}_0] \times 100\%$, where OD_0 (optical density) stands for reaction absorbance of the control and OD_1 is the absorbance of the sample. Each experiment was repeated three times with resveratrol serving as the positive control. The IC_{50} value, commonly known as the concentration of tested sample with half-maximal reaction, was determined using Excel linear regression of serial scavenging percent values vs concentrations.

α -Glucosidase assay: The inhibitory assay for α -glucosidase reaction was carried out similarly to our prior protocol [15, 16]. The sample was dissolved in DMSO to produce a stock of 400 mg/ml. In a 100-mM phosphate buffer (pH 6.8), a series of dilutions to final concentrations of 256, 64, 16, and 4 $\mu\text{g/ml}$ were made for each sample. A reaction mixture containing sample, 100-mM phosphate buffer (pH 6.8), and α -glucosidase (0.4 U/ml) was pre-incubated for 10 min at 37°C in a 96-well plate. As a substrate, 2.5 mM of pNPG (p-nitrophenyl- α -D-glucopyranoside) solution was added to the combination. The reaction was halted by adding Na_2CO_3 0.2 M after 30 min of incubation at 37°C. The number of released p-nitrophenol from pNPG was calculated at 410 nm by an Elisa reader. Acarbose was used as a positive control, and each assay was carried out three times.

Similar to above, the IC_{50} value stands for the concentration of compound exhibiting 50% inhibition of α -glucosidase activity under the assay condition.

AChE assay: The AChE inhibitory assay was performed based on the modified Ellman's method [17]. The sample was dissolved in DMSO (20 mg/ml) and sodium phosphate buffer to final concentrations of 128.0, 32.0, 8.0, 2.0, and 0.5 mg/ml. In a 96-well plate, the mixture of 25 μl of the prepared sample as described above was added to 25 μl PBS, 25 μl of 0.22 U/ml AChE, and 125 μl of 3 mM DTNB (5-5'-dithiobis-2-nitrobenzoic) and pre-incubated for 15 min at 25°C. The substrate acetylthiocholine iodide (ATCI) (25 μl of 15 mM ATCI) was then added. Hydrolytic reaction of ATCI catalysed by AChE was carried out at 412 nm by an Elisa reader. Each experience was repeated three times and donepezil was used as a positive control.

Cytotoxic assay: The MTT experiment was used to assess the cytotoxic activity of EtOH extract and isolated compounds **1-4** against human cancer cell lines KB, LU-1, Hep-G2, and MCF-7 [18]. Cells were grown in DMEM (Dulbecco's Modified Eagle Medium) medium containing 10% fetal bovine serum (FBS), 1% Penicillin and Streptomycin, and 1% L-glutamine

at 37°C in a humidified environment of 5% CO_2 . Samples were dissolved in DMSO to produce a stock of 20 mg/ml and each compound with final concentrations of 256, 64, 16 and 4 $\mu\text{g/ml}$ was then prepared from this stock.

On 96-well plates, cells were separated with trypsin, planted with 3×10^4 cells/ml in each well, then treated with varying doses of material. The controls were cells that had not been treated. After 72 h of treatment, each well was treated with an MTT solution (10 μl , 5 mg/ml) of phosphate buffer for 4 h until intracellular purple formazan crystals were evident. The MTT was removed and replaced with a 100- μl DMSO solution. An Elisa reader at 540 nm was used to determine the OD of the solution. ODs from the three duplicate experiments were used to calculate the inhibition ratio. Ellipticine was used as a positive control.

3. Results and discussion

The phytochemical investigation of the EtOH extract of *C. longa* rhizomes, collected in Bac Giang, Vietnam, has led to the isolation of four compounds **1-4**. Based on NMR and MS spectral analyses, together with a comparison with literature, the chemical structures of these compounds were elucidated as curcumin (**1**), demethoxycurcumin (**2**), bisdemethoxycurcumin (**3**), and cyclocurcumin (**4**) (Fig. 1) [19].

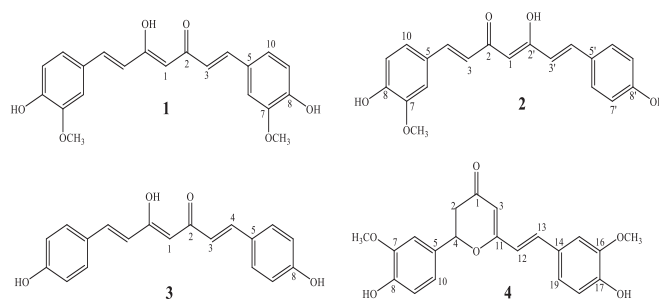


Fig. 1. The chemical structures of the isolated compounds 1-4.

Regarding quantitative analysis, an optimized strategy of the HPLC-DAD system was carried out to obtain the three separated peaks with R_t of 14.390 min (compound **1**), 13.760 min (compound **2**), and 13.117 (compound **3**) on the chromatogram, as compared with those of *C. longa* extract (Fig. 2). The calibration curve of each compound was established from at least six appropriate concentrations in triplicate by plotting the peak areas versus the concentration and good linearity was found in the test ranges, which ranged from 0.9986 to 0.9990 (Table 1). The LODs and LOQs were in the range of 0.7108-0.9265 mg/mg and 2.1538-2.8075 mg/mg, respectively. Most importantly, the contents of compounds **1-3** found in the *C. longa* extract was identified to be 7.215 ± 0.101 , 3.927 ± 0.031 , and 2.255 ± 0.049 mg/g, respectively. As mentioned above, pharmacological values of *C. longa* are becoming more and more involved in the presence of curcuminoids. As compared to

literature, compounds **1-3** are dominate in Vietnamese *C. longa* extract, and higher than those of Indonesian *C. aeruginosa*, *C. heyneana*, *C. manga*, and *C. soloensis* extracts [20] as well as Indian *C. longa* extract [21].

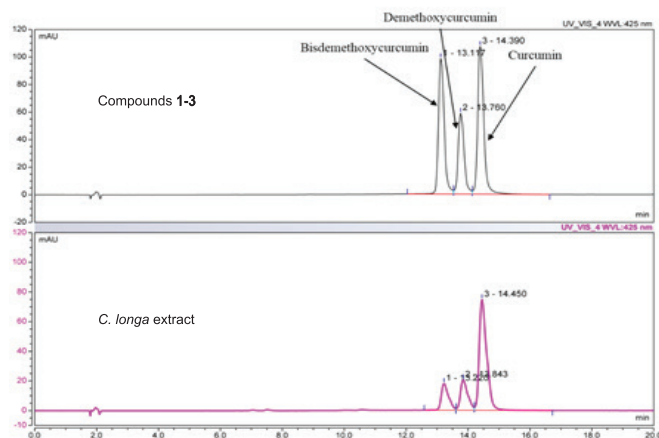


Fig. 2. The representative chromatograms at UV 254 nm of a mixture of compounds **1-3** and *C. longa* extract.

Table 1. Retention time, regression equation, R^2 , LOQ, LOD, and content of compounds **1-3**.

No	R_t (min)	Regression equation	R^2	LOD ($\mu\text{g/g}$)	LOQ ($\mu\text{g/g}$)	Content in extract (mg/g)
1	14.390	$y=0.170x-0.242$	0.9986	0.9095	2.7562	7.215 ± 0.101
2	13.760	$y=0.091x-0.184$	0.9989	0.7108	2.1538	3.927 ± 0.031
3	13.117	$y=0.152x-0.512$	0.9990	0.9265	2.8075	2.255 ± 0.049

The EtOH extract and its isolated curcuminoids **1-4** were further subjected to antioxidative and α -glucosidase inhibitory assays (Table 2). Regarding the antioxidative assay, the EtOH extract showed free radical scavenging capability with an IC_{50} value of 25.6 ± 1.15 $\mu\text{g/ml}$. Curcuminoids **1-4** are now considered promising antioxidants since they possess remarkable free DPPH radical scavenging. Their IC_{50} values ranged from 9.23 ± 0.2 to 14.6 ± 0.3 $\mu\text{g/ml}$ as compared with that of the positive control resveratrol (IC_{50} 11.5 ± 0.09 $\mu\text{g/ml}$). The deletion of one methoxy group has caused a larger IC_{50} value when compared between compounds **1** and **2**. However, the deletion of two methoxy groups in compound **3** would help to reduce the IC_{50} value.

Considering the α -glucosidase inhibitory assay, the EtOH extract exhibited inhibitory activity with an IC_{50} value of 45.8 ± 2.1 $\mu\text{g/ml}$. Curcuminoids **1-4** also induced significant activity. The IC_{50} values of compounds **1-4** were 8.7 ± 0.2 , 14.91 ± 0.23 , 10.46 ± 0.3 , and 15.54 ± 0.32 $\mu\text{g/ml}$, respectively. Notably, these values are much less than that of the positive control acarbose (IC_{50} 169.14 ± 3.2 $\mu\text{g/ml}$). However, in contrast to antioxidative activity, compound **1** with the lowest IC_{50} value showed better activity than compounds **2** and **3** due to the number of methoxy groups.

Regarding the AChE assay, the EtOH extract showed activity with an IC_{50} value of 46.45 ± 3.9 $\mu\text{g/ml}$ (Table 2). As compared to donepezil (IC_{50} 0.035 ± 0.01 $\mu\text{g/ml}$), the IC_{50} values of the isolated compounds occurred in following the order: **3** (IC_{50} 9.32 ± 0.42 $\mu\text{g/ml}$) < **2** (IC_{50} 16.52 ± 1.81 $\mu\text{g/ml}$) < **1** (IC_{50} 29.27 ± 2.1 $\mu\text{g/ml}$) < **4** (IC_{50} 38.12 ± 3.45 $\mu\text{g/ml}$). Therefore, it can be concluded that methoxylation is the main cause for the IC_{50} increase.

Table 2. DPPH radical scavenging, α -glucosidase, and AChE inhibitory activities.

No.	IC_{50} values ($\mu\text{g/m}$)		
	DPPH radical scavenging	α -Glucosidase inhibition	AChE inhibition
1	9.23 ± 0.2	8.7 ± 0.2	29.27 ± 2.1
2	10.78 ± 0.25	14.91 ± 0.23	16.52 ± 1.81
3	8.09 ± 0.15	10.46 ± 0.3	9.32 ± 0.42
4	14.6 ± 0.3	15.54 ± 0.32	38.12 ± 3.45
EtOH extract	25.6 ± 1.15	45.8 ± 2.1	46.45 ± 3.9
References	Resveratrol: 11.5 ± 0.09	Acarbose: 169.14 ± 3.2	Donepezil: 0.035 ± 0.01

The search for cytotoxic agents from natural plants is one of the most important components of drug development. In this regard, we applied an MTT assay to reveal potential cytotoxicity of the EtOH extract of the Vietnamese *C. longa* and its isolated compounds **1-4**. The EtOH extract was associated with IC_{50} values ranging from 22.73 ± 1.08 to 77.71 ± 2.64 $\mu\text{g/ml}$ against four cancer cell lines KB, Hep, Lu, and MCF7 (Table 3). Curcuminoids **1-4** also exhibited moderate to weak cytotoxic activities. Compound **2** had induced moderate activity against the three cancer cell lines KB, Hep, and MCF7, but showed weak activity against cancer cell line Lu. In another case, compound **4** presented IC_{50} values of 122.63 - 157.93 $\mu\text{g/ml}$ towards three cancer cell lines KB, Hep, and MCF7, but failed to control cancer cell line Lu (IC_{50} >256.0 $\mu\text{g/ml}$). Compounds **1-2** exhibited cytotoxicity better than compound **3** due to the presence of methoxy groups.

Table 3. Cytotoxic activity of the EtOH extract and isolated compounds **1-4**.

No.	IC_{50} values ($\mu\text{g/ml}$)			
	KB	Hep	Lu	MCF7
1	91.90 ± 3.25	65.90 ± 2.36	134.48 ± 6.64	103.62 ± 4.53
2	36.57 ± 1.78	40.30 ± 2.54	129.68 ± 5.76	59.53 ± 3.78
3	134.92 ± 6.65	98.62 ± 3.85	156.44 ± 7.14	118.86 ± 5.95
4	124.55 ± 6.37	122.63 ± 5.76	>256	157.93 ± 7.26
EtOH	32.96 ± 1.23	22.73 ± 1.08	77.71 ± 2.64	40.20 ± 1.78
Ellipticine	0.36 ± 0.03	0.35 ± 0.03	0.38 ± 0.03	0.30 ± 0.02

Conclusions

Chromatographic separation of the EtOH extract of Vietnamese *C. longa* rhizomes resulted in the isolation and determination of four curcuminoids including curcumin (**1**), demethoxycurcumin (**2**), bisdemethoxycurcumin (**3**), and cyclocurcumin (**4**). Compound **1** was a major compound with 7.215 ± 0.101 mg/g extract, whereas values of 3.927 ± 0.031 and 2.255 ± 0.049 mg/g were found in compounds **2** and **3**, respectively. Curcuminoids **1-4** are now promising antioxidative and α -glucosidase inhibitory agents in *C. longa* extract because their IC_{50} values are comparable to that of resveratrol and acarbose. Both the EtOH extract and compounds **1-4** also exhibited AChE inhibition and cytotoxicity against four cancer cell lines KB, Hep. Lu, and MCF7. Methoxylation has greatly affected biological results...

CRedit author statements

Thi Thu HaNguyen, Thi Tu Anh Le, Thanh Tra Nguyen: Experiments and data analysis; The Son Ninh: Writing the article.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] C.A.C. Araujo, L.L. Leon (2012) "Biological activities of *Curcuma longa* L.", *Mem. Inst. Oswaldo Cruz.*, **96**(5), pp.723-728, DOI: 10.1590/s0074-02762001000500026.
- [2] S.J. Park, C.V. Garcia, G.H. Shin, et al. (2018) "Improvement of curcuminoid bioaccessibility from turmeric by a nanostructured lipid carrier system", *Food Chemistry*, **251**, pp.51-57, DOI: 10.1016/j.foodchem.2018.01.071.
- [3] J. Miquel, A. Bernd, J.M. Sempere, et al. (2002) "The curcuma antioxidants: Pharmacological effects and prospects for future clinical use. A review", *Archives of Gerontology and Geriatrics*, **34**(1), pp.37-46, DOI: 10.1016/s0167-4943(01)00194-7.
- [4] V. Krup, H. Prakash, A. Harini (2013), "Pharmacological activities of turmeric (*Curcuma longa* linn): A review", *Journal of Homeopathy and Ayurvedic Medicine*, **2**, DOI: 10.4172/2167-1206.1000133.
- [5] H.P.T. Ammon, M.A. Wahl (1991), "Pharmacology of *Curcuma longa*", *PlantaMedica*, **57**(1), pp.1-7, DOI: 10.1055/s-2006-960004.
- [6] L. Labban (2014), "Medicinal and pharmacological properties of Turmeric (*Curcuma longa*): A review", *International Journal of Pharmaceutical and Biomedical Science*, **5**, pp.17-23.
- [7] Z. Ma, A. Shayeghanpour, D.R. Brocks, et al. (2007), "High-performance liquid chromatography analysis of curcumin in rat plasma: Application to pharmacokinetics of polymeric micellar formulation of curcumin", *Biomedical Chromatography*, **21**(5), pp.546-552, DOI: 10.1002/bmc.795.
- [8] M.M. Sanagi, U.K. Ahmad, R.M. Smith (1993), "Application of supercritical fluid extraction and chromatography to the analysis of Turmeric", *Journal of Chromatography Science*, **31**(1), pp.20-25, DOI: 10.1093/chromsci/31.1.20.
- [9] H.P. Lestari, S. Martono, R. Wulandari, et al. (2017), "Simultaneous analysis of curcumin and demethoxycurcumin in *Curcuma xanthorrhiza* using FTIR spectroscopy and chemometrics", *International Food Research Journal*, **24**(5), pp.2097-2101.
- [10] F. Sahnea, M. Mohammadi, G.D. Najafpour, et al. (2017), "Enzyme-assisted ionic liquid extraction of bioactive compound from turmeric (*Curcuma longa* L.): Isolation, purification and analysis of curcumin", *Industrial Crop and Products*, **95**, pp.686-694, DOI: 10.1016/j.indcrop.2016.11.037.
- [11] N.T. Thoa, N.H. Dang, D.H. Giang, et al. (2020), "Metabolomics approach for discrimination and quality control of natural and commercial *Fallopia multiflora* products in Vietnam", *International Journal of Analytical Chemistry*, **2020**, DOI: 10.1155/2020/8873614.
- [12] I.E. Abdelsamed, H. Maichi, V.T. Nguyen, et al. (2020), "A new cerebroside from the endophytic fungus *Ophiocordyceps longissima*: Structural-electronic and antioxidant relations. Experimental and DFT calculated studies", *Journal of Molecular Structure*, **1200**, DOI: 10.1016/j.molstruc.2019.127061.
- [13] T.S. Ninh, K. Mari, T.H. Tran, et al. (2019), "Chemical constituents of the Vietnamese plants *Dalbergia tonkinensis* Prain and *Cratoxylum formosum* (Jack) Dyer in Hook and their antioxidative activities", *Medicinal Chemistry Research*, **28**(9), pp.1441-1447, DOI: 10.1007/s00044-019-02383-9.
- [14] T.T.H. Nguyen, V.C. Pham, T.T. Nguyen, et al. (2020), "Chemical constituents from methanolic extract of *Garcinia mackeaniana*", *Vietnam Journal of Science and Technology*, **58**(4), pp.411-418, DOI: 10.15625/2525-2518/58/4/14680.
- [15] T.T. Nguyen, V.T. Nguyen, V.C. Pham, et al. (2021), "Chemical constituents from the leaves of *Syrax argenteifolius* H.L. Li and their biological activities", *Phytochemistry Letters*, **41**, pp.70-73, DOI: 10.1016/j.phytol.2020.11.003.
- [16] L.T.T. Anh, N.T. Son, N.V. Tuyen, et al. (2021), "Antioxidative and α -glucosidase inhibitory constituents of *Polyscias guilfoylei*: Experimental and computational assessments", *Molecular Diversity*, DOI: 10.1007/s11030-021-10206-6.
- [17] T.T.H. Nguyen, et al. (2021), "A new flavonoid from the leaves of *Garcinia mckeaniana* Craib and α -glucosidase and acetylcholinesterase inhibitory activities", *Natural Product Research*, DOI: 10.1080/14786419.2021.191601.
- [18] N.T.T. Ha, P.V. Cuong, N.T. Tra, et al. (2020), "Cytotoxic naphthoquinones from *Diospyros fleuryana* leaves", *Discovery Phytomedicine*, **7**(1), pp.42-46, DOI: 10.15562/phytomedicine.2019.117.
- [19] V. Sueth-Santiago, J.B.B. Moraes, E.S.S. Alves, et al. (2016), "The effectiveness of natural diarylheptanoids against *Trypanosoma cruzi*: Cytotoxicity, ultrastructural alterations and molecular modeling studies", *PLOS ONE*, **11**(9), DOI: 10.1371/journal.pone.0162926.
- [20] R. Bos, T. Windono, H.J. Woerdenbag, et al. (2007) "HPLC-photodiode array detection analysis of curcuminoids in *Curcuma* species indigenous to Indonesia", *Phytochemical Analysis*, **18**(2), pp.118-122, DOI: 10.1002/pca.959.
- [21] G.K. Jayaprakash, L.J.M. Rao, K.K. Sakariah (2002), "Improved HPLC method for the determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin", *Journal of Agriculture and Food Chemistry*, **50**(13), pp.3668-3672, DOI: 10.1021/jf025506a.

Study on the effects of plasticiser types and contents on physicochemical properties of HPMC/Shellac composite films

Thu Trang Pham^{1*}, Thanh Tung Nguyen¹, Thi Thu Ha Pham¹, Trung Duc Nguyen¹, Van Khoi Nguyen¹, Quang Huy Nguyen¹, Cong Hoan Do¹, Vu Thang Tran¹, Thi Phuong Hoang¹, Phan Hang Nguyen²

¹Institute of Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Street, Nghia Do Ward, Cau Giay District, Hanoi, Vietnam
²Higher Education Department, Ministry of Education and Training, 35 Dai Co Viet Street, Le Dai Hanh Ward, Hai Ba Trung District, Hanoi, Vietnam

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Abstract:

The objective of this work was to study the effects of different plasticisers [glycerol (G), propylene glycol (PG), polyethylene glycol 400 (PEG 400)] at different contents (10-30% compared to hydroxypropyl methylcellulose (HPMC)) on the properties of HPMC/shellac composite films. Sensory, mechanical properties, and surface morphology were used to evaluate changes in the composite films by adding different plasticisers. The results showed that with the addition of plasticisers, the films became more transparent and flexible. As the plasticiser content increased, the tensile strength and elastic modulus of the films decreased. At a plasticiser content of 20%, the water vapor permeability (WVP) of the composite films reached its minimum value. The SEM images showed that the HPMC/shellac composite film containing 20% G had the smoothest surface, and the components of this film were uniformly distributed.

Keywords: composite film, hydroxypropyl methylcellulose, plasticiser, shellac.

Classification number: 2.2

1. Introduction

In recent years, our country's agricultural production has made enormous progress but lacks sustainability. Given that vegetables and fruits have water contents around 80-90% of their total weight, they are very perishable [1], which leads to high post-harvest losses of agricultural products. Indeed, more than 25% of fruits and more than 30% of vegetables are lost due to lack of post-harvest technology. Therefore, the technology of preserving vegetables and fruits in order to prolong storage times while maintaining their commercial value has been the focus of research and development by scientists. Among the methods of preserving fruits and vegetables being researched and used today, biopolymers are very interesting not only because they have outstanding advantages over petroleum-based polymer films, but because of their biodegradable and environmentally-friendly properties. A biopolymer film is a thin material layer used to coat the surface of vegetables/fruits or to replace the natural protective wax and provide a moisture and oxygen barrier. This film is placed directly on the

fruit surface by dipping, spraying, or sweeping to create a modified atmosphere (MA). The semi-permeable film formed on the surface of the vegetables/fruits restricts their respiration and controls moisture loss, as well as limits the release of active compounds such as antioxidants, flavours, or antibacterial agents [2]. Such films have been used to maintain quality and prolong the shelf life of some fresh fruits such as citrus fruits (oranges, lemons, and tangerines), apples, and cucumbers. They have advantages such as retention of pigments, sugars, acids, and aromas, as well as reduction of mass loss, maintenance of quality during transportation and storage, improved consumer appeal, and prolonging the shelf life [3]. Coating materials are commonly used from materials of biological origin and certified as safe for humans such as proteins, polysaccharides, and lipids.

HPMC is one of the matrix materials used directly on the surface of fruits and vegetables because it has good film forming ability, is odourless, tasteless, has good air permeability, and retains the product scent. However, the disadvantage of HPMC is that it is hydrophilic, so recent

*Corresponding author: Email: thutrang90vhh@gmail.com

research directions aim to combine natural and synthetic waxes such as beeswax, shellac, paraffin wax, etc. into film formulae to improve water vapor barrier properties as well as combine the beneficial properties of both film-forming materials. In addition, plasticisers are also added to increase the flexibility of the film [4-6], the most commonly used plasticisers are polyols such as sorbitol, G, PG, and PEG 400. Therefore, this paper focuses on evaluating the influence of different plasticisers on the physico-chemical properties of HPMC/shellac composite films.

2. Materials and methods

2.1. Materials

HPMC E15 resin was produced by Zhejiang Joinway Pharmaceutical Co. Ltd., (China) and dewaxed shellac was supplied by Raj Kumar Shellac Industries (India), both of which are food grade. Other chemicals: G, PG, PEG 400), lauric acid, absolute ethanol are all pure chemicals made in China and used directly without refining.

2.2. Methods

2.2.1. Preparation of the HPMC/shellac composites

- To prepare the colloidal solution of HPMC, 5 g HPMC was dispersed in 80 ml of distilled water at 80°C and stirred at rate of 200 rpm until completely dissolved. Then the solution was lowered to 40-50°C and the plasticisers (G, PG, PEG 400) were added with weights of 0.5-1.5 g (content of 10-30% as compared to HPMC) and stirring was continued at 200 rpm for 120 min.

- To prepare the emulsification of the shellac, 0.1 g shellac and 0.01 g lauric acid were put into a beaker containing 20 ml of absolute ethanol, and the mixture was stirred at 200 rpm for 120 min and then filtered through Whatman filter paper No.5.

- The shellac emulsion was slowly poured into the HPMC solution, and the mixture was stirred at 300 rpm for 180 min to obtain a composite film forming solution.

To evaluate the properties of the HPMC/shellac composite films, 6 ml of the film-forming solution was put into a petri dish (diameter of 100 mm) and then placed in an oven and dried at 40°C until dry. After drying, the film was removed from the petri dish and stored in a desiccator for at least 24 h before measurements and testing. The symbols of the film samples are summarised in Table 1.

Table 1. The symbols of film samples.

Sample symbols	Plasticizer	Content of plasticiser as compared with HPMC (%)
0.5 G	Glycerol	10
1 G		20
2 G		30
0.5 PG	Propylene glycol	10
1 PG		20
2 PG		30
0.5 PEG	Polyethylene glycol	10
1 PEG		20
2 PEG		30

2.2.2. Characterisation

The surface morphology and fracture surface morphology of the HPMC/shellac composite films were investigated by using a JEOL SM-6510 LV device (Japan). The surface of the sample was coated with a thin gold layer by vacuum evaporation to increase contrast. The mechanical properties were measured on a BP-1068 instrument according to ASTM D882 with a tensile speed of 10 mm/min. WVP was determined according to ASTM E96.

3. Results and discussion

3.1. Sensory evaluation of films

Sensory evaluation is a simple, effective tool that gives information about the appearance, colour, and durability, which could be related to other properties such as mechanical properties, surface morphology and WVP to select suitable film features. Photographs of composite films using different plasticisers at different concentrations are shown in Fig. 1.

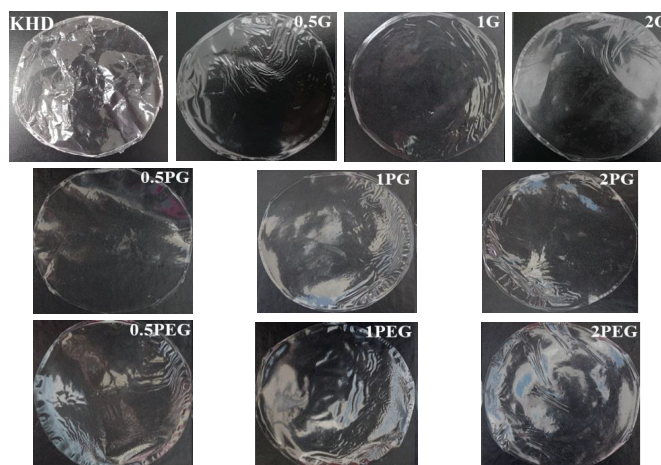


Fig. 1. Photograph of the HPMC/shellac composite films without plasticiser (KHD) and with various plasticisers.

The results showed that in the absence of plasticisers the films were brittle, hard, fragile, and difficult to peel. This was because both the main film-forming materials were HPMC and shellac, which had -OH groups forming intramolecular and intermolecular H bonds. When adding plasticisers to the film, the film became more transparent and glossier, and the surface of film was smoother.

When using G as a plasticiser, the film with 10% G was still brittle and the film with 20% G was flexible and unbroken, while the film with 30% content was too flexible, difficult to form, and viscous. This could be because with the same film forming formulation, 10% G, was not enough to fully plasticize HPMC and at 30% content, the excess G molecules had migrated to the film surface thus forming a sticky and viscous film [7, 8].

Just like G, the film with 10% PG was not flexible and broke easily when peeled off. The films with 20% PG was flexible and did not break when peeled off, while the film with 30% PG was too flexible and presented oil scum on the surface of the film after drying. However, the films containing PG plasticiser were often weak because of the weak polarization of PG [9].

Particularly, the surface of the films containing the PEG 400 plasticiser with all three concentrations of 10, 20 and 30% was smooth and did not break during the peeling process. However, the film with 10% PEG 400 was still brittle, and the film with 30% PEG 400 gave a very flexible film, presented oil scum on the surface of the film, and they had less elasticity than the 1 PEG film. With 20% content, the film was glossy, beautiful, and had good tensile strength and elongation at break [10]. Therefore, it could be seen that PEG, a plasticiser with a small molecular mass, easily interacted with the polymer chains and increased the flexibility of the films.

Thus, when using plasticisers, the activity and flexibility of the polymer chains were improved due to interaction between the polymer chains and the plasticiser, which increased the molecular mobility. However, with the same plasticiser content of 20%, the film containing the G plasticiser was more elastic and flexible.

3.2. Surface morphology of films

Since plasticisers contain polar -OH groups, it was possible to strengthen interactions between surface of

polymer and water molecules by reducing the polymer matrix density and increasing the degree of polymer chain flexibility. Surface and fracture surface SEM images of HPMC/shellac films are shown in Figs. 2-4.

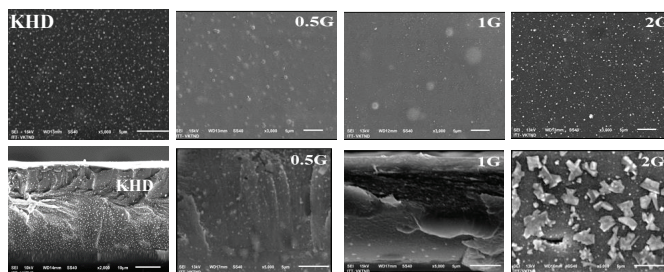


Fig. 2. SEM images of surface (top) and fracture surface (bottom) of the films using G plasticiser.

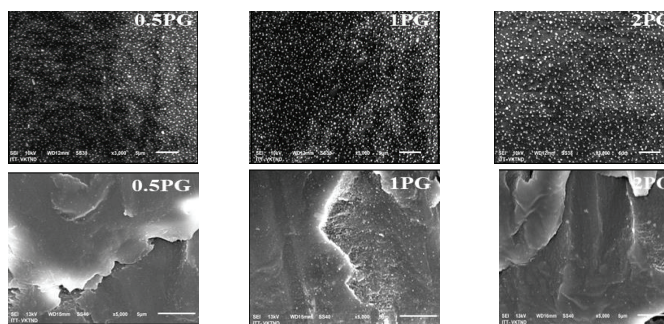


Fig. 3. SEM images of surface (top) and fracture surface (bottom) of the films using PG plasticiser.

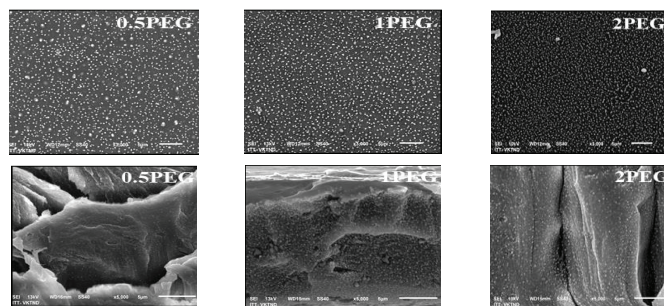


Fig. 4. SEM images of surface (top) and fracture surface (bottom) of the films using PEG plasticiser.

Observing surface and fracture surface SEM images of HPMC/shellac composite films, it was found that in the absence of plasticisers, the film surface was rough and defects appeared on the film surface. At the fracture surface, there were discontinuities in the polymer matrix structure and capillaries and pores appeared. When using plasticisers, the components of the film dispersed into each other more evenly. This might be because plasticisers acted as spacers between polymer chains thereby reducing the intermolecular forces and increasing the flexibility of the polymer chains [11]. In

all three plasticisers, it was found that the components in the film were most evenly distributed with 20% content. This proved that 10% plasticiser content was not sufficient enough to plasticize other components in the film. Meanwhile, at 30% content, the plasticiser carries other components to the surface and causes the appearance of particles. It was also found that increasing the plasticiser concentration increased the diffusion rate of the components in the film and when the diffusion rate was high, it led to the migration of plasticisers out of the polymer matrix [9].

Comparisons of surface SEM images of the films using different plasticisers showed that the films with 20% G plasticiser had the smoothest surface, small particles, and plasticized film components.

3.3. The mechanical properties of the composite films

The mechanical properties of the HPMC/shellac composite films with different plasticisers are summarised in Table 2. The results showed that when the plasticiser content increased, the tensile strength and elastic modulus of the films decreased with all three plasticisers. When the plasticiser content was increased from 10 to 30%, the tensile strength of films decreased from 25.23 to 16.49 MPa for films containing G, from 29.45 to 21.12 MPa for films containing PG, and from 32.54 to 15.83 MPa for films containing PEG 400. Meanwhile, the elongation at break of the films increased with increasing content of plasticisers. This could be explained that the addition of plasticisers made polymer chains more flexible by replacing polymer-polymer interactions with polymer-plasticiser interactions [5].

Table 2. The mechanical properties of films with different plasticisers.

Samples	Tensile strength (MPa)	Elongation at break (%)	Elastic modulus ($\times 10^{-2}$ MPa)
0.5 G	25.23	11.89	10.92
1 G	17.02	28.41	2.93
2 G	16.49	32.62	1.74
0.5 PG	29.45	3.91	17.37
1 PG	26.85	7.70	15.37
2 PG	21.12	15.47	12.08
0.5 PEG	32.54	17.29	14.37
1 PEG	24.45	26.56	8.32
2 PEG	15.83	32.00	1.60

Comparing the mechanical properties of the films when using different plasticisers, it could be seen that the mechanical properties of the HPMC/shellac composite

films did not change much by using the PG plasticiser. This could be because PG has a lower polarity than G and PEG 400, so it had less interaction with film components and formed lower flexibility films [12].

The results also showed that when using a G plasticiser, the tensile strength and elastic modulus of the films were the lowest, while the elongation at break was the highest. This proved that the plasticizing ability of G was better than that of PG and PEG 400. This was due to G having a much lower molecular weight than PEG 400, so it was easier to penetrate among polymer chains.

3.4. The WVP of films

The WVP of the HPMC/shellac composite films when using plasticisers at content of 10-30% is summarised in Table 3. The results showed that the WVP of the films with plasticisers was lower than that of the control film without plasticisers. With all plasticisers (G, PG, PEG 400), the films with 20% plasticiser content had a lower WVP than those with 10 and 30% plasticiser content. It is possible that when using 10% content, plasticiser content was not sufficient enough to fully plasticize the film components and thus the components were not uniformly dispersed into each other as indicated in the surface morphology. Therefore, the water vapor resistance of these films was lower. At 20% content, the plasticisers were residual and could combine with itself to open the polymer structure resulting in an increase to the WVP of the film.

Table 3. WVP of films with different plasticisers [g.mm/m².day.kPa].

Plasticiser content (%)	WVP of films		
	G	PG	PEG 400
0	19.65		
10	12.01	11.21	13.79
20	9.87	10.86	11.58
30	11.01	11.56	12.47

Comparing the three types of plasticisers, it was found that when the plasticiser content was 20%, the films using G had the lowest WVP. This might be due to the fact that G has the smallest molecular size, so it could easily penetrate between the polymer chains, so the plasticizing efficiency was higher, and the film components were uniformly dispersed with smaller sizes than PG and PEG 400 plasticisers.

4. Conclusions

Three plasticisers with different concentrations improved the mechanical properties and reduced the WVP of HPMC/shellac composite films. The presence of

plasticisers helped the components of the film to disperse into each other more evenly resulting in a clearer and smoother film surface. Among the three plasticisers, G, PG, and PEG 400, G with a content of 20% was the most effective plasticiser for HPMC/shellac composite films.

CRedit author statement

Thu Trang Pham, Thanh Tung Nguyen, Thi Thu Ha Pham: Conceptualisation, Methodology, Software, Resources, Writing - Review and Editing; Trung Duc Nguyen, Van Khoi Nguyen, Quang Huy Nguyen, Cong Hoan Do: Data curation, Software, Writing original draft preparation; Vu Thang Tran, Thi Phuong Hoang, Phan Hang Nguyen: Supervision, Software, Validation.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] R.K. Dhall (2013), "Advances in edible coatings for fresh fruits and vegetables: A review", *Critical Reviews in Food Science and Nutrition*, **53**(5), pp.435-450, DOI: 10.1080/10408398.2010.541568.
- [2] R. Sothornvit, J.M. Krochta (2000), "Plasticiser effect on oxygen permeability of beta-lactoglobulin films", *Journal of Agricultural and Food Chemistry*, **48**(12), pp.6298-6302, DOI: 10.1021/jf000836l.
- [3] E.O. Peris, O. Dvir, O. Feygenberg, et al. (2002), "Production of acetaldehyde and ethanol during maturation and modified atmosphere storage of litchi fruit", *Postharvest Biology and Technology*, **26**(2), pp.157-165, DOI: 10.1016/S0925-5214(02)00024-8.
- [4] M.B.P. Gago, C. Rojas, M.A. DelRio (2002), "Effect of lipid type and amount of edible hydroxypropyl methylcellulose-lipid composite coatings used to protect postharvest quality of mandarins cv. Fortune", *Food Chemistry and Toxicology*, **67**(8), pp.2903-2910, DOI: 10.1111/j.1365-2621.2002.tb08836.x.
- [5] F. Labouffie, M. Hémati, A. Lamure, et al. (2013), "Effect of the plasticiser on permeability, mechanical resistance and thermal behaviour of composite coating films", *Powder Technology*, **238**, pp.14-19, DOI: 10.1016/j.powtec.2012.07.035.
- [6] S.A.V. Chamorro, M.B. Pérez-Gago, M.Á. Río, et al. (2009), "Effect of antifungal hydroxypropyl methylcellulose (HPMC)-lipid edible composite coatings on postharvest decay development and quality attributes of cold-stored 'Valencia' oranges", *Postharvest Biology and Technology*, **54**(2), pp.72-79, DOI: 10.1016/j.postharvbio.2009.06.001.
- [7] M.A. Silva, et al. (2009), "Alginate and pectin composite films crosslinked with Ca²⁺ ions: Effect of the plasticiser concentration", *Carbohydrate Polymer*, **77**(4), pp.736-742, DOI: 10.1016/j.carbpol.2009.02.014.
- [8] C.G. Otoni, R.J.A. Bustillos, H.M.C. Azeredo, et al. (2017), "Recent advances on edible films based on fruits and vegetables - A review", *Comprehensive Reviews in Food Science and Food Safety*, **16**(5), pp.1151-1169, DOI: 10.1111/1541-4337.12281.
- [9] B. Panda, A.S. Parihar, S. Mallick (2014), "Effect of plasticiser on drug crystallinity of hydroxypropyl methylcellulose matrix film", *International Journal of Biological Macromolecules*, **67**, pp.295-302, DOI: 10.1016/j.ijbiomac.2014.03.033.
- [10] R. Sothornvit, J.M. Krochta (2005), "Plasticisers in edible films and coatings", *Innovations in Food Packaging*, **23**, pp.403-433, DOI: 10.1016/B978-012311632-1/50055-3.
- [11] C.A.R. Bastida, M. M. Polo, G. Velazquez, et al. (2004), "Effect of plasticiser, pH and hydration on the mechanical and barrier properties of Zein and ethylcellulose films", *Ciencia e Tecnologia de Alimentos*, **4**(4), pp.251-256, DOI: 10.1080/11358120409487767.
- [12] R. Sothornvit, J.M. Krochta (2001), "Plasticiser effect on mechanical properties of P-lactoglobulin films", *Journal of Food Engineering*, **50**(3), pp.149-155, DOI: 10.1016/S0260-8774(00)00237-5.

Interaction between a complex fluid flow and a rotating cylinder

Cuong Mai Bui¹, Tinh Xuan Ho^{2*}

¹University of Technology and Education, The University of Danang, 41 Le Duan Street, Hai Chau 1 Ward, Hai Chau District, Da Nang City, Vietnam

²Department of Computational Engineering, Vietnamese - German University, Ring Road 4, Quarter 4, Thoi Hoa Ward, Ben Cat Town, Binh Duong Province, Vietnam

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Abstract:

The flow of a thixotropic Bingham material past a rotating cylinder is studied under a wide range of Reynolds and Bingham numbers, thixotropic parameters, and rotational speeds. A microstructure transition of the material involving breakdown and recovery processes is modeled using a kinetic equation, and the Bingham-Papanastasiou model is employed to represent shear stress-strain rate relations. Results show that the material's structural state at equilibrium depends greatly on the rotational speed and the thixotropic parameters. A layer around the cylinder resembling a Newtonian fluid is observed, in which the microstructure is almost completely broken, the yield stress is negligibly small, and the apparent viscosity approximates that of the Newtonian fluid. The thickness of this Newtonian-like layer varies with the rotational speed and the Reynolds number, but more significantly with the former than with the latter. In addition, the lift and moment coefficients increase with the rotational speed. These values are found to be close to those of the Newtonian fluid as well as of an equivalent non-thixotropic Bingham fluid. Many other aspects of the flow such as the flow pattern, the unyielded zones, and strain rate distribution are presented and discussed.

Keywords: Bingham, computational fluid dynamics (CFD), non-Newtonian fluid, thixotropy, yield stress.

Classification number: 2.3

1. Introduction

Non-Newtonian liquids such as sediment [1-4], fresh concrete, and cement [5-7] have rheologically complex characteristics, which can include viscoplasticity, typically of a Bingham type, and thixotropy. Bingham materials flow when an applied shear stress (τ) is greater than a threshold value (τ_0 i.e., yield stress). Otherwise, they behave like a solid. Thixotropy is a characteristic associated with the materials' microstructure that can be broken down and/or built up under shearing conditions. The breakdown process increases their flowability, while the recovery process does the opposite. Good reviews about thixotropy can be found in, e.g., H.A. Barnes (1997) [8], J. Mewis, et al. (2009) [9], and most recently R.G. Larson, et al. (2019) [10]. In a flow field of these materials, solid-like zones where $\tau \leq \tau_0$ can be formed known as unyielded zones; beyond these zones where $\tau > \tau_0$ the materials are yielded and hence behave like liquids.

Thixotropic Bingham liquids are encountered in numerous applications in which interaction between complex liquids and a moving object can be presented. For Newtonian fluids, the fluid-solid interaction is a

classical problem in fluid mechanics and has extensively been studied [11-13]. However, only a limited number of works have been found for Non-Newtonian fluids. While a majority of these works dealt with stationary cylinders, only a few examined rotating cylinders.

With a stationary cylinder, D.L. Tokpavi, et al. (2008) [14] investigated the creeping flow of viscoplastic fluid. Size and shape of unyielded zones were found to depend on the Oldroyd number (Od) at low values and asymptote those at $Od = 2 \times 10^5$. S. Mossaz, et al. (2010, 2012) [15-17] explored both numerically and experimentally a yield-stress fluid flow over a stationary cylinder. The flow was laminar with and without a recirculation wake. Aspects such as size of the recirculation wake and unyielded zones were investigated. Recently, Z. Ouattara, et al. (2018) [18] performed a rigorous study of a cylinder translating near a wall in a still Herschel-Bulkley liquid. The flow was at a Reynolds number of $Re \sim 0$ and both numerical and experimental approaches were employed. Effects of Od and cylinder-wall gap on drag force were reported.

Regarding the flow over a rotating cylinder, several works have been performed, for example, with a shear-thinning viscoelastic fluid [19] and shear-thinning power-

*Corresponding author: Email: tinh.hx@vgu.edu.vn

law fluid [20] at $Re \leq 40$. Results of flow pattern as well as drag and lift forces were reported to depend on Re , the cylinder's rotational speed, and the shear-thinning behaviour. In addition, P. Thakur, et al. (2016) [21] explored a yield stress flow over a wide range of Bingham numbers, i.e., $0 \leq Bn \leq 1000$. Flow aspects such as streamlines, yield boundaries, and unyielded zones were reported. It was stated that flow morphology at $Bn=1000$ and at Re in the range of 0.1-40 was identical. Most recently, M.B. Khan, et al. (2020) [22] carried out an intensive study of flow and heat transfer characteristics of a FENE-P-type viscoelastic fluid over a rotating cylinder. It was found that an inertio-elastic instability was induced at low rotational speeds that destabilized the flow; however, at high speeds, this instability gradually diminished and the flow became steady at $Re=60$ and 100. For the convection heat transfer, a correlation for the Nusselt number was proposed.

It is worth noting that the fluid in all of the aforementioned works is non-thixotropic. With a thixotropic material, as its microstructure and rheology can change under shearing conditions, its flow behaviours would become more complex than those of a simple yield-stress fluid. Indeed, in the flow of a thixotropic Bingham fluid past a stationary cylinder at $Re=45$ and $Bn=0.5$ and 5, reported by A. Syrakos, et al. (2015) [23], thixotropic parameters were found to significantly affect the flow field, especially the location and size of the unyielded or yielded zones.

In this work, we aim to further explore the flow behaviours of this type of fluid. In particular, we investigate the interaction of a thixotropic Bingham fluid with a rotating cylinder over a relatively wide range of Re , i.e., $Re=20-100$, and a dimensionless rotational speed of up to 5. Such a flow is expected to span from steady to unsteady laminar regimes. Special focus will be on a fluid layer surrounding the cylinder where the strain rate is large because of the rotation. Within this layer, the microstructure can be substantially broken resulting in an apparent viscosity as small as plastic viscosity, and thus the fluid can behave like a Newtonian one. This layer's effects on hydrodynamic forces will be examined.

2. Theory background

2.1. Governing equations

The mass and momentum equations for the fluid flow are, respectively, as follows:

$$\nabla \cdot \underline{u} = 0 \quad (1)$$

$$\frac{\partial(\rho \underline{u})}{\partial t} + \rho \underline{u} \cdot \nabla \underline{u} = \nabla \cdot \underline{\sigma} \quad (2)$$

where $\underline{u}$ is the velocity, ρ the fluid density and $\underline{\sigma} = -p\underline{I} + \underline{\tau}$ the total stress tensor. Moreover, p is the pressure, $\underline{I}$ the unit tensor, and $\underline{\dot{\gamma}}$ the deformation rate tensor defined as

$$\dot{\gamma}_{ij} = \frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i}.$$

For a Newtonian fluid, $\underline{\tau} = \mu \underline{\dot{\gamma}}$, and for Bingham fluid, it is modeled as:

$$\begin{cases} \underline{\tau} = \left(K + \frac{\tau_0}{\dot{\gamma}} \right) \underline{\dot{\gamma}} & \text{if } \tau > \tau_0 \\ \dot{\gamma} = 0 & \text{if } \tau \leq \tau_0 \end{cases} \quad (3)$$

Here, K is the consistency, $\dot{\gamma} = \sqrt{\frac{1}{2} \underline{\dot{\gamma}} : \underline{\dot{\gamma}}}$ is the strain rate

tensor's magnitude, and $\tau = \sqrt{\frac{1}{2} \underline{\tau} : \underline{\tau}}$ is the intensity of the extra stress noting that $\dot{\gamma}$ and τ are scalars. As this model is discontinuous at $\tau = \tau_0$, regularization methods such as Papanastasiou's technique (1987) [24] and bi-viscosity approximations [25, 26] can be utilized to avoid singular possibilities. The former approach was proven to be more computationally reliable and efficient compared to bi-viscous ones [27]. It is hence employed in this work as:

$$\underline{\tau} = \left(K + \frac{\tau_0 [1 - \exp(-m\dot{\gamma})]}{\dot{\gamma}} \right) \underline{\dot{\gamma}} \quad (4)$$

with m being the regularization parameter, which takes on a value of 40000 in this work. Note that when $m \rightarrow \infty$, Eq. (4) approaches Eq. (3) for an ideal HB fluid.

The Reynolds number is defined as $Re = \rho u_\infty D / K$ and the Bingham number as $Bn = \tau_y D / K u_\infty$ where D is the diameter of the cylinder, u_∞ the far field velocity, and τ_y the maximum yield stress. Furthermore, a dimensionless rotational speed α_r is defined as $\alpha_r = \omega D / 2u_\infty$ with ω being the angular speed.

Thixotropy is modeled using a dimensionless structural parameter, λ , which takes on a value between 0 (completely unstructured) and 1 (fully structured). Specifically, its evolution follows a kinetic equation mimicking a reversible chemical reaction as [28]:

$$\frac{\partial \lambda}{\partial t} + \underline{u} \cdot \nabla \lambda = \alpha(1 - \lambda) - \beta \lambda \dot{\gamma} \quad (5)$$

where α and β are, respectively, the recovery and breakdown parameters. Accordingly, the first and the second terms on the right-hand side of Eq. (5) represent the recovery and the breakdown phenomena. The yield stress is determined as $\tau_0 = \lambda \tau_y$ [29] with τ_y being the yield stress at $\lambda=1$. When $\lambda=0$, $\tau_0=0$, and the fluid becomes Newtonian.

2.2. Computational implementation

A two-dimensional (2D) computational domain employed in this work is shown in Fig. 1. It is a circular domain with a diameter $D_\infty = 200D$. The inlet velocity

condition is applied to the front half of the domain's boundary while outlet pressure is applied to the rear half. In addition, a no-slip boundary condition is applied to the cylinder's surface. A structured mesh consisting of 92000 elements is generated in the domain. Results for strain rate profiles at several positions are shown in Fig. 2 and it is obvious that the mesh of 92000 elements is sufficient. Computation is carried out using ANSYS FLUENT augmented with User-Defined Functions (UDF) taking into account Eqs. (4) and (5). As Re is relatively low, i.e., $20 \leq Re \leq 100$, the viscous-laminar model is employed.

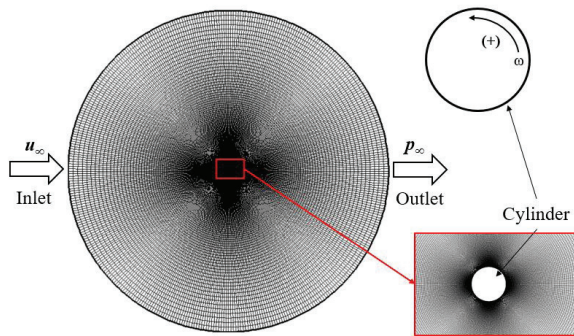


Fig. 1. Computational domain and mesh.

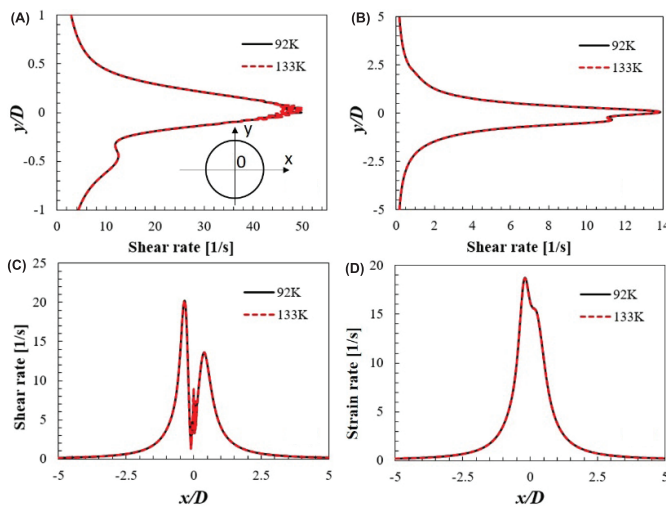


Fig. 2. Comparison of strain rate profiles along (A) $x=0.501D$, (B) $x=0.51D$, (C) $y=-0.501D$, and (D) $y=-0.51D$ at $Re=100$, $Bn=0.5$, $\alpha_r=5$, $\alpha=0.05$ and $\beta=0.05$ between a mesh of 92000 and a mesh of 133000 elements.

3. Results and discussion

3.1. Validation

For a stationary cylinder, results for the streamline pattern, the near-field unyielded zones, and the structural parameter of a thixotropic Bingham liquid at $Re=45$ and $Bn=0.5$ are shown in Fig. 3. Here, the breakdown

parameter is $\beta=0.05$, and the recovery parameter takes on various values as $\alpha=0.01$, 0.05 , and 0.1 . It is observed that under these conditions, the flow around the cylinder is in a steady laminar regime with a flow recirculation wake behind the cylinder. With a greater value of α , the fluid is more structured (large λ) especially inside the recirculation wake. The wake becomes smaller, whereas the unyielded zones become larger when α increases. These trends are well in line with those at the same conditions reported by A. Syrakos, et al. (2015) [23].

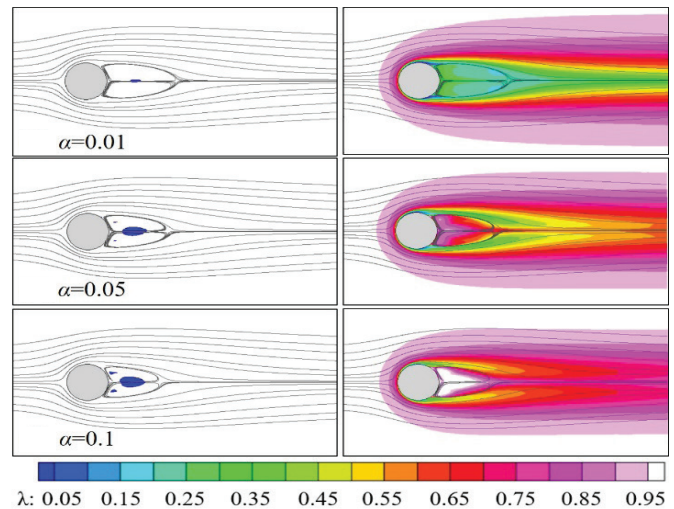


Fig. 3. Unyielded zones (left, dark areas) and distribution of λ (right) of a thixotropic flow at $Re=45$, $Bn=0.5$, $\beta=0.05$, and different values of α . Streamlines are shown on both sides; the cylinder is stationary.

For a rotating cylinder, results for drag (C_d) and lift (C_l) coefficients of a Newtonian fluid are compared with existing data. This is done for $\alpha_r=1$ and $Re=20$, 40 , and 100 , and the results are presented in Table 1. It is noted that C_l can be positive or negative depending on the rotation direction; however, only its magnitude is shown. As can be seen, good agreement is achieved for all the cases. Furthermore, flow field morphology of a (non-thixotropic) Bingham liquid at $\alpha_r=0.5$ and $Re=0.1$, 20 , and 40 is presented in Fig. 4. Size and shape of the near-field unyielded and yielded zones are found to be in great agreement with those obtained by P. Thakur, et al. (2016) [21].

Table 1. C_d and C_l of a Newtonian fluid on a rotating cylinder at $\alpha_r=1$.

	Present work		Reference data	
	C_d	C_l	C_d	C_l
$Re=20$	1.83	2.73	1.84 [20], 1.84 [12]	2.75 [20], 2.72 [12]
$Re=40$	1.32	2.59	1.32 [20], 1.32 [12]	2.60 [20], 2.60 [12]
$Re=100$	1.10	2.49	1.10 [12], 1.11 [11]	2.50 [12], 2.50 [11]

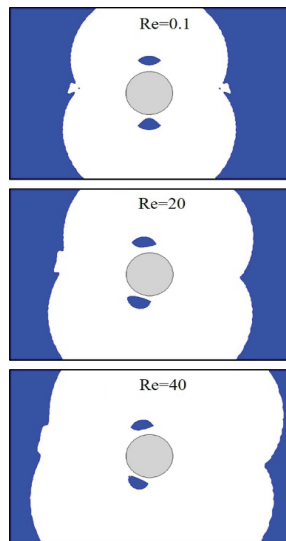


Fig. 4. Flow morphology of Bingham fluid at $\alpha_r=0.5$, $Bn=10$, and $Re=0.1, 20$, and 40 . Two unyielded zones are located above and below the cylinder.

3.2. Effect of the rotational speed

The effect of α_r on the flow field at $Re=20, 45$, and 100 is investigated in this section. To this end, various values of α_r ranging from 0 to 5 are realized. All the simulations are conducted at $Bn=0.5$, and with the thixotropic parameters of $\alpha=0.05$ and $\beta=0.05$. Results for the streamlines and the near-field unyielded zones are shown in Fig. 5. It is obvious that when the cylinder is stationary ($\alpha_r=0$), the flow is symmetrical at $Re=20$ and 45 .

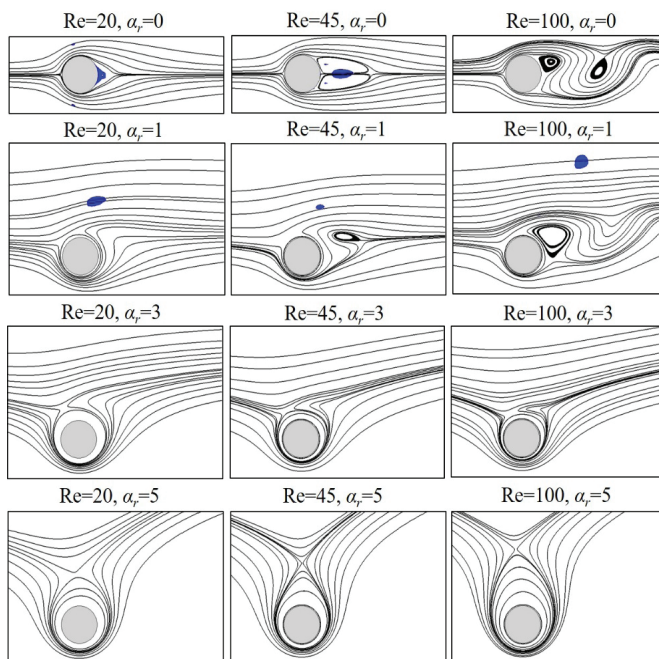


Fig. 5. Streamline pattern and unyielded zones (dark areas) at different rotational speeds (rows) and Re (columns).

A static, rigid zone is observed at $Re=20$, whereas three moving unyielded zones appear in the recirculation bubble behind the cylinder at $Re=45$. This finding is in line with A. Syrakos, et al. (2015) [23]. When the cylinder rotates ($\alpha_r \neq 0$), the symmetry no longer exists, and the rigid zones are pushed upward and away from the cylinder along the rotation direction. These zones are indeed not seen in proximity to the cylinder at $\alpha_r=3$ and 5 . At $Re=100$ (the highest Re investigated), the flow past the stationary cylinder is unsteady with periodic vortex shedding behind the cylinder. In addition, no rigid zones are observed near the cylinder at any rotational speeds.

Contours of the vorticity magnitude are shown in Fig. 6. As can be seen, the vortex shedding manifests only at $Re=100$ and $\alpha_r=0$ and 1 although the vortex pattern is somewhat pushed upward at $\alpha_r=1$. The flow becomes steady at greater rotational speeds, i.e., $\alpha_r=3$ and 5 .

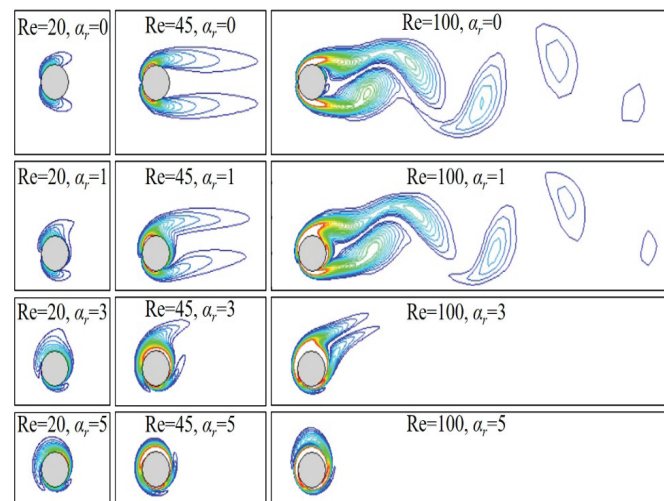


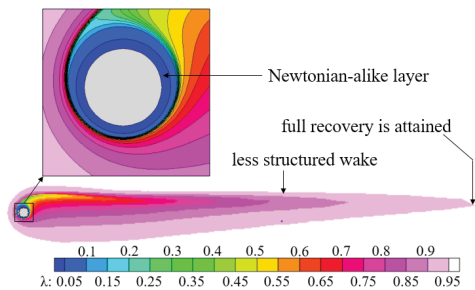
Fig. 6. Contours of the vorticity magnitude at different rotational speeds (rows) and Re (columns).

Results for the Strouhal number, $St=fD/u_\infty$, where f is the vortex frequency, at $\alpha_r=0, 0.5$, and 1 are provided in Table 2. It is noticed that St (thus f) of the non-thixotropic Bingham flow is smaller than that of the thixotropic Bingham and Newtonian flows at the same α_r . This trend of St can be attributed to the viscous effect, which is supposed to be greatest in non-thixotropic flows and smallest in Newtonian flows. In addition, St is found to slightly increase as α_r increases, especially for Bingham flows since their viscous effect becomes less important. It is worth mentioning that our results for the Newtonian flow at $\alpha_r=0$ match perfectly with the experimental results of E. Berger, et al. (1972) [30] ($St=0.16-0.17$) and C. Williamson (1989) [31] ($St=0.164$).

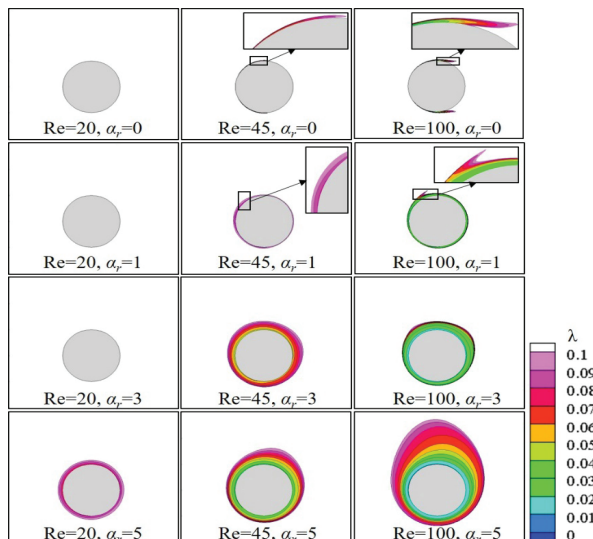
Table 2. Strouhal number St at $Re=100$ and $Bn=0.5$.

Fluid	$\alpha_r=0$	$\alpha_r=0.5$	$\alpha_r=1$
Newtonian	0.163	0.165	0.165
Thixotropic Bingham	0.160	0.165	0.165
Non-thixotropic Bingham	0.152	0.156	0.160

Furthermore, the distribution of the structural parameter λ at equilibrium is shown in Fig. 7 for $Re=45$ and $\alpha_r=5$. The material is found to be substantially broken and becomes little structured ($\lambda \leq 0.05$) in a small region surrounding the cylinder. As the broken material passes the cylinder and moves to the downstream, its structure is gradually recovered and it reaches a fully structured state far behind the cylinder.

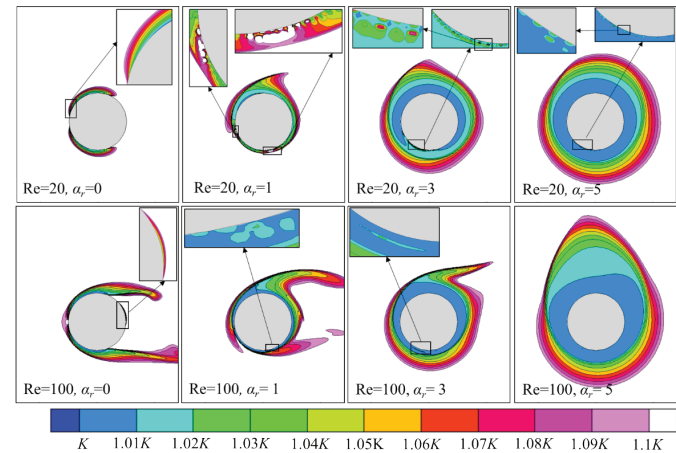

Fig. 7. Distribution of the structural parameter at $Re=45$, $Bn=0.5$, and $\alpha_r=5$.

Additionally, the distribution of λ in close proximity to the cylinder is shown in Fig. 8 for various values of Re and α_r . It is noted that only $\lambda \leq 0.1$ is shown. A Newtonian-like layer is defined as $\lambda \leq 0.01$, which is equivalent to 99% of the microstructure having been broken, making the fluid essentially behave like a Newtonian one. This


Fig. 8. Distribution of λ around the cylinder at different values of α_r (rows) and Re (columns).

layer turns out to be very thin and noticeable only at high values of Re (e.g., 45 and 100) and great rotational speeds (e.g., $\alpha_r=3$ and 5).

The apparent viscosity of the Newtonian-like layer is expected to approach that of a Newtonian fluid, which is K according to Eq. (4). The results for the distribution of the apparent viscosity are presented in Fig. 9 in detail, which it is cut off at 1.1K.


Fig. 9. Distribution of the apparent viscosity at $Re=20$ (top row) and $Re=100$ (bottom row) and different values of α_r .

It is obvious that the viscosity is not uniform, and in general it increases from the surface of the cylinder to the outside. For the stationary cylinder ($\alpha_r=0$), the viscosity transition is quite smooth. However, for the rotating cylinder, the viscosity distribution is not continuous as small islands of greater viscosity appear within zones of small and constant viscosity. This phenomenon takes place below or on the lower part of the cylinder where two fluid motions meet and surpass each other. One fluid motion is caused by the rotation of the cylinder the other is the incoming flow. It is worth mentioning that the velocity of the former changes its direction as it flows along the surface. Fluid deformation is therefore expected to rapidly change from one point to another and can take on negative or positive values. As a consequence, the strain rate magnitude, defined as $\dot{\gamma} = \sqrt{\frac{1}{2} \dot{\gamma} : \dot{\gamma}}$, can be non-continuous as well as apparent viscosity. As Re and/or α_r increases, the viscosity distribution becomes more monotonous; indeed, at $Re=100$ and $\alpha_r=5$, the mentioned viscosity islands are not found.

The non-continuous distribution of strain rate is observed also with Newtonian and non-thixotropic

Bingham fluids, as evident from Fig. 10 for $Re=45$ and $\alpha_r=5$. In addition, it is noticed that the strain rate distribution of the three fluids in close proximity to the cylinder is almost identical, which can be attributed to the high rotational speed and thus high shear.

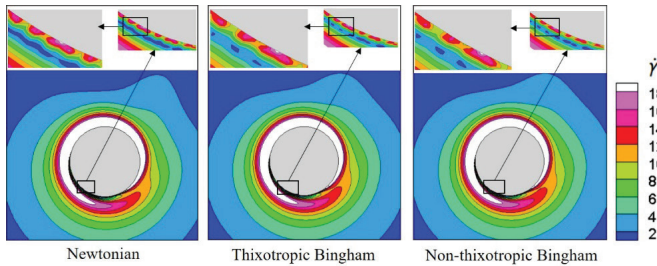


Fig. 10. Distribution of the strain rate for different fluids at $Re=45$ and $\alpha_r=5$.

The Newtonian-like layer can be alternatively defined using the apparent viscosity, that is, $\mu_{app} \leq 1.01K$. This definition is pertinent to non-thixotropic fluids. Accordingly, as can be observed from Fig. 9, the thickness of this layer increases significantly as α_r increases, however, the effect of Re is less important. It is noteworthy that the two approaches (structure-wise and viscosity-wise) to defining the Newtonian-like layer result in a deviation of its thickness. Nevertheless, this follows the same trend as Re and/or α_r are varied (see Figs. 8 and 9).

3.3. Effect of the thixotropic parameters

Simulations for $Re=45$, $Bn=0.5$, $\alpha_r=1$, and varying α and β (in the range from 0.001 to 1) are conducted. Here, focus is paid on the structural state λ in the region around the cylinder.

The distribution of λ at equilibrium is shown in Fig. 11 for different values of α , and that of the apparent viscosity is also shown therein. It is obvious that the material is more structured when α is greater, i.e., a greater structural recovery rate compared with the breakdown rate. Accordingly, the Newtonian-like layer defined by $\lambda \leq 0.01$ is thinner and becomes hardly observed for $\alpha=1$. The same trend is observed when it is defined by $\mu_{app} \leq 1.01K$.

In a similar manner, the effect of β representing the breakdown rate is demonstrated in Fig. 12. As can be expected, it is opposite to the effect of α . The Newtonian-like layer can be clearly observed for $\beta=1$ but hardly noticed for $\beta=0.001$. Like the previous case and as mentioned earlier, the Newtonian-like layer is somewhat thicker and thus easier to be noticed when defined by $\mu_{app} \leq 1.01K$ than by $\lambda \leq 0.01$.

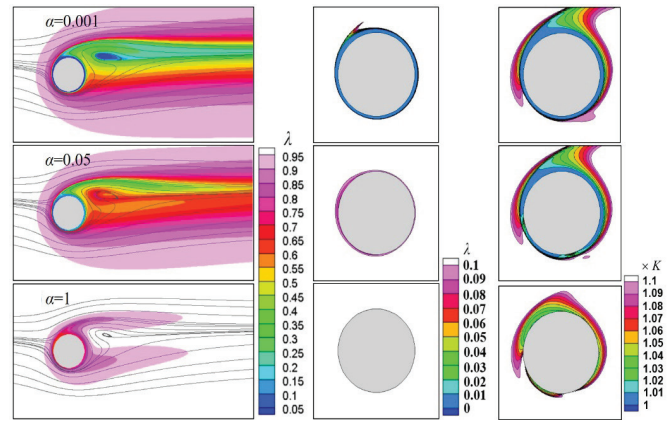


Fig. 11. Distributions of λ (left and middle) and apparent viscosity (right) for $\beta=0.05$ and various values of α ; $Re=45$, $Bn=0.5$, and $\alpha_r=1$.

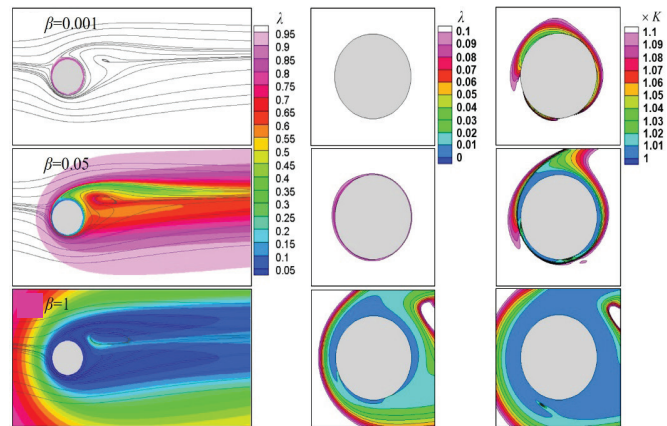


Fig. 12. Distributions of λ (left and middle) and apparent viscosity (right) for $\alpha=0.05$ and various values of β ; $Re=45$, $Bn=0.5$, and $\alpha_r=1$.

3.4. Effect of Bn

The effect of the Bingham number on the thixotropic flow at $Re=45$ and 100 is examined here. To that end, simulations for $Bn=1$, 2, and 5 are performed. The other parameters are kept constant, that is, $\alpha=0.05$, $\beta=0.05$, and $\alpha_r=1$. Results for the streamline pattern and the unyielded zones are presented in Fig. 13. It is observed that no static rigid zones are formed under these conditions, similar to the case of $Bn=0.5$ presented in Fig. 5. Moving rigid zones are found to scatter in the flow field. They are closer to the cylinder at higher Bn . At $Re=100$, the flow regime is found to transition from a non-stationary laminar regime at $Bn=1$ to a stationary one at $Bn=2$ or higher. In addition, Fig. 14 shows the distribution of λ and the vorticity contours at $Re=100$ and $Bn=1$ and 2. It is noticed that the material is less structured in the wake of the cylinder, especially in areas of great vorticity. As Bn increases the wake (especially its less structured core resembling a tail) becomes narrower.

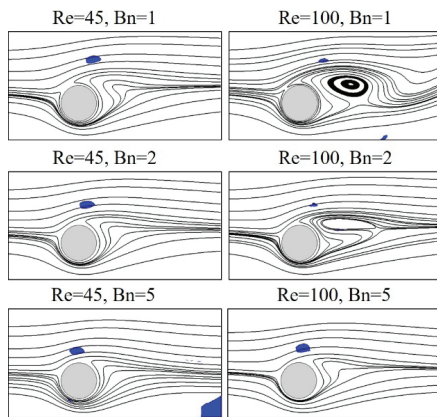


Fig. 13. Streamline pattern and unyielded zones (dark areas) of the thixotropic flow at $Re=45$ (left) and 100 (right), and different values of Bn ; $\alpha_r=1$.

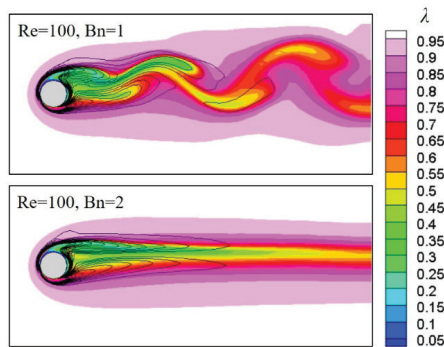


Fig. 14. Distribution of λ at $Bn=1$ and 2 ; $Re=100$ and $\alpha_r=1$. Vorticity contours are also shown.

Furthermore, the distribution of the apparent viscosity is shown in Fig. 15. It is obvious that at a relatively low rotational speed, i.e., $\alpha_r=1$, viscosity islands are found to exist and the Newtonian-like layer is not continuous, substantially thin, and becomes negligible as Bn increases to as high as 5 .

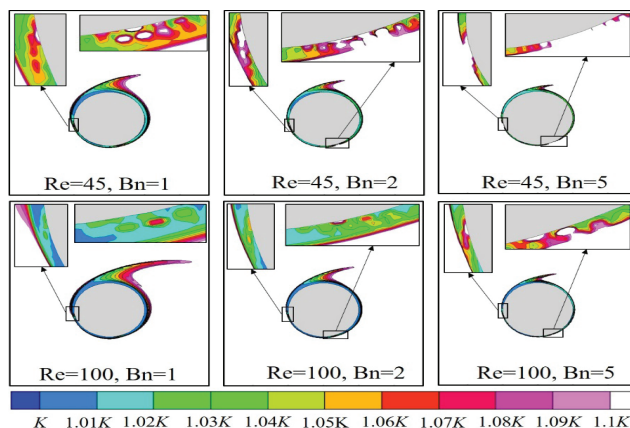


Fig. 15. Apparent viscosity at different values of Bn and $Re=45$ (top row) and 100 (bottom row).

3.5. Hydrodynamic forces

Results for C_d , C_p and C_m are presented in Fig. 16 for various values of Re , Bn , and α_r . It is noted that $C_m = M / (0.5\rho u_\infty^2 AL)$ is the moment coefficient with M being the moment about z -axis, A the reference area, and L the length of the cylinder.

At the same Bn and rotational speed, the drag coefficient is found to be smaller at higher Re . At $\alpha_r=1$, it increases approximately linearly with Bn with a slope being greater for $Re=45$ than for $Re=100$. In addition, it is noticed that the drag coefficient has a minimum value at $\alpha_r=3$ for all Re conducted. S.K. Panda, et al. (2010) [20] also observed a similar trend for power-law liquids. However, more research may be needed for a better understanding of its governing mechanisms.

It is worth mentioning that as the rotation of the cylinder is counter-clockwise, C_l and C_m are always negative. Their magnitude (positive) is found to increase with increasing the rotational speed. The effect of Re on C_l is relatively small at $\alpha_r \leq 3$ and significant at higher α_r . Unlike C_d , C_l does not change its trend at this critical speed. The magnitude of C_m is seen to increase linearly with increasing α_r and Bn ; this trend is more pronounced at smaller Re than at higher Re .

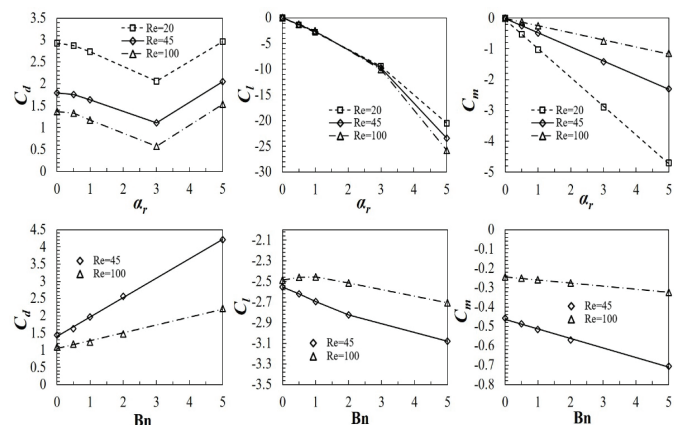


Fig. 16. C_d , C_p and C_m versus α_r at $Bn=0.5$ (top row) and versus Bn at $\alpha_r=1$ (bottom row).

A comparison of the hydrodynamic coefficients between Newtonian, thixotropic, and non-thixotropic Bingham fluids at $Re=45$ is presented in Fig. 17. It is noticed that C_d of the thixotropic fluid is somewhat smaller than that of the non-thixotropic fluid. They are both at $Bn=0.5$, however, as the microstructure of the former can be broken, its yield stress and thus apparent viscosity reduce especially in regions surrounding the cylinder and its wake. C_d of the equivalent Newtonian

fluid is considerably smaller. A negligible difference between C_l and C_m among the three fluids is observed. It is worth mentioning that the strain rate distribution of these fluids at $\alpha_r=5$ in proximity to the cylinder is almost identical (see Fig. 10). The lift and moment coefficients can thus be postulated to be dictated by the fluid layer around the cylinder, which is typically the Newtonian-like layer.

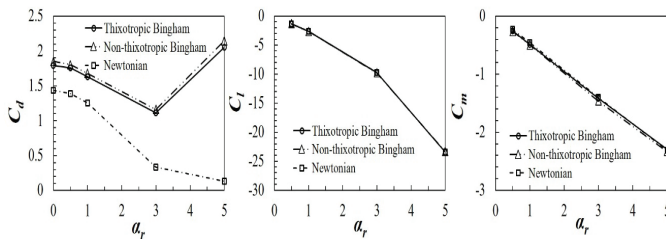


Fig. 17. Comparison of C_d , C_p and C_m between three types of fluid at $Re=45$, $Bn=0.5$, and various values of α_r .

Furthermore, Fig. 18 shows the distribution of the static pressure coefficient, $C_p = 2(p - p_0)/(\rho u_\infty^2)$, on the cylinder's surface for various values of α_r . It is noticed that the pressure curve is symmetrical only for the case of stationary cylinder and at relatively low Re , i.e., $Re=20$ and 45 . At $Re=100$, the flow becomes unsteady with periodic vortex shedding behind the cylinder and the C_p curve at any particular time instant is not necessarily symmetrical although it can be if averaged over a long enough time. For the case of a rotating cylinder ($\alpha_r \geq 0$), the symmetry is completely lost, and a minimum value of C_p is observed at $\sim 270^\circ$. This minimum value decreases (negative) significantly with increasing rotational speed. Accordingly, the lift force (pointing downward) increases considerably as the rotational speed increases, which agrees with the C_l - α_r curve shown in Fig. 16.

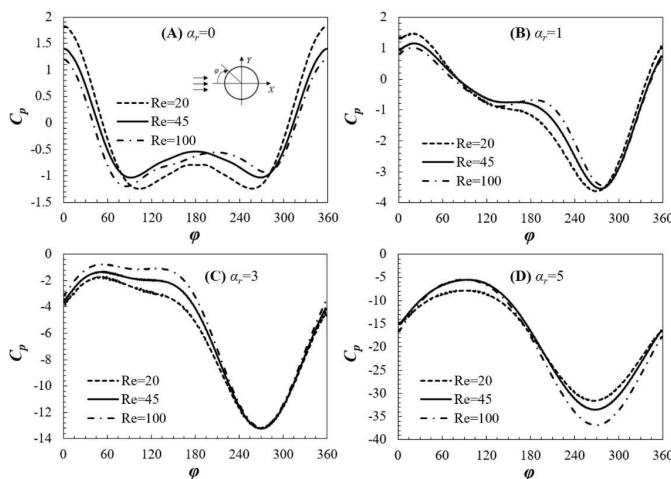


Fig. 18. Distribution of the static pressure on the cylinder's surface at various values of α_r ; $Bn=0.5$.

4. Conclusions

The flow of a thixotropic Bingham fluid over a rotating cylinder has been studied using a numerical approach. The effects of the rotational speed, thixotropic parameters, Bn , and Re on the flow behaviours were investigated. Under the conditions realized, e.g., $Re=20$ – 100 , $Bn \leq 5$, and $\alpha_r \leq 5$, the flow was laminar and steady except for the case of $Re=100$, $Bn=0.5$, and $\alpha_r=1$ where it was unsteady with vortex shedding behind the cylinder.

The thixotropic material was less structured at higher rotational speeds. A region of low λ was observed around the cylinder, in which the yield stress and the apparent viscosity were small, and the fluid was believed to behave like a Newtonian one. Two definitions of the Newtonian-like layer were proposed, that is, $\lambda \leq 0.01$ and $\mu_{app} \leq 1.01K$. Its thickness was found to greatly depend on the rotational speed (i.e., greater at higher α_r) and, at relatively smaller extent, on the thixotropic parameters Re and Bn .

Results of C_d , C_p and C_m were reported and discussed. They were found to significantly depend on the rotational speed, Re , and Bn . The magnitude of C_l and C_m increases with α_r and Bn , however, C_d was found to change its trend as it obtained a minimum value at $\alpha_r=3$. More importantly, C_l and C_m of the Newtonian, thixotropic, and non-thixotropic Bingham fluids at $Re=45$ and $Bn=0.5$ were found to be close to one another and this was attributable to the Newtonian-like layer.

CRediT author statements

Cuong Mai Bui, Thanh Xuan Ho: Experiments and data analysis, Writing the article.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] M.I. Carretero, M. Pozo (2009), "Clay and non-clay minerals in the pharmaceutical industry: Part I. Excipients and medical applications", *Applied Clay Science*, **46**(1), pp.73-80, DOI: 10.1016/j.clay.2009.07.017.
- [2] J. Berlamont, M. Ockenden, E. Toorman, et al. (1993), "The characterisation of cohesive sediment properties", *Coastal Engineering*, **21**(1), pp.105-128, DOI: 10.1016/0378-3839(93)90047-C.
- [3] G. Chapman (1949), "The thixotropy and dilatancy of a marine soil", *Journal of The Marine Biological Association of the United Kingdom*, **28**(1), pp.123-140, DOI: 10.1017/S0025315400055247.

- [4] V. Osipov, S. Nikolaeva, V. Sokolov (1984), "Microstructural changes associated with thixotropic phenomena in clay soils", *Geotechnique*, **34(3)**, pp.293-303, DOI: 10.1680/geot.1984.34.3.293.
- [5] R. Shaughnessy III, P.E. Clark (1988), "The rheological behavior of fresh cement pastes", *Cement Concrete Research*, **18(3)**, pp.327-341, DOI: 10.1016/0008-8846(88)90067-1.
- [6] B. Min, L. Erwin, H. Jennings (1994), "Rheological behaviour of fresh cement paste as measured by squeeze flow", *Journal of Materials Science*, **29(5)**, pp.1374-1381, DOI: 10.1007/BF00975091.
- [7] J.E. Wallevik (2009), "Rheological properties of cement paste: Thixotropic behavior and structural breakdown", *Cement Concrete Research*, **39(1)**, pp.14-29, DOI: 10.1016/j.cemconres.2008.10.001.
- [8] H.A. Barnes (1997), "Thixotropy - A review", *Journal of Non-Newtonian Fluid Mechanics*, **70(1-2)**, pp.1-33, DOI: 10.1016/S0377-0257(97)00004-9.
- [9] J. Mewis, N.J. Wagner (2009), "Thixotropy", *Advances in Colloid Interface Science*, **147**, pp.214-227, DOI: 10.1016/j.cis.2008.09.005.
- [10] R.G. Larson, Y. Wei (2019), "A review of thixotropy and its rheological modeling", *Journal of Rheology*, **63(3)**, pp.477-501, DOI: 10.1122/1.5055031.
- [11] D. Stojkovic, M. Breuer, F. Durst (2002), "Effect of high rotation rates on the laminar flow around a circular cylinder", *Physics of Fluids*, **14(9)**, pp.3160-3178, DOI: 10.1063/1.1492811.
- [12] S.B. Paramane, A. Sharma (2009), "Numerical investigation of heat and fluid flow across a rotating circular cylinder maintained at constant temperature in 2-D laminar flow regime", *International Journal of Heat Mass Transfer*, **52(13-14)**, pp.3205-3216, DOI: 10.1016/j.ijheatmasstransfer.2008.12.031.
- [13] S. Karabelas (2010), "Large eddy simulation of high-Reynolds number flow past a rotating cylinder", *International Journal of Heat and Fluid Flow*, **31(4)**, pp.518-527, DOI: 10.1016/j.ijheatfluidflow.2010.02.010.
- [14] D.L. Tokpavi, A. Magnin, P. Jay (2008), "Very slow flow of Bingham viscoplastic fluid around a circular cylinder", *Journal of Non-Newtonian Fluid Mechanics*, **154(1)**, pp.65-76, DOI: 10.1016/j.jnnfm.2008.02.006.
- [15] S. Mossaz, P. Jay, A. Magnin (2010), "Criteria for the appearance of recirculating and non-stationary regimes behind a cylinder in a viscoplastic fluid", *Journal of Non-Newtonian Fluid Mechanics*, **165(21-22)**, pp.1525-1535, DOI: 10.1016/j.jnnfm.2010.08.001.
- [16] S. Mossaz, P. Jay, A. Magnin (2012a), "Non-recirculating and recirculating inertial flows of a viscoplastic fluid around a cylinder", *Journal of Non-Newtonian Fluid Mechanics*, **177-178**, pp.64-75, DOI: 10.1016/j.jnnfm.2012.04.008.
- [17] S. Mossaz, P. Jay, A. Magnin (2012b), "Experimental study of stationary inertial flows of a yield-stress fluid around a cylinder", *Journal of Non-Newtonian Fluid Mechanics*, **189-190**, pp.40-52, DOI: 10.1016/j.jnnfm.2012.10.001.
- [18] Z. Ouattara, P. Jay, D. Bleses, et al. (2018), "Drag of a cylinder moving near a wall in a yield stress fluid", *AIChE Journal*, **64(11)**, pp.4118-4130, DOI: 10.1002/aic.16220.
- [19] P. Townsend (1980), "A numerical simulation of Newtonian and visco-elastic flow past stationary and rotating cylinders", *Journal of Non-Newtonian Fluid Mechanics*, **6(3-4)**, pp.219-243, DOI: 10.1016/0377-0257(80)80004-8.
- [20] S.K. Panda, R. Chhabra (2010), "Laminar flow of powerlaw fluids past a rotating cylinder", *Journal of Non-Newtonian Fluid Mechanics*, **165(21-22)**, pp.1442-1461, DOI: 10.1016/j.jnnfm.2010.07.006.
- [21] P. Thakur, S. Mittal, N. Tiwari, et al. (2016), "The motion of a rotating circular cylinder in a stream of Bingham plastic fluid", *Journal of Non-Newtonian Fluid Mechanics*, **235**, pp.29-46, DOI: 10.1016/j.jnnfm.2016.06.013.
- [22] M.B. Khan, C. Sasmal, R. Chhabra (2020), "Flow and heat transfer characteristics of a rotating cylinder in a FENE-P type viscoelastic fluid", *Journal of Non-Newtonian Fluid Mechanics*, **282**, DOI: 10.1016/j.jnnfm.2020.104333.
- [23] A. Syrakos, G.C. Georgiou, A.N. Alexandrou (2015), "Thixotropic flow past a cylinder", *Journal of Non-Newtonian Fluid Mechanics*, **220**, pp.44-56, DOI: 10.1016/j.jnnfm.2014.08.008.
- [24] T.C. Papanastasiou (1987), "Flows of materials with yield", *Journal of Rheology*, **31(5)**, pp.385-404, DOI: 10.1122/1.549926.
- [25] C. Beverly, R. Tanner (1989), "Numerical analysis of extrudate swell in viscoelastic materials with yield stress", *Journal of Rheology*, **33(6)**, pp.989-1009, DOI: 10.1122/1.550042.
- [26] S.S.P. Kumar, A.V. Quesada, M. Ellero (2020), "Numerical investigation of the rheological behavior of a dense particle suspension in a biviscous matrix using a lubrication dynamics method", *Journal of Non-Newtonian Fluid Mechanics*, **281**, DOI: 10.1016/j.jnnfm.2020.104312.
- [27] Y. Fan, N.P. Thien, R.I. Tanner (2001), "Tangential flow and advective mixing of viscoplastic fluids between eccentric cylinders", *Journal of Fluid Mechanics*, **431**, pp.65-89, DOI: 10.1017/S0022112000002998.
- [28] F. Moore (1959), "The rheology of ceramic slip and bodies", *J. Trans. Brit. Ceram. Soc.*, **58**, pp.470-492.
- [29] E.A. Toorman (1997), "Modelling the thixotropic behaviour of dense cohesive sediment suspensions", *Rheologica Acta.*, **36(1)**, pp.56-65, DOI: 10.1007/BF00366724.
- [30] E. Berger, R. Wille (1972), "Periodic flow phenomena", *Annual Review of Fluid Mechanics*, **4(1)**, pp.313-340, DOI: 10.1146/annurev.fl.04.010172.001525.
- [31] C. Williamson (1989), "Oblique and parallel modes of vortex shedding in the wake of a circular cylinder at low Reynolds numbers", *Journal of Fluid Mechanics*, **206**, pp.579-627, DOI: 10.1017/S0022112089002429.

Comparative studies of relaxor-ferroelectric $\text{Pb}_{0.92}\text{La}_{0.08}(\text{Zr}_{0.52}\text{Ti}_{0.48})\text{O}_3$ thin films deposited by pulsed laser deposition and sol-gel spin coating

Thi Ha Dang^{1,2,3*}, Thi Doan Tran¹, Duc Minh Nguyen^{1,4}, Van Truong Do², Ngoc Hung Vu¹

¹International Training Institute for Materials Science, Hanoi University of Science and Technology, 1 Dai Co Viet Street, Bach Khoa Ward, Hai Ba Trung District, Hanoi, Vietnam

²School of Mechanical Engineering, Hanoi University of Science and Technology, 1 Dai Co Viet Street, Bach Khoa Ward, Hai Ba Trung District, Hanoi, Vietnam

³Vietnam National University of Forestry, Xuan Mai Town, Chuong My District, Hanoi, Vietnam

⁴MESA+ Institute for Nanotechnology, University of Twente, Drienerlolaan 57522 NB Enschede, Netherlands

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Abstract:

In this study, the energy storage properties of relaxor $\text{Pb}_{0.92}\text{La}_{0.08}(\text{Zr}_{0.52}\text{Ti}_{0.48})\text{O}_3$ (PLZT) thin films grown on Pt/Si substrates using pulsed laser deposition (PLD) and sol-gel methods were investigated. The PLZT thin films deposited by PLD possessed a columnar growth microstructure and mixed orientations of (100) and (110) while a dense microstructure and preferred orientation of (100) were achieved by sol-gel deposition. Although the electric breakdown strength (E_{BD}) of the sol-gel-deposited PLZT thin films ($E_{\text{BD}}=2200$ kV/cm) was slightly higher than that of the films deposited using PLD ($E_{\text{BD}}=2100$ kV/cm), the PLD-deposited PLZT thin films had a larger recoverable energy storage density (U_{reco}) and energy storage efficiency (η), which can be explained by its slimmer polarization loop, higher maximum polarization, and lower remanent polarization. At corresponding E_{BD} values, the U_{reco} and η values were 33.2 J/cm³ and 67.5% for the PLD-deposited films and 27.5 J/cm³ and 62.2% for the sol-gel-deposited films. Although the sol-gel-deposited PLZT thin films had lower energy storage performance, the sol-gel method remains a promising method for the fabrication of thin films for dielectric energy storage applications due to its unique advantages of low fabrication cost, simple preparation process, and easy control of chemical composition.

Keywords: energy storage performance, pulsed laser deposition, relaxor ferroelectrics, sol-gel, thin film capacitors.

Classification number: 2.3

1. Introduction

Dielectric capacitors have attracted great attention as pulse-power energy storage devices due to their high power density, fast charge/discharge speed, and excellent thermal and cycling stabilities compared with other energy storage systems such as fuel, chemical batteries, and electromechanical capacitors [1-5]. Indeed, dielectric thin film capacitors with improved performance are becoming eagerly demanded as microelectronics devices are rapidly developing toward miniaturization, light weight, and easy integration. Moreover, dielectric materials in film form usually show an increased electric breakdown strength, which gives rise to larger energy storage density as compared with their bulk-ceramic counterpart [3, 6]. Among dielectric thin film capacitors, relaxor-ferroelectric (RFE) thin films have been extensively investigated due to their slim polarization

hysteresis (P - E) loop, low remanent polarization (P_r), low coercive field (E_c), and high breakdown strength (E_{BD}). These properties are a result of a phase transition from a long-range ordered polar state with macroscopic domains in normal ferroelectrics (FEs) to a short-range polar state with nano-scale domains (known as polar nanoregions, or PNRs) in RFEs [7-10], which makes them good candidates for energy storage applications [11-14]. Another feature that distinguishes FEs from RFEs is the frequency dependence of the dielectric constant versus temperature. Dielectric studies on FEs indicate a narrow dielectric constant peak at a high transition temperature (T_m), which corresponds to the maximum value of the dielectric constant. Meanwhile, the existence of a broadened phase transition peak in the temperature-dependent dielectric constant is observed in RFEs [15-17].

*Corresponding author: Email: danghaktek@gmail.com

In general, the volumetric energy storage density (U_{store}), recoverable energy storage density (U_{reco}), and energy storage efficiency (η) of a dielectric capacitor can be calculated from the P - E loop as follows [18]:

$$U_{store} = \int_0^{P_{max}} E dP \quad (1)$$

$$U_{reco} = \int_{P_r}^{P_{max}} E dP \quad (2)$$

$$\eta(\%) = 100 \times U_{reco} / U_{store} \quad (3)$$

where P_r and P_{max} are the remanent polarization and maximum polarization (polarization at the maximum applied electrical field E_{max}), respectively.

There are various methods that can be used to fabricate perovskite-oxide ferroelectric thin films such as PLD [19, 20], electron beam deposition [21], sputtering [22], and sol-gel spin coating [23-25]. Among these methods, ferroelectric thin films grown by PLD exhibit excellent ferroelectric and piezoelectric properties [26-29]. However, all methods except sol-gel spin coating require advanced and expensive equipment. Therefore, sol-gel spin coating is particularly suitable for ferroelectric thin film fabrication because of its simplicity, low cost, and, more importantly, easy-to-control stoichiometry and composition in multicomponent films.

In this study, we compared the ferroelectric and energy storage properties of relaxor $Pb_{0.92}La_{0.08}(Zr_{0.52}Ti_{0.48})O_3$ (PLZT) thin films grown on (111)Pt/Ti/SiO₂/Si(100) (Pt/Si) substrates using PLD and sol-gel spin coating. The Pt/Si substrates were chosen because they are widely used in the production of microsystems with ferroelectric films and are well suited to industrial applications [30]. The relaxor behaviour of PLZT films is influenced by the frequency dispersion of the dielectric constant and the increase in T_m with increasing frequency [31]. Experimental results illustrate that E_{BD} slightly improves with a dense microstructure that is accompanied by a degradation of P_{max} and a slight increase of P_r in sol-gel-deposited PLZT thin films. Finally, the U_{reco} and η values achieved in PLZT thin films deposited by PLD were 33.2 J/cm³ and 67.5% (at E_{BD} =2100 kV/cm), that of the sol-gel method were 27.5 J/cm³ and 62.2% (at E_{BD} =2200 kV/cm).

2. Experimental procedure

2.1. Fabrication of Pt/Si substrate

For the Pt/Si substrate, a 15-nm-thick Ti adhesive layer and a 100-nm-thick Pt bottom electrode were deposited at room temperature by sputtering on a 500-nm-thick SiO₂ layer formed on a Si(100) substrate using wet oxidation at 1150°C.

2.2. Pulsed laser deposition of PLZT thin films

The PLZT thin films were deposited on Pt/Si substrates from a stoichiometric target ($Pb_{0.92}La_{0.08}(Zr_{0.52}Ti_{0.48})O_3$) using PLD. The base pressure of the PLD vacuum chamber was below 10⁻⁶ mbar. The optimized deposition conditions of the PLZT thin film were a laser repetition rate 10 Hz, energy density 2.5 J/cm², oxygen pressure 0.1 mbar, and substrate temperature 600°C [28]. After deposition, the films were cooled to room temperature in a 1-bar oxygen atmosphere with at a ramp rate of 8°C/minute.

2.3. Sol-gel spin coating of PLZT thin films

The precursor materials selected for the fabrication of the PLZT thin film by sol-gel consisted of lead acetate trihydrate ($Pb[CH_3COO]_2 \cdot 3H_2O$), lanthanum nitrate ($La[NO_3]_3$), titanium *iso*-propoxide ($Ti[i-OPr]_4$), and zirconium *n*-propoxide ($Zr[n-OPr]_4$). These materials were dissolved in 2-methoxyethanol (MOE) solvent with 15% lead-excess to compensate for lead loss during annealing. The 0.4 M PLZT precursor solution was dripped onto the Pt/Si substrate and spin coated at 2000 rpm for 30 s, followed by pyrolysis at 400°C for 10 min. The number of spin coatings were controlled to achieve a desired thickness, and each coating was about 50 nm thick. Finally, the films were annealed at 650°C for 60 min in air. The final thickness of the films was about 250 nm. More information on sol-gel spin coating of PLZT thin films [32]. The schematic diagram of the PLZT thin film fabricated by the sol-gel method is shown in Fig. 1.

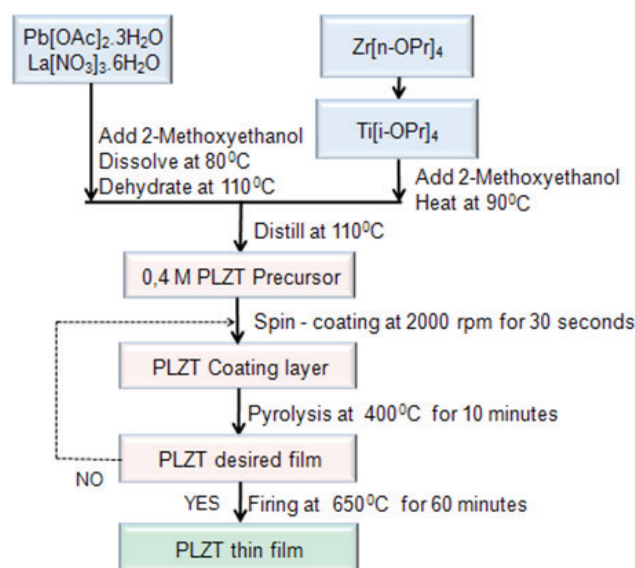


Fig. 1. The schematic representation of the sol-gel spin coating process of PLZT thin films.

2.4. Fabrication of thin film capacitors

For electrical measurements, the capacitors (size: $200 \times 200 \mu\text{m}^2$) were patterned by a standard photolithography process and structured by argon-beam etching of the Pt top electrodes (100 nm thick), and wet etching of the PLZT films with *HF-HCl* solution to expose the bottom electrodes.

2.5. Analysis and characterization

The crystallographic properties of the thin films were analysed by X-ray diffraction (XRD) θ - 2θ scans using a PANalytical X-ray diffractometer (Malvern PANalytical) that used $\text{Cu-K}\alpha$ radiation with a wavelength of $1.5405 \text{ \AA}$. Atomic force microscopy (Bruker Dimension Icon) and cross-sectional high-resolution scanning electron microscopy (HRSEM, Zeiss-1550, Carl Zeiss Microscopy GmbH) were performed to investigate the surface morphology, microstructure, and thickness of the as-grown thin films. The polarization-electric field (P - E) hysteresis loops and switching current-electric field (I_{sw} - E) curves were collected with using the dynamic hysteresis measurement (DHM) of the ferroelectric module of an aixACCT TF-2000 Analyzer (aixACCT Systems GmbH).

3. Results and discussion

The XRD pattern shown in Fig. 2A reveals that all the PLZT thin films grown on Pt/Si substrates have the desired perovskite phase without the pyrochlore phase within the detection limit of the XRD. The PLZT thin film deposited by PLD had a (100)/(110) mixed orientation, meanwhile a (100)/(111) mixed orientation was observed in the sol-gel-deposited PLZT thin film. A qualitative analysis of the thin film orientation was calculated as:

$$\%(h00) = 100 \times \frac{(I_{(100)} + I_{200})}{(I_{(100)} + I_{(110)} + I_{(111)} + I_{200})}$$

where h ($=1, 2$) is identical to the one of Miller (hkl) indices, and I is the intensity of a peak position of the PLZT thin films. The percentages of the (100), (110), and (111) orientations of the sol-gel-deposited PLZT thin film were 63.3, 5.2, and 31.5%, respectively, and 61.5, 38.5, and 0% for the PLZT thin film deposited by PLD. The out-of-plane lattice parameters derived from the corresponding (200) orientation were about 4.027 and 4.042 $\text{\AA}$, respectively, for the films deposited using sol-gel and PLD methods. As the out-of-plane lattice parameters in the PLZT thin films were close to the a -axis lattice parameters in corresponding PLZT bulk ceramics [33], therefore, it was concluded that the PLZT thin films grown on Pt/Si substrates in this study contained mainly a -domains.

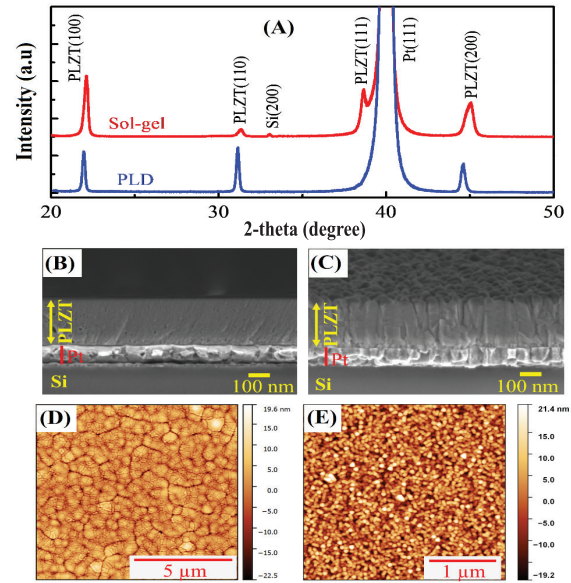


Fig. 2. (A) XRD patterns, (B, C) cross-sectional SEM, and (D, E) surface topographic AFM images of PLZT thin films deposited by sol-gel (B, D) and PLD methods (C, E).

The microstructures of PLZT thin films observed from the cross-sectional SEM and topographic AFM images are shown in Figs. 2B-2E. Figures 2B and 2C indicate that the sol-gel-deposited PLZT thin film had a dense structure while the PLD-deposited film exhibited a columnar structure. The thickness of the PLZT thin films was about 250 nm. The root-mean-square (RMS) surface roughness measured from the AFM images were about 4.4 nm and 7.1 nm for the films deposited by sol-gel and PLD, respectively. The RMS roughness combined with cross-sectional SEM reveal that the films with columnar structure had a larger surface roughness.

Figure 3 shows the P - E hysteresis loops and I_{sw} - E switching curves of PLZT thin films measured at 1000 kV/cm and 1 kHz. As seen in Fig. 3A, all the P - E loops were quite narrow with low P_r and small E_c values. The P_{max} , P_r , and E_c values were 36.04, 6.98 $\mu\text{C}/\text{cm}^2$, and 29.4 kV/cm for the PLZT films deposited by sol-gel and 39.81, 2.06 $\mu\text{C}/\text{cm}^2$, and 42.1 kV/cm for those deposited by PLD. The P_r and E_c values of both films were significantly lower than those of normal ferroelectric $\text{Pb}(\text{Zr}_{0.52}\text{Ti}_{0.48})\text{O}_3$ (PZT) thin films, i.e., $P_r \approx 20.65 \mu\text{C}/\text{cm}^2$ and $E_c \approx 160 \text{ kV}/\text{cm}$ measured at 1000 kV/cm and 1 kHz, meanwhile, the P_{max} value ($\approx 46.28 \mu\text{C}/\text{cm}^2$) was slightly higher [32]. In normal FEs, polarization domains have a polarization vector that points along the direction of the applied electric field. The rotation of dipoles is considered to be the main contribution to the total polarization (or P_{max} at the maximum applied field of 1000 kV/cm in this case). When the external electric field is removed, most of the spontaneous polarization will retain the poled direction, which results in a large P_r in normal ferroelectric P - E

loops. In contrast, the long-range ordered polar state in RFEs is destroyed, leading to the formation of a short-range polar state with PNRs, which can spontaneously return back to their initial short-range ordered state and result in a small P_r in P - E loops [34-36]. These above data confirm the existence of relaxor behaviour in the PLZT thin films in this study.

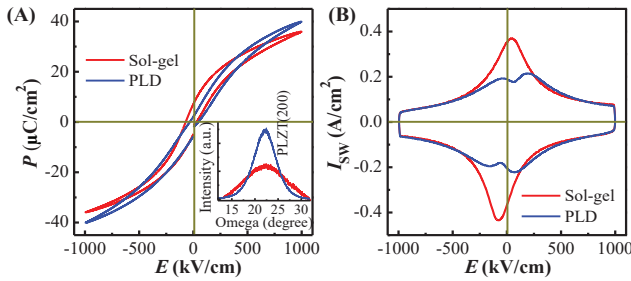


Fig. 3. (A) P - E hysteresis loops and (B) I_{sw} - E switching current curves of PLZT thin films deposited by sol-gel and PLD methods. Inset in Fig. 3A shows the rocking curves (omega scans) of the PLZT(200) peaks.

Figure 3B presents the corresponding I_{sw} - E switching current curves of the PLZT thin films. Another characteristic feature of RFEs is the presence of double-switching peaks corresponding to the coercive field, which was observed in the low electric field region of the sol-gel-deposited PLZT thin films. Meanwhile, in the low electric field region of the I_{sw} - E curve of the PLD-deposited thin films, four peaks were observed, which are similar to the I_{sw} - E curve of antiferroelectric films but with much higher E_c values [37]. Indeed, the PLD-deposited thin films did exhibit antiferroelectric-like switching behaviour [18]. The difference observed in the I_{sw} - E curves of the PLZT thin films in this study can be explained by the change in the crystalline quality of the films. The full width at half maximum (FWHM) of the rocking curve of the PLZT(200) peaks in Fig. 3A inset were 9.7 and 5.5°, respectively, for the PLZT thin films deposited by sol-gel and PLD. The FWHM value is a measure of the range over which the lattice structure in the different grains tilts with respect to the film normal [38] and is therefore an indication of the homogeneity of the films. The crystalline quality of the PLD-deposited PLZT thin films was much better than those deposited by sol-gel. The previous paper indicated that PLZT thin films of better crystalline quality have a larger P_m and lower P_r , as well as a higher intensity of the four switching peaks [20].

In general, the diffuseness factor can be used to describe the degree of relaxor behaviour. The diffuseness factor varies from 1 (normal FEs) to 2 (ideal RFEs) [39, 40]. The diffuseness factor in RFEs can be attributed to the film quality in which RFE thin films with higher

crystalline quality exhibit a larger diffuseness factor or stronger relaxor behaviour [41].

Similar to Fig. 4 (A, B) shows that both energy storage densities (U_{store} and U_{reco}) and the energy storage efficiency (η) of the PLD-deposited PLZT thin films measured at 1000 kV/cm and 1 kHz are higher than those deposited by sol-gel. This was due to the larger ($P_{max} - P_r$) value and slimmer P - E loop that was just discussed above. Under an applied field of 1000 kV/cm , the U_{reco} and η values were 9.7 J/cm^3 and 69.7%, and 13.3 J/cm^3 and 74.3%, respectively, for the PLZT thin films deposited by sol-gel and PLD.

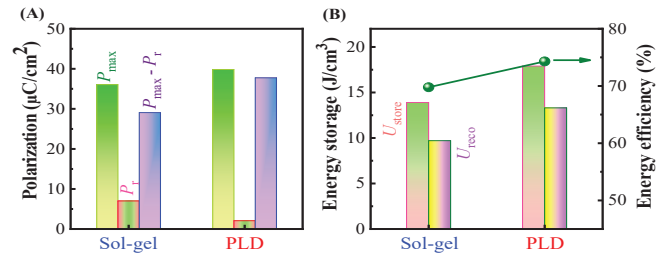


Fig. 4. (A) Polarization (P_{max} , P_r and $P_{max} - P_r$), (B) energy storage density (U_{store} and U_{reco}), and energy efficiency (η) of PLZT thin films deposited by sol-gel and PLD. The data were derived from the corresponding P - E loops in Fig. 3A.

Together with the enhanced recoverable energy storage density and energy storage efficiency, a large electric breakdown strength (E_{BD}) is also necessary to achieve high energy storage density for practical applications. E_{BD} values can be determined from the measurement of a series of capacitors made from each sample by using the Weibull distribution [6, 42-44]:

$$X_i = \ln(E_i) \quad (4)$$

$$Y_i = \ln\left(-\ln\left[1 - \frac{i}{n+1}\right]\right) \quad (5)$$

where E_i is the breakdown field of capacitor i in a series of n devices of the same sample type. The breakdown fields are in increasing order, which reflects the decreasing survival chance for a specific field estimated by the value $S_i = (1 - i/(n+1))$. The Weibull distribution plots for the breakdown strength of the PLZT thin films are shown in Fig. 5A. Fig. 5B illustrates the thin film capacitors before and after breaking. The critical breakdown strength (taking E_0 as E_{BD}) of a specific type of capacitor is extracted from the intercept of each line with the line $Y=0$ [12]. As can be seen from Fig. 5A, the E_{BD} values are about 2200 kV/cm and 2100 kV/cm for the PLZT thin films deposited by sol-gel and PLD, respectively. Compared with the PLD-deposited PLZT thin films with a columnar structure, the dense structure leads to a slight increase in breakdown strength in the sol-gel-deposited PLZT thin films.

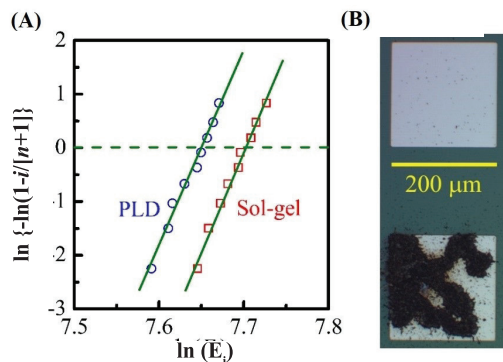


Fig. 5. (A) Weibull distribution of breakdown strength for the capacitors of PLZT thin films deposited by sol-gel and PLD and (B) optical images of thin film capacitors before (upper) and after breaking (lower).

Figure 6 compares the P - E hysteresis loops of the PLZT thin films measured at 1000 kV/cm and their corresponding E_{BD} values. Similar to the P - E loops measured at 1000 kV/cm, the P - E loop of PLD-deposited PLZT thin films at E_{BD} (2100 kV/cm) were slimmer than those deposited by sol-gel (E_{BD} =2200 kV/cm). Moreover, the P_{max} of the P - E loop measured at the E_{BD} of the PLD-deposited PLZT thin films was higher than that of the sol-gel PLZT thin films, even as a lower E_{BD} value was achieved in the PLD-deposited films. As a result, the U_{reco} and η values, measured at their corresponding E_{BD} , were 27.5 J/cm³ and 62.2% for the PLZT thin films deposited by sol-gel and 33.2 J/cm³ and 67.5% for those deposited by PLD.

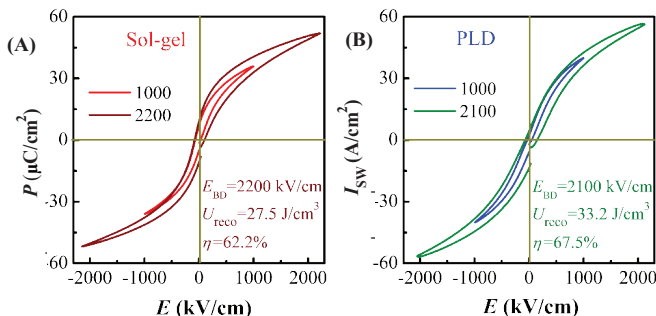


Fig. 6. P - E loops of the PLZT thin films deposited by (A) sol-gel and (B) PLD measured at 1000 kV/cm and their corresponding E_{BD} values.

4. Conclusions

In summary, we studied the ferroelectric properties and energy storage performance of relaxor PLZT thin films deposited on Pt/Si substrates by PLD and sol-gel spin coating. The sol-gel-deposited PLZT thin films with dense structure exhibited a mixed orientation of (100) and (111), meanwhile a mixed orientation of (100) and (110) was observed in the columnar PLZT thin films deposited by PLD. We found that the relaxor behaviour was enhanced with improvements in the crystalline

quality of the film. On the other hand, the PLD-deposited PLZT thin films had stronger relaxor behaviour with a slimmer P - E loop and lower P_r value due to its higher crystalline quality. Together with a larger P_m , higher U_{reco} and η values were achieved in the PLD-deposited PLZT thin films. Despite the even lower values of U_{reco} (-17%) and η (-8%) obtained in the sol-gel-deposited PLZT thin films, sol-gel can be still an effective method for the development of thin film capacitors for energy storage applications because of their low cost, great versatility in composition, simplicity, and scalable fabrication technique.

CRediT author statement

Thi Ha Dang, Thi Doan Tran, Duc Minh Nguyen, Van Truong Do, Ngoc Hung Vu: Data curation, Software, Visualisation, Investigation, Writing, Methodology, Supervision, Revising the manuscript.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] K. Han, N. Luo, S. Mao, et al. (2019), "Realizing high low-electric-field energy storage performance in AgNbO₃ ceramics by introducing relaxor behaviour", *Journal of Materials*, **5**(4), pp.597-605, DOI: 10.1016/j.jmat.2019.07.006.
- [2] K. Yao, S. Chen, M. Rahimabady, et al. (2011), "Nonlinear dielectric thin films for high-power electric storage with energy density comparable with electrochemical supercapacitors", *IEEE Transactions on Ultrasonics, Ferroelectrics, Frequency Control*, **58**(9), pp.1968-1974, DOI: 10.1109/TUFFC.2011.2039.
- [3] H. Palneedi, M. Peddigari, G.T. Hwang, et al. (2018), "High-performance dielectric ceramic films for energy storage capacitors: Progress and outlook", *Advanced Functional Materials*, **28**(42), DOI: 10.1002/adfm.201803665.
- [4] X. Hao (2013), "A review on the dielectric materials for high energy storage application", *Journal of Advanced Dielectrics*, **3**(1), DOI: 10.1142/S2010135X13300016.
- [5] F. Li, J. Zhai, B. Shen, et al. (2018), "Recent progress of eco-friendly perovskite-type dielectric ceramics for energy storage applications", *Journal of Advanced Dielectrics*, **8**(6), DOI: 10.1142/S2010135X18300050.
- [6] M.D. Nguyen, E.P. Houwman, M.T. Do, et al. (2020a), "Relaxor-ferroelectric thin film heterostructure with large imprint for high energy storage performance at low operating voltage", *Energy Storage Materials*, **25**, pp.193-201, DOI: 10.1016/j.ensm.2019.10.015.
- [7] V.V. Shvartsman, D.C. Lupascu (2012), "Lead-free relaxor ferroelectrics", *Journal of the American Ceramic Society*, **95**(1), pp.1-26, DOI: 10.1111/j.1551-2916.2011.04952.x.
- [8] L.E. Cross (1994), "Relaxorferroelectrics: An overview", *Ferroelectrics*, **151**(1), pp.305-320, DOI: 10.1080/00150199408244755.
- [9] V. Shvartsman, W. Kleemann, J. Dec, et al. (2006), "Diffuse phase transition in BaTi_{1-x}Sn_xO₃ ceramics: An intermediate state between ferroelectric and relaxor behaviour", *Journal of Applied Physics*, **99**(12), DOI:10.1063/1.2207828.

- [10] M.D. Nguyen, G. Rijnders (2020b), "Comparative study of piezoelectric response and energy storage performance in normal ferroelectric, antiferroelectric and relaxor-ferroelectric thin films", *Thin Solid Films*, **697**, DOI: 10.1016/j.tsf.2020.137843.
- [11] A. Kursumovic, W.W. Li, S. Cho, et al. (2020), "Lead-free relaxor thin films with huge energy density and low loss for high temperature applications", *Nano Energy*, **71**, DOI: 10.1016/j.nanoen.2020.104536.
- [12] M.D. Nguyen (2021), "Ultrahigh energy storage performance in lead-free BZT thin films by tuning relaxor behaviour", *Materials Research Bulletin*, **133**, DOI: 10.1016/j.materresbull.2020.111072.
- [13] S. Ji, Q. Li, D. Wang, et al. (2021), "Enhanced energy storage performance and thermal stability in relaxor ferroelectric $(1-x)\text{BiFeO}_3$ - $x(0.85\text{BaTiO}_3$ - $0.15\text{Bi}(\text{Sn}_{0.5}\text{Zn}_{0.5})\text{O}_3$) ceramics", *Journal of the American Ceramic Society*, **104**(6), DOI: 10.1111/jace.17705.
- [14] Z. Hu, B. Ma, S. Liu, et al. (2014), "Relaxor behaviour and energy storage performance of ferroelectric PLZT thin films with different Zr/Ti ratios", *Ceramics International*, **40**(1), pp.557-562, DOI: 10.1016/j.ceramint.2013.05.139.
- [15] A. Kumar, K.J. Raju, A. James (2017), "Diffuse phase transition in mechanically activated $(\text{Pb}_{1-x}\text{La}_x)(\text{Zr}_{0.60}\text{Ti}_{0.40})\text{O}_3$ electro-ceramics", *Journal of Materials Science: Materials in Electronics*, **28**(1), pp.13928-13936, DOI: 10.1007/s10854-017-7242-6.
- [16] A. Pramanick, S. Nayak (2021), "Perspective on emerging views on microscopic origin of relaxor behaviour", *Journal of Materials Research*, DOI: 10.1557/s43578-020-00010-7.
- [17] V. Shvartsman, J. Dec, Z. Xu, et al. (2008), "Crossover from ferroelectric to relaxor behaviour in $\text{BaTi}_{1-x}\text{Sn}_x\text{O}_3$ solid solutions", *Phase Transitions*, **81**(11-12), pp.1013-1021, DOI: 10.1080/01411590802457888.
- [18] M.D. Nguyen, E.P. Houwman, G. Rijnders (2018a), "Energy storage performance and electric breakdown field of thin relaxor ferroelectric PLZT films using microstructure and growth orientation control", *The Journal of Physical Chemistry C*, **122**(27), pp.15171-15179, DOI: 10.1021/acs.jpcc.8b04251.
- [19] M.D. Nguyen, E.P. Houwman, M. Dekkers, et al. (2016), "Enhanced energy storage density and energy efficiency of epitaxial $\text{Pb}_{0.9}\text{La}_{0.1}(\text{Zr}_{0.55}\text{Ti}_{0.45})\text{O}_3$ relaxor-ferroelectric thin-films deposited on silicon by pulsed laser deposition", *APL Materials*, **4**(8), DOI: 10.1063/1.4961636.
- [20] M.D. Nguyen, C.T. Nguyen, H.N. Vu, et al. (2018b), "Controlling microstructure and film growth of relaxor-ferroelectric thin films for high breakdown strength and energy storage performance", *Journal of the European Ceramic Society*, **38**(1), pp.95-103, DOI: 10.1016/j.jeurceramsoc.2017.08.027.
- [21] B. Panda, S. Ray, A. Dhar, et al. (1996), "Electron beam deposited lead-lanthanum-zirconate-titanate thin films for siliconbased device applications", *Journal of Applied Physics*, **79**(2), pp.1008-1012, DOI: 10.1063/1.360887.
- [22] H. Adachi, K. Wasa (1991), "Sputtering preparation of ferroelectric PLZT thin films and their optical applications", *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*, **38**(6), pp.645-655, DOI: 10.1109/58.108865.
- [23] X. Hao, Y. Wang, J. Yang, et al. (2012), "High energy storage performance in $\text{Pb}(\text{La}(\text{Ti}(\text{Zr})\text{O})\text{relaxor ferroelectric thin films}$ ", *Journal of Applied Physics*, **112**(11), DOI: 10.1063/1.4768461.
- [24] L. Zhang, X. Hao, J. Yang, et al. (2013), "Large enhancement of energy storage properties of compositional graded $(\text{Pb}_{1-x}\text{La}_x)(\text{Zr}_{0.65}\text{Ti}_{0.35})\text{O}_3$ relaxor ferroelectric thick films", *Applied Physics Letters*, **103**(11), DOI: 10.1063/1.4821209.
- [25] S. Tong, B. Ma, M. Narayanan, et al. (2013), "Lead lanthanum zirconate titanate ceramic thin films for energy storage", *ACS Applied Materials & Interfaces*, **5**(4), pp.1474-1480, DOI: 10.1021/am302985u.
- [26] M. Dinescu (2010), "PLD of piezoelectric and ferroelectric materials", *Laser - Surface Interactions for New Materials Production*, Springer, pp.307-330, DOI: 10.1007/978-3-642-03307-0_14.
- [27] K.K. Sahoo, R. Katoch, K. Brajesh, et al. (2020), "Improved ferroelectric response of pulsed laser deposited BiFeO_3 - PbTiO_3 thin films around morphotropic phase boundary with interfacial PbTiO_3 buffer layer", *Journal of Applied Physics*, **127**(6), DOI: 10.1063/1.5110335.
- [28] M.D. Nguyen, E. Houwman, M. Dekkers, et al. (2014), "A fast room-temperature poling process of piezoelectric $\text{Pb}(\text{Zr}_{0.45}\text{Ti}_{0.55})\text{O}_3$ thin films", *Science of Advanced Materials*, **6**(2), pp.243-251, DOI: 10.1166/sam.2014.1708.
- [29] C.T.Q. Nguyen, M.D. Nguyen, M. Dekkers, et al. (2014), "Process dependence of the piezoelectric response of membrane actuators based on $\text{Pb}(\text{Zr}_{0.45}\text{Ti}_{0.55})\text{O}_3$ thin films", *Thin Solid Films*, **556**, pp.509-514, DOI: 10.1016/j.tsf.2014.01.007.
- [30] M.D. Nguyen (2021), "Tuning of microstructure and piezoelectric response of laser ablated $\text{Pb}(\text{Zr,Ti})\text{O}_3$ films by varying the oxygen deposition pressure", *Ferroelectrics*, **573**(1), pp.42-51, DOI: 10.1080/00150193.2021.1890462.
- [31] B. Ma, Z. Hu, S. Liu, et al. (2013), "Temperature-dependent dielectric nonlinearity of relaxor ferroelectric $\text{Pb}_{0.92}\text{La}_{0.08}\text{Zr}_{0.52}\text{Ti}_{0.48}\text{O}_3$ thin films", *Appl. Phys. Lett.*, **102**(20), DOI: 10.1063/1.4807665.
- [32] H.T. Dang, T.T. Trinh, C.T. Nguyen, et al. (2019), "Enhancement of relaxor behaviour by La doping and its influence on the energy storage performance and electric breakdown strength of ferroelectric $\text{Pb}(\text{Zr}_{0.52}\text{Ti}_{0.48})\text{O}_3$ thin films", *Materials Chemistry Physics*, **234**, pp.210-216, DOI: 10.1016/j.matchemphys.2019.06.005.
- [33] P.S. Janrao, V. Mathe (2017), "Phase tuning in lanthanum doped lead zirconate titanate near morphotropic phase boundary using chemical co-precipitation route and their dielectric properties", *Journal of Materials Science: Materials in Electronics*, **28**(1), pp.1078-1085, DOI: 10.1007/s10854-016-5631-x.
- [34] Q. Hu, X. Wei (2019), "Abnormal phase transition and polarization mismatch phenomena in BaTiO_3 -based relaxor ferroelectrics", *Journal of Advanced Dielectrics*, **9**(5), DOI: 10.1142/S2010135X19300020.
- [35] L. Yang, J. Xu, Q. Li, et al. (2016), "High piezoelectricity associated with crossover from nonergodicity to ergodicity in modified $\text{Bi}_{0.5}\text{Na}_{0.5}\text{TiO}_3$ relaxor ferroelectrics", *Journal of Electroceramics*, **37**(1), pp.23-28, DOI: 10.1007/s10832-016-0036-z.
- [36] Z. Yang, H. Du, L. Jin, et al. (2019), "Realizing high comprehensive energy storage performance in lead-free bulk ceramics via designing an unmatched temperature range", *Journal of Materials Chemistry*, **7**(48), pp.27256-27266, DOI: 10.1039/C9TA11314B.
- [37] M.D. Nguyen, G. Rijnders (2018c), "Electric field-induced phase transition and energy storage performance of highly-textured PbZrO_3 antiferroelectric films with a deposition temperature dependence", *Journal of The European Ceramic Society*, **38**(15), pp.4953-4961, DOI: 10.1016/j.jeurceramsoc.2018.07.026.
- [38] H. Funakubo, M. Dekkers, A. Sambri, et al. (2012), "Epitaxial PZT films for MEMS printing applications", *APL Materials*, **37**(11), pp.1030-1038, DOI: 10.1557/mrs.2012.271.
- [39] Z. Shen, X. Wang, B. Luo, et al. (2015), " BaTiO_3 - BiYbO_3 perovskite materials for energy storage applications", *Journal of Materials Chemistry A*, **3**(35), pp.18146-18153, DOI: 10.1039/C5TA03614C.
- [40] C. Yang, Y. Han, C. Feng, et al. (2020), "Toward multifunctional electronics: Flexible NBT-based film with a large electrocaloric effect and high energy storage property", *ACS Applied Materials & Interfaces*, **12**(5), pp.6082-6089, DOI: 10.1021/acsami.9b21105.
- [41] J. Ouyang (2019), *Nanostructures in Ferroelectric Films for Energy Applications*, Elsevier, 386pp.
- [42] H. Pan, J. Ma, J. Ma, et al. (2018), "Giant energy density and high efficiency achieved in bismuth ferrite-based film capacitors via domain engineering", *Nature Communications*, **9**(1), pp.1-8, DOI: 10.1038/s41467-018-04189-6.
- [43] Y. Wang, J. Cui, L. Wang, et al. (2017), "Compositional tailoring effect on electric field distribution for significantly enhanced breakdown strength and restrained conductive loss in sandwich-structured ceramic/polymer nanocomposites", *Journal of Materials Chemistry*, **5**(9), pp.4710-4718, DOI: 10.1039/C6TA10709E.
- [44] Q. Yuan, G. Li, F.Z. Yao, et al. (2018), "Simultaneously achieved temperature-insensitive high energy density and efficiency in domain engineered BaTiO_3 - $\text{Bi}(\text{Mg}_{0.5}\text{Zr}_{0.5})\text{O}_3$ lead-free relaxor ferroelectrics", *Nano Energy*, **52**, pp.203-210, DOI: 10.1016/j.nanoen.2018.07.055.

Effective treatment for symptomatic brain tumours and arteriovenous malformation in children by radiosurgery with a rotating gamma system

Khoa Mai Trong¹, Phuong Pham Cam^{2*}, Luan Nguyen Duc², Hung Nguyen Quang²,
Phuong Dao Manh², Thai Pham Van¹

¹Hanoi Medical University, 1 Ton That Tung Street, Khuong Thuong Ward, Dong Da District, Hanoi, Vietnam

²The Nuclear Medicine and Oncology Centre, Bach Mai Hospital, 78 Giai Phong Street, Phuong Mai Ward, Dong Da District, Hanoi, Vietnam

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Abstract:

To assess the effectiveness of radiosurgery with a rotating gamma system (RGS) for paediatric brain tumours and arteriovenous malformation (AVM), this work was carried out on 123 patients with brain tumours or AVM received radiosurgery at Bach Mai hospital. The median dose was 13 Gy from a range of 8 to 20 Gy. Endpoints include the effects of increased intracranial pressure, seizures, hemiplegia, and tumour size on the syndrome. Results exhibited that the percentages of patients with complete response, partial response, and disease progression out of 59 patients with headache accounted for 11.9, 69.5, and 18.6%, respectively. Of the 20 patients with seizures, 30% had complete response, 65% partial response, and 5% stable disease. Regarding brain tumour size, 9 months after treatment, those with complete response, partial response, stable disease, and disease progression made up 18.7, 50.4, 14.6, and 16.3% of the total patients, respectively. There was no statistically significant effect of radiation dose on the results. This study showed a high percentage of children with brain tumours and AVM undergoing radiosurgery with a RGS had symptomatic responses after treatment.

Keywords: arteriovenous malformation, brain tumours in children, headache, hemiplegia, radiosurgery, rotating gamma system, seizures, symptom relief.

Classification number: 3.2

1. Introduction

Brain tumours are the second most common group of tumours in children following only after hematologic cancers. This group of diseases originates from an abnormal proliferation of nerve cells, astrocytes, dendritic cells, or neuroblastoma. Brain tumours may also be secondary from many types of cancer elsewhere in the body. Depending on the location, size, and nature of the tumour, the primary treatment for a brain tumour may be surgery, radiotherapy, or chemotherapy. In addition, surgery is difficult to conduct in cases of brainstem tumours or tumours in some other locations because serious complications can occur. In those cases, radiosurgery using a rotating gamma knife (RGK) is a safe and effective option that has been proven in many studies [1-3]. Meanwhile, brain AVM includes lesions that are defined by the presence of arteriovenous (AV) shunting through a nidus of coiled and tortuous vascular connections that connect feeding arteries to draining veins [4]. Brain AVM is considered congenital vascular lesions that can be present at any age. AVM was reported

to have a higher rate of rupture than in adults [5]. RGK radiosurgery is one of the most effective treatment methods for this disease, which has been known for a long time [6].

Today, along with advances in medicine, many new types of radiotherapy machines and methods have been introduced to focus the dose on lesions while minimizing the radiation dose to surrounding healthy tissues. Since then, radiation therapy for the treatment of brain tumours and AVM in children has been increasingly studied. Radiation therapy plays an important role especially for patients with large tumours, deep tumour sites, or tumours on important organs such as the brainstem, ventricles, etc., which are all very difficult places to perform surgery. Gamma knife radiosurgery (GKRS) has been used by Swedish professor Lar Leksell to treat brain tumours since 1968 with very good results. The gamma knife system used by Lar Leksell at that time contained 201 sources of Cobalt-60 arranged on a spherical hood containing directional envelopes. These radiation sources

*Corresponding author: Email: phamcamphuong@gmail.com

were directed through directional gates so that the beams were focused on the pathological point in the brain [7, 8]. The RGS uses 30 sources of Cobalt-60 symmetrically distributed around a hemisphere between latitude angles of 13 to 43° as measured from the sagittal plane. The sources within the single housing helmet rotate at a speed of approximately 2 to 4 rotations per minute. Inside the source hemisphere, a concentric collimating hemisphere is co-rotating with the sources and exposes the desired beam diameter such as 4, 8, 14, or 18 mm. The rotating sources of RGS simulate an infinite number of beams and promote extremely high target-to-surface dose ratios [9]. Since July 2007, the Nuclear Medicine and Oncology Centre at Bach Mai hospital has used the Gamma ART 6000, an American radiosurgery system, which is a hybrid with features of both the gamma knife and LINAC radiosurgery systems to treat brain tumours and AVM. More than 6000 patients, including children, with brain tumours and intracranial diseases have been treated with the RGK. This group has conducted a thesis to assess the role of this treatment method in the treatment of brain tumours and intracranial diseases in children.

2. Patients and study methods

From July 2007 to December 2020, 123 paediatric patients (≤ 15 years old) included in this prospective interventional study received radiosurgery for brain tumours and intracranial diseases with RGS at the Nuclear Medicine and Oncology Centre, Bach Mai hospital, Hanoi. The patients had symptomatic tumours of the brain or AVM. Their lesions had specific features on magnetic resonance imaging (MRI), computed tomography (CT), or magnetic resonance spectroscopy (MRS) [10, 11]. Specifically, the lesions had a maximum diameter of 3 cm, and in a few cases, we accepted tumours with sizes of no greater than 5 cm with no severe comorbidity. The ages of the patients were no greater than 15 years old. The patients were without pregnancy or lactation. There were indications for the subjects to be treated with RGK given by the Hospital Medical Council. All patients gave consent to join in the study.

In this study, we used the Gamma ART 6000, an American Radiosurgery system. We gave patients a single dose of radiosurgery from 8 to 20 Gy. The dose was defined to the outer margin of the tumour representing 50% isodose. The radiation oncologists decided the specific radiation dose for each patient. We then grouped patients according to the treated dose with a threshold of 14 Gy (median value of the range from 8 to 20 Gy) to test whether the radiation dose affected the treatment outcome.

The primary endpoint was a response in clinical symptoms in a follow-up period of 9 months after treatment, which included the syndrome of increased intracranial pressure, seizures, and hemiplegia. When compared to symptoms before treatment, the patient might have either complete response (complete relief of symptoms), partial response (partial relief of symptoms), stable disease (no change in symptoms), or progressive disease (an increase in symptoms). Another study endpoint was the change in the size of tumours on the MRI scans after 9 months.

The data were coded and analysed by SPSS 16.0 using statistical algorithms. A p-value of less than 0.05 was considered significant.

3. Results

3.1. Patient characteristics

Out of 123 paediatric patients that met the criteria in this study, 45 patients were female (36.6%) and 78 patients were male (63.4%). The median age was 10 years in a range from 4 to 15 years. Diagnosis beginning with the highest rate: astrocytoma in 29 patients (23.6%), AVM in 20 patients (16.3%), pineal gland tumour in 18 patients (14.6%), and brainstem tumour in 14 patients (11.4%). The tumour was in the temporal lobe in 21 patients (17.1%), in the frontal lobe in 20 patients (16.3%), in the pineal gland in 18 patients (14.6%), and other positions accounted for less percentage.

3.2. Clinical symptoms prior to RGK

The clinical symptoms of the 123 patients are shown in Table 1. Prior to RGS irradiation, 59 patients (48%) had a significant headache, 20 patients (16.3%) had significant seizures, and 17 patients (13.8%) had significant hemiplegia.

Table 1. Clinical symptoms prior to RGK.

Symptom	Number of patients	Proportion (%)
Increased intracranial pressure	59	48.0
Seizures	20	16.3
Hemiplegia	17	13.8
Puberty early	4	3.3
Double vision	3	2.4
Blurred vision	7	5.7
Cross-eyed	1	0.8
Drooping eyelids	4	3.3
Tinnitus	1	0.8
Cerebellum syndrome	6	4.8
Absence of consciousness	1	0.8
Total	123	100.0

3.3. Response at 9 months after RGS irradiation

Of the 59 patients who suffered significant intracranial pressure prior to RGS irradiation, the rates of complete response, partial response, and disease progression at 9 months after RGS irradiation were 11.9, 69.5, and 18.6%, respectively (Fig. 1).

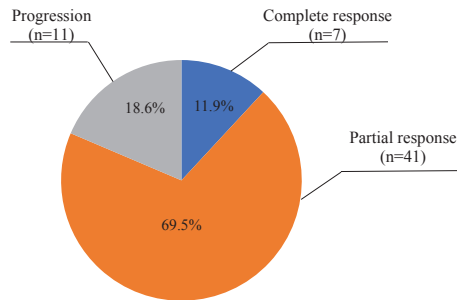


Fig. 1. Syndrome of increased intracranial pressure: response at 9 months after RGS irradiation.

Of the 20 patients who experienced seizures prior to irradiation, 6 patients (30%) achieved complete response, 13 patients (65%) had partial response, and one patient (5%) maintained stable disease at 9 months following RGS irradiation, respectively (Fig. 2).

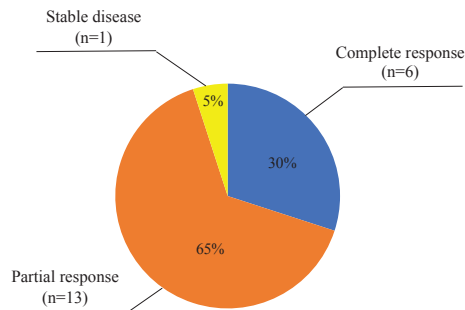


Fig. 2. Seizures: response at 9 months after rotating gamma system (RGS) irradiation.

Of the 17 patients who experienced hemiplegia prior to irradiation, 3 patients (17.6%) achieved complete response, 10 patients (58.9%) had partial response, and 4 patients (23.5%) had disease progression at 9 months following RGS irradiation, respectively (Fig. 3).

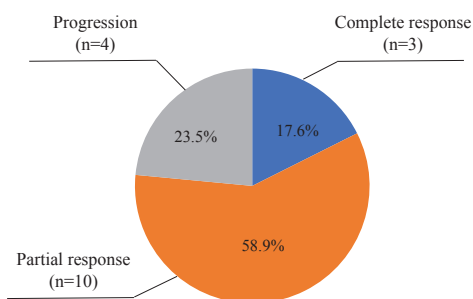


Fig. 3. Hemiplegia: response at 9 months after RGS irradiation.

In terms of sizes of the tumours 9 months after treatment: we achieved complete response in 23 out of the 123 patients (18.7%), partial response (with median decrease in size by 20%) in 62 patients (50.4%), and stable disease in 18 patients (14.6%). In 20 patients (16.3%), the lesion appeared larger than prior to RGS irradiation. Brainstem tumours had the highest rate of recurrence, which occurred in 13 out of 14 patients (92.8%). Patients with AVM had the best response. Of the 20 patients who experienced AVM prior to irradiation, at 9 months following RGS irradiation, 12 patients (60%) achieved a complete response, 5 patients (25%) had a partial response, and 3 patients (23.5%) maintained stable disease (Fig. 4). About side effects after radiosurgery, 27.6% of patients had symptoms of increased intracranial pressure. However, the symptoms were controlled with medical treatment.

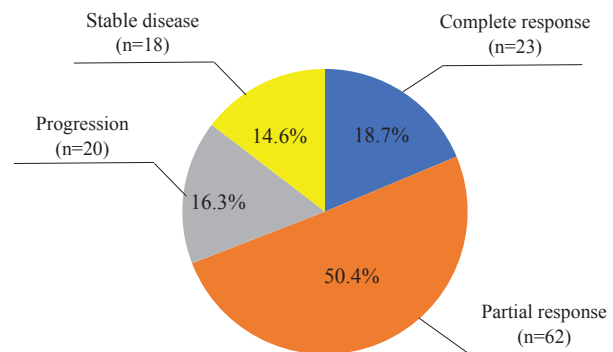


Fig. 4. Size tumours: response at 9 months after RGS irradiation.

3.4. Response rates related to different doses of rotating gamma system radiosurgery

Complete response was defined as complete relief of symptoms or complete disappearance of the brain tumours and intracranial diseases. The overall response was defined as complete or partial symptom relief or decrease in size of the brain tumours and intracranial diseases. p-values were calculated with the Chi-square test. The best response to RGS irradiation regarding the syndromes of increased intracranial pressure, seizures, hemiplegia, and decrease of tumour size were compared for RGS doses of ≤ 14 Gy (n=58), and > 14 Gy (n=65). The results of this subgroup analysis are summarised in Table 2. The rotating gamma system dose had no significant impact on outcomes.

Table 2. Treatment response according to the radiation dose threshold of 14 Gy.

Endpoint	≤14 Gy	>14 Gy	p-value
<i>Syndrome of increased intracranial pressure</i>			
Complete response rate	3.4% (2/59)	8.5% (5/59)	0.17
Overall response rate	39% (23/59)	42.4% (25/59)	0.45
<i>Seizures</i>			
Complete response rate	10% (2/20)	20% (4/20)	0.14
Overall response rate	20% (4/20)	75% (15/20)	0.27
<i>Hemiplegia</i>			
Complete response rate	0% (0/17)	17.6% (3/17)	0.02
Overall response rate	17.6% (3/17)	58.8% (10/17)	0.03
<i>Brain tumours size</i>			
Complete response rate	4.9% (6/123)	13.8% (17/123)	0.003
Overall response rate	29.1% (36/123)	39.8% (49/123)	0.21
<i>AVM size</i>			
Complete response rate	2% (2/20)	50% (10/20)	0.47

4. Discussion

Brain tumours ranked second in childhood cancers (only after hematologic cancers), account for 15% of cancers in children under 16 years of age. Childhood central nervous system tumour incidence varies by country from 1.12-5.14 cases per 100,000 persons, with the highest rate of incidence being in the United States [12]. Tumour resection surgery is the first treatment of choice. However, it is difficult to achieve a R0 resection in many cases due to the high risk of complications. For these cases, the indication for alternative treatment with radiosurgery using RGK is reasonable and has been proven to be safe and effective in many studies [1-5]. The classical radiosurgery system and accelerated radiotherapy were introduced in the late 1960s and 1980s, respectively. The RGK radiosurgery system incorporates the advantages of both methods and was introduced in the late 1990s [9]. This system is currently only available in a few large treatment centres around the world as well as in Vietnam. At the Nuclear Medicine and Oncology Centre, Bach Mai hospital, there have been more than 6,000 patients including children with brain tumours and AVM treated with the RGK.

Rotating gamma radiosurgery resulted in very high rates of symptomatic response in syndromes of increased intracranial pressure or seizures. Overall complete and partial response rates were 81.4 and 95%, respectively, in which AVM and astrocytoma had the highest response rates. Of the 29 patients with astrocytoma 9 months after surgery, 24 patients had a partial response (82.8%). In 20 patients with AVM, the response rate was 85% where 12 patients achieved complete response and 8 patients had partial response, which accounts for 60 and 40%, respectively.

In terms of tumour size, an overall response was observed in 69.1% of patients. However, in 16.3% of the cases, lesions appeared larger than prior to RGS irradiation. Patients with AVM and astrocytoma had the best response. Of the 20 patients who experienced AVM prior to irradiation, 12 patients (60%) achieved complete response, 5 patients (25%) had partial response, and 3 patients (23.5%) maintained stable disease, respectively at 9 months following RGS irradiation. In 29 patients with astrocytoma, 16 patients (55.2%) had partial response, 8 patients (27.6%) maintained stable disease, and 5 patients (17.2%) had disease progression 9 months after radiosurgery. Response to treatment depends on many factors including the grade of malignancy of the lesion. That is probably the reason why AVM, benign lesions, had better responses than other tumours. Besides, the location of the lesion is also a factor related to the effectiveness of treatment. Perhaps this is why brainstem tumours are generally less responsive.

When comparing RGS doses of ≤14 Gy and >14 Gy, it is seen that dose had no significant impact on outcomes. One reason for this may be that the study subjects were not a diagnostically homogeneous group. With each type of brain tumour, and especially with different histopathology, different tumour locations likely have different responses to radiation therapy. Therefore, in order to better understand the dose-response relationship in paediatric brain tumour patients, further studies in each disease group should be conducted.

Other studies also showed the effectiveness of radiosurgery with AVM and brain tumours in children. A study from the University of Pittsburgh reviewed 135 children treated with GKRS. The median GKRS prescription dose to the nidus margin was 20 Gy (from 15 to 25 Gy), which correlated with the median maximum target dose of 40 Gy (from 30 to 50 Gy). A reduced dose was prescribed for large AVMs and niduses located in an eloquent area as per published risk/benefit prediction curves based on the 12 Gy volume. Paediatric AVM obliteration rates at 3, 4, 5, and 10 years were 45, 64, 67, and 72%, respectively [6]. In 2017, P.C.Phuong, et al. (2017) [13] reported a mean reduction in tumour size from 1.87 to 1.15 cm after 36 months of treatment when performing radiosurgery with a dose of 12 Gy for 37 patients with low-grade brainstem glioma. Based on these results, RGK should be considered in selected patients with astrocytomas. The most appropriate indication may be for patients who are inoperable or have residual lesion after surgery. We may need more research to confirm this claim. C.G. Hadjipanayis, et al. (2002) [14] conducted a study at the University of Pittsburgh on 37 patients with unresectable or recurrent pilocytic astrocytomas. In the study, 68% of the patients were 18 years old or younger.

The median radiation dose was 15 Gy and 48.6% of the patients achieved a complete or partial response. Neurological symptoms worsened in only 2 patients after radiosurgery. In another study, J. Boethius, et al. (2002) [15] performed radiosurgery on 19 patients including 16 children with radiation doses from 9 to 20 Gy (median of 10 Gy). The author followed the group of patients for a median time of 4.7 years and concluded that tumour size was moderately reduced in 85% of patients while the disease was controlled in 18 out of 19 patients. J.A. Barcia, et al. (1994) [16] studied radiosurgery performed with the mean dose of 21.7 Gy in 16 patients with inoperable low-grade gliomas. The author reported that 50% of tumours had a complete response and 31% had partial response or stable disease.

When researching the literature, there were a few studies with comparable indicators as those presented in this study. For example, B. Mirza, et al. (2010) [17] treated 6 children with AVMs and 12 children with tumours using stereotactic radiotherapy and radiosurgery. They reported a rate of 83% in control or regression in patients with tumours, and all children with AVMs. In 2003, A. Hirth, et al. [18] conducted a study of 12 children with cerebral or skull base tumours with a mean tumour margin dose of 13.8 Gy. After a mean follow-up of 78.6 months, they found that seven patients remained stable.

The overall response rates in our present study were higher than other studies. This showed that radiosurgery using RGK is an effective treatment for brain tumours and intracranial diseases. It should be added, however, that there may be a risk of bias since the studies are retrospective.

5. Conclusions

In conclusion, this study shows that treatment with a RGK system helps children with brain tumours and intracranial diseases achieve high symptom response rate and reduce tumour size after radiosurgery. Therefore, it is a suitable alternative treatment when surgical removal of the entire tumour is not possible.

CRedit author statement

Khoa Mai Trong, Phuong Pham Cam, Luan Nguyen Duc, Hung Nguyen Quang, Phuong Dao Manh, Thai Pham Van: Data curation, Software, Visualisation, Investigation, Writing, Methodology, Supervision, Revising the manuscript.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] A.S. Plant, J. Pathare, D. Engelhart, et al. (2018), "Safety and efficacy of gamma knife radiosurgery for pediatric brain tumours", *Neuro-Oncology*, **20**(2), DOI: 10.1093/neuonc/noy059.536.
- [2] D. Weintraub, C.-P. Yen, Z. Xu, et al. (2012), "Gamma knife surgery of pediatric gliomas", *J. Neurosurg. Pediatr.*, **10**(6), pp.471-477, DOI: 10.3171/2012.9.PEDS12257.
- [3] N. Massager (2012), "Gamma knife radiosurgery", *Rev. Med. Brux.*, **33**(4), pp.367-370 (in French).
- [4] M. Gaballah, P.B. Storm, D. Rabinowitz, et al. (2014), "Intraoperative cerebral angiography in arteriovenous malformation resection in children: A single institutional experience", *J. Neurosurg. Pediatr.*, **13**(2), pp.222-228, DOI: 10.3171/2013.10.PEDS13291.
- [5] T.E. Darsaut, R. Guzman, M.L. Marcellus, et al. (2011), "Management of pediatric intracranial arteriovenous malformations: Experience with multimodality therapy", *Neurosurgery*, **69**(3), pp.540-556, DOI: 10.1227/NEU.0b013e3182181c00.
- [6] J.H. Suh, G.H. Barnett (2003), "Stereotactic radiosurgery for brain tumours in pediatric patients", *Technology in Cancer Research & Treatment*, **2**(2), pp.141-146, DOI: 10.1177/153303460300200210.
- [7] L. Leksell (1951), "The stereotactic method and radiosurgery of the brain", *Acta. Chir Scand.*, **102**(4), pp.316-319.
- [8] L. Leksell (1983), "Stereotactic radiosurgery", *J. Neurol. Neurosurg. Psychiatry*, **46**(9), pp.797-803, DOI: 10.1136/jnnp.46.9.797.
- [9] A. Wu (1992), "Physics and dosimetry of the gamma knife", *Neurosurgery Clinics of North America*, **3**(1), pp.35-50.
- [10] L. Yin, L. Zhang (2013), "Correlation between MRI findings and histological diagnosis of brainstem glioma", *Can. J. Neurol. Sci.*, **40**(3), pp.348-354, DOI: 10.1017/s0317167100014293.
- [11] J.C. Flickinger, D. Kondziolka, L.D. Lunsford, et al. (2000), "Development of a model to predict permanent symptomatic postradiosurgery injury for arteriovenous malformation patients", *Int. J. Radiat. Oncol. Biol. Phys.*, **46**(5), pp.1143-1186, DOI: 10.1016/s0360-3016(99)00513-1.
- [12] K.J. Johnson, J. Cullen, J.S.B. Sloan, et al. (2014), "Childhood brain tumour epidemiology: A brain tumour epidemiology consortium review", *Cancer Epidemiol. Biomarkers Prev.*, **23**(12), pp.2716-2736, DOI: 10.1158/1055-9965.EPI-14-0207.
- [13] P.C. Phuong, N.Q. Hung, T.B. Ngoc, et al. (2017), "Rotating gamma system irradiation: A promising treatment for low-grade brainstem gliomas", *In Vivo*, **31**(5), pp.957-960.
- [14] C.G. Hadjipanayis, D. Kondziolka, P. Gardner, et al. (2002), "Stereotactic radiosurgery for pilocytic astrocytomas when multimodal therapy is necessary", *J. Neurosurg.*, **97**(1), pp.56-64, DOI: 10.3171/jns.2002.97.1.0056.
- [15] J. Boethius, E. Ulfarsson, T. Rahn, et al. (2002), "Gamma knife radiosurgery for pilocytic astrocytomas", *J. Neurosurg.*, **97**(5), pp.677-680, DOI: 10.3171/jns.2002.97.supplement.
- [16] J.A. Barcia, J.L.B. Salorio, C. Ferrer, et al. (1994), "Stereotactic radiosurgery of deeply seated low grade gliomas", *Acta Neurochir Suppl.*, **62**, pp.58-61, DOI: 10.1007/978-3-7091-9371-6_12.
- [17] B. Mirza, A. Mønsted, J. Harding, et al. (2010), "Stereotactic radiotherapy and radiosurgery in pediatric patients: Analysis of indications and outcome", *Childs Nerv. Syst.*, **26**(12), pp.1785-1793, DOI: 10.1007/s00381-010-1134-x.
- [18] A. Hirth, P.H. Pedersen, R. Baardsen, et al. (2003), "Gamma-knife radiosurgery in pediatric cerebral and skull base tumours", *Med. Pediatr. Oncol.*,

Nitrogen-containing heterocyclic compounds from the roots of *Callerya speciosa*

Duc Thien Dao¹, Quoc Thang Le², Thanh Tam Nguyen^{1,3*}

¹Institute of Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Street, Nghia Do Ward, Cau Giay District, Hanoi, Vietnam

²Department of Chemistry, Hue University of Education, 34 Le Loi Street, Hue City, Thua Thien Hue Province, Vietnam

³Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Street, Cau Giay District, Hanoi, Vietnam

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Abstract:

Callerya speciosa (Champ.) Schot. [synonym: *Millettia speciosa* Champ.] belongs to the family Leguminosae, which grows in Southeast Asia, in the tropical and subtropical forests of Hainan Island, southern mainland China and in the north areas of Vietnam. *C. speciosa* have been used as a traditional medicine for the treatment of fever, cough, headache, backache, rheumatism, chronic bronchitis, and nephritis. The presence of phenolics, phenolic glycosides, pterocarpanes, flavonoids, isoflavonoids, sterols, and chromones in this species have been reported. In this study, the four nitrogen-containing heterocyclic compounds uridine (1), 2-(β -D-glucopyranosyl)-3-isoxazolin-5-one (2), adenosine (3), and hypaphorine (4) were isolated from the n-butanol extract of the roots of *Callerya speciosa* collected in Vietnam. The compounds were isolated and purified by silica gel, Sephadex LH-20 and RP-18 column chromatography. As a result, their structures were characterized on the basis of extensive NMR, mass spectroscopic analyses, and comparison with reported values. This is the first report on the isolation of compounds 1–4 from *Callerya speciosa*.

Keywords: *Callerya speciosa*, Leguminosae, nitrogen-containing heterocyclic compounds.

Classification number: 3.3

1. Introduction

Callerya speciosa (Champ.) Schot. [synonym: *Millettia speciosa* Champ.] is a valuable medicinal plant belonging to the family Leguminosae, which is widely distributed in Southeast Asia in the tropical and subtropical forests of Hainan Island and southern mainland China [1]. *C. speciosa* is also found in the north areas of Vietnam such as Tuyen Quang, Bac Kan, Bac Giang, and Phu Tho. In recent years, *C. speciosa* has been planted more frequently in areas of Vietnam as its roots have been used as a traditional medicine for the treatment of fever, cough, headache, backache, rheumatism, chronic bronchitis, and nephritis [2]. Previous phytochemical studies on this species revealed the presence of phenolics, phenolic glycosides, pterocarpanes, flavonoids, isoflavonoids, sterols, and chromones [3–9]. In a previous work, we reported the isolation and structural characterization of a new oleanane triterpenoid along with three known compounds from the ethyl acetate extract of the *C. speciosa* roots [10]. In a further investigation of chemical constituents from the n-butanol extract of the roots of this species, we isolated four nitrogen-containing compounds including uridine (1), 2-(β -D-glucopyranosyl)-3-isoxazolin-5-one (2), adenosine (3), and hypaphorine (4), and their structures were fully characterized.

2. Materials and methods

2.1. General experiment procedure

1D- and 2D-NMR spectra were acquired on a Bruker Avance 500 Ultrashield NMR Spectrometer. ESI-MS was measured on an Agilent LC-MSD-Trap SL. Thin layer chromatography was carried on silica gel 60 F254 (0.25 mm, Merck) and reversed phase RP18 F254S (0.25 mm, Merck) plates. Column chromatography was performed using silica gel 60 (230–400 mesh, Merck), YMC RP-18 resins (30–50 μ m, Fuji Silysia Chemical Ltd), and Sephadex LH-20 gel (Amersham Pharmacia Biotech).

2.2. Plant material

The plant material was collected in the Tan Yen district, Bac Giang province, in March of 2018. The sample identification was done by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST), and a voucher specimen was preserved in the Laboratory of Natural Products Research, Institute of Chemistry, VAST.

2.3. Extraction and isolation

The powdered roots of *C. speciosa* (870 g) were extracted with 95% methanol three times at room

*Corresponding author: Email: nttam@ich.vast.vn

temperature. The extracts were filtered, combined, and concentrated under reduced pressure. The obtained residue was successively dissolved with water and re-extracted in turn with ethyl acetate and n-butanol. The organic solvents were concentrated to give 4.6 g and 9.7 g of the corresponding extracts.

The n-butanol extract was fractionated by silica gel column chromatography eluted with CH_2Cl_2 :MeOH:H₂O (4:1:0.1 - 1.5:1:0.2, v/v) to give 7 fractions. Fraction 2 was re-purified by silica gel column (CH_2Cl_2 :MeOH:H₂O = 4:0.8:0.1, v/v), then Sephadex LH-20 column (MeOH) to afford compound **1** (4 mg). Fraction 4 was re-purified on a silica gel column (CH_2Cl_2 :MeOH:H₂O = 3:1:0.1, v/v), then Sephadex LH-20 column (MeOH) to give compounds **2** (3 mg) and **3** (4 mg). Fraction 6 was chromatographed on a reversed phase silica gel (RP-18) column (MeOH:H₂O = 1:1), then silica gel column (CH_2Cl_2 :MeOH:H₂O = 2.5:1:0.1, v/v) to yield compound **4** (7 mg).

Uridine (1):

Yellowish solid. ESI-MS: m/z 267.2 $[\text{M}+\text{Na}]^+$. ¹H-NMR (500 MHz, CD₃OD): δ_{H} 8.02 (1H, d, $J=7.0$ Hz, H-6), 5.92 (1H, d, $J=4.0$ Hz, H-1'), 5.71 (1H, d, $J=7.0$ Hz, H-5), 4.21-4.19 (1H, m, H-2'), 4.18-4.16 (1H, m, H-3'), 4.03-4.02 (1H, m, H-4'), 3.86 (1H, dd, $J=10.0$, 2.0 Hz, H-5a'), 3.75 (1H, dd, $J=10.0$, 2.0 Hz, H-5b'). ¹³C-NMR (125 MHz, CD₃OD): δ_{C} 166.19 (C-4), 152.47 (C-2), 142.73 (C-6), 102.66 (C-5), 90.75 (C-1'), 86.37 (C-4'), 75.72 (C-2'), 71.31 (C-3'), 62.28 (C-5').

2-(β -D-glucopyranosyl)-3-isoxazolin-5-one (2):

Colourless solid. ESI-MS: m/z 248.1 $[\text{M}+\text{H}]^+$. ¹H-NMR (500 MHz, CD₃OD): δ_{H} 8.44 (1H, d, $J=3.0$ Hz, H-3), 5.33 (1H, d, $J=3.0$ Hz, H-4), 4.92 (1H, d, $J=7.5$ Hz, H-1'), 3.87-3.85 (1H, m, H-6a'), 3.69-3.67 (1H, m, H-6b'), 3.61-3.60 (1H, m, H-2'), 3.46 (1H, t, $J=7.5$ Hz, H-3'), 3.41-3.40 (1H, m, H-5'), 3.36 (1H, d, $J=7.5$ Hz, H-4'). ¹³C-NMR (125 MHz, CD₃OD): δ_{C} 173.96 (C-5), 154.73 (C-3), 90.86 (C-4), 90.43 (C-1'), 80.39 (C-5'), 78.67 (C-3'), 73.85 (C-2'), 70.93 (C-4'), 62.46 (C-6').

Adenosine (3):

White solid. ESI-MS: m/z 268.1 $[\text{M}+\text{H}]^+$. ¹H-NMR (500 MHz, CD₃OD): δ_{H} 8.32 (1H, s, H-8), 8.20 (1H, s, H-2), 5.99 (1H, d, $J=6.5$ Hz, H-1'), 4.76 (1H, dd, $J=6.0$, 5.5 Hz, H-2'), 4.35 (1H, dd, $J=5.0$, 3.0 Hz, H-3'), 4.19 (1H, dd, $J=3.5$, 3.0 Hz, H-4'), 3.91 (1H, dd, $J=12.5$, 3.0 Hz, H-5a'), 3.77 (1H, dd, $J=12.5$, 3.0 Hz, H-5b'). ¹³C-NMR (125 MHz, CD₃OD): δ_{C} 157.41 (C-6), 153.52 (C-2), 150.02 (C-4), 142.01 (C-8), 121.24 (C-5), 91.26 (C-1'), 88.17 (C-4'), 75.48 (C-2'), 72.65 (C-3'), 63.47 (C-5').

Hypaphorine (4):

Colourless solid. ESI-MS: m/z 247.3 $[\text{M}+\text{H}]^+$. ¹H-NMR (500 MHz, CD₃OD): δ_{H} 7.67 (1H, dd, $J=6.5$, 1.0 Hz, H-5), 7.39 (1H, dd, $J=6.5$, 1.0 Hz, H-8), 7.23 (1H, s, H-2), 7.15 (1H, dt, $J=6.0$, 1.0 Hz, H-7), 7.09 (1H, dt, $J=6.0$, 1.0 Hz, H-6), 3.91 (1H, t, $J=6.0$ Hz, H-11), 3.44 (2H, d, $J=6.0$ Hz, H-10), 3.29 [9H, s, $-\text{N}^+(\text{CH}_3)_3$]. ¹³C-NMR (125 MHz, CD₃OD): δ_{C} 171.34 (C-12), 137.98 (C-9), 128.37 (C-4), 125.16 (C-2), 122.63 (C-7), 120.07 (C-6), 119.22 (C-5), 112.55 (C-8), 109.21 (C-3), 80.58 (C-11), 52.73, 52.71, 52.69 [$-\text{N}^+(\text{CH}_3)_3$], 24.62 (C-10).

3. Results and discussion

Compound **1** was obtained as yellowish solid. The ¹³C-NMR and HSQC spectra of compound **1** revealed resonances for nine carbons of which four carbons [δ_{C} 166.19 (C-4), 152.47 (C-2), 142.73 (C-6), 102.66 (C-5)] were assigned to a uracil and five carbons [δ_{C} 90.75 (C-1'), 86.37 (C-4'), 75.72 (C-2'), 71.31 (C-3'), 62.28 (C-5')] to a ribofuranosyl unit. The ¹H-NMR spectrum showed signals of a pair of doublets at δ_{H} 8.02 (1H, d, $J=7.0$ Hz, H-6) and 5.71 (1H, d, $J=7.0$ Hz, H-5); a ribofuranose with an anomeric proton signal at δ_{H} 5.92 (1H, d, $J=4.0$ Hz, H-1'); and other sugar protons in regions δ_{H} 4.21-3.75 ppm. The HMBC spectrum showed correlations from H-1' (δ_{H} 5.92) to C-2 (δ_{C} 152.47) and C-6 (δ_{C} 142.73) indicating uracil linked to a ribofuranose via a β -N1-glycosidic bond. The molecular formula of **1** was deduced to be C₉H₁₂N₂O₆ based on NMR data and an ESI-MS pseudo-molecular ion peak at m/z 267.2 $[\text{M}+\text{Na}]^+$. The ¹H- and ¹³C-NMR data from compound **1** were consistent with uridine in literature [11]. Therefore, compound **1** was elucidated to be uridine.

Compound **2** was isolated as colourless solid. The ¹H-NMR spectrum of compound **2** shows typical signals of isoxazolin-5-one with two doublets of H-3 and H-4 at δ_{H} 8.44 and 5.33, respectively ($^3J_{3,4}=3.0$ Hz). In addition, the signals of a β -D-glucopyranosyl unit with an anomeric proton signal at δ_{H} 4.92 (1H, d, $J=7.5$ Hz, H-1') and complex proton signals in the region of δ_{H} 3.87-3.36 were also observed. Corresponding to the ¹H-NMR spectrum, the ¹³C-NMR of **2** showed one carbonyl (δ_{C} 173.96), two methine carbons (δ_{C} 154.73, 90.86), and a glucose unit (δ_{C} 90.43, 80.39, 78.67, 73.85, 70.93, and 62.46). The Heteronuclear Multiple Bond correlation (HMBC) correlation between H-1' (δ_{H} 4.92) and C-3 (δ_{C} 154.73) were detected. A molecular formula of C₉H₁₃NO₇ was determined for compound **2** on the basis of an ion peak $[\text{M} + \text{H}]^+$ at m/z 248.1 in ESI-MS and NMR data.

Based on this evidence and comparison with the reported data [12], the structure of **2** was determined to be 2-(β -D-glucopyranosyl)-3-isoxazolin-5-one.

Compound **3** was isolated as a white solid. The ^1H - and ^{13}C -NMR spectra of **3** showed the presence of an adenine unit with three quaternary carbons at δ_{C} 157.41 (C-6), 150.02 (C-4), and 121.24 (C-5) and two methane groups [δ_{H} 8.32 (1H, s, H-8), δ_{C} 142.01 (C-8); δ_{H} 8.20 (1H, s, H-2), δ_{C} 153.52 (C-2)]. In addition, the signals of a ribofuranosyl moiety with four methane groups at δ_{H} 5.99 (1H, d, $J=6.5$ Hz, H-1'), δ_{C} 91.26 (C-1'); δ_{H} 4.76 (1H, dd, $J=6.0, 5.5$ Hz, H-2'), δ_{C} 75.48 (C-2'); δ_{H} 4.35 (1H, dd, $J=5.0, 3.0$ Hz, H-3'), δ_{C} 72.65 (C-3'); δ_{H} 4.19 (1H, dd, $J=3.5, 3.0$ Hz, H-4'), δ_{C} 88.17 (C-4')], and one methylene group at δ_{H} 3.91 (1H, dd, $J=12.5, 3.0$ Hz, H-5a'), 3.77 (1H, dd, $J=12.5, 3.0$ Hz, H-5b'), δ_{C} 63.47 (C-5') were observed. The HMBC correlations from H-1' (δ_{H} 5.99) to C-4 (δ_{C} 150.02), and C-8 (δ_{C} 142.01) suggested that an adenine attached to a ribose sugar molecule via a β -N₉-glycosidic bond. Its molecular formula was established as $\text{C}_{10}\text{H}_{13}\text{N}_5\text{O}_4$ by a combination of NMR data and an ESI-MS pseudo-molecular ion peak at m/z 268.1 $[\text{M}+\text{H}]^+$. From the above spectral data, the structure of **3** was determined to be adenosine. The ^1H - and ^{13}C -NMR data (in CD_3OD) of **3** resemble those of adenosine in literature [13].

Compound **4** was obtained as a colourless solid. The ^1H -NMR spectrum of **4** exhibited signals characteristic of 3-substituted indole skeleton with four aromatic vicinal protons of a disubstituted benzene ring at δ_{H} 7.67 (1H, dd, $J=6.5, 1.0$ Hz, H-5), 7.39 (1H, dd, $J=6.5, 1.0$ Hz, H-8), 7.15 (1H, dt, $J=6.0, 1.0$ Hz, H-7), 7.09 (1H, dt, $J=6.0, 1.0$ Hz, H-6); and one singlet at δ_{H} 7.23 (1H, s, H-2). The signals of the side chain including three protons at δ_{H} 3.91 (1H, t, $J=6.0$ Hz, H-11), 3.44 (2H, d, $J=6.0$ Hz, H-10); and three methyl groups at δ_{H} 3.29 (9H, s) were also observed. The ^{13}C -NMR spectrum of **4** showed one carbonyl group (δ_{C} 171.34), five sp^2 methines (δ_{C} 125.16, 122.63, 120.07, 119.22, 112.55), three sp^2 quaternary carbons (δ_{C} 137.98, 128.37, 109.21), one nitrogen-bearing sp^3 methine (δ_{C} 80.53), one sp^3 methylene (δ_{C} 24.62), and three methyls (δ_{C} 52.73, 52.71, 52.69). The HMBC correlations between the methyl protons at δ_{H} 3.29 with these methyl carbons at δ_{C} 52.73, 52.71, 52.69, and methine carbon at δ_{C} 80.53 suggested these three methyls connected together with one nitrogen and $-\text{N}^+(\text{CH}_3)_3$ group linked with methine carbon C-11. In addition, the HMBC spectrum exhibited correlations from protons H-10 (δ_{H} 3.44) to carbons C-2 (δ_{C} 125.16), C-3 (δ_{C} 109.21), C-4 (δ_{C} 128.37) and C-12 (δ_{C} 171.34); from H-11 (δ_{H} 3.91) to C-3 (δ_{C} 3.91), and C-12 (δ_{C} 171.34). The molecular formula

of **4** was determined to be $\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_2$ from ESI-MS molecular ion peak at m/z 247.3 $[\text{M}+\text{H}]^+$ and NMR data. Based on this evidence and comparison with the reported values in literature [14], compound **4** was determined to be hypaphorine (Fig. 1).

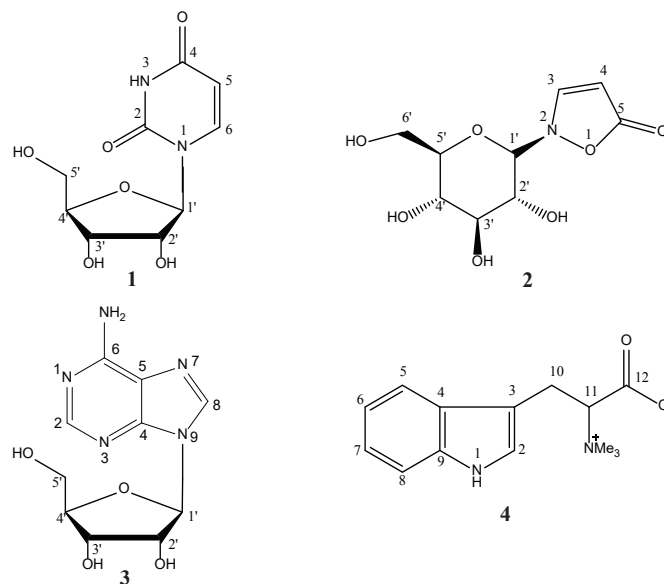


Fig. 1. Structures of compounds 1-4.

4. Conclusions

Column chromatography of the n-butanol extract of *Callerya speciosa* roots resulted in the isolation of four nitrogen-containing heterocyclic compounds: uridine (**1**), 2-(β -D-glucopyranosyl)-3-isoxazolin-5-one (**2**), adenosine (**3**), and hypaphorine (**4**). The chemical structures of compounds **1-4** were established by MS and NMR. All these compounds were isolated from this species for the first time.

CRedit author statements

Dao Duc Thien: Methodology, Data curation, Writing original draft preparation; Le Quoc Thang: Writing original draft preparation; Nguyen Thanh Tam: Writing - Reviewing and Editing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] L. Li, Z. Li, K. Li, et al. (2013), "Development and characterization of EST-SSR markers in the Chinese medicinal plant *Callerya speciosa* (Fabaceae)", *Applications in Plant Sciences*, **1**, DOI: 10.3732/aps.1200345.
- [2] D.T. Loi (2004), *Vietnamese Medicinal Plants and Ethnomedical Herbs*, Medical Publishing House, Hanoi, 1276pp (in Vietnamese).
- [3] T. Yin, G. Tu, Q. Zhang, et al. (2008), "Three new phenolic glycosides from the caulis of *Millettia speciosa*", *Magnetic Resonance in Chemistry*, **46(4)**, pp.387-391, DOI: 10.1002/mrc.2189.
- [4] T. Yin, H. Liang, B. Wang, et al. (2010), "A new flavonol glycoside from *Millettia speciosa*", *Fitoterapia*, **81**, pp.274-275, DOI: 10.1016/j.fitote.2009.09.013.
- [5] D.L. Chen, Y.Y. Liu, G.X. Ma, et al. (2005), "Two new rotenoids from the roots of *Millettia speciosa*", *Phytochemistry Letters*, **12**, pp.196-199, DOI: 10.1016/j.phytol.2015.04.003.
- [6] T. Uchiyama, M. Furukawa, S. Isobe, et al. (2003), "New oleanane-type triterpene saponins from *Millettia speciosa*", *Heterocycles*, **60(3)**, pp.655-661, DOI: 10.3987/COM-02-9678.
- [7] P Ding, J.Y Qiu, G. Ying, et al. (2014), "Chemical constituents of *Millettia speciosa*", *Chinese Herbal Medicines*, **6(4)**, pp.332-334, DOI: 10.1016/S1674-6384(14)60051-4.
- [8] M.Q. Fu, G.S. Xiao, Y.J. Xu, et al. (2016), "Chemical constituents from roots of *Millettia speciosa*", *Chinese Herbal Medicines*, **8(4)**, pp.385-389, DOI: 10.1016/S1674-6384(16)60068-0.
- [9] V.D. Loi, P.G. Nam, N.T. Vung (2017), "Chromones isolated from root of *Callerya speciosa* Schot. growing in Thai Nguyen province", *Journal of Medicinal Materials*, **22**, pp.302-305.
- [10] D.D. Thien, N.T. Tam, N.T.H. Anh, et al. (2020), "A new oleanane triterpenoid from the roots of *Callerya speciosa*", *Letters in Organic Chemistry*, **17(5)**, pp.388-392, DOI: 10.2174/1570178616666190806124038.
- [11] N.T.H. Van, N.T.H. Anh, T.V Sung, et al. (2005), "Studies on chemical composition of *Angelica sinensis*'s V - The nitrogen-containing compounds", *Vietnam Journal of Chemistry*, **43(5)**, pp.1-6.
- [12] T. Becker, P Kartikey, C. Paetz, et al. (2015), "Synthesis and photosensitivity of isoxazolin-5-one glycosides", *Organic & Biomolecular Chemistry*, **13(13)**, pp.4025-4030, DOI: 10.1039/c5ob00244c.
- [13] V.G. Nebieridze, A.V. Skhirtladze, E.P. Kemertelidze, et al. (2017), "Nucleosides from *Tribulus terrestris*", *Chemistry of Natural Compounds*, **53**, pp.1010-1011, DOI: 10.1007/s10600-017-2188-8.
- [14] H. Bel-Kassaoui, D. Lamnaouer, A. Jossang, et al. (2008), "Role of hypaphorine in the toxicity of *Astragalus lusitanicus*", *Natural Product Research*, **22(5)**, pp.453-457, DOI: 10.1080/14786410701591986.

Fabrication and structural characterisation of self-crosslinking hydrogel based on Vietnamese sources of chitosan and alginate

Tin Dai Luong^{1,2}, An Tran-My Le^{1,2}, Nhan Thien Vo^{1,2}, Thi Hiep Nguyen^{1,2*}

¹School of Biomedical Engineering, International University - Ho Chi Minh city, Quarter 6, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam

²Vietnam National University - Ho Chi Minh city, Quarter 6, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam

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Abstract:

Hydrogel materials have attracted extensive research over the last few decades, especially in tissue engineering, due its many comparable properties with native tissue. This study proposes a new approach to hydrogel fabrication that combines oxidized alginate (OA) into N,O-carboxymethyl chitosan (NOCC) and an oxidized hyaluronan-based hydrogel system employing low cost, domestically sourced chitosan and alginate. Hydrogel fabrication parameters including component ratio and hydrogel concentration were studied. Fourier transform infrared spectroscopy (FT-IR) analysis was applied to confirm the chemical structure of the modified materials. Fabricated hydrogel samples were assessed by cross-sectional surface morphology, equilibrium swelling degree, and *in vitro* degradation behaviour. The results revealed NOCC and oxidized hyaluronic acid (OHA)-OA component contributions toward hydrogel properties, a threshold of raising total polymer content within the network, and a comparison with previous formulations using unmodified alginate. Using low cost domestic resources is a promising way to reduce fabrication expenses, and further work should be performed to evaluate the potential of this cost-effective hydrogel for the tissue engineering field.

Keywords: carboxymethyl chitosan, oxidized alginate, oxidized hyaluronan, self-crosslinking hydrogel, Vietnamese materials.

Classification number: 3.6

1. Introduction

Hydrogels are unique materials with several desirable properties and they have been applied to a wide range of applications, especially those in the biomedical field [1]. Besides their practical uses as contact lenses and wound dressings, hydrogels have also received considerable attention as a promising scaffolding material for tissue engineering. This area of research aims to create an alternative source of functional grafts to overcome the current gap between transplantation supply and demand [2]. Specifically, hydrogels are formed by crosslinking hydrophilic polymer chains in water, thus, they not only possess an aqueous environment resembling a native extracellular matrix, but also a porous structure for nutrient and waste diffusion during cell culture. Furthermore, control over the transformation from a polymer solution into a gel structure enables, first, minimally invasive injection into complicated defects, secondly, homogeneous delivery of cell and bioactive factors, and, lastly, its potential use as a specialized ink for developing 3D bio-printing technology [3].

Although synthetic polymers are reproducible and exhibit high mechanical stability, only a few of them satisfy the requirements of biocompatibility and appropriate biodegradation, such as poly(ethylene glycol) and poly(N-isopropylacrylamide) [4]. Moreover, synthetic polymer-based hydrogels usually lack the necessary bioactivity while their crosslinking nature, especially polymerization reactions, might employ cytotoxic reagents. Therefore, natural polymers have been extensively targeted as hydrogel components alone or in combination with synthetic polymers [5]. Different crosslinking methods could be employed for natural polymer-based hydrogels, such as the exposure to external stimuli [6], the conventional use of small bi-functional crosslinking agents [7], or the combination of reactive polymers [8]. In the last stated method, two or more hydrogel components with reactive functional groups are prepared independently and allowed to react upon combination. This self-crosslinking approach helps to control the intensity of external factors or the use of additional cytotoxic cross-linkers. Among different types of reactions, Schiff-base reactions stand out as they take place

*Corresponding author: Email: nthiep@hcmiu.edu.vn

under the physiological conditions with rapid kinetics [9]. This reaction requires one component with nucleophilic amino groups ($-NH_2$) and another with electrophilic aldehyde groups ($-CHO$), both of which react with each other to form covalent Schiff-base bonds ($C=N$).

Chitosan, a linear polysaccharide derived from natural chitin by a deacetylation step, is a common material with amino functional groups for Schiff-base reaction-based hydrogels [10, 11]. Chitosan has demonstrated good biocompatibility and low immunogenicity in previous studies. To overcome its drawback of low solubility at neutral pH, chitosan can be grafted with additional carboxylate anions ($-COO^-$) or quaternized to obtain permanently protonated amino groups ($-NH_3^+$). NOCC belongs to the former modification technique and is a common constituent for Schiff-base crosslinking reactions since sufficient amino groups remain compared to other grafting techniques targeting N positions. Following chitosan, a second component with aldehyde groups can be achieved by periodate oxidation. It is necessary that the polymer possesses vicinal hydroxyl groups along their chains for the oxidation to take place [9]. Hyaluronic acid (HA), or hyaluronan, is a linear natural polysaccharide that satisfies such a requirement. It exists in the extracellular matrix of human connective tissues, especially cartilage and synovial fluid [12]. Short HA chains have been hypothesized to mediate many biological functions related to proliferation and differentiation of chondrocytes and pro-inflammatory response during wound healing [13]. Modified chitosan has been crosslinked with OHA, such as succinyl chitosan for chondrocyte encapsulation [14], or NOCC for tissue adhesive and dermal wound healing [15, 16]. Despite good healing results, most of these hydrogel formulations exhibit rapid degradation, especially under *in vivo* conditions due to the enzymatic hydrolysis of HA [15]. Therefore, improving the stability of hydrogel containing HA is indispensable to apply this potential biomaterial for tissue engineering field.

Alginate is another linear polysaccharide derived from brown algae with desirable biocompatibility, biodegradability, and low-immunogenicity [17]. It can also be oxidized to crosslink with modified chitosan and has been further combined with bioactive compounds such as albumin for soft tissue engineering [18] and curcumin for dermal wound healing [19]. As a strategy to improve NOCC-OHA hydrogel formulation mentioned above, unmodified alginate was blended into a hydrogel network and an increase in overall stability and degradation time was observed even though the hydrogel concentration remained constant [20]. The given hypothesis was the creation of a more balanced amount of charged groups for a synergistic

polyelectrolyte complexation. There is no follow-up study on the effect of oxidizing alginate, which might promote overall crosslink density inside the network and let OA form stronger covalent bonds with NOCC instead of electrostatic interaction.

Herein, a self-crosslinking hydrogel was fabricated based on NOCC, OHA, and OA (Fig. 1). Besides investigating the potential of incorporating OA into the NOCC-OHA network, domestic sources of chitosan and alginate were also targeted to make use of their abundant resource in Vietnam. Different parameters in the hydrogel fabrication process were evaluated including the component ratios and the concentration of polymer content. Modified structures were analysed by FT-IR and viscosity measurements while fabricated hydrogel samples were characterized by scanning electron microscopy (SEM) observation, equilibrium swelling degree, and *in vitro* degradation tests.

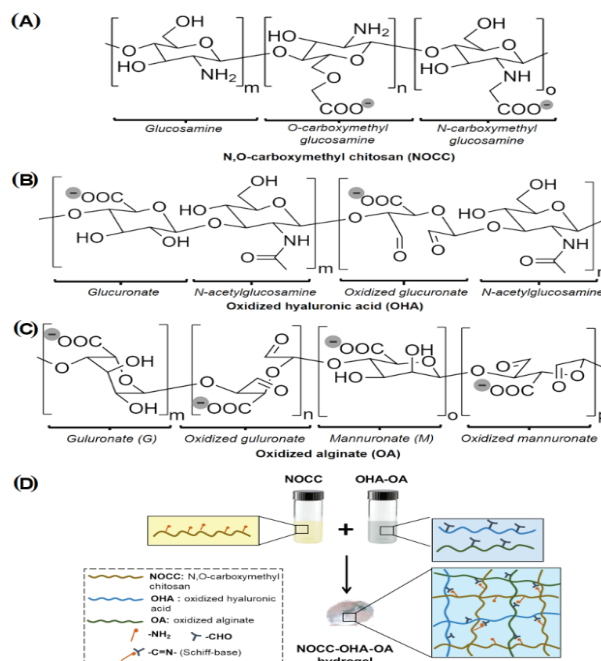


Fig. 1. Chemical structures of (A) NOCC, (B) OHA, (C) OA, and (D) crosslinking mechanism of NOCC-OHA-OA hydrogel.

2. Materials and methods

2.1. Materials

Chitosan derived from shrimp shells with high, medium, or low molecular weight were purchased from Vietnam Food JSC. (Vietnam). HA was purchased from Shandong Bouliga Biotechnology Co., Ltd. (China). Alginate derived from brown seaweed *Sargassum mclurei* was supplied by Nha Trang Institute of Technology Research and Application (Vietnam). Chloroacetic acid was purchased from HiMedia Laboratories Pvt. Ltd. (India). Sodium metaperiodate

(≥99%) was purchased from Thermo Fisher Scientific Inc. (UK). Dialysis bag (MWCO of 14 kDa) was purchased from Sigma-Aldrich Co., LLC (USA). Isopropyl alcohol, sodium hydroxide, hydrochloric acid, and ethylene glycol were purchased from Xilong Chemical Ltd. (China).

2.2. Methods

2.2.1. Synthesis and characterization of hydrogel components

Synthesis of NOCC: chitosan from a Vietnamese source with high, medium, or low molecular weights were modified into NOCC based on a prior study [21]. Briefly, 1 g of chitosan was suspended in 5 ml of isopropyl alcohol. Then, 5 ml of aqueous sodium hydroxide solution was added and the entire mixture was placed at room temperature for an hour. Next, 5 ml of chloroacetic acid in isopropyl alcohol was added 5 times every 5 min. The molar ratio between chloroacetic acid and sodium hydroxide was fixed at 0.42 based on previous study, and different concentrations of chloroacetic acid were investigated for optimal solubility. Specifically, the weights of used sodium hydroxide and chloroacetic acid were investigated between 2.5, 3.125, and 3.75 g. Heat (60°C) was applied at the time of chloroacetic acid addition, and the reaction lasted for a total of 3 h. After the reaction step, the mixture was filtered to obtain the solid part. This part was dissolved in distilled water and neutralized with hydrochloric acid solution. The neutral solution was then dialyzed for 3 d against distilled water. After that, it was frozen overnight and lyophilized for at least 36 h using a Freezone 6 l Benchtop Freeze Dry System (Labconco, USA). The obtained NOCC was kept at 2-8°C.

Synthesis of OHA and OA: OHA was synthesized from HA based on a previous study with slight modification [14]. At first, a solution of 1% (w/v) HA was prepared at room temperature. Next, an aqueous solution of sodium metaperiodate (NaIO_4) with 40% molar equivalence to HA dimers was prepared in dark condition and added dropwise into the HA solution. The mixture was stirred for 2 h at room temperature with a fully covered container to avoid light exposure. After that, ethylene glycol with 4-fold molar equivalence to NaIO_4 was added to quench residual oxidizing reagents. The mixture was stirred for one more hour under the same conditions. The sample was then dialyzed for 3 days against distilled water. The obtained solution was frozen overnight and lyophilized for at least 36 h to achieve the OHA product, which was stored at 2-8°C.

Alginate derived from Vietnamese brown seaweed was used to synthesize OA by a similar oxidation process to OHA. Briefly, a solution of 1% (w/v) alginate was prepared. Then, an aqueous solution of NaIO_4 with 20% molar equivalence to alginate monomers was prepared without light exposure and allowed to react with alginate. The

conditions for reaction, dialysis, and freeze-drying steps were similar to the OHA modification protocol. Obtained OA was also stored at 2-8°C.

FT-IR analysis: the chemical structures of the modified components were determined using FT-IR spectroscopy by a Vertex 70v spectrometer (Bruker, USA). The measurement was carried out at room temperature on freeze-dried components in the wavenumber range of 4000-600 cm^{-1} against a KBr membrane background.

Viscosity evaluation: the viscosity of NOCC or OHA-OA precursor solutions at different concentrations were evaluated. NOCC solutions were made by dissolving freeze-dried NOCC in distilled water at 3, 4, or 5% (w/v). OHA-OA solutions were made with the same range of concentrations as the NOCC solutions by dissolving lyophilized OHA and OA with a weight ratio of 1:1. The viscosity was determined at room temperature using a DVE-RV viscometer (Brookfield, USA) equipped with a small sample adaptor (SC4-28).

2.2.2. Fabrication and characterization of NOCC-OHA-OA hydrogel

Fabrication of hydrogel samples: each pair of NOCC and OHA-OA solutions at a specified concentration (3, 4, or 5% w/v) were gently combined at a specified volume ratio to enable crosslinking. Five volume ratios of 3:1, 2:1, 1:1, 1:2, and 1:3 were investigated for the hydrogel at 3% concentration from which a narrower range of volume ratios were chosen for the other two concentrations of 4 and 5%. Formation of hydrogel was confirmed by macroscopic observation.

FT-IR analysis: the prepared hydrogel samples were frozen and lyophilized, then investigated with FT-IR under the same conditions as modified components above.

Cross-sectional surface morphology analysis: the cross-sectional surface morphology of hydrogel samples was observed using SEM. Briefly, each freeze-dried sample was sliced to reveal the inner cross-sectional surface. This surface was then coated with a thin layer of gold using a Smart Coater (JEOL, Japan) and observed using a JSM-IT100 microscope (JEOL, Japan) at 10 kV. The pore diameters were analysed using ImageJ software (USA) ($n=3$, 40 measurements per sample). The results were displayed as mean±standard deviation.

Equilibrium swelling degree measurement: the equilibrium swelling degree of the hydrogel samples in a hydrated state were measured gravimetrically. At first, each sample was prepared and placed in a 24-well plate. Its initial weight was denoted as W_0 . The hydrogel was then immersed completely in phosphate-buffered saline (PBS) (1 X, pH=7.4) and incubated at 37°C. Each sample was weighed every hour until it reached the equilibrium state. The weight

at that point was denoted as W_s . The swelling degree was calculated using the following equation:

$$\text{Equilibrium swelling degree (\%)} = (W_s - W_0) / W_0 \times 100.$$

The experiment was done in triplicate. The results were displayed as mean $\pm$ standard deviation.

2.2.3. In vitro degradation assessment

The degradation of each hydrogel sample was measured gravimetrically under simulated physiological conditions of PBS (1 X, pH=7.4) and 37°C as in the previous experiment. Briefly, the initial hydrogel weight was denoted as W_0 . Then, the weight was measured every 24 h for 9 days, recorded as W_t (weight on day t). The PBS medium was changed every 3 day. The percentage of remaining weight (W_d , %) at each time point was calculated using the following equation:

$$W_d (\%) = W_t / W_0 \times 100.$$

The test was conducted in triplicate. The results were expressed as the mean $\pm$ standard deviation.

2.2.4. Statistical analysis

All statistics were performed using OriginPro (USA). Results were reported as mean $\pm$ standard deviation (SD). One-way ANOVA was used to compare the effect of one variable between multiple groups, while two-way ANOVA and Tukey's post hoc test were applied to compare effects of multiple variables. The level of significance was set at $p < 0.05$.

3. Results and discussion

3.1. Synthesis of NOCC

Although direct employment of chitosan has been explored in previous studies like crosslinking with benzaldehyde-grafted zinc phthalocyanines [22] or cuminaldehyde [23], the use of acidic solvents to dissolve raw chitosan is not appropriate for cell encapsulation to target tissue engineering. Carboxymethylation was thus performed to acquire a more soluble chitosan derivative NOCC. This substitution reaction took place between a nucleophilic O atom of hydroxyl or N atom of amino groups on chitosan and electrophilic C atom of chloroacetic acid in a polar protic solvent of water and isopropanol [24]. The conditions for this reaction might vary according to the source of chitosan, as each source differs in many respects especially molecular weight, degree of deacetylation, and crystallinity. Our group has determined that the quantities of chloroacetic acid and sodium hydroxide are key parameters of the reaction, which affects the solubility of obtained NOCC [25]. Altering the chloroacetic acid amount changes the grafting degree of charged carboxymethyl groups onto chitosan chains. Besides, an alkaline environment facilitates this grafting reaction, so the amount of base should be raised whenever the amount of acid reactant is increased to

ensure a sufficiently high pH value. For imported high-cost chitosan, the most suitable amounts of both acid and base were found to be about 2.5 g per 1 g of chitosan, which yields NOCC product that is soluble up to 5% concentration (w/v) [25]. Based on that result, an investigation into reactant quantities was conducted using Vietnamese low-cost chitosan with different molecular weights. Solubility of obtained NOCC was evaluated after the filtered product reached the dissolution and neutralization steps.

Regarding the high molecular weight source, a wide range from 1.25 to 5 g of reactants were investigated. Representative images of the NOCC solution formulated with 2.5, 3.75, and 5 g of reactant are shown in Fig. 2. Although the solubility of NOCC improved with the increase in reactant, aggregates of insoluble chitosan were observed in the solution with 5 g reactant. For the medium molecular weight source, more distinct results were obtained for a smaller range of 2.5, 3.125, and 3.75 g of reactant. A transition from 2.5 to 3.125 g showed lower amounts and smaller sizes of insoluble chitosan flakes. These observed aggregates were also reduced compared to previous samples from the high molecular weight source, which might be attributed to the higher reaction efficiencies of smaller polymer chains. A further increase to 3.75 g did not demonstrate significant difference in solubility. With regard to the low molecular weight source, repeated amounts of 2.5 and 3.125 g of reactant were applied. However, the solubility of the obtained NOCC did not considerably exceed that of the medium molecular weight. Therefore, this source of chitosan was chosen to reinforce the hydrogel network compared to lower one, while the quantity of 3.125 g of chloroacetic acid and base was employed instead of 3.75 g for a milder use of chemicals with equivalent result. The small amount of aggregates was filtered before the dialysis step to collect solely NOCC products.

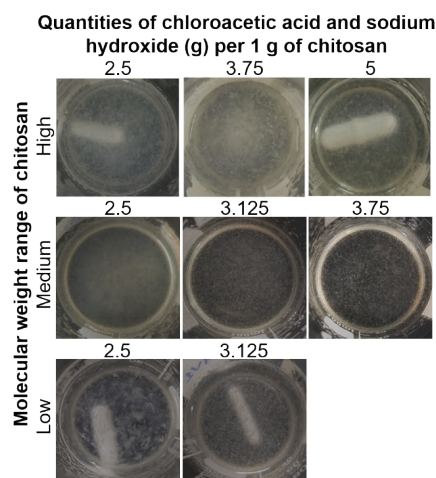


Fig. 2. Top view of NOCC solution obtained from chitosan with high, medium, and low molecular weight using different quantities of chloroacetic acid and sodium hydroxide (g) per 1 g of raw chitosan in the reaction.

FT-IR spectroscopy was used to confirm the modification of chitosan into NOCC. The spectrum of NOCC (Fig. 3) demonstrated emerging broad bands at 1580 and 1403 cm^{-1} of -COO^- antisymmetric and symmetric stretching vibrations, which confirms the appearance of grafted carboxylate groups. These signals overlapped with some original bands of chitosan such as the C=O stretching vibration of amide I (1649 cm^{-1}) and N-H bending vibration of the primary amino group and amide II (1568 cm^{-1}) [26]. Although carboxymethyl groups were partially grafted on reactive amino groups for Schiff-base reactions, a previous investigation [24] showed that this modification took place mostly at the O-position instead of the N-position.

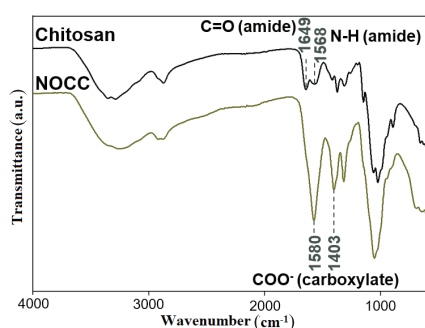


Fig. 3. FT-IR spectra of chitosan and NOCC.

3.2. Synthesis of OHA and OA

HA and alginate were oxidized to OHA and OA, respectively, to participate in the crosslinking reaction with NOCC via aldehyde groups. Periodate served as the oxidizing agent that selectively converted vicinal diols along polysaccharide chains into separate di-aldehydes. Unlike the synthesis of NOCC, the molar ratio was fixed between this oxidizing agent and saccharide unit containing vicinal diol, thus, the same amounts of reactants were employed regardless of material source.

As shown in Fig. 4, the spectra of both OHA and OA mostly resembled the original unmodified spectra with the exception of a new shoulder peak around 1730 cm^{-1} representing the C=O stretching vibration of aldehyde. Normally, a C=O stretching vibration yields strong signal owing to the large dipole moment of the bond nature. However, several nearby hydroxyl groups along carbohydrate chains could react with free aldehydes to establish reversible hemiacetals, which lowers the signal intensity. This observation was comparable to FT-IR results from other published studies that showed shoulder peak of aldehyde group between 1721 and 1740 cm^{-1} for oxidized hyaluronan and alginate [15, 27].

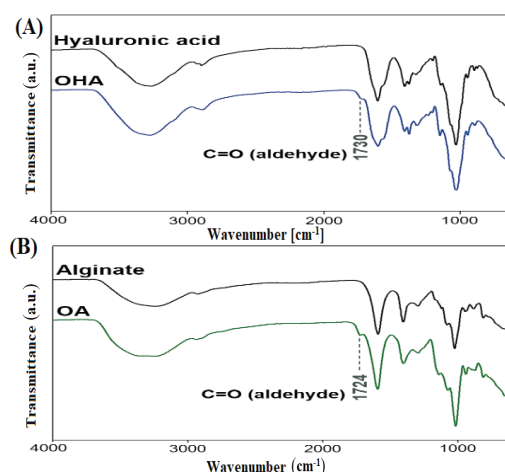


Fig. 4. FT-IR spectra of (A) HA and OHA and (B) alginate and OA.

An oxidation degree of 40% for OHA was chosen based on previous studies that compared a close range of degrees around 40% and showed that 40% yielded closer actual values [15] and more desirable properties including biocompatibility [16]. A corresponding degree of 20% was chosen for alginate, as each monomer unit on the alginate structure could be oxidized rather than a dimer unit like HA. Periodate-based oxidation is associated with partial chain cleavage because the obtained aldehyde derivative is more susceptible to alkaline β -elimination and radical depolymerization compared with the original polysaccharide [28]. Therefore, a choice of low oxidation degrees could prevent this chain cleavage problem.

3.3. Viscosity evaluation of NOCC and OHA-OA solutions

NOCC and OHA-OA solutions were prepared at different concentrations ranging from 3 to 5% (w/v) for viscosity measurements. OHA and OA were dissolved together at a weight ratio of 1:1 as they both possess aldehyde groups and were expected to react simultaneously with NOCC upon combination. As shown in Fig. 5, an increase in concentration of both solutions led to an increase in viscosity, which was expected. The transition from 3 to 5% led to a 10-fold increment in viscosity for NOCC and 6-fold increment for OHA-OA. In comparison, OHA-OA solutions showed lower viscosity values than NOCC solutions at all concentrations, which was mostly attributed to the difference in intermolecular forces throughout the polymer network of each solution. NOCC possesses both positively charged protonated amino groups (-NH_3^+) and negatively charged carboxylate groups (-COO^-) at near neutral pH, which form numerous electrostatic interactions between NOCC chains. On the other hand, both OHA and OA only exhibit carboxylate anions possessing weak electrostatic interactions with mobile cations like Na^+ .

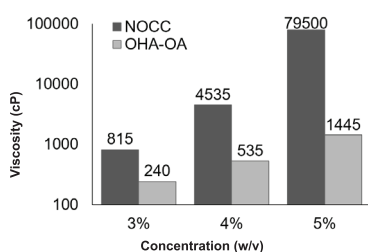


Fig. 5. Viscosity of NOCC and OHA-OA solutions at different concentrations.

3.4. Fabrication of hydrogel samples

Following the preparation of NOCC and OHA-OA solutions, different hydrogel samples were fabricated by combining these precursor solutions at a predetermined concentration and volume ratio. The influence of concentration on the hydrogel network were investigated between 3, 4, and 5% (w/v). Regarding the next parameter, as exact moles of amino and aldehyde groups on large polymer molecules are hard to accurately quantify, a common alternative utilizing a component ratio within the network to reflect molar ratio of those functional groups was employed. When the components were combined at an actual molar ratio close to 1:1, the gel network reached its highest crosslink density.

For 3% hydrogel, a wide range of volume ratios were examined between the NOCC and OHA-OA solutions, specifically, 3:1, 2:1, 1:1, 1:2, and 1:3. The hydrogel structure gradually stabilized from a 3:1 ratio to 1:1 ratio, and then became weaker for ratios 1:2 and 1:3. As the two extremes (3:1 and 1:3 ratios) showed unstable hydrogel shapes with long gelation time, the three intermediate ratios of 2:1, 1:1, and 1:2 were used to investigate the 4 and 5% concentrations. At 4% concentration, the increases in viscosities of two precursor solutions started to hinder the handling step of hydrogel combination and mixing. At this concentration, the 1:1 and 2:1 ratios appeared equivalently stable. Regarding the 5% concentration, a 2:1 ratio demonstrated a more stable structure compared to the 1:1 and 1:2 ratios. Considering that the same component ratio should yield equivalent molar ratios regardless of concentration factor, the shifting stability from a 1:1 ratio of 3% and 4% hydrogel to a 2:1 ratio of 5% hydrogel might be attributed to an additional effect from the hydrogel component. The NOCC solution at 5% concentration showed a large viscosity variance with OHA-OA solution and thus its higher proportion might partially contribute to the stability of the network by physical interactions alongside expected covalent crosslinking.

A representative spectra of hydrogel samples at 3% concentration and volume ratios of 1:2, 1:1, and 2:1 are shown in Fig. 6. All hydrogel spectra seemed to inherit most of the peaks from its constituents including NOCC, OHA, and OA. The desirable C=N peak of a Schiff-base bond around 1650 cm^{-1} [29, 30] was not distinctly observed, as it

was overlapped by the adjacent stronger and broader -COO⁻ antisymmetric stretching peak of carboxylate around 1590 cm^{-1} . Nonetheless, the small carbonyl band of aldehyde around 1730 cm^{-1} from the OHA and OA spectra disappeared from the hydrogel spectra, which is evidence that these aldehyde groups participated in the crosslinking reaction. Besides this covalent bond, other physical interactions are also established in the hydrogel network despite not being observed through FT-IR technique including hydrogen bonds and electrostatic interactions between polar or charged groups like hydroxyl, carboxyl, amino, and carbonyl.

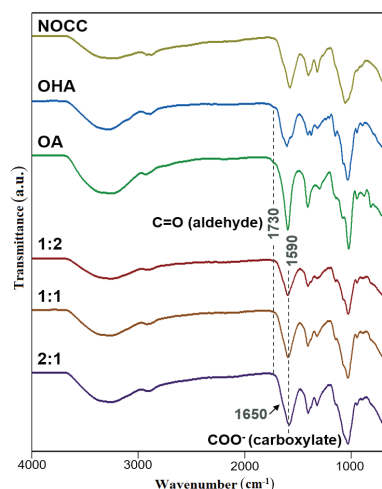


Fig. 6. FT-IR spectrum of NOCC, OHA, OA, and hydrogel samples at 3% concentration and volume ratios of 1:2, 1:1, and 2:1 between NOCC and OHA-OA solutions.

3.5. Cross-sectional surface morphology analysis

Morphologies of the cross-sectional surfaces were observed using SEM, and the representative images are presented in Fig. 7. By subjecting hydrogel samples to deep freezing and lyophilization, the inner ice crystals sublimated, leaving a porous network of polymer chains behind. An investigation into surface smoothness, pore shape, and pore size of the cross-sections reflects corresponding homogeneity of the crosslinking process and stiffness of the hydrogel system.

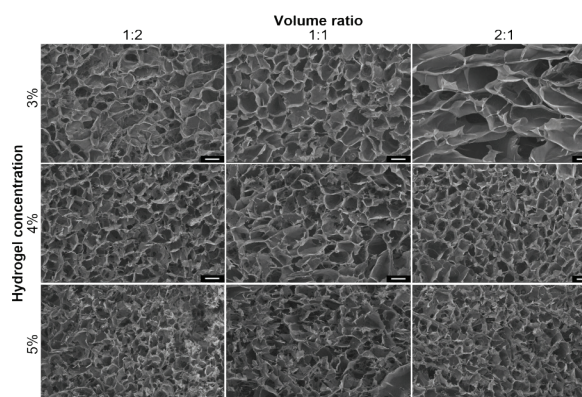


Fig. 7. SEM images of cross-sectional surface of hydrogel samples at magnification of 100X (scale bar of 100 μm).

Overall, the samples exhibited distinct pores to be examined. Among the three tested concentrations, the 3% sample showed more stable pore structures. Appearance of disrupted small holes on the pore surface, along with more disarranged pore shapes, could be observed on the 4 and 5% samples. This heterogeneity might be attributed to a more difficult mixing step at higher viscosity. Besides the influence of concentration, volume ratio was another factor that affected pore size and surface stability according to SEM results. Samples at a 1:2 ratio demonstrated a higher chance of interruption throughout the surface, and a larger diameter was seen for the 2:1 ratio at 3% concentration. In contrast, the other two ratios exhibited higher stability, especially for samples at 3 and 4% concentrations.

A more detailed analysis of pore size was made by measuring the pore diameter of the obtained images and then expressing the results in terms of size distribution and mean value comparison, as shown in Figs. 8 and 9. Considering the distribution curve of all samples, most of them showed a homogeneous range of pore sizes, except for 3% sample at 2:1 ratio. This sample also exhibited a significant increase in average pore diameter compared to other ratios of the same concentration (168 μm versus 103 and 105 μm). Based on these observations, the sample with 1:1 ratio seemed to be the structure with highest crosslink density for 3% concentration owing to its lower pore size, homogeneous size range, and more intact surface. The sample with 4% concentration and 1:1 ratio demonstrated significantly higher pore diameter compared to the 1:2 and 2:1 ratios. Combined with previously observed surface morphology, a shift of stability towards the 2:1 ratio could be noticed as its surface showed less disruptive areas than the 1:2 ratio. Lastly, samples at 5% concentration showed no difference in mean pore diameter between tested ratios, which might indicate their close stiffness.

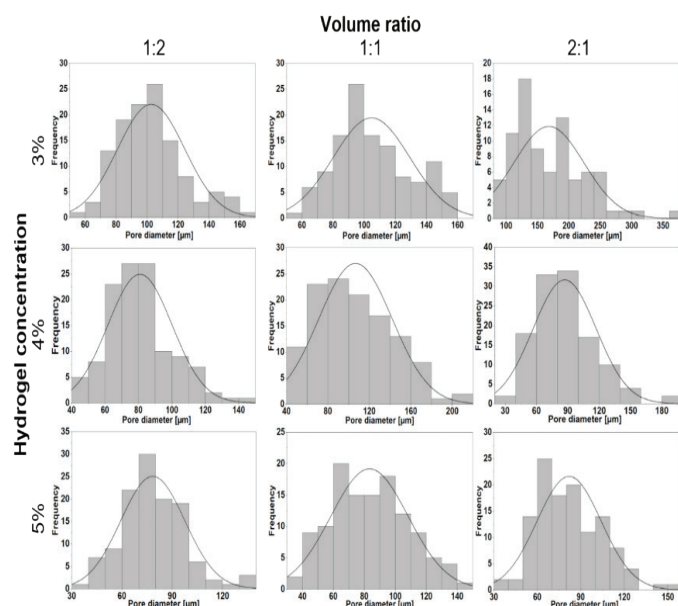


Fig. 8. Pore diameter distribution of cross-sectional surface of hydrogel samples (n=120).

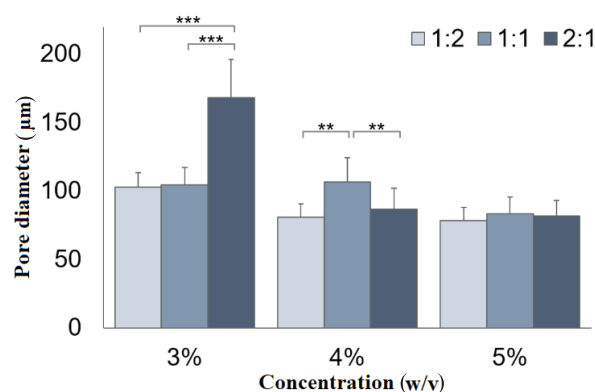


Fig. 9. Pore diameter (μm) of cross-sectional surface of hydrogel samples (values=mean $\pm$ standard deviation, n=120) (**p<0.01, ***p<0.001).

By comparing samples with similar volume ratios but different concentrations, the influence of concentration on hydrogel morphology was assessed. A significant decrease in pore diameter was noticed when increasing sample concentration from 3 to 5% for all ratios (p<0.001). This could be explained by the increasing polymer content in the network leading to more a stable and stiffer structure.

3.5.1. Equilibrium swelling degree measurement

Hydrogel volume change under physiological environment served as an essential factor for practical application of hydrogel. For example, a structure enlarged too much might exceed permissible space at defect site. Swelling behaviour in simulated physiological condition of PBS 1 X at 37°C was monitored for all hydrogel samples. The calculated equilibrium swelling degrees of those samples are presented in Fig. 10. An increase in NOCC component within hydrogel network seemed to correlate with a corresponding increase in hydrogel swelling capacity for 3 and 5% samples. Specifically, 3% hydrogel with 1:2 ratio reached 87% swelling degree, which is significantly lower than 149% of 1:1 ratio. Similarly, the equilibrium swelling degree was 121% for 5% hydrogel with 1:2 ratio, and

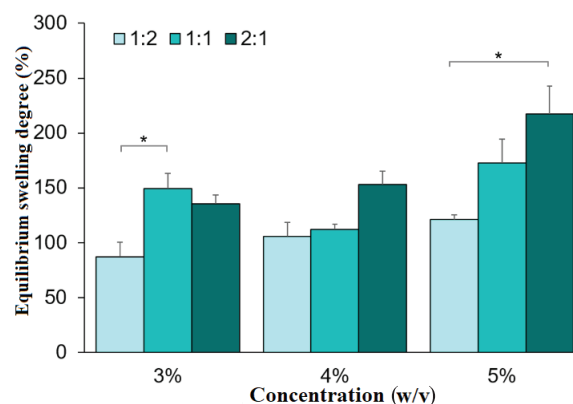


Fig. 10. Equilibrium swelling degree of hydrogel samples at physiological conditions (values=mean $\pm$ standard deviation, n=3) (*p<0.05).

a significant increase to 217% was observed for 2:1 ratio. The likely explanation for this phenomenon is that the higher NOCC proportion is, the higher amount of charged groups exists inside the hydrogel network. This is associated with a higher inward diffusion from surrounding liquid. This result did not demonstrate the same trend as previous pore diameter graph. Therefore, the swelling capacity of hydrogel based on charge-carrying polysaccharides might depend mostly on inherent characteristic of composition inside hydrogel structure rather than crosslink density and stiffness as in SEM result. Another evidence for the claim above is that altering concentration showed no significant effect on the equilibrium swelling degree of hydrogel regarding all tested volume ratios.

3.5.2. *In vitro* degradation assessment

Degradation kinetic is a crucial characteristic to consider the balance between degradation rate of scaffold and ECM deposition rate of host cells. Degradation behaviour of hydrogel samples under physiological condition was plotted against the function of incubation time over the course of 9 days as shown in Fig. 11. After reaching equilibrium swelling with the surrounding environment, hydrolysis of Schiff-base bonds and glycosidic bonds took place, which led to eventual degradation of the polymer network. It is noticeable that 3% hydrogel degraded much more rapidly over

6 days compared to the other two concentrations. No significant difference was observed between 4 and 5% hydrogels, as their weights remained approximately 30% compared to the first day. Despite the fact that higher stiffness was observed for 5% hydrogel based on cross-sectional morphology result, an increase in polymer content was not associated with benefits of longer degradation, which might indicate 5% as a threshold for concentration of the proposed hydrogel system. This threshold might be partly attributed to the massive increase in viscosity of precursor solutions, which probably hindered efficient interaction and crosslinking between reactive polymers. On the ninth day, although there was no significant effect of component ratios on degradation rate of the 5% hydrogel, a significantly higher weight remaining was observed for the same with ratio of 2:1 at 4% hydrogel. This sign of improved stability correlated with the result of cross-sectional surface morphology above, despite that this sample may attain high equilibrium swelling degree within the first few hours.

Based on results of morphology, swelling, and degradation behaviour, the ratios of constituents and hydrogel concentration affected the stability of acquired hydrogel network. A concentration of 4% was sufficient to impart higher crosslink density and longer degradation duration without facing many difficulties with handling the highly viscous precursor solutions. A volume ratio of 2:1, which is associated with a higher proportion of NOCC within the network, demonstrated the best stability of obtained hydrogels at 4% concentration. Increasing NOCC content possibly balanced the molar ratios of reactive groups, and simultaneously added more rigid polymeric coils into the structure compared to weaker chains of OHA and OA. Although the incorporation of OA showed faster degradation compared to unmodified alginate in a previous study [20] in which all investigated formulations remained more than 40% of their initial weight on the ninth day, further work should be implemented to fully understand other aspects such as injectability and biocompatibility. One probable explanation for the observed higher degradation kinetic is the cleavage of alginate chains due to the oxidation process, as stated previously.

4. Conclusions

In summary, NOCC-OHA-OA hydrogels were fabricated based on Schiff-base reaction and characterized in this research, with alteration of firstly component ratios between NOCC and OHA-OA, and secondly the concentrations of hydrogel from 3 to 5%. Different conditions were investigated to synthesize low-cost NOCC from a Vietnamese source, and 3.125 g of both chloroacetic acid reactant and base were chosen. Chemical structures of modified materials were confirmed using FT-IR spectroscopy. Although no direct Schiff-base bond signal was observed on the hydrogel's FT-IR spectra, the disappearance of the aldehyde signal and macroscopic observation of gelation confirmed the formation of the hydrogel. Although higher concentration contributed higher polymer content for the hydrogel network, 4% was determined as a sufficient concentration to avoid high viscosity concerns. In terms of component ratio, higher NOCC content appeared to impart

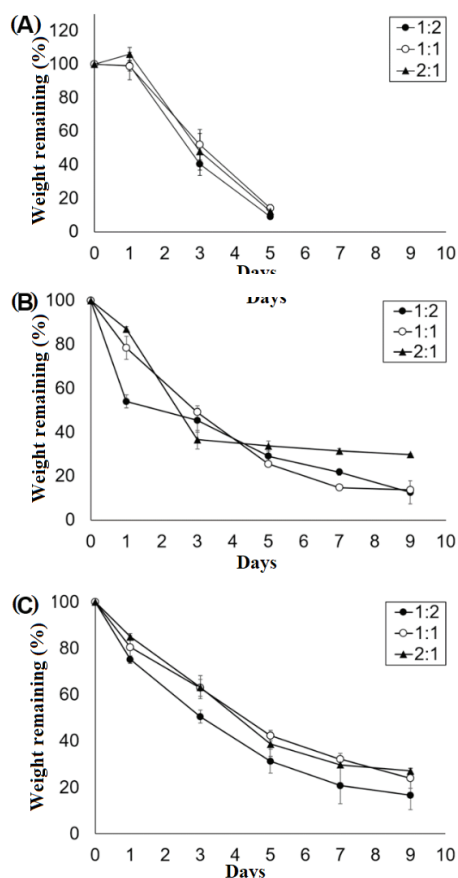


Fig. 11. Degradation kinetic of hydrogel samples at concentration of (A) 3%, (B) 4%, and (C) 5% in physiological conditions within 9 days (values=mean±standard deviation, n=3).

higher stability into the gel structure and improve long-term degradation, although the corresponding high swelling degree should be considered as well. Based on the obtained results in this study, further works on injectability and biocompatibility should be performed to, first, compare this formulation with one employing unmodified alginate and, second, evaluate this potential polysaccharide-based injectable hydrogel for applications in tissue engineering.

CRedit author statement

Tin Dai Luong, An Tran-My Le, Nhan Thien Vo: Data curation, Software, Visualisation, Investigation, Writing; Thi Hiep Nguyen: Methodology, Supervision, Revising the manuscript.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] E. Calo, V.V. Khutoryanskiy (2015), "Biomedical applications of hydrogels: A review of patents and commercial products", *European Polymer Journal*, **65**, pp.252-267, DOI: 10.1016/j.eurpolymj.2014.11.024.
- [2] F.J. O'Brien (2011), "Biomaterials & scaffolds for tissue engineering", *Materials Today*, **14**(3), pp.88-95, DOI: 10.1016/S1369-7021(11)70058-X.
- [3] I. Armentano, M. Dottori, E. Fortunati, et al. (2010), "Biodegradable polymer matrix nanocomposites for tissue engineering: A review", *Polymer Degradation and Stability*, **95**(11), pp.2126-2146, DOI: 10.1016/j.polymdegradstab.2010.06.007.
- [4] D.M. Radulescu, I.A. Neacsu, A.M. Grumezescu, et al. (2022), "New insights of scaffolds based on hydrogels in tissue engineering", *Polymers*, **14**(4), DOI: 10.3390/polym14040799.
- [5] Y.D. Taghipour, V.R. Hokmabad, A.R.D. Bakhshayesh, et al. (2020), "The application of hydrogels based on natural polymers for tissue engineering", *Current Medicinal Chemistry*, **27**(16), pp.2658-2680, DOI: 10.2174/0929867326666190711103956.
- [6] M. Ghorbani, J. Ai, M.R. Nourani, et al. (2017), "Injectable natural polymer compound for tissue engineering of intervertebral disc: *In vitro* study", *Materials Science and Engineering: C*, **80**, pp.502-508, DOI: 10.1016/j.msec.2017.06.007.
- [7] W.Y. Su, Y.C. Chen, F.H. Lin (2010), "Injectable oxidized hyaluronic acid/adipic acid dihydrazide hydrogel for nucleus pulposus regeneration", *Acta Biomaterialia*, **6**(8), pp.3044-3055, DOI: 10.1016/j.actbio.2010.02.037.
- [8] F. Yu, X. Cao, L. Zeng, et al. (2013), "An interpenetrating HA/G/CS biomimetic hydrogel via Diels - Alder click chemistry for cartilage tissue engineering", *Carbohydrate Polymers*, **97**(1), pp.188-195, DOI: 10.1016/j.carbpol.2013.04.046.
- [9] J. Xu, Y. Liu, S.H. Hsu (2019), "Hydrogels based on Schiff base linkages for biomedical applications", *Molecules*, **24**(16), DOI: 10.3390/molecules24163005.
- [10] J. Zhang, W. Xia, P. Liu, et al. (2010), "Chitosan modification and pharmaceutical/biomedical applications", *Marine Drugs*, **8**(7), pp.1962-1987, DOI: 10.3390/md8071962.
- [11] R. Antony, T. Arun, S.T.D. Manickam (2019), "A review on applications of chitosan-based Schiff bases", *International Journal of Biological Macromolecules*, **129**, pp.615-633, DOI: 10.1016/j.ijbiomac.2019.02.047.
- [12] C. Chung, J.A. Burdick (2009), "Influence of three-dimensional hyaluronic acid microenvironments on mesenchymal stem cell chondrogenesis", *Tissue Engineering Part A*, **15**(2), pp.243-254, DOI: 10.1089/ten.tea.2008.0067.
- [13] G. Abatangelo, V. Vindigni, G. Avruscio, et al. (2020), "Hyaluronic acid: Redefining its role", *Cells*, **9**(7), DOI: 10.3390/cells9071743.
- [14] H. Tan, C.R. Chu, K.A. Payne, et al. (2009), "Injectable *in situ* forming biodegradable chitosanhyaluronic acid based hydrogels for cartilage tissue engineering", *Biomaterials*, **30**(13), pp.2499-2506, DOI: 10.1016/j.biomaterials.2008.12.080.
- [15] L. Li, N. Wang, X. Jin, et al. (2014), "Biodegradable and injectable *in situ* cross-linking chitosan-hyaluronic acid based hydrogels for postoperative adhesion prevention", *Biomaterials*, **35**(12), pp.3903-3917, DOI: 10.1016/j.biomaterials.2014.01.050.
- [16] N.T.P. Nguyen, L.V.H. Nguyen, N.M.P. Tran, et al. (2019), "The effect of oxidation degree and volume ratio of components on properties and applications of *in situ* cross-linking hydrogels based on chitosan and hyaluronic acid", *Materials Science and Engineering C*, **103**, DOI: 10.1016/j.msec.2019.04.049.
- [17] K.Y. Lee, D.J. Mooney (2012), "Alginate: Properties and biomedical applications", *Progress in Polymer Science*, **37**(1), pp.106-126, DOI: 10.1016/j.progpolymsci.2011.06.003.
- [18] L. Xing, J. Sun, H. Tan, et al. (2019), "Covalently polysaccharide-based alginate/chitosan hydrogel embedded alginate microspheres for BSA encapsulation and soft tissue engineering", *International Journal of Biological Macromolecules*, **127**, pp.340-348, DOI: 10.1016/j.ijbiomac.2019.01.065.
- [19] X. Li, S. Chen, B. Zhang, et al. (2012), "*In situ* injectable nano-composite hydrogel composed of curcumin, N, O-carboxymethyl chitosan and oxidized alginate for wound healing application", *International Journal of Pharmaceutics*, **437**(1-2), pp.110-119, DOI: 10.1016/j.ijpharm.2012.08.001.
- [20] A.N.M. Le, T.T. Nguyen, K.L. Ly, et al. (2020), "Modulating biodegradation and biocompatibility of *in situ* crosslinked hydrogel by the integration of alginate into N, O-carboxymethyl chitosan-aldehyde hyaluronic acid network", *Polymer Degradation and Stability*, **180**, DOI: 10.1016/j.polymdegradstab.2020.109270.
- [21] X.G. Chen, H.J. Park (2003), "Chemical characteristics of O-carboxymethyl chitosans related to the preparation conditions", *Carbohydrate Polymers*, **53**(4), pp.355-359, DOI: 10.1016/S0144-8617(03)00051-1.
- [22] A.R. Karimi, A. Khodadadi, M. Hadizadeh (2016), "A nanoporous photosensitizing hydrogel based on chitosan cross-linked by zinc phthalocyanine: An injectable and pH-stimuli responsive system for effective cancer therapy", *RSC Advances*, **6**(94), pp.91445-91452, DOI: 10.1039/C6RA17064A.
- [23] S. Sharma, P. Jain, S. Tiwari (2020), "Dynamic imine bond based chitosan smart hydrogel with magnified mechanical strength for controlled drug delivery", *International Journal of Biological Macromolecules*, **160**, pp.489-495, DOI: 10.1016/j.ijbiomac.2020.05.221.
- [24] V. Mourya, N.N. Inamdara, A. Tiwari (2010), "Carboxymethyl chitosan and its applications", *Advanced Materials Letters*, **1**(1), pp.11-33, DOI: 10.5185/amlett.2010.3108.
- [25] N.T.P. Nguyen, L.V.H. Nguyen, N.M.P. Tran, et al. (2017), "Synthesis of cross-linking chitosan-hyaluronic acid based hydrogels for tissue engineering applications", *International Conference on the Development of Biomedical Engineering in Vietnam*, **63**, pp.671-675, DOI: 10.1007/978-981-10-4361-1_115.
- [26] E.M. Dahmane, M. Taourirt, N. Eladlani, et al. (2014), "Extraction and characterization of chitin and chitosan from *Parapenaeus longirostris* from Moroccan local sources", *International Journal of Polymer Analysis and Characterization*, **19**(4), pp.342-351, DOI: 10.1080/1023666X.2014.902577.
- [27] L. Pescosolido, S. Miatto, C.D. Meo, et al. (2010), "Injectable and *in situ* gelling hydrogels for modified protein release", *European Biophysics Journal*, **39**(6), pp.903-909, DOI: 10.1007/s00249-009-0440-2.
- [28] K.A. Kristiansen, A. Potthast, B.E. Christensen (2010), "Periodate oxidation of polysaccharides for modification of chemical and physical properties", *Carbohydrate Research*, **345**(10), pp.1264-1271, DOI: 10.1016/j.carres.2010.02.011.
- [29] S. Celik, F. Ozkok, A.E. Ozel, et al. (2021), "Synthesis, FT-IR and NMR characterization, antibacterial and antioxidant activities, and DNA docking analysis of a new vanillin-derived imine compound", *Journal of Molecular Structure*, **1236**, DOI: 10.1016/j.molstruc.2021.130288.
- [30] G. Das, D.B. Shinde, S. Kandambeth, et al. (2014), "Mechanosynthesis of imine, β -ketoenamine, and hydrogen-bonded imine-linked covalent organic frameworks using liquid-assisted grinding", *Chemical Communications*, **50**(84), pp.12615-12618, DOI: 10.1039/C4CC03389B.

Current nanocarriers in therapeutic improvement of Andrographolide

Van Hong Nguyen*, An Hong Nguyen Pham

Pharmaceutical Engineering Lab, School of Biomedical Engineering, International University, Vietnam National University - Ho Chi Minh City, Quarter 6, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam

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Abstract:

Currently, herbs have been investigated for novel bioactivities and improvement of traditional therapeutic effectiveness. Among them, *Andrographis paniculata*, with the active component andrographolide, is well recognized as an anti-inflammatory, anti-bacterial, anti-cancer, anti-hypertensive, anti-hyperglycaemic, hepatoprotective, and antihyperlipidemic compound. However, andrographolide has poor physicochemical properties, suffers first - pass metabolism that limits its medical application. In this review article, potential nanocarriers for andrographolide delivery including nanovesicles, inorganic nanoparticles, lipid-based nanoparticles, polymeric nanoparticles, nanocrystals, nanoemulsions, and microemulsions are presented. Formula ingredients, preparation methods and bioactivity effect investigation for each andrographolide nano type are clarified. Moreover, the improvement of the poor bioavailability characteristics, as well as the boosting the therapeutic effectiveness of andrographolide in clinical trials by nanotechnology are described. Besides, the broadening in administration routes of andrographolide nano drug delivery systems, including oral, injection, pulmonary, or dermal are taken into account.

Keywords: *A. paniculata*, Andrographolide, herbal, nanocarriers, therapeutic effect.

Classification number: 3.6

1. Introduction

In the discovery of novel drugs, natural compounds have impressive properties and characteristics such as better therapeutic effects as well as low toxicity compared to synthesized agents. Andrographolide (AG) is the representative bioactive chemical of the plant *Andrographis paniculata* that is currently being explored in the pharmaceutical industry. This compound proposes significant beneficial advantages such as antibacterial [1], anti-inflammation, cancer prevention, and neuroprotective properties (see Fig. 1) [2]. Antimicrobial activity is the most prominent effect, which has been applied to the treatment of infectious diseases since ancient times, for example, for gastrointestinal inflammation-related diseases and respiratory infection [3]. Also, AG can be considered as an antiviral agent [4], especially express inhibition of SARS-CoV-2 in Calu-3, which are human lung epithelial cells [5]. Promising treatments of AG could be confirmed by numerous current clinical trials on lung infection, arthritis, rheumatoid, gastrointestinal cancer, migraine, cognitive impairment, COVID-19 virus, and so on.

AG is a white crystalline powder with an extremely bitter flavour. At neutral to basic pH, Andrographolide is unstable and hydrolyses to an inert product. The high hydrophobicity ($\log P=2.632\pm0.135$), poor water solubility (3.29 ± 0.73 mg/ml), and fast transportation by P-glycoprotein out of the cells are all factors that contribute to the poor bioavailability of AG. Therefore, it is important to study methodologies to improve therapeutic treatment with AG. Many pharmaceutical techniques such as solid dispersion [6], inclusion complex [7], and nanotechnology [8] have been investigated to improve the solubility and permeability of AG.

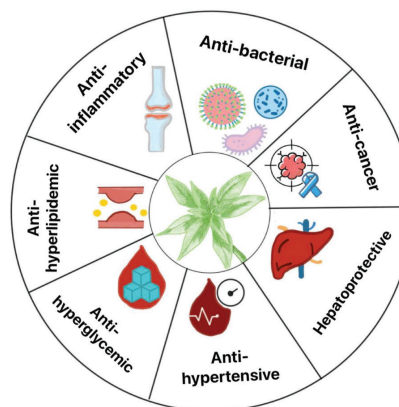


Fig. 1. Therapeutic effects of Andrographolide.

Nanotechnology is considered the most promising strategy for drug delivery systems, especially for natural compounds and specifically for AG [9]. The incorporation of natural herbs in nanoplatforms is necessary for the enhancement of biocompatibility, the prolongation of drug circulation in living bodies, and the ease of permeability through the cell membrane for effective treatments. In particular, AG nano formulations show better results in bioavailability, targeting effect, and safety [10]. Hence, modern drug delivery systems loading AG through various uptake routes, which could improve solubility, stability, absorption, and overcome resistance mechanisms in tumour cells are summarized.

*Corresponding author: Email: nhvan@hcmu.edu.vn

2. Nano drug delivery systems loading Andrographolide

Nanostructures including inorganic nanoparticles (NPs), polymeric NPs, lipid-based NPs, micelles, and nanovesicles are employed for delivery of AG to boost the therapeutic effects on various types of diseases.

2.1. Inorganic NPs

Inorganic NPs are non-toxic, biocompatible, as well as hydrophilic and highly stable in comparison with organic ones [11]. Besides, inorganic NPs have received a lot of attention as drug or gene delivery vehicles because of their high cellular absorption ability, low immunogenic response, and low cytotoxicity. Inorganic NPs include calcium phosphate, silica, iron oxide, magnesium phosphate, gold, and other metals. The electromagnetic, optical, and catalytic properties of metal NPs such as gold and silver NPs are known to be influenced by their shape and size. The targetability and controlled release of these NPs were obtained through physicochemical modification [8] mainly by conjugating with organic components, making them exceedingly biocompatible. The combination of inorganic NP design with multidisciplinary approaches enables future applications of AG in drug delivery and tissue engineering [11].

Mesoporous silica NPs (MSNs) have a highly organized mesoporous structure that may give an exceptionally high specific surface area and aperture volume, which are suited for drug loading among different inorganic NPs, especially AG. Therefore, AG powder was loaded onto MSNs, obtained size around 100 nm, with a significantly high loading capacity (LC) of $22.38 \pm 0.71\%$. Furthermore, *in vitro* evaluation proved that AG MSNs could decrease post-inflammatory factors by about 40%. Moreover, AG-loaded-MSNs were designed as a pH-responsive system, which enable these NPs to break down in an acidic osteoarthritis environment. With a phospholipid coating layer, MSNs as nanocarriers demonstrated good lubricating capability in the treatment of osteoarthritis [8] (Table 1).

2.2. Polymeric NPs

Polymeric NPs are one of the most popular types of nanocarriers, which contain natural polymers like chitosan, alginate, gelatine, and synthesis polymers such as polylactide (PLA) [21] and copolymers like polylactide-co-glycolide (PLGA) [22]. Synthetic polymers with high batch-to-batch repeatability and purity make it easier to change the pattern of drug release from polymeric NPs [23].

In one study, a cationic-modified PLGA was used to fabricate biopolymeric NPs loading *A. paniculata* extraction in 95% ethanol. Briefly, the preparation of the NPs is conducted with PLGA, Pluronic F-127, and chloroform, then collected by ultracentrifugation. AG NPs had an encapsulation efficiency (EE) value of $43.39 \pm 0.33\%$ in a 200-nm size range. The AG polymeric NPs had an important impact on the induction of CCl_4 activity by the increase of antioxidant enzymes in the liver at more than 50% effectiveness compared to the blank AG ones [15]. Besides,

Table 1. Andrographolide nanoparticle excipients.

Nano-types	Excipients	Size (nm)	PDI	Zeta potential (mV)	EE (%)	LC (%)	Reference
Vesicles	Liposomes: Soybean lecithin Cholesterol Mannitol	77.91 ± 22.91	0.22 ± 0.04	-56.13 ± 3.33	94.77 ± 1.77	6.70 ± 0.69	[12]
	Niosomes: Span 60 Cholesterol Caprylocaproyl macrogol-8 glycerides	$125-226$	<0.1	-34.02 ± 1.40	97.75 ± 1.28	N/A	[13, 14]
Inorganic NPs	Cetyltrimethyl ammonium bromide (CTAB) 2-ethoxyethanol Aqueous ammonia Tetraethyl orthosilicate (TEOS)	100	<0.1	-20.93 ± 3.40	43.39 ± 0.33	22.38 ± 0.71	[8]
Polymeric NPs	PLGA Chitosan Pluronic F-127	229.7 ± 17.17	0.234 ± 0.02	34.4 ± 1.87	43.39 ± 0.33	N/A	[15]
SLNs	Egg lecithin Tween-80 Anhydrous alcohol Glycerol-monostearate Compritol ATO888 Mannitol	286.1 ± 8.03	0.34 ± 0.03	-20.5 ± 0.3	91.00 ± 0.09	3.49 ± 0.03	[16]
Nano-emulsion	Cocunut oil Sesame oil Jojoba oil Polysorbate 80 Sorbitan oleate 80 Propylene glycol	225.2 ± 3.2	0.59 ± 0.02	-8.52 ± 2.14	93.76 ± 0.03	0.27 ± 0.00	[17, 18]
Micro-emulsion	Tween 80 Isopropyl myristate Glycerol Sorbitol Arachis oil Soybean oil	$15.9-18.6$	0.173	22.90 ± 31.01	N/A	N/A	[19]
Micelles	NH_2 -PEG- NH_2 polymer PLGA-COOH Dicyclohexyl-carbodiimide N-hydroxysuccinimide	80	<0.18	-3.5	92.1 ± 0.98	8.4 ± 0.04	[20]

chitosan-modified AG NPs on hepatic antioxidant enzymes were also fabricated to enhance the bioactivities of AG [24]. Poorly soluble chemicals may have significant adverse effects when systemically given over long periods of time. Incorporation of medicinal compounds in the hydrophobic cavity of NPs produces the desired outcomes *in vitro* and *in vivo*. Furthermore, the procedures for synthesizing polymers, which can have a variety of side effects and toxicity when using chemical methods, should be considered [25].

2.3. Micelles

Polymeric micelles, which are generated by the self-assembly of amphiphilic blocks or grafted copolymers, have the following advantages such as suitability for delivery of both hydrophilic and hydrophobic bioactive chemicals, biodegradability, biocompatibility, and an extended lifecycle *in vivo* through a “stealth effect”, which allows them to be more precise in active drug targeting [26].

In recent years, numerous types of copolymers have been developed. Because of their biocompatibility and biodegradability, as well as their ability to control drug release, amphiphilic copolymers of PEG and PLGA are mainly used for polymeric micelle formation. In *in vitro* cytotoxicity experiments on MAD-MB-231 cells, even at high concentrations, blank PLGA-PEG-PLGA micelles exhibited no cytotoxicity. Besides, AG polymeric micelles by modified PLGA-PEG-PLGA copolymers had sufficient size, less than 200 nm with no aggregation or adhesion, as well as EE and loading capacity (LC) values of $92.1 \pm 0.98\%$, $8.4 \pm 0.04\%$, respectively. There was no significant change in

size or zeta-potential after 15-day storage of AG micelles. When compared to free AG formulations, the proportion of apoptotic cells produced by AG micelles was substantially greater. Based on all of these results, such as the cytotoxicity, cell cycle block, and cell apoptosis assays, it was found that AG-loaded micelles had a stronger effect on MAD-MB-231 cells [20] (Fig. 2).

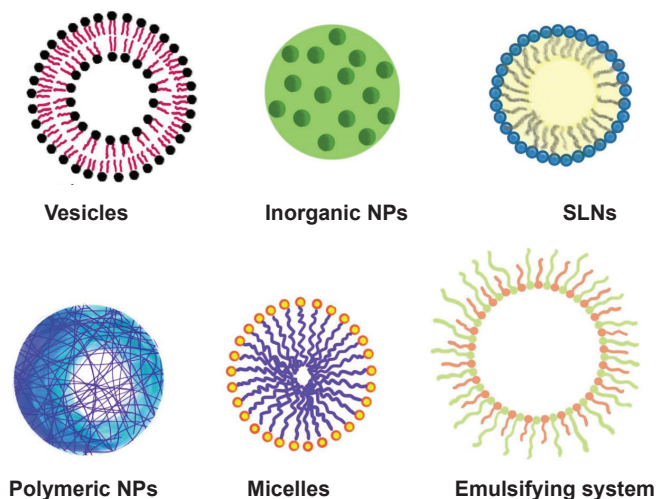


Fig. 2. Classification of Andrographolide NPs.

3. Nanovesicles

3.1. Liposomes

Liposomes are phospholipid bilayer nanovesicles specifically designed for advanced permeability through cell membranes [27]. Additionally, other benefits of liposomes are extreme biocompatibility, non-toxicity, and practicality to the human body, especially for injection route. However, liposomes have low stability in liquid form, and are prone to drug leakage due to the lipophilic layer being easily absorbed through membrane, gut wall of the GI tract, or the hydrophilic compound being dissolved in the blood flow [28].

Liposomes encapsulating AG were developed as multilayer-multilamellar structures with phosphatidylethanolamine, cholesterol, and diacetyl phosphate [29]. In one study, AG powder was loaded into a liposomal system of small size (approximately 78 nm) and produced a high encapsulation efficiency value of 94%. AG liposomes were prepared with the injection method and then freeze-dried to obtain liposomal AG dry powder inhalers (LADPIs). AG liposomes were small and stable with a mean size of 77.91 nm and zeta potential of -56.13 mV, high encapsulation efficiency (EE) of $94.77 \pm 1.77\%$ and drug loading (DL) of $6.70 \pm 0.69\%$. Liposomes were well recovered after re-hydration of LADPIs and were suitable for pulmonary delivery [12]. In another study, the characterization determined that the EE and LC of AG in liposomes were $79.03 \pm 6.23\%$ and $2.91 \pm 0.09\%$, respectively. The EE value of these AG liposomes were not as high as the previous liposomes for lungs as there was other lipids in the formulation that would affect the encapsulation [30].

3.2. Niosomes

Niosomes are bilayer vesicles made of non-ionic surfactants with amphiphilic substances that increase drug loading. As opposed to liposomes, niosomes are extremely stable, require no refrigeration, and have a moderate production cost. Niosomes enable loading of both hydrophilic and hydrophobic agents, which enhance the solubility and mobility of weakly water soluble pharmaceutical agents through phospholipid layers. Moreover, niosomes can modify membrane permeability, osmosis absorption, and biocompatibility of bioactives resulting in better therapeutic effectiveness [31].

AG-loaded niosomes were fabricated for wound healing applications. Previously, AG was extracted using water and ethanol as solvents. Then, that extraction was loaded into a niosome formulation first, and then mixed into a Carbopol gel at a suitable concentration. Among various types of NPs, AG niosomes seem to have the highest EE value at $97.75 \pm 1.28\%$ with water extract and $97.21 \pm 1.89\%$ with 70% ethanol extract. Moreover, AG niosomes also had a good size range of 125-226 nm. AG niosomal gel enhanced the viability of human skin fibroblast cells, and recovered 100% wound healing closure on rats [13].

4. Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) are being developed into colloidal carriers, which are a composition of lipid ingredients and water-soluble surfactants. SLNs shield active ingredients from oxidation by encapsulating them and delivering a controlled release of a drug over time. Moreover, SLN preparation methods are simple and avoid the need for harmful solvents. Additionally, the adherence of SLNs to the gut wall and the absorption enhancing impact of lipids on pharmacological activities were mostly responsible for the absorption enhancing effect of orally administered SLNs [32].

AG-SLNs were prepared by high pressure homogenization using Compritol 888 ATO and glyceryl monostearate (GMS) as lipid carriers and lecithin and Tween-80 as surfactants. The resulting SLN morphology was spherical or oval, and no drug crystals occurred. AG-SLNs had an EE value of $91.00 \pm 0.09\%$ and DL value of $3.49 \pm 0.03\%$. Transformation of AG-SLNs into solid form by lyophilization with mannitol without changes in size range or morphology. AG-SLNs enhanced AG bioavailability, about 2.41 times greater than the AG suspension, as well as antihyperlipidemic efficacy by enhancing AG solubility, stability in the gut, and altering AG transport mode in Caco-2 cells. Lipid-lowering effects of AG were obviously boosted by SLN, suggesting that AG-SLNs might improve AG oral absorption and therefore improve the lipid-lowering impact [16].

In another study, AG-SLNs were fabricated for the transportation of drugs through the blood brain barrier by emulsion [33]. Briefly, Compritol 888 ATO and Brij 78 were dissolved in solvents with various ratios of AG (2.5, 5 and 10%),

which was then added dropwise to PBS at pH 7.4 under magnetic stirring. The highest EE and LC percentages of AG-SLNs at this level were $92.4 \pm 1.77\%$ and $8.4 \pm 0.16\%$, respectively. AG-SLNs in drying powder or suspension form are stable for 30 days at 4°C and 25°C , respectively. The results of *in vivo* experiments demonstrated that AG-SLNs could cross the blood-brain barrier and reach brain tissues, hence boosting AG penetration. From the great characteristics of this development, SLNs are potential systems to improve the treatment of neurodegenerative illnesses of AG.

4.1. Micro-emulsions

Microemulsions appear as transparent solutions with particle sizes less than 100 nm and are physically stable under thermodynamic conditions by using a high concentration of surfactants. In one study, the formulation of AG microemulsions contained an oil phase with isopropyl myristate, arachis oil, and soybean oil. The co-surfactants were absolute alcohol, glycerol, and sorbitol, and the aqueous phase was bi-distilled water. Tween 80 was chosen as the primary surfactant due to its low cytotoxicity and stability across pH and ionic strength ranges. In this design, with the amount (w/w) of surfactant Tween 80 at 25%, the oil phase at 2.5%, and alcohol at 50%, the optimized oil-in-water microemulsion had a size of nearly 16 nm. Furthermore, the AG microemulsion has low oral toxicity, as demonstrated by the rat study [19]. In another study, AG in ethanolic extract was loaded in a microemulsion prepared by cholesterol and isopropyl myristate as the oil phase, bile salts, lecithin as a surfactant, and RH-40 and ethanol as co-surfactants. The resulting microemulsion had a size above 19 nm, and an improved bioavailability of AG over 6 times greater than pure ethanolic extract on rabbits [34].

4.2. Nanoemulsions

Nanoemulsions are submicron-sized colloidal particle systems with diameter ranges between 10 and 1,000 nm. Oil-in-water AG-loaded nanoemulsions (AG-NE) were made to improve AG effectiveness for skin treatment. In the study, AG-NE had a reasonable EE of $93.76 \pm 0.03\%$. However, the LC was only $0.27 \pm 0.00\%$. A combination of Tween 80 and Span 80 was used as an emulsifier with a ratio of 5:1 at 10% w/w. AG and AG-NE were tested for cytotoxicity in two types of cells, which are non-melanoma skin cancer cells (A-431 cells) and normal skin fibroblast cells (HFF-1 cells) at concentrations of 30-100 $\mu\text{g/ml}$. Both the NE base and AG-NE had nanometre-sized droplets, were able to resist the effect of centrifugal force. With the formulation of the nanoemulsion, the bioavailability of AG for oral route was enhanced by about 8 times more than AG suspension [18]. For the further investigation of leishmaniasis treatment, and especially for the intraperitoneal injection, nano systems of emulsions for AG were prepared using a solvent evaporation technique to evaluate the different ratios between the infection proportions of treated and untreated macrophages. Moreover, they used vitamin E as the stabilizer for the protection of the drugs and enhancement of the

permeability through cell membranes [35]. Over the course of 3 months, the size of this AG-NE did not significantly change, and the quantity of loaded AG remained the same [36] (Table 2).

Table 2. Route administration of Andrographolide nanocarriers.

Route	AG bioactivities	Bioactivity	Reference
Pulmonary delivery	Liposomal AG dry powder to cure of <i>S. aureus</i> -induced pneumonia.	Antibacterial	[12]
	In diabetic rats without insulin, increasing glucose consumption lowers plasma glucose.		[37]
Oral treatment	Reduces plasma glucose levels and increases β -endorphin as the immunoreactivity.		[38]
	Lower blood glucose and preserve pancreatic beta cells at the same time.	Anti-hyperglycaemic	[39]
	Decrease the levels of serum creatinine, serum urea nitrogen, urinary albumin excretion, serum urea, and blood glucose in induced diabetic rats.		[40]
Gavage-force feeding route	The extraction of AG protects against 5 myocardial damages in high fat diet-induced obese mice models.	Anti-hypertensive	[41]
Intragastric administration	The bioavailability and antihyperlipidemic activity were improved by AG-SLNs by increasing the solubility and stability of AG in the intestine and by changing its transport mode in Caco-2 cell.	Anti-hyperlipidaemic	[16]
	AG exhibited a significant inhibition on the mouse model by lowering macrophage inflammatory protein (MIP-2) mRNA.	Anti-inflammation	[42]
Intraperitoneally	Against oxidative stress-induced liver damage.	Hepatoprotective	[15]
	Inhibits NF- κ B and MAPK-mediated inflammatory responses.	Anti-inflammation	[43]
	Inhibits gastric cancer cell proliferation and metastasis of the gastric cell line SGC7901.	Anticancer	[44]
-	Enhances the expression pattern of proapoptotic proteins like caspase-3 and caspase-9, which suggest the involvement of the mitochondrial apoptotic pathway in the chemo preventive effect.	Anticancer	[45]
	Induced cell cycle arrest and apoptotic death in MDA-MB-231 breast cancer cells without affecting growth of normal cell.	Anticancer	[46]

5. Therapeutic effects of Andrographolide

5.1. Anti-bacterial

The antibacterial bioactivity of AG is related to reduction of pathogens and regulation of the immune system to inhibit inflammatory actions. AG is considered as a potential inhibitor of NF- κ B, which is one of the specific antibacterial responses [47]. Early attempts to treat arthritis and leishmaniasis with AG and synthesized prodrugs failed due to solubility and distribution issues. In previous studies, the AG- β -cyclodextrin inclusion complex performed effective treatment against *S. aureus* pneumonia [48]. Since the discovery of its antiviral and antibacterial properties, AG was delivered by nanocarrier in the treatment of parasitic diseases [49]. In the *in vivo* test of hamster models, AG liposomes decorated with macrophage mannosyl-fucosyl receptors showed potential treatment of the leishmaniasis infection as well as better safety on liver and kidney in hamster models [29]. In another study, AG liposomes reduced the bacterial activity, lessened lung injury, and inhibited the inflammatory reactions caused by bacterial pneumonia. Noticeably, AG liposomal in inhaler powder form expressed higher anti-*S. aureus* lung effects *in vivo* than a ten-fold

dose of AG, as well as penicillin. Hence, AG has the ability to regulate the immune response in order to maintain the antibacterial effect while inhibiting the inflammatory response [12].

5.2. Anti-inflammatory

AG may affect inflammatory responses related glycolysis, which can suppress inflammation and cancer growth [50]. Hypoxia, anaerobic glycolysis, and lactification occur due to the increase of cell metabolic activity in the inflammatory state. A pH-responsive, mesoporous, silica-loaded AG, coated with phospholipid to enhance lubricant ability for local treatment of osteoarthritis has been proposed. The improvement of AG effectiveness on osteoarthritis by this nanocarrier was confirmed by IL-1-induced tissue in a rat model [8]. AG was also loaded into niosomes, followed by incorporating gel to enhance the treatment of the wound. Macroscopic assessments were conducted in the initial wound areas to compare the AG-loaded niosomal gel and blank gel. The AG niosomal gel showed less inflammation and less scarring within 3 days. From day 7 to day 10 of the experiment, the rat wound had no irritation or red spots on the skin and its hair rapidly regrew under the treatment of AG niosomal gel. The anti-inflammatory and anti-oxidant properties of AG could eliminate oxidative stress molecules, boost wound closure, leading to improve the vascular channel generation and the epithelial cell proliferation [51]. The anti-inflammatory impact of AG was significantly increased by microemulsions, which were shown to have two-fold greater bioavailability than AG tablets [19].

5.3. Anti-hyperglycemic

AG has been proven for the treatment of high glucose in blood by reducing levels of glucose and albumin while preserving pancreatic beta cells and increasing beta-endorphins as immunoreactivity [40]. AG was placed in a nano-emulsion and nano-suspension with Tween 20, PEG 400, and Capryol 90 in a specific ratio [10]. The results indicated a significant decrease in blood glucose levels in rat models within 8 days when treated with AG nano-emulsion and nano-suspension [52]. From the *in vitro* tests of pancreatic beta cells, these AG nano-emulsions and suspensions showed the capability of insulin secretion by immunoreactive analysis as well as high biocompatibility and bioavailability [53].

5.4. Anti-hypertensive

A. paniculata extraction protected mice from myocardial damage in high fat diet mice models, which indicated AG's effects of reducing blood pressure and treatment of cardiac hypertrophy [54]. Besides, AG could decrease air proinflammatory cell adhesion, lung irritation, lung damage, as well as lower the levels of protein and mRNA in the cytokine serum and bronchoalveolar fluid [43]. Additionally, AG was promoted for the enhancement of therapeutic effects in the treatment of pulmonary hypertension by oral route in mouse models based on the results of decreasing oxidative stress and the pathways of inflammatory cells [55].

5.5. Anti-hyperlipidemic

AG and *A. paniculata* are well known for their antidiabetic and antihyperlipidemic effects [56]. In comparison with AG suspensions, the SLNs show better bioavailability by increasing the solubility and stability of AG in the intestines [16], which strengthen proof of antihyperlipidemic effects of AG [30]. Specifically, SLNs sustained the release of AG for up to 24 h compared to 8 h for AG solution *in vitro*, and bioavailability increased by about 2.5 times *in vivo*. Furthermore, the key mechanisms for increased AG-SLNs bioavailability were clarified including an increase in solubility, a reduction in intestinal tract metabolism (particularly in the duodenum), improved colon absorption, and a change in transport mode. AG lipid-lowering action is boosted by AG-SLNs, suggesting that they are a potential cargo for AG via oral uptake [16].

5.6. Hepatoprotective

Antiviral medicines, steroids, and vaccinations are especially harmful to the liver when used in long-term treatments like antitubercular, antiretroviral, or anticancer chemotherapy. AG pre-treatment considerably protected the liver from the reduction of the quantity and quality of bile caused by paracetamol. In conscious rats and anesthetized guinea pigs, the increases in bile flow, bile salt, and bile acids reveal that AG had a significant choleretic effect that is dependent on the dosage that increases from 4.8% to 73% [57]. The studies of hepatoprotective effects of AG in rats were demonstrated by the acute hepatitis induced in them [58]. Liver-protective effect of *A. paniculata* was improved by polymeric micelles, which were formed by modified poly(lactic-co-glycolic) NPs in CCl₄ acute liver injury models [15]. Chitosan-modified AGNPs increased AG activity on hepatic antioxidant enzymes [24]. Furthermore, chitosan AGNPs were shown to protect the liver against mitochondrial liver damage. Many other factors have been linked to increased hepatoprotection including the overexpression of various antioxidative enzymes and the replenishment of glutathione in the liver. Cytokine modulation by chitosan AGNPs improved CCl₄-damaged liver architecture recovery. As a result, newly designed AG polymeric NPs broadened the treatment of oxidative stress and hepatocellular inflammation. To allow entry into clinical trials, more pharmacokinetic studies of similarly designed nanosystems are needed, and one is currently underway [15].

5.7. Anti-cancer

In cancer cells, AG produces cell cycle apoptosis without affecting other healthy cell growth [59]. PLGA-PEG-PLGA micelles increased cellular uptake of AG by more than twofold, resulting in significantly higher toxicity on breast cancer MAD-MB-231 cells *in vitro* and nearly threefold AUC in rats [20]. AG liposomes were prepared to treat breast cancer cells and subjected to wound healing and trans-well experiments. In wound healing experiments, liposome-loaded AG showed a significantly lower migratory capacity of 4T1 cells than the other groups. In the trans-well experiment, for the loaded liposome group, a comparable reduction of breast cancer cell invasion was consistently seen [30].

6. Future perspective

AG poses a significantly large number of good advantages in disease treatments, especially proved by human trials. However, low solubility and stability may be a barrier to pharmaceutical product design along with the hard, bitter flavour that is an additional complication in AG formulation optimization. Therefore, there are demands for scientists to overcome those issues of AG, which is the prospective active agent for infectious illnesses, specifically for the COVID-19 pandemic. Besides, from Table 3, the exact amount of pure AG, and not only the *A. paniculata* extract, needs to be published in papers, the product label, and product description for a better understanding and the correct use of AG by consumers. Nanotechnology has been shedding light on the widening and realistic application of AG. Promising products containing AG will go to the market earlier with a tremendous increase in calling clinical trials for various treatments such as neuroprotective, hepatoprotective, migraine, antibacterial-related diseases, anti-inflammation related diseases, COVID-19 and so on.

Table 3. Supplemental products containing Andrographolide.

Function	Product	Dosage form	Dose
Immune support	Bixa Botanical	Capsule	450 mg <i>A. paniculata</i> extract
	Maple Life Science	Capsule	400 mg <i>A. paniculata</i> extract
	Medi Herb: Andrographis complex	Tablet	100 mg <i>A. paniculata</i> extract
Liver functions support	Tefroliv Forte Tablet	Tablet	60 mg <i>A. paniculata</i> extract
	Stimuliv Syrup	Syrup	60 mg <i>A. paniculata</i> extract
Treatment of respiratory tract, skin, and intestinal infections	Regenerative Nutrition	Capsule	400 mg <i>A. paniculata</i> extract
Cough, bronchitis treatment	Traphaco: Xuyen Tam Lien	Capsule	160 mg <i>A. paniculata</i> extract
Prevent bronchitis infection	OPG: Xuyen Tam Lien nasal and throat spray	Spray	<i>A. paniculata</i> extract

7. Conclusions

AG possesses various therapeutic activities like liver protection, analgesic properties, anti-inflammation, as well as antihyperglycemic and anti-tumour properties. However, the harsh taste, low water solubility, considerable liver first-pass degradation, and low oral bioavailability has urges the development of AG formulations. Advancements in nano drug delivery systems have already strengthened and broadened the usage of AG through various administrations such as pulmonary delivery, oral route, skin application, and so on. Numerous nanocarriers have been prepared including inorganic NPs, polymeric NPs, micelles, nanovesicles, SLNs, and nano- and micro- emulsions, which show effectiveness in the improvement of AG's physicochemical properties as well as bioavailability *in vitro* and *in vivo*. However, remaining challenges include maintaining structural integrity, enhancing biofluid stability, as

well as improving biocompatibility and manufacturing ease and safety while reducing adverse effects and increasing efficacy.

CRedit author statements

Van Hong Nguyen, An Hong Nguyen Pham: Conceptualisation, Methodology, Data curation, Formal analysis, Writing - Review & Editing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] L. Zhang, M. Bao, B. Liu, et al. (2020), "Effect of Andrographolide and its analogs on bacterial infection: A review", *Pharmacology*, **105**(3-4), pp.123-134, DOI: 10.1159/000503410.
- [2] R. Hossain, C. Quispe, J.H. Bravo, et al. (2022), "Neurobiological promises of the bitter diterpene lactone Andrographolide", *Oxidative Medicine and Cellular Longevity*, DOI: 10.1155/2022/3079577.
- [3] D.W. Wang, Y.J. Xiang, Z.L. Wei, et al. (2021), "Andrographolide and its derivatives are effective compounds for gastrointestinal protection: A review", *European Review for Medical and Pharmacological Sciences*, **25**(5), pp.2367-2382, DOI: 10.26355/eurrev_202103_25276.
- [4] R. Latif, C.Y. Wang (2020), "Andrographolide as a potent and promising antiviral agent", *Chinese Journal of Natural Medicines*, **18**(10), pp.760-769, DOI: 10.1016/S1875-5364(20)60016-4.
- [5] S. Jeon, M. Ko, J. Lee, et al. (2020), "Identification of antiviral drug candidates against SARS-CoV-2 from FDA-approved drugs", *Antimicrobial Agents and Chemotherapy*, **64**(7), pp.e00819-e00820, DOI: 10.1128/AAC.00819-20.
- [6] C.C. Yen, Y.K. Liang, C.-P. Cheng, et al. (2020), "Oral bioavailability enhancement and anti-fatigue assessment of the Andrographolide loaded solid dispersion", *International Journal of Molecular Sciences*, **21**(7), DOI: 10.3390/ijms21072506.
- [7] H. Gao, Y. Su, W. Wang, et al. (2021), "Integrated computer-aided formulation design: A case study of Andrographolide/cyclodextrin ternary formulation", *Asian Journal of Pharmaceutical Sciences*, **16**(4), pp.494-507, DOI: 10.1016/j.ajps.2021.03.006.
- [8] M. He, Z. Qin, X. Liang, et al. (2021), "A pH-responsive mesoporous silica nanoparticles-based drug delivery system with controlled release of Andrographolide for OA treatment", *Regenerative Biomaterials*, **8**(4), DOI: 10.1093/rb/rbab020.
- [9] V. Dhapte, V. Pokharkar (2019), "Chapter 13 - Nanosystems for drug delivery: Design, engineering, and applications", *Green Synthesis, Characterization and Applications of Nanoparticles*, Elsevier, pp.321-345, DOI: 10.1016/B978-0-08-102579-6.00013-7.
- [10] Y. Syukri, R. Martien, E. Lukitaningsih, et al. (2018), "Novel self-nano emulsifying drug delivery system (SNEDDS) of Andrographolide isolated from *Andrographis paniculata* nees: Characterization, *in-vitro* and *in-vivo* assessment", *Journal of Drug Delivery Science and Technology*, **47**, pp.514-520, DOI: 10.1016/j.jddst.2018.06.014.
- [11] W. Paul, C.P. Sharma (2020), "Inorganic nanoparticles for targeted drug delivery", *Biointegration of Medical Implant Materials*, pp.333-373, DOI: 10.1016/B978-0-08-102680-9.00013-5.
- [12] M. Li, T. Zhang, L. Zhu, et al. (2017), "Liposomal Andrographolide dry powder inhalers for treatment of bacterial pneumonia via anti-inflammatory pathway", *International Journal of Pharmaceutics*, **528**(1-2), pp.163-171, DOI: 10.1016/j.ijpharm.2017.06.005.
- [13] R. Jamaludin, N.M. Daud, R.S.R. Sulong, et al. (2021), "Andrographis paniculata-loaded niosome for wound healing application: Characterisation and *in vivo* analyses", *Journal of Drug Delivery Science and Technology*, **63**, DOI: 10.1016/j.jddst.2021.102427.

- [14] F. Nowroozi, A. Almasi, J. Javidi, et al. (2018), "Effect of surfactant type, cholesterol content and various downsizing methods on the particle size of niosomes", *Iranian Journal of Pharmaceutical Research*, **17**(Suppl. 2), pp.1-11.
- [15] P. Roy, S. Das, R.G. Auddy, et al. (2014), "Engineered Andrographolide nanosystems for smart recovery in hepatotoxic conditions", *International Journal of Nanomedicine*, **9**, pp.4723-4735, DOI: 10.2147/IJN.S65262.
- [16] T. Yang, H.H. Sheng, N.P. Feng, et al. (2013), "Preparation of Andrographolide-loaded solid lipid nanoparticles and their *in vitro* and *in vivo* evaluations: Characteristics, release, absorption, transports, pharmacokinetics, and antihyperlipidemic activity", *Journal of Pharmaceutical Sciences*, **102**(12), pp.4414-4425, DOI: 10.1002/jps.23758.
- [17] N. Sooksai, W. Treesuppharat, S. Theeramunkong, et al. (2019), "Andrographolide-loaded nanoemulsion and its activity against non-melanoma skin cancer cells", *Key Engineering Materials*, **819**, pp.139-144, DOI: 10.4028/www.scientific.net/KEM.819.139.
- [18] C.C. Yen, Y.C. Chen, M.T. Wu, et al. (2018), "Nanoemulsion as a strategy for improving the oral bioavailability and anti-inflammatory activity of Andrographolide", *International Journal of Nanomedicine*, **13**, pp.669-680, DOI: 10.2147/IJN.S154824.
- [19] H. Du, X. Yang, H. Li, et al. (2012), "Preparation and evaluation of Andrographolide-loaded microemulsion", *Journal of Microencapsulation*, **29**(7), pp.657-665, DOI: 10.3109/02652048.2012.680508.
- [20] J. Zhang, Y. Li, W. Gao, et al. (2014), "Andrographolide-loaded PLGA-PEG-PLGA micelles to improve its bioavailability and anticancer efficacy", *Expert Opinion on Drug Delivery*, **11**(9), pp.1367-1380, DOI: 10.1517/17425247.2014.924503.
- [21] O. Escalona-Rayó, P. Fuentes-Vázquez, S. Jardon-Xicotencatl, et al. (2019), "Rapamycin-loaded polysorbate 80-coated PLGA nanoparticles: Optimization of formulation variables and *in vitro* anti-glioma assessment", *Journal of Drug Delivery Science and Technology*, **52**, pp.488-499, DOI: 10.1016/j.jddst.2019.05.026.
- [22] M. Szczęch, K. Szczepanowicz (2020), "Polymeric core-shell nanoparticles prepared by spontaneous emulsification solvent evaporation and functionalized by the layer-by-layer method", *Nanomaterials*, **10**(3), DOI: 10.3390/nano10030496.
- [23] I. Khan, K. Saeed, I. Khan (2019), "Nanoparticles: Properties, applications and toxicities", *Arabian Journal of Chemistry*, **12**(7), pp.908-931, DOI: 10.1016/j.arabjc.2017.05.011.
- [24] A. Makhlof, Y. Tozuka, H. Takeuchi (2011), "Design and evaluation of novel pH-sensitive chitosan nanoparticles for oral insulin delivery", *European Journal of Pharmaceutical Sciences*, **42**(5), pp.445-451, DOI: 10.1016/j.ejps.2010.12.007.
- [25] A. Kahru, H.C. Dubourguier (2010), "From ecotoxicology to nanoeotoxicology", *Toxicology*, **269**(2-3), pp.105-119, DOI: 10.1016/j.tox.2009.08.016.
- [26] M.C.I.M. Amin, A.M. Butt, M.W. Amjad, et al. (2017), "Polymeric micelles for drug targeting and delivery", *Nanotechnology-Based Approaches for Targeting and Delivery of Drugs and Genes*, Elsevier, pp.167-202, DOI: 10.1016/B978-0-12-809717-5.00006-3.
- [27] S.K. Sahoo, V. Labhasetwar (2003), "Nanotech approaches to drug delivery and imaging", *Drug Discovery Today*, **8**(24), pp.1112-1120, DOI: 10.1016/S1359-6446(03)02903-9.
- [28] A. Gabizon, D. Goren, R. Cohen, et al. (1998), "Development of liposomal anthracyclines: From basics to clinical applications", *Journal of Controlled Release*, **53**(1-3), pp.275-279, DOI: 10.1016/S0168-3659(97)00261-7.
- [29] J. Sinha, S. Mukhopadhyay, N. Das, et al. (2000), "Targeting of liposomal Andrographolide to *L. donovani* - infected macrophages *in vivo*", *Drug Delivery*, **7**(4), pp.209-213, DOI: 10.1080/107175400455137.
- [30] X. Kang, Z. Zheng, Z. Liu, et al. (2018), "Liposomal codelivery of doxorubicin and Andrographolide inhibits breast cancer growth and metastasis", *Molecular Pharmaceutics*, **15**(4), pp.1618-1626, DOI: 10.1021/acs.molpharmaceut.7b01164.
- [31] R. Khan, R. Irchhaiya (2016), "Niosomes: A potential tool for novel drug delivery", *Journal of Pharmaceutical Investigation*, **46**(3), pp.195-204, DOI: 10.1007/s40005-016-0249-9.
- [32] E. Salah, M.M. Abouelfetouh, Y. Pan, et al. (2020), "Solid lipid nanoparticles for enhanced oral absorption: A review", *Colloids and Surfaces B: Biointerfaces*, **196**, DOI: 10.1016/j.colsurfb.2020.111305.
- [33] G. Graverini, V. Piazzi, E. Landucci, et al. (2018), "Solid lipid nanoparticles for delivery of Andrographolide across the blood-brain barrier: *in vitro* and *in vivo* evaluation", *Colloids and Surfaces B: Biointerfaces*, **161**, pp.302-313, DOI: 10.1016/j.colsurfb.2017.10.062.
- [34] L. Xiao-Yan, X. Niu, Q.J. Feng, et al. (2016), "A new biocompatible microemulsion increases extraction yield and bioavailability of *Andrographis paniculata*", *Chinese Journal of Natural Medicines*, **14**(9), pp.683-691, DOI: 10.1016/S1875-5364(16)30081-4.
- [35] S. Mondal, et al. (2013), "In vitro susceptibilities of wild and drug resistant *Leishmania donovani* amastigote stages to Andrographolide nanoparticle: Role of vitamin E derivative TPGS for nanoparticle efficacy", *PLOS One*, **8**(12), DOI: 10.1371/journal.pone.0081492.
- [36] P. Roy, S. Das, R.G. Auddy, et al. (2013), "Engineered Andrographolide nanoparticles mitigate paracetamol hepatotoxicity in mice", *Pharmaceutical Research*, **30**(5), pp.1252-1262, DOI: 10.1007/s11095-012-0964-5.
- [37] B.C. Yu, C.R. Hung, W.C. Chen, et al. (2003), "Antihyperglycemic effect of Andrographolide in streptozotocin-induced diabetic rats", *Planta Medica*, **69**(12), pp.1075-1079, DOI: 10.1055/s-2003-45185.
- [38] B.C. Yu, C.K. Chang, C.F. Su, et al. (2008), "Mediation of δ -endorphin in Andrographolide-induced plasma glucose-lowering action in type I diabetes-like animals", *Naunyn-Schmiedeberg's Archives of Pharmacology*, **377**(4), pp.529-540, DOI: 10.1007/s00210-007-0240-0.
- [39] Z. Zhang, J. Jiang, P. Yu, et al. (2009), "Hypoglycemic and beta cell protective effects of Andrographolide analogue for diabetes treatment", *Journal of Translational Medicine*, **7**(1), DOI: 10.1186/1479-5876-7-62.
- [40] J. Xu, Z. Li, M. Cao, et al. (2012), "Synergetic effect of Andrographis paniculata polysaccharide on diabetic nephropathy with Andrographolide", *International Journal of Biological Macromolecules*, **51**(5), pp.738-742, DOI: 10.1016/j.ijbiomac.2012.06.035.
- [41] Y.L. Hsieh, M.A. Shibu, C.K. Li, et al. (2016), "Andrographis paniculata extract attenuates pathological cardiac hypertrophy and apoptosis in high-fat diet fed mice", *Journal of Ethnopharmacology*, **192**, pp.170-177, DOI: 10.1016/j.jep.2016.07.018.
- [42] G.F. Dai, J. Zhao, Z.W. Jiang, et al. (2011), "Anti-inflammatory effect of novel Andrographolide derivatives through inhibition of NO and PGE2 production", *International Immunopharmacology*, **11**(12), pp.2144-2149, DOI: 10.1016/j.intimp.2011.09.010.
- [43] S. Peng, N. Hang, W. Liu et al. (2016), "Andrographolide sulfonate ameliorates lipopolysaccharide-induced acute lung injury in mice by down-regulating MAPK and NF- κ B pathways", *Acta Pharmaceutica Sinica B*, **6**(3), pp.205-211, DOI: 10.1016/j.apsb.2016.02.002.
- [44] L. Dai, G. Wang, W. Pan (2017), "Andrographolide inhibits proliferation and metastasis of SGC7901 gastric cancer cells", *Biomed Research International*, DOI: 10.1155/2017/6242103.
- [45] R.R. Malla, S. Kumari, K.G.K. Deepak, et al. (2019), "Chapter 7 - Terpenoids as potential targeted therapeutics of pancreatic cancer: Current advances and future directions", *Breaking Tolerance to Pancreatic Cancer Unresponsiveness to Chemotherapy*, Elsevier, pp.111-116, DOI: 10.1016/B978-0-12-817661-0.00007-X.
- [46] H. Sukardiman, A. Widyawaruyanti, Sismindari, et al. (2007), "Apoptosis inducing effect of Andrographolide on TF-47 human breast cancer cell line", *African Journal of Traditional, Complementary and Alternative Medicines*, **4**(3), pp.345-351, DOI: 10.4314/ajtcam.v4i3.31228.
- [47] L. Ma, X. Liu, H. Liang, et al. (2012), "Effects of 14- α -lipoyl Andrographolide on quorum sensing in *Pseudomonas aeruginosa*", *Antimicrobial Agents and Chemotherapy*, **56**(12), pp.6088-6094, DOI: 10.1128/AAC.01119-12.
- [48] T. Zhang, L. Zhu, M. Li, et al. (2017), "Inhalable Andrographolide- β -cyclodextrin inclusion complexes for treatment of *Staphylococcus aureus* pneumonia by regulating immune responses", *Molecular Pharmaceutics*, **14**(5), pp.1718-1725, DOI: 10.1021/acs.molpharmaceut.6b01162.
- [49] S. Pund, A. Joshi (2017), "Chapter 23 - Nanoarchitectures for neglected tropical protozoal diseases: Challenges and state of the art", *Nano-and Microscale Drug Delivery Systems*, Elsevier, pp.439-480, DOI: 10.1016/B978-0-323-52727-9.00023-6.
- [50] W.S.D. Tan, W. Liao, S. Zhou, et al. (2017), "Is there a future for Andrographolide to be an anti-inflammatory drug? Deciphering its major mechanisms of action", *Biochemical Pharmacology*, **139**, pp.71-81, DOI: 10.1016/j.bcp.2017.03.024.
- [51] E.B. Kurutas (2015), "The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state", *Nutrition Journal*, **15**(1), DOI: 10.1186/s12937-016-0186-5.
- [52] P. Li, A. Tan, C.A. Prestidge, et al. (2014), "Self-nanoemulsifying drug delivery systems for oral insulin delivery: *in vitro* and *in vivo* evaluations of enteric coating and drug loading", *International Journal of Pharmaceutics*, **477**(1-2), pp.390-398, DOI: 10.1016/j.ijpharm.2014.10.039.
- [53] R. Balamurugan, V. Durairamian, S. Ignacimuthu (2011), "Antidiabetic activity of γ -sitosterol isolated from *Lippia nodiflora* L. in streptozotocin induced diabetic rats", *European Journal of Pharmacology*, **667**(1-3), pp.410-418, DOI: 10.1016/j.ejphar.2011.05.025.
- [54] X. Nie, C. Shen, J. Tan, et al. (2021), "Andrographolide attenuates established pulmonary hypertension via rescue of vascular remodeling", *Biomolecules*, **11**(12), DOI: 10.3390/biom11121801.
- [55] J. Chen, A. Peters, C.L. Papke, et al. (2017), "Loss of smooth muscle α -actin leads to NF- κ B- dependent increased sensitivity to angiotensin II in smooth muscle cells and aortic enlargement", *Circulation Research*, **120**(12), pp.1903-1915, DOI: 10.1161/CIRCRESAHA.117.310563.
- [56] T. Yang, H.X. Shi, Z.T. Wang, et al. (2013), "Hypolipidemic effects of Andrographolide and neoAndrographolide in mice and rats", *Phytotherapy Research*, **27**(4), pp.618-623, DOI: 10.1002/ptr.4771.
- [57] B. Shukla, P.K. Visen, G.K. Patnaik, et al. (1992), "Choleretic effect of Andrographolide in rats and guinea pigs", *Planta Medica*, **58**(2), pp.146-149, DOI: 10.1055/s-2006-961416.
- [58] S.G. Wynn, B.J. Fougere (2007), "Veterinary herbal medicine: A systems- based approach", *Veterinary Herbal Medicine*, pp.291-409, DOI: 10.1016/B978-0-323-02998-8.50024-X.
- [59] M. Banerjee, S. Chattopadhyay, T. Choudhuri, et al. (2016), "Cytotoxicity and cell cycle arrest induced by Andrographolide lead to programmed cell death of MDA-MB-231 breast cancer cell line", *Journal of Biomedical Science*, **23**(1), DOI: 10.1186/s12929-016-0257-0.

Investigation of the effect of alpha wave-containing music on the cognitive performance and brain activity of university students

Lam Quang Nguyen¹, Hoang Minh Anh An¹, Vu Quang Thinh Tran¹, Phuc Hoang Anh Le¹,
Van Luan Tran¹, Mai Thao Le¹, Trong Kien Nguyen², Thi Thanh Huong Ha^{1*}

¹International University, Vietnam National University - Ho Chi Minh City, Quarter 6, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam

²Posts and Telecommunications Institute of Technology, 11 Nguyen Dinh Chieu Street, Da Kao Ward, District 1, Ho Chi Minh City, Vietnam

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Abstract:

Cognition is one of the most vital brain functions, which ranges from assisting students in recalling what they have been taught to assisting us in concentrating while accomplishing chores. Nowadays, many jobs require advanced cognitive abilities, particularly among high school and university students. As a result, preserving or improving our cognitive abilities has become necessary in modern society. Alpha-wave music, which targets the brain frequency range from 8 to 12 Hz, is a promising approach to boosting cognitive performance. The purpose of this study is to evaluate the immediate, short term, or long term effects of alpha wave music on the cognitive performance and brain activity of students. In an online experiment, two groups of students were recruited and randomized into control and experimental groups. The experimental group was required to listen to alpha music for two weeks (here called the intervention). Participants from both groups will have their cognitive performance assessed before, immediately after, and two weeks following the intervention. In the immediate effect experiment, another group of participants have their alpha relative power evaluated while performing different tasks. The results of the online experiment revealed that feedback from participants listening to alpha music are mostly positive, while the response time of cognitive tasks indicated an improvement trend ($p < 0.05$). The positive potential of alpha music was also confirmed with the immediate effect study where the alpha power was higher during alpha music exposure than during the remaining sessions. In conclusion, this study provided preliminary evidence to support the implementation of alpha music intervention to improve the cognitive function of students.

Keywords: alpha music, cognitive performance, electroencephalography, university students.

Classification number: 3.6

1. Introduction

Cognitive function is a set of human capacities such as concentration, memory, mathematical calculations, and verbal or non-verbal language decisions, which all imperatively contribute to the learning and working process. Undoubtedly students face complex examinations and competition pressure from peers and frequently utilize cognitive functions throughout their academic performance and daily life. Consequently, their productivity and academic score may gradually decrease without proper techniques to enhance their cognitive performance in these environments [1, 2]. To ameliorate those adverse effects, continuous training to strengthen and enhance cognitive abilities has been implemented to accelerate students' working productivity [3, 4]. Therefore, finding a feasible and effective intervention is demanded by students to improve their cognitive functions.

Alpha wave-containing music is considered a promising solution, which applies the principle of alpha brainpower within the frequency range of 8 Hz to 12 Hz. Fundamentally, the alpha wave represents states of relaxation and consciousness that manifests itself as decreased anxiety and increased creativity [5]. The alpha wave-containing music operates based on binaural beats, which comprise two distinct sound frequencies transmitting into each ear, for instance, 200 Hz in the right ear and 210 Hz in the left ear. Eventually, an alpha wave of 10 Hz is generated by the brain when the listeners are listening to the music in an enclosed environment by using earphones or headphones [1, 6]. In the relationship between cognitive performance and brain activity, alpha waves in the frontoparietal area have a strong connection with short-term memory [7], working memory [8, 9], and analytical capabilities [10]. Furthermore, some studies suggest that individuals in the

*Corresponding author: Email: htthuong@hcmu.edu.vn

experimental group could considerably raise their upper alpha power (9.5-12 Hz) when listening to alpha music whereas the control group did not [11, 12], which indicates an increase in the relaxation state of the experimental group. However, there has not been a meticulous investigation of how alpha music improves the cognitive performance of the Vietnamese population, especially university students. Moreover, previous studies have not investigated the changes in brain wave activity when listening to alpha music versus other music genres.

Therefore, this study aims to assess the effect of alpha music therapy on improving the cognitive function of Vietnamese university students in the short-term and the long-term aspects. By using cognitive tasks, we will examine the performance outcomes of the applied tasks after listening to alpha music for at least 30 minutes/day for 14 days. Moreover, subjective responses to alpha music would also be collected from the experimental group. Regarding the immediate effect experiment, the comparison of alpha relative power in alpha music listening sessions with control music can examine the instantaneous effect of alpha music on the subject. From the obtained results, a conclusion of whether alpha music could be applied broadly in academic settings can be formed.

2. Methodology

2.1. Experimental design

The experiment included two types of data collection: an online experiment that evaluates short term and long term effects using remote data collection and an immediate effect experiment using on-site data collection, which assesses the Electroencephalography (EEG) signal. The protocol used for the immediate effect subjects would not be applied in the online experiment and vice versa.

2.1.1. Online experiment: The experimental group and control group data collection were conducted on an online platform under the supervision of technicians due to the impact of the Covid-19 (Fig. 1A). Subjects were required to attend three evaluating sessions, and the protocol was similar in each session: They were asked to perform two cognitive tasks: operation span task (12 trials) and matrix span task (16 trials) (see section 3). After the first session (week 1) finished, the experimental group was asked to listen to the alpha wave containing music for two weeks (14 days) for at least 30 minutes each day before the start of the second session (week 3). After the week 3 data collection, the experimental group was required to stop listening to alpha music. The third session (week 5) was conducted to examine the sustained effect of alpha music.

2.1.2. Immediate effect experiment: Subjects participated in only one session, in which EEG acquisition was recorded using Philips Respironics Alice 6 and Sleepware G3 application. Before conducting the experiment, subjects were required to listen to alpha music for one week (seven days) for at least 30 minutes per day at home. Subsequently, they attended the eight

stages of EEG data collection: bio-calibration, resting phase (3 minutes), listening to control music, 1st operation span task, 1st 30-second rest, listening to alpha music, 2nd operation span task, 2nd 30-second rest (Fig. 1B). Regarding the “control music”, subjects were allowed to choose music based on preference, for which the song duration was limited to 5 minutes. After finishing listening to the chosen music, they would do the operation span task (12 trials) and rest for 30 seconds afterward. Sequentially, subjects listened to alpha wave-containing music for the same period (5 minutes) and repeated the same task, then ended with a 30-second resting phase.

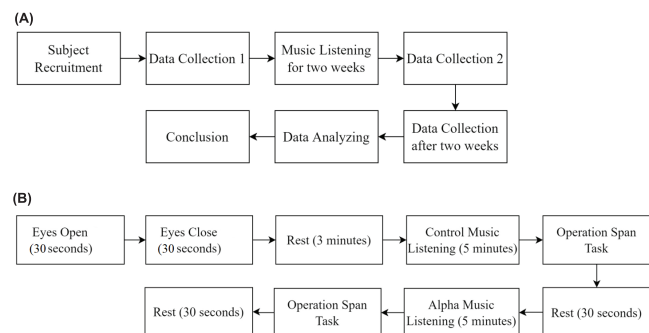


Fig. 1. (A) Data collection protocol for the short term and long term experiments and (B) data collection protocol for the immediate effect experiment.

2.2. Subject recruitment

Forty students with age range from 18 to 22 years old studying at International University were recruited (male $n=15$, mean age=20.5 years old; female $n=32$, mean age=19 years old). All participants met the following criteria: right-handed, with no visual impairment, non-smoking, and not using substances like depressants, inhalants, or stimulants, which have detrimental effects on the nervous system. After signing the consent form, the 40 subjects who participated in the online experiment were randomly assigned to two groups: the experimental group, which listens to alpha music ($n=20$) and the control group that will not listen to alpha music ($n=20$). In the final data collection session (week 5), the total number of remaining subjects was 20 ($n=10$ for the experimental group and $n=10$ for the control group).

On the other hand, the immediate effect experiment included seven subjects (immediate group) who listened to alpha music while recording EEG data ($n=7$) and met the same requirements as the online experiment.

This study was approved by the Institutional Review Board - IRB of the School of Biomedical Engineering, International University, Vietnam National University, Ho Chi Minh city.

2.3. Cognitive tasks

The content of the operation span and matrix span tasks primarily focuses on testing comprehensive cognitive functions, including memorizing capacities and calculation skills, which students frequently use in their academic performance and daily life.

Since the main scope of the study is to estimate the improvement of cognitive functions of students after listening to alpha music, these chosen tasks are suitable for our experiment [13].

The operation span task aims to evaluate the memory and attention capacity of the subjects, and it consists of two tasks (remembering and arithmetic tasks) (Figs. 2A and 3A) [13-15]. In the remembering task, the screen displays a random number to memorize. Subsequently, the arithmetic task shows a mathematical calculation and requires subjects to confirm accuracy by pressing the left and right arrows on the laptop keyboard. The process is repeated for the following rounds. After reaching the final round, the subjects are asked to type the numbers that appeared in chronological order. The first trial begins with three rounds, then the numbers and calculations gradually increase after two trials, ending after 12 trials.

The matrix span task examines spatial, memory, and attention ability (non-verbal memory) [13-15]. In this task, participants must memorize the location of a coloured box that randomly appears on the screen and use their mouse to click the exact location of the box on a blank 4x4 grid, in chronological order. After every three trials, the number of coloured boxes increased by one unit and the experiment ended after 16 trials (Figs. 2B and 3B). In both tasks, subjects were informed of which trial they were attempting and the accuracy of their answers at the top of the screen (Figs. 3C and 3D).

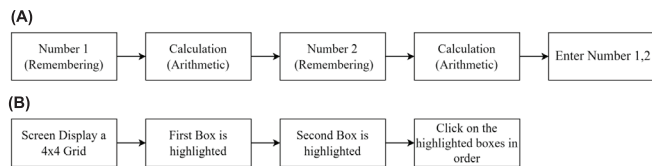


Fig. 2. (A) Operation span task and diagram of the first three trials; (B) Matrix span task and diagram of the first three trials.

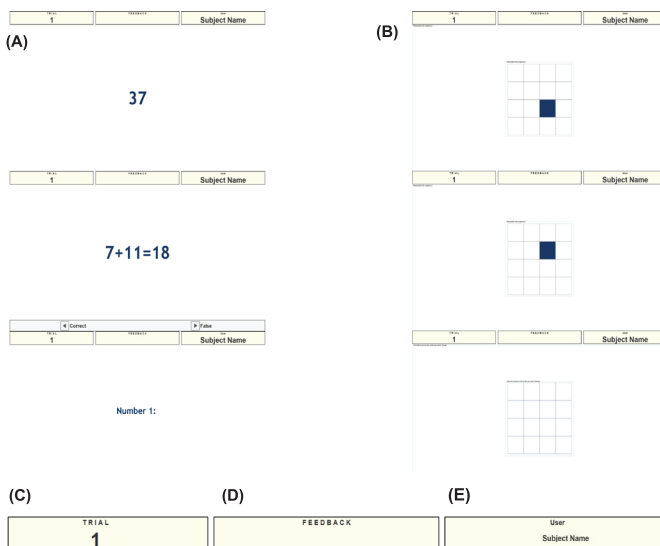


Fig. 3. Illustration of (A) operation span task and (B) matrix span task; header bar for the two cognitive tasks with (C) trial numerical order, (D) feedback from subject's answer, and (E) subject's name or identification number.

2.4. EEG signal acquisition

For the immediate effect study, a Philips Respironics Alice 6 (Fig. 4A) device was utilized for EEG recording. Ten main channels (Fig. 4B), namely, Fp1, Fp2, F7, F8, F3, F4, T5, T6, O1, O2, EOG1, and EOG2, were selected. Each channel represents a brain area: Fp stands for Pre-frontal; F stands for Frontal; T stands for Temporal; O stands for Occipital; and EOG for Electrooculogram.

During EEG acquisition, subjects were asked to restrain body movements to limit electromyography (EMG) noises. Moreover, continuous supervision during data recording was necessary to detect any possible abnormalities in the recorded channels and guarantee their stability for subsequent noise removal.

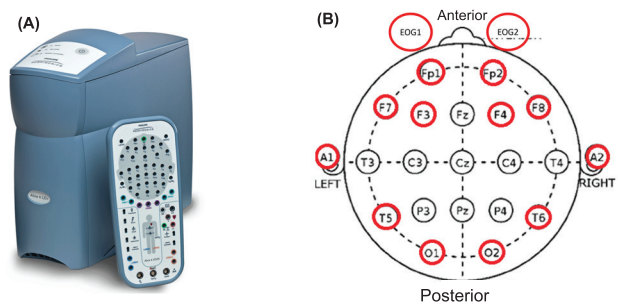


Fig. 4. (A) The ALICE 6 Device and (B) the selected EEG channels for measurement [16].

2.5. Signal processing and EEG power calculation

During the EEG acquisition, several instances of noise were caused by eye blinking (EOG noise) as well as head orientation and neck muscle electromyography (EMG noises), which can lead to incorrect data and an inaccurate calculation of EEG power. Consequently, the designed protocol utilized the EEGLAB toolbox (shown in Fig. 5), which enhances signal quality and aids the EEG power computing accuracy by implementing noise removal techniques. A bandpass filter was the first filter technique applied and it confines the collected data within a desired frequency range, which is between 0.5 and 45 Hz in this study. The reason for choosing these limits is that they cancel out noise contributions beyond the neurological range (EMG noise). The sequential noise filter technique uses independent component analysis (ICA), which involves separating a variety of signals (independent signal sources) into statistically-independent and non-Gaussian signal components [17]. After applying both techniques, the data was segmented to calculate the relative alpha band power. Based on the protocol, the segmented sessions are 30-s rest; 3-min rest; music listening - either control or alpha music; operation span task; and eyes open/eyes closed.

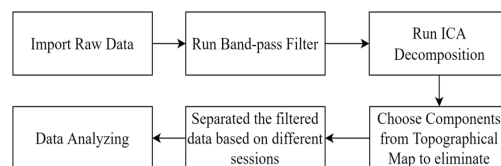


Fig. 5. Electroencephalogram noise filtering protocol.

An EEG's relative power is a fundamental EEG feature, which is defined as the ratio between the total power of all bands and the overall energy intensity of an electrode on a specific region at different frequency bands [18-20]. In addition, the relative power is frequency band power expressed as a percentage of the total signal power. Specifically, the calculation of alpha relative power in this study will enable an understanding of the percentage of total signal power in the alpha frequency range in different sessions [21]. The "EEGLAB" toolbox in MATLAB was utilized for calculating the relative power of each brain wave in each session. The frequencies are defined as follows: Delta from 1 to 4 Hz, Theta from 4 to 8 Hz, alpha from 8 to 12 Hz, and beta from 12 to 30 Hz.

$$\text{Alpha band relative power} = \frac{\text{Alpha band absolute power}}{\text{Total absolute power}}$$

2.6. Statistical analysis

The statistical analysis was performed in GraphPad Prism 8.0 software. Two-way ANOVA and multiple comparisons were applied to compare performance scores and response time in the cognitive tasks between the experimental group and control group. The statistics used to evaluate the (sustained) impact of the intervention confirmed a difference between the two groups and the results in each session within a group. For the EEG data, the analysis of the relative power is illustrated based on observations of the trendlines and a comparison of each session of EEG alpha relative power. This is because statistical analysis cannot be used due to the inadequate number of subjects. The analyses described below demonstrated significant results with $p < 0.05$, which is represented by the star (*) in column graphs (see Fig. 7D).

3. Results

3.1. Students' feedback after listening to alpha music

The purpose of collecting a student's subjective estimation about the alpha music was to understand the personal reaction of the student to the intervention. Most of the experimental subjects gave positive feedback after two weeks, such as increased relaxing state, eased mind, and improved sleep quality (Fig. 6). On the other hand, a few subjects (15%) reported being uncomfortable with the intervention while others gave neutral feedback (15%) while listening to the alpha music. Detailed feedback is included in Supplementary Table 1.

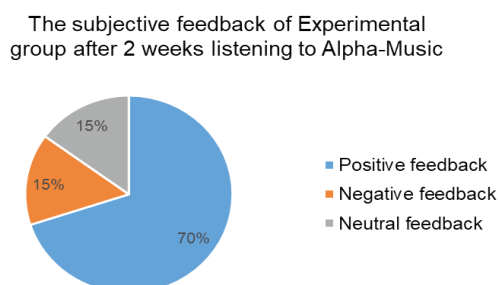


Fig. 6. Pie chart of feedback from the experimental group after listening to alpha music for two weeks.

3.2. The effect of alpha music intervention on improving cognitive function

Because the main goal of the study was to observe the cognitive improvement of students, we hypothesized that the short-term and long-term effects of alpha music in enhancing cognitive functions could be demonstrated after 14 days of listening to alpha music [10]. To validate this hypothesis, a designed a set of cognitive tasks including operation span task and matrix span task and applied Two-way ANOVA to confirm the effects of our proposed intervention are done.

3.2.1. Short term effect

Arithmetic task-operation span task: There were no differences detected in the accuracy of the task for the experimental group during the two-week alpha music listening period. Average accuracy for the experimental group in weeks 1 and 3 were 40.15 ± 1.46 ; 40.15 ± 1.63 , respectively; $p > 0.99$. On the other hand, the accuracy marginally decreased for the control group. The average accuracy for the control group was 40.6 ± 1.79 ; 40.35 ± 1.39 , respectively, $p = 0.78$ (Fig. 7A).

Unlike the remembering task's results, both groups displayed a reduction in completed time; however, only the experimental group showed a significant reduction. The average completion time for the experimental group before and after listening to alpha music was 2.34 ± 0.73 ; 2.10 ± 0.69 with $p = 0.05$ (Fig. 7D). Based on the significant decrease in response time with the experimental group, we can conclude that the mathematic calculation skills of the subjects mildly improved.

Remembering task-operation span task: Within the experimental group, there was an increasing trend in performance accuracy after listening to alpha music for two weeks. Nevertheless, the increase was not significant according to ANOVA analysis. The average scores in weeks 1 and 3 were 35.40 ± 6.082 and 36.25 ± 3.837 , respectively, with $p = 0.724$. On the other hand, a mild reduction in the control group's average score was shown, which is insignificant. The average scores for weeks 1 and 3 were 33.65 ± 5.613 and 33.05 ± 7.323 , respectively, while $p = 0.85$ (Fig. 7B).

In contrast to the accuracy, both the control and experimental groups exhibited a decreasing trendline in the average task completion time, however both were statistically insignificant. Specifically, the average response time in the control group for weeks 1 and 3 were 2.14 ± 0.56 and 1.99 ± 0.44 , respectively; $p = 0.145$. Also, the average response time in the experimental group was 2.08 ± 0.86 and 2.00 ± 0.72 , respectively; $p = 0.62$ (Fig. 7E). In conclusion, no effect of alpha music was observed in the experimental group from the remembering task.

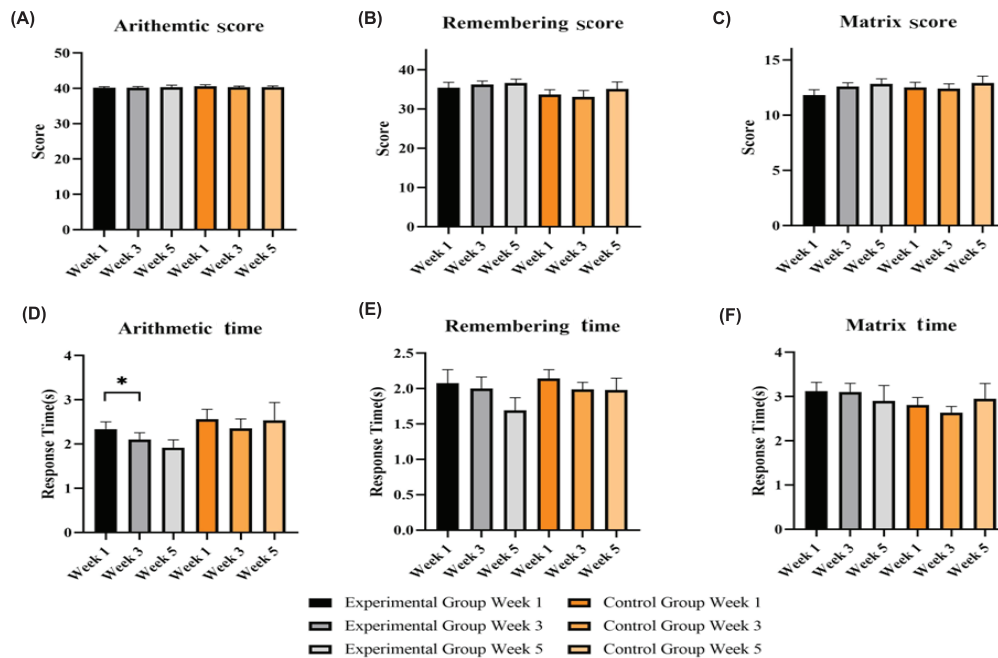


Fig. 7. Results from the accuracy of (A) arithmetic task, (B) remembering task, and (C) matrix span task. Response time of (D) arithmetic task, (E) remembering task, and (F) matrix span task.

Matrix span task: The mean scores of the experimental and control groups exhibited different trends. While the control group's average score moderately dropped, the experimental group has risen to a notable degree. Average scores for the experimental and control groups within a 2-week duration were 11.8 ± 2.2 and 12.6 ± 1.47 ; 12.5 ± 2.11 and 12.4 ± 1.96 ; with $p=0.21$ and $p=0.97$; respectively (Fig. 7C). The same trend in the response time after a two-week intervention, which was observed in the remembering task, was also recorded in the matrix span task. Both groups demonstrated a reduction in task completion time. The average response time for experimental group and control group within the 2-week duration were 3.12 ± 0.90 and 3.10 ± 0.88 ; 2.81 ± 0.77 and 2.64 ± 0.61 ; with $p=0.99$ and $p=0.61$; respectively (Fig. 7F). Overall, the results from the matrix span task did not indicate any effect from alpha music listening in the experimental group.

3.2.2. Long term effect

The long term effects of alpha music intervention on improving cognitive functions were validated throughout the utilized cognitive tasks (operation span task and matrix span task). According to Fig. 7, no difference in trendlines were observed between week 1 and week 5 and week 3 and week 5.

3.3. Electroencephalogram signal and power-immediate effect experiment

3.3.1. Noise filtering efficacy: After applying noise removal techniques like band-pass filter and ICA, the peaks caused by eye movements, which are found at the end of the EEG raw signal (Fig. 8A), were completely removed or dampened (Fig. 8B). The pre-processing step, therefore, enhanced the quality of the EEG signal.

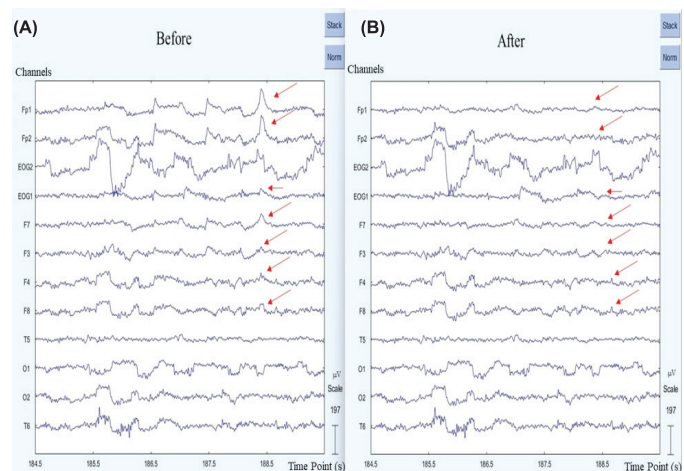


Fig. 8. Signal quality and noise cancellation: (A) before and (B) after signal processing.

3.3.2. Alpha-band relative power: This evaluation was conducted on seven subjects. Five of them show higher alpha band relative during alpha music listening compared to other sessions (rest sessions, tasks sessions, and control-music listening sessions) in all ten channels (Fig. 9). Specifically, the average relative power for the alpha music session is higher than the control music session (Supplementary Table 2). In addition, a comparison of the average power in each channel (i.e., which takes the average power from all subjects in that one specific channel) also yielded similar results. Analysing the relative power in alpha music and control music with 3-minute rest confirms that the power is much higher for alpha music. To summarize, the instantaneous effect of alpha music on alpha

band relative power was observed based on the comparison between the alpha music listening session with other sessions.

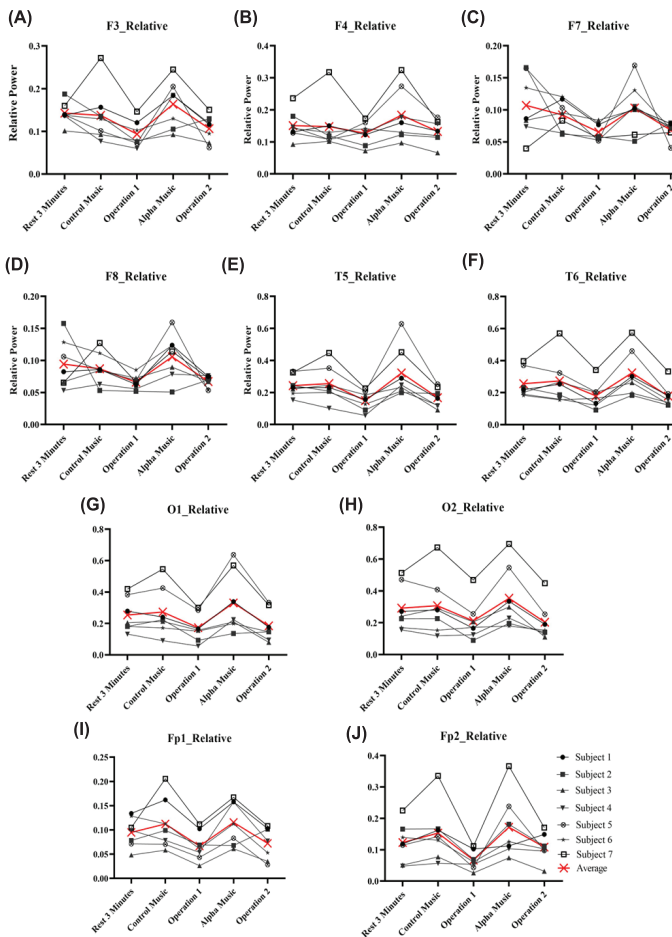


Fig. 9. EEG relative power of alpha band over the ten channels: (A) F3; (B) F4; (C) F7; (D) F8; (E) T5; (F) T6; (G) O1; (H) O2; (I) Fp1; (J) Fp2 of seven subjects in different stages (rest 3 min, control music listening, operation task 1, alpha music listening, operation task 2).

4. Discussion

4.1. The effect of alpha music on the enhancement of cognitive function

After examining the effect of alpha music therapy on the subject's cognitive performance, the results have achieved the following positive effects: Considering the accuracy, although no significant changes were noticed between the first and third week, an increasing pattern was noticed in the experimental group, with the average task scores increased after alpha music listening. On the other hand, looking at the response time, only the arithmetic task result showed a significant decrease in the task completion time, suggesting that mathematical skills and working memory performance have been significantly ameliorated. Regarding the two remaining tasks, the trend remains indifferent. Another positive outcome of the experiment is the student feedback, in which approximately 70% of the responses are positive. To be

more specific, they reported that their concentration had been improved, resulting in increased performance in their daily lives (Fig. 6). Contradictory to the short-term effect, the results of the long-term effect do not show any specific pattern, which can be due to the limited number of listening days and minutes [10].

4.2. The immediate effect of alpha music on the brain response

Regarding the immediate effect experiment, even though the number of participants in this study is relatively small (only seven subjects involved), the majority have shown an increase in their relative alpha power during the alpha music listening session in approximately all channels. Moreover, their relative alpha power is higher than other sessions, including task sessions, the 30-second rest sessions, and notably, the 3-minute rest session and the control music (preferred music) session. The result indicates that listening to alpha music can immediately affect the EEG power in subjects, which is consistent with previous studies involving the effect of alpha music on EEG [22]. In addition, the increase in the average relative power of all ten channels suggests that alpha music can positively enhance the alpha power on frontal, occipital, and temporal lobes. Moreover, the increase in alpha band power during the listening of alpha music can lead to the enhancement of cognitive performance. Additionally, this study confirms the better effect of listening to alpha music on subjects brain wave, rather than listening to the music type chosen by the participants or resting silently for three minutes.

4.3. Limitations

During the experiment, the study encountered the following challenges that can contribute to a minimal effect of the intervention on cognitive function. First of all, the number of subjects is relatively low in both experiments. Particularly, there were only 20 subjects in each group for the online experiment. Moreover, only ten subjects from each group participated in the long-term experiment. Meanwhile, there were only seven participants in the immediate effect experiment. The reason for the lack of subject numbers was due to the COVID-19 outbreak in Ho Chi Minh city (2021) and restricted commuting post-social distance, which prevented the broadcast of our project to potential candidates (for the online experiment), and the mobility of subjects joining the Offline experiment (immediate effect experiment). Secondly, with the online experiment conducted close to a Vietnamese national holiday and final exams, 60% of the participants reported that their daily workloads were arduous. Therefore, they tended to complete their assigned tasks at a competitively faster rate without focusing on their performance accuracy, which led to increasing inaccuracy.

5. Conclusions

From all yielded results of the two experiments (online and immediate effect), it can be concluded that alpha music positively affects the cognitive skills and mood of subjects. In addition, listening to alpha music can have an instantaneous effect on alpha band relative power. However, an improvement in experiment protocol and new approaches are needed for more apparent results.

The preliminary findings have pointed to a potent application of alpha wave-containing music therapy for a variety of working contexts.

Supplementary

Supplementary Table 1. Students' feedback after listening to alpha music for two weeks.

Positive feedback	Negative feedback	Neutral feedback
Ease the mind, and feel more alert (4 subjects)	Music sounds frightening, leading to an emptiness feeling (1 subject)	Music is normal (3 subjects)
Feel quite relaxed, reduce stress (7 subjects)	Making sleeper more than wanted (2 subjects)	
Easier to fall into sleep (3 subjects)		

Supplementary Table 2. Average relative power of alpha wave on ten channels in the different states.

	Rest-3 min	Control music	Task 1	Rest 2	Alpha music	Task 2	Rest 3
F3	0.14	0.14	0.09	0.13	0.16	0.11	0.14
F4	0.15	0.15	0.13	0.16	0.18	0.13	0.17
F7	0.11	0.09	0.07	0.08	0.1	0.07	0.09
F8	0.09	0.09	0.07	0.09	0.11	0.07	0.1
Fp1	0.09	0.11	0.07	0.09	0.12	0.07	0.1
Fp2	0.12	0.15	0.1	0.16	0.17	0.11	0.14
O1	0.25	0.27	0.17	0.26	0.33	0.18	0.27
O2	0.29	0.31	0.21	0.27	0.35	0.2	0.25
T5	0.24	0.26	0.15	0.22	0.32	0.17	0.25
T6	0.26	0.27	0.18	0.23	0.32	0.18	0.25
Average power	0.18± 0.02	0.18± 0.03	0.12± 0.02	0.17± 0.02	0.22± 0.03	0.13± 0.02	0.18± 0.02

CRedit author statement

Lam Quang Nguyen, Hoang Minh Anh An, Vu Quang Thinh Tran: Data curation, Software, Visualisation; Phuc Hoang Anh Le, Van Luan Tran, Mai Thao Le, Trong Kien Nguyen, Thi Thanh Huong Ha: Investigation, Writing, Methodology; Thi Thanh Huong Ha: Supervision, Revising the manuscript.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] A. Stauder, B.K. Thege, M.E. Kovács, et al. (2010), "Worldwide stress: Different problems, similar solutions? Cultural adaptation and evaluation of a standardized stress management program in Hungary", *International Journal of Behavioral Medicine*, **17**(1), pp.25-32, DOI: 10.1007/s12529-009-9054-4.

[2] S.H. Seo, J.T. Lee (2010), "Stress and EEG", *Convergence and Hybrid Information Technologies*, DOI: 10.5772/9651.

[3] A. Lauenroth, A.E. Ioannidis, B. Teichmann (2016), "Influence of combined physical and cognitive training on cognition: A systematic review", *BMC Geriatrics*, **16**(1), DOI: 10.1186/s12877-016-0315-1.

[4] M.W. Eysenck, N. Derakshan, R. Santos, et al. (2007), "Anxiety and cognitive performance: Attentional control theory", *Emotion*, **7**(2), pp.336-353, DOI: 10.1037/1528-3542.7.2.336.

[5] C. Martindale, D. Hines, L. Mitchell, et al. (1984), "EEG alpha asymmetry and creativity", *Personality and Individual Differences*, **5**(1), pp.77-86, DOI: 10.1016/0191-8869(84)90140-5.

[6] J.M. Hedlun, J.C. Webster, J.M. Hedlun (1950), "On the frequency limits of binaural beats", *Journal of the Acoustical Society of America*, **22**(4), pp.468-473, DOI: 10.1121/1.1906629.

[7] W.P. Nan, J.P. Rodrigues, J. Ma, et al. (2012), "Individual alpha neurofeedback training effect on short term memory", *International Journal of Psychophysiology*, **86**(1), pp.83-87, DOI: 10.1016/j.ijpsycho.2012.07.182.

[8] C. Escolano, M. Aguilar, J. Minguez (2011), "EEG-based upper alpha neurofeedback training improves working memory performance", *Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, pp.2327-2330, DOI: 10.1109/iembs.2011.6090651.

[9] J.J. Hsueh, T.S. Chen, J.J. Chen, et al. (2016), "Neurofeedback training of EEG alpha rhythm enhances episodic and working memory", *Human Brain Mapping*, **37**(7), pp.2662-2675, DOI: 10.1002/hbm.23201.

[10] S.W. Pheeah, H. Nisar (2017), "EEG-based alpha neurofeedback training for mood enhancement", *Australasian Physical & Engineering Sciences in Medicine*, **40**(2), pp.325-336, DOI: 10.1007/s13246-017-0538-2.

[11] B. Zoefel, R.J. Huster, C.S. Herrmann (2011), "Neurofeedback training of the upper alpha frequency band in EEG improves cognitive performance", *NeuroImage*, **54**(2), pp.1427-1431, DOI: 10.1016/j.neuroimage.2010.08.078.

[12] S. Hanslmayr, P. Sauseng, M. Doppelmayr, et al. (2005), "Increasing individual upper alpha power by neurofeedback improves cognitive performance in human subjects", *Applied Psychophysiology and Biofeedback*, **30**(1), pp.1-10, DOI: 10.1007/s10484-005-2169-8.

[13] R. Colom, I. Rebollo, F.J. Abad, et al. (2006), "Complex span tasks, simple span tasks, and cognitive abilities: A reanalysis of key studies", *Memory & Cognition*, **34**, pp.158-171, DOI: 10.3758/BF03193395.

[14] J.M. Stone, J.N. Towse (2015), "A working memory test battery: Javabased collection of seven working memory tasks", *Journal of Open Research Software*, **3**(1), DOI: 10.5334/jors.br.

[15] C.C.V. Bastian, A. Locher, M. Ruffin (2013), "Tatool: A Java-based open-source programming framework for psychological studies", *Behavior Research Methods*, **45**, pp.108-115, DOI: 10.3758/s13428-012-0224-y.

[16] Y. Zhao, S.A. Billings, H. Wei, et al. (2012), "Tracking time-varying causality and directionality of information flow using an error reduction ratio test with applications to electroencephalography data", *Physical Review E*, **86**(5), DOI: 10.1103/PhysRevE.86.051919.

[17] G.R. Naik, D.K. Kumar (2011), "An overview of independent component analysis and its applications", *Informatica*, **35**, pp.63-81.

[18] S. Machado, C.E. Portella, J.G. Silva, et al. (2007), "Changes in quantitative EEG absolute power during the task of catching an object in free fall", *Arquivos de Neuro-Psiquiatria*, **65**(3A), pp.633-636, DOI: 10.1590/s0004-282x2007000400017.

[19] H.J. Niemmarkt, W. Jennekens, J.W. Pasman, et al. (2011), "Maturational changes in automated EEG spectral power analysis in preterm infants", *Pediatric Research*, **70**(5), pp.529-534, DOI: 10.1203/PDR.0b013e31822d748b.

[20] R. Yuvaraj, M. Murugappan, N.M. Ibrahim, et al. (2014), "On the analysis of EEG power, frequency and asymmetry in Parkinson's disease during emotion processing", *Behavioral and Brain Functions*, **10**(1), DOI: 10.1186/1744-9081-10-12.

[21] R.B. Govindan, A. Massaro, G. Vezina, et al. (2017), "Does relative or absolute EEG power have prognostic value in HIE setting?", *Clinical Neurophysiology: Official Journal of The International Federation of Clinical Neurophysiology*, **128**(1), pp.14-15, DOI: 10.1016/j.clinph.2016.10.094.

[22] K. Vijayalakshmi, S. Sridhar, P. Khanwani (2010), "Estimation of effects of alpha music on EEG components by time and frequency domain analysis", *International Conference on Computer and Communication Engineering (ICCC'E'10)*, pp.1-5, DOI:10.1109/ICCC'E.2010.5556761.

Potential land for agriculture development in three coastal districts of Ben Tre province in the Mekong delta, Vietnam

Chi Nguyen Phan¹, Tuan Huy Vuong¹, That Loc Ton², Thanh Vu Pham^{1*}

¹College of Environment and Natural Resources, Can Tho University, Campus II, 3/2 Street, Hung Loi Ward, Ninh Kieu District, Can Tho City, Vietnam

²Duy Tan University, 254, Nguyen Van Linh Street, Thac Gian Ward, Thanh Khe District, Da Nang City, Vietnam

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Abstract:

The research objective was to determine the suitability of land regarding physical and economic conditions as a basis to support managers in planning effective agricultural development strategies that are suitable and sustainable for land potential in a future affected by climate change. In this research, physical and socio-economic data were collected by field surveys, household interviews (330 households), and agricultural manager discussions. Additionally, the methodology of land suitability evaluation was based on Food and Agriculture Organization (FAO) (1976 & 2007) and Geographic Information System (GIS) tools, which were used to determine suitability classifications for land-use types (under different physical and socio-economic conditions) in three coastal districts within the Ben Tre province. The results showed that agricultural production status in the research area was divided into three agro-ecological zones including the freshwater zone, brackish water zone, and saltwater zone of which the highest rate of agricultural land area was aquaculture. The next major proportion of agricultural area belonged to land for planting perennial crops and land for planting annual crops. Besides, 7 and 8 land suitability zones were determined, respectively, depending on physical and economic conditions, for 11 main land-use types. This research contributes toward basic knowledge that aims to support local decision makers in their transformation of agricultural land and adaptation of salinization in the future.

Keywords: agro-ecological zones, Ben Tre province, potential land, salinization, sustainable agriculture.

Classification number: 5.1

1. Introduction

In recent years, the Mekong delta has been heavily affected by climate change events such as prolonged drought, erratic rain, unseasonal rain, local flooding, and saltwater intrusion, which all disturb the agricultural production of farmers [1-3]. Ben Tre is one provinces in Vietnam that will be most affected and vulnerable to climate change in Southeast Asia [4, 5].

The coastal zone in Ben Tre province consists of three coastal districts, namely, Binh Dai, Ba Tri, and Thanh Phu. This zone has a diverse ecosystem with three main agro-ecological zones including the freshwater zone, brackish water zone, and saltwater zone. The river system comprises a relatively dense network of river courses that receive freshwater and sediment from Hau and Tien rivers (two main parts of Mekong river) [6].

Recently, salinization status has become more complex and has strongly affected agriculture production in the coastal zones of the Ben Tre province [7]. In addition, small-scale farms still occupy a large ratio of land there. Potential

land for agriculture has not been suitably used yet, which leads to conflict in local land use processes and especially in boundary areas between freshwater and saltwater zones [8]. Besides, to ensure the economic development of Ben Tre and the increasing requirements for the quality of agricultural products were about product quality when society was developed [9]. In addition, saline intrusion and sea-level rise affected crop yield and quality [10]. At the same time, land resources were degraded due to the abuse of chemical fertilizers and pesticides used for the farmer's agricultural intensification [11-13].

Regarding the above issues, research was conducted to assess the suitability of land and its natural and economic conditions for agricultural land use types as a basis to support managers with rationally orienting land use as well as economically and effectively exploiting land resources in the coastal areas of the Ben Tre province under the impact of future climate change. Specifically, this study (1) performs physical land suitability evaluations; (2) provides quantitative economic land suitability evaluations, and (3) integrates physical-economic land suitability evaluations.

*Corresponding author: Email: pttvu@ctu.edu.vn

2. Methods

2.1. Study scope

This study selected Ben Tre's three coastal districts including Thanh Phu, Binh Dai, and Ba Tri districts (Fig. 1) as a case study area because it is an area that has been heavily affected by saline intrusion and sea level rise, which strongly affects the type of agricultural land use [10].

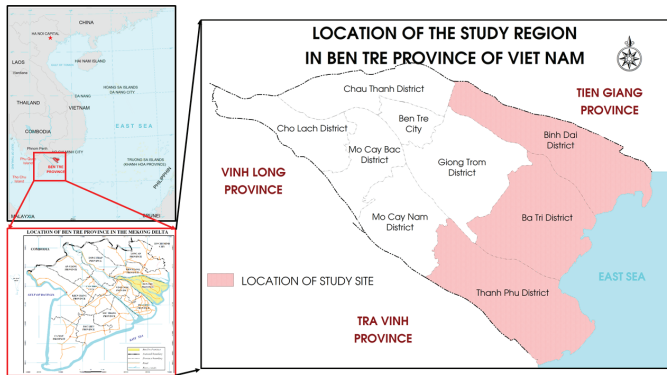


Fig 1. Location of study site.

2.2. Data collection and analysis

Secondary data collection: Data and documents used including agricultural land-use status, present land use map, land use planning, and other relation data that were collected in study area of Ben Tre's coastal zone.

Primary data collection: Field surveys were conducted to determine major available agricultural land use. In addition, the study was conducted by random interviews with 330 farmer households, and the number of questionnaires was distributed to eleven land use types in Ben Tre's three coastal districts (30 households/land use type) [14]. The information collected includes farmers' agricultural production conditions such as investment costs, profits, advantages and disadvantages in production of farmers. Three PRA (participatory rural appraisal) with local farmers and agricultural manager discussions were conducted to collect extra data and validate household interview data.

Data analysis method: Collected data was aggregated by Microsoft Excel to support the process of analysing, evaluating, and comparing data about total investment costs, profits, and capital efficiency (benefit/cost) of land use types, which was based on economic suitability analysis for each land use type. The maps were normalized on coordinate system VN 2000/WGS 84 zone 48 (6 Degrees). Next, the maps were digitized, overlapped, and edited by Mapinfo software to establish thematic maps.

2.3. Data processing and evaluation method

The research used was land suitability evaluation methodology [15, 16] that determines physical and economic

land suitability, respectively, for local major land use types (Fig. 2.)

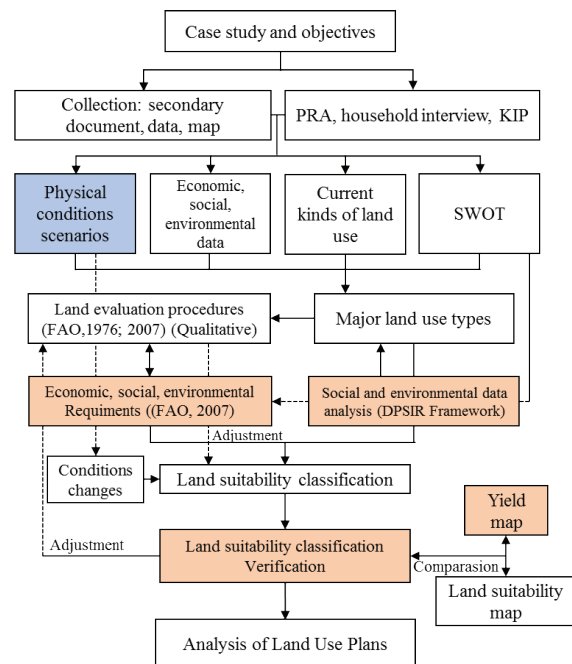


Fig. 2. Framework of land suitability evaluation methodology.

3. Results and discussion

3.1. Land mapping units

The result of PRA with local farmers, agricultural manager discussions, and surveys showed that five land characteristics strongly affected agriculture production in the coastal zone of Ben Tre province including soil types, salinity, time of salinity duration, depth of occurrence of sulfuric horizon, and depth of occurrence of sulfidic horizon. Based on these characteristics, five single information maps were established, respectively, and overlapped together to delineate a Land-mapping units map that has twenty land-units in three coastal districts of Ben Tre's coastal zone from year 2018 (Table 1 and Fig. 3). Of which, land unit No. 19 had the largest area (34.94%) occupying 36,451.3 ha, and there were characteristics of saline soil, water salinity greater than 16‰, and salinity time was 12 month, not alum. Based on this data, these land units could be suitable for the development of land use types of the brackish and saline ecological sub-region. In contrast, land unit No. 16 had the smallest area (0.01%) occupying 13.4 ha, and possessed characteristics of saline soil, non-salinated, and not alum. This land unit could develop land use types belonging to a freshwater ecological sub-region. However, attention should be paid to the leaching of salinity in the soil. The characteristics of the remaining land units and the area distribution of each land unit are detailed in Table 1.

Table 1. The information of land units in three coastal districts of the Ben Tre province.

LMUs	Soil types	Salinity (‰)	Salinity duration (month)	Depth of occurrence of sulfuric horizon (cm)	Depth of occurrence of sulfidic horizon (cm)	Area (ha)	Ratio (%)
1	AR	0	0	None	None	2,999.8	2.88
2	FL	0	0	None	50-100	306.3	0.29
3	FL	0	0	50-100	>100	3,318.2	3.18
4	FL	0-≤16	6	None	50-100	754.0	0.72
5	FL	0-≤16	6	50-100	>100	979.2	0.94
6	FL	>16	12	50-100	>100	255.7	0.25
7	GL	0	0	None	None	13,524.5	12.96
8	GL	0-≤16	6	None	None	10,035.7	9.62
9	GL	>16	12	None	None	2,941.7	2.82
10	GL	0	0	None	None	10,956.0	10.50
11	GL	0	0	100-150	>150	10,291.6	9.86
12	GL	0-≤16	6	None	None	1,099.9	1.05
13	GL	0-≤16	6	100-150	>150	1,894.7	1.82
14	GL	>16	12	None	None	89.3	0.09
15	GL	>16	12	100-150	>150	440.3	0.42
16	SC	0	0	None	None	13.4	0.01
17	SC	0-≤16	6	None	None	7,673.5	7.36
18	SC	0-≤16	6	100-150	>150	280.9	0.27
19	SC	>16	12	None	None	36,451.3	34.94
20	SC	>16	12	100-150	>150	19.1	0.02

Land Map Units (LMUs), Arenosol (AR), Fluvisol (FL), Gleysol (GL), Solonchak (SC).

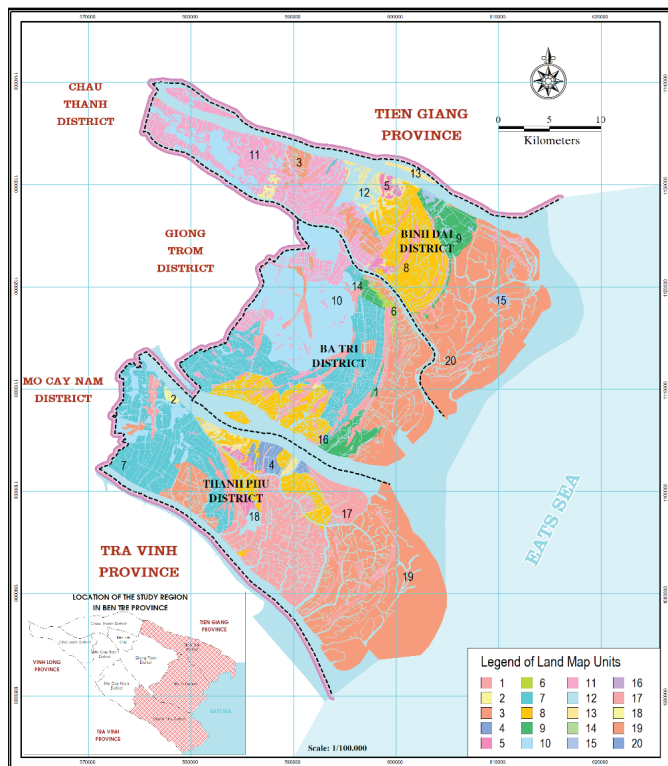


Fig. 3. Map of land units in three districts of the coastal zone of the Ben Tre province.

3.2. Physical land suitability evaluation

Eleven major land use types with cultivation calendar (Fig. 4) were considered to identify as potential development in study area via a data collection process allocated in three agro-ecological zones such as the freshwater zone, brackish water zone, and salt water zone. The identification and description of the land use types (Table 2) is considered an essential part of the evaluation procedure. In particular, the conversion of agricultural land use by farmers is often affected by physical conditions such as saline intrusion, erratic weather, and economic conditions such as consumption markets, product selling prices, and labour resources for different land use types.

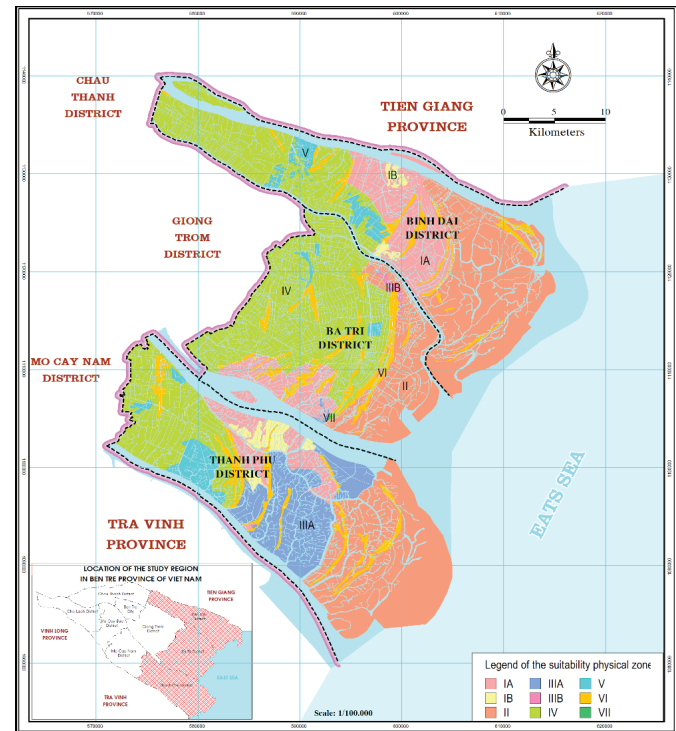


Fig. 4. Map of the suitability of physical zones for land use types in three coastal districts of the Ben Tre province.

Table 2. Cultivation calendar of land use types.

Month (dl)/Land use types	1	2	3	4	5	6	7	8	9	10	11	12
Triple rice	→	→	→	→	→	→	→	→	→	→	→	→
Double rice	→	→	→	→	→	→	→	→	→	→	→	→
Double rice - Cash crops	→	→	→	→	→	→	→	→	→	→	→	→
Rice - Shrimp	→	→	→	→	→	→	→	→	→	→	→	→
Coconut - Shrimp (Crayfish)	→	→	→	→	→	→	→	→	→	→	→	→
Coconut	→	→	→	→	→	→	→	→	→	→	→	→
Cash crops	→	→	→	→	→	→	→	→	→	→	→	→
Fruits	→	→	→	→	→	→	→	→	→	→	→	→
Aquacultural	→	→	→	→	→	→	→	→	→	→	→	→
Salt	→	→	→	→	→	→	→	→	→	→	→	→
Shrimp - Forest	→	→	→	→	→	→	→	→	→	→	→	→

Source: Field survey, 2018.

Land characteristics were used to delineate Land-mapping units. The research identified three land qualities that were believed to have significant effects on the types of land use under consideration. Land qualities are described by means of the land characteristics below:

- Nutrient availability (fertilizer - soil types).
- Soil toxicity (depth of occurrence of sulfuric horizon and depth of occurrence of sulfidic horizon).
- Salinity hazards (salinity, salinity duration).

Each type of land use has different requirements, which refer to the set of land qualities that determine the production and management conditions of types of land use. Therefore, limitations are land qualities and their expression by means of diagnostic criteria adversely affect a type of land use. Land evaluation is essential in order to ensure suitability classes and show the suitability of each land mapping unit for each relevant type of land use.

The process of land suitability classification is the fitness of a given land mapping unit for a defined use. Land suitability classes reflect degrees of suitability [16, 17]. Suitability classes are recognized within the order suitable, and definitions may be appropriate in a qualitative classification: Class S1 (highly suitable): >80% maximum yield; class S2 (moderately suitable): >40-80% maximum yield; class S3 (marginally suitable): 20-40% maximum yield; and class N (not suitable): <20% maximum yield,

The result of physical land suitability evaluation showed that seven land suitability zones were determined as follows: suitability zone I occupied 14,482.50 ha (13.85%) with highly suitable (S1), moderately suitable (S2) for rice - shrimp, shrimp, salt production and shrimp - forest; suitability zone II occupied 3,490.30 ha (34.29%), the largest proportion in study area, with highly suitable for land use types in saltwater (shrimp, salt, shrimp - forest). This zone had highly salinity and constant salinity durations because it is close to East Sea; suitability zone III occupied 7,929.17 ha (7.58%) with highly suitable (S1) for shrimp, salt and moderately suitable (S2) for shrimp - forest. Allocated mainly in the Thanh Phu district, suitability zone IV occupied 34,770.71 ha (33.5%), which is in a freshwater zone. Therefore, this zone was suitable with triple rice, double rice, double rice - cash crops, cash crops, fruits, coconut - shrimp (giant freshwater shrimp). Mainly in Ba Tri district, suitability zone V occupied 3,624.50 ha, which was also suitable for freshwater land use. Allocated mainly in the Thanh Phu and Binh Dai districts, suitability zone VI was highly suitable (S1) for coconut, fruits, and cash crops. However, soil conditions were limited as this zone is not able to be developed for rice, rice - cash crop, or coconut - shrimp. Suitability zone VII was the lowest area, which occupied 0.01%. This zone was limited by salinity so that it was not suitable for any land use types.

Based on physical land suitability evaluation, the land use types that are suitable for each agro-ecological zones are identified. The

results of physical land suitability evaluation for major land use types in Ben Tre's coastal zone had different suitability classes. Soil and salinity were major limitations of land characteristics, which adversely affected the type of land use.

Limitations of land qualities in suitability zones need to provide land improvements that cause beneficial changes in the qualities of the land itself. Additionally, a combination between local agricultural planning and improvement of land use are also necessary to offer suitable solutions with a choice of land use type.

3.3. Quantitative economic land suitability evaluation

Based on the results of physical land suitability evaluation, this study conducted quantitative economic land suitability evaluations for eleven major land use types, which were chosen in Ben Tre's three coastal zones. Land suitability classification of economic criteria of land use types was generated by economic interview data of farmers (Table 3.)

Table 3. Factor rating of economic land suitability classification of land use types.

Criteria	Decentralization of economic factor			
	S1	S2	S3	N
Revenue	>74.64	55.98-74.64	37.32-55.98	<37.32
Benefit - cost ratio (BCR)	>1.75	1.32-1.75	0.88-1.32	<0.88

Highly suitable (S1), moderately suitable (S2), marginally suitable (S3), not suitable (N).

The result of economic land suitability evaluation was determined to consist of eight suitability zones such as suitability zone I (Fig. 5), which was highly suitable for cash crops and fruits occupying 6,624.25 ha, and suitability zone II, which occupies 1,733.14 ha. For revenue, this zone was highly suitable (S1) for salt production. However, regarding BCR, it was moderately suitable (S2) for shrimp - forest, marginally suitable (S3) for shrimp and salt, and was not suitable (N) for the rest of land use types. Suitability zone III occupied 255.66 ha. For the revenue, this zone was highly suitable (S1) for salt production. Regarding BCR, it was moderately suitable (S2) for salt production and shrimp - forest and marginally suitable (S3) for shrimp. Suitability zone IV occupied 34,772.10 ha. For the revenue, this zone was highly suitable (S1) for coconut - shrimp, cash crops and fruits, and marginally suitable (S3) for triple rice, double rice - cash crops, and coconut. Regarding BCR, it was highly suitable (S1) for coconut, cash crops, and fruits, and moderately suitable (S2) for coconut - shrimp and marginally suitable (S3) for triple rice, double rice. Suitability zone V occupied 13,030.3 ha. For the revenue, this zone was highly suitable (S1) for salt production and moderately suitable (S2) for rice - shrimp. Regarding BCR, it was moderately suitable (S2) for shrimp and shrimp - forest, and was marginally suitable (S3) for salt production. Suitability zone VI occupied 39,941.55 ha. In revenue, this zone was highly suitable (S1) for salt production. Regarding BCR, it was highly suitable (S1)

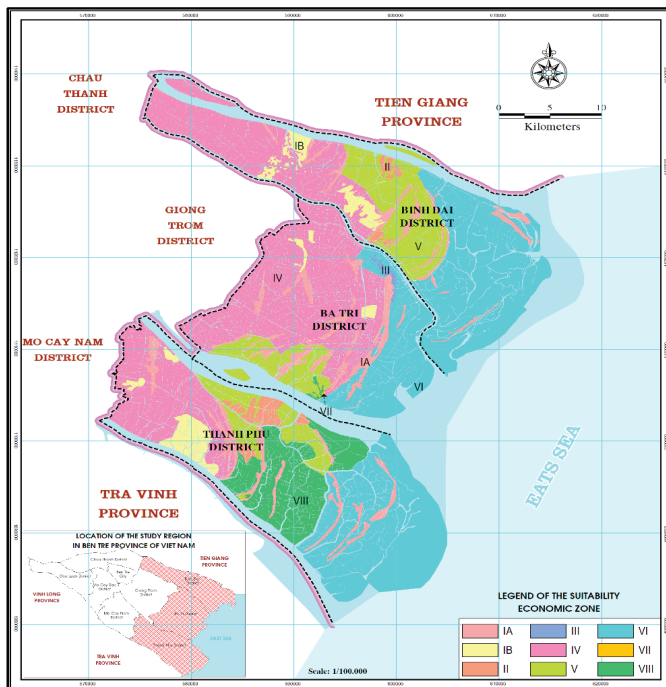


Fig. 5. Map of the suitability economic zone for land use types in the three coastal districts of the Ben Tre province.

for shrimp - forest and moderately suitable (S2) for shrimp and salt. Suitability zone VII gave the lowest proportion in economic land suitability evaluation (about 13.36 ha). For the revenue, this zone was moderately suitable (S2) and was marginally suitable (S3) for cash crops, fruits, coconut - shrimp. Regarding BCR, it was highly suitable (S1) for coconut and marginally suitable (S3) for cash crops and fruits. Suitability zone VIII occupied 7,954.41 ha. For the revenue, this zone was highly suitable (S1) for salt production. Regarding BCR, it was moderately suitable (S2) for shrimp, shrimp - forest, and marginally suitable (S3) for salt.

The result of economic land suitability evaluation (revenue and BCR) for the eleven main land use types in the study area showed that most of the suitability zones were marginally suitable (S3) or not suitable (N) for triple rice, double rice, and double rice - cash crops in economic conditions. Salt production was a land use type that demonstrated high suitability (S1) in five suitability zones. However, price of salt was high at the present time, and one needs to consider economic efficiency of salt production in the future.

3.4. Integrated physical-economic land suitability evaluation

On the basis of economic land suitability and physical land suitability of land units, the results have identified eight land suitability zones integrating economic and physical condition for eleven land use types in Ben Tre's three coastal districts, and the distribution of land suitability zones is shown in Fig. 6.

The results showed that land suitability zone I was physically not suitable (N) for freshwater land use types such as: triple rice, double rice, double rice mixed cash-crop, coconut, and fruits due to the influence of saline intrusion conditions. In contrast, it was

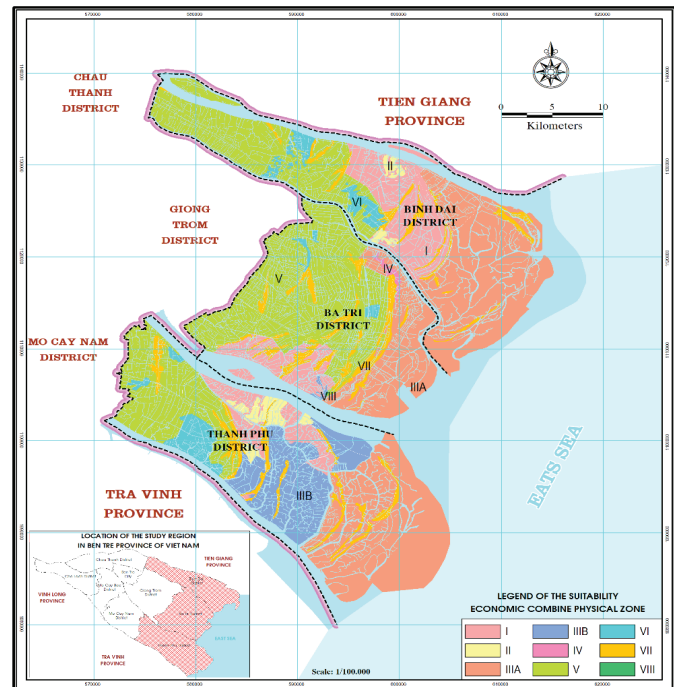


Fig 6. Map of the suitability of combined economic and physical zones for land use types in three coastal districts of the Ben Tre province.

physically suitable from moderately (S2) to highly suitable (S1) for land use types such as salt production, shrimp - forest, rice - shrimp, and shrimp. However, profit suitability of land use types ranged from marginally suitable (S3) to not suitable (N) due to risks from epidemics, consumer markets, and product selling prices. Because these land use types had low initial investment capital, the efficiency of capital presented moderately suitable (S2) in suitability zone I, which occupied 13,030.3 ha. Similar to suitable zone I, suitable zone II has the potential for development of land use types in brackish and saline ecological zones, and the suitability was physically moderately suitable (S2) or not suitable (N) for profit. The efficiency of capital had suitability ranging from moderately (S2) to highly (S1) suitable for land use types such as salt production and shrimp - forest. The area of suitability zone II was about 1,733.1 ha. Suitability zone III has been divided into two ecological sub-zones (IIIA and IIIB) and because of this it was physically marginally suitable (S3) for land use type of rice-shrimp, and it was highly suitable (S1) for shrimp, salt production, and shrimp-forest (IIIA) and moderately suitable (S2) to ecological sub-zone IIIB.

In addition, the benefit over cost of both sub-zones had suitability from moderately (S2) to highly suitable (S1) for the above land use types, and profit suitability was marginal (S3) to not suitable (N). The area of zone III was about 47,896.0 ha. Suitability zone IV was an area that was physically marginally suitable (S3) for rice - shrimp, and moderately suitable (S2) for land use types such as shrimp, salt production, and shrimp - forest. However, the profitability was not suitable (N) for all land use types, the capital efficiency was highly suitable (S1) for the land use type of salt production, and shrimp-forest had moderate suitability (S2). Suitability zone IV had a low area of suitable zones occupying only 225.7 ha. Meanwhile,

suitability zone V occupied 34,772.1 ha and this area was highly suitable (S1) for land use types of the freshwater ecological sub-zone such as triple rice, double rice, double rice - cash crops, coconut, and coconut mixed crayfish, cash crops, and fruits. The remaining land use types were not suitable (N). However, the economic indicators of profit and efficiency of capital in this region were of a well-developed ability for land use types like coconut mixed crayfish, coconuts, cash crops, and fruits. Suitability zone VI was an area that had a developed ability for land use types like the suitability zone V. However, the physical suitability of land use types were reduced to moderately suitable (S2), and the economic suitability was the development suitability for land use types of cash crops and fruits. Suitability zone VI occupied 3,624.5 ha. The suitability zone VII was a zone that was physically marginally suitable (S3) for land use types such as triple rice, double rice, double rice - cash crop, and was economically not suitable (N). However, the area was moderately (S2) to highly suitable (S1) for land use types of coconut mixed crayfish, coconut, cash crops, and fruits in regards to both physical and economic factors. Suitability zone VII occupied 2,999.8 ha. Suitability zone VIII was physically marginally suitable (S3) for land use types of the freshwater ecoregion and not suitable (N) for the remaining land use types. Economic suitability of zone VIII has profit high suitability (S1) for cash crops and fruits, marginally suitable (S3) for coconut mixed crayfish, and was not suitable for development for the remaining land use types. However, the investment efficiency for the development of use types was not suitable (N) for land use types. The distribution area of suitability zone VIII occupied the least among the suitability zones (13.4 ha).

Based on the combined physical and economic suitability zones for land use types in Ben Tre's three coastal districts, managers could receive help to plan agricultural production zones that are suitable for physical and farmer's economic conditions that are aimed at bringing the highest production efficiency, to build concentrated production areas with homogeneous quality, and bring into play the potential and available advantages of the zones. In addition, the land use types chosen to be suitable for each soil, water, and climate conditions will support the sustainable and effective structural transformation of the agricultural sector in Ben Tre's coastal districts.

4. Conclusions

Twenty land mapping units were identified by five single maps by their physical conditions. Seven and eight suitability zones, respectively, for eleven major land use types in Ben Tre's three coastal districts were identified by physical land suitability evaluations and economic land suitability evaluations. In addition, the results identified eight land suitability zones combining quantitative economic and physical conditions for land use types in Ben Tre's three coastal districts.

The results contribute to the basic of knowledge that supports agricultural managers in land use planning in the future and offers solutions (infrastructure and non-infrastructure) for adaptation to complex salinization.

CRedit author statement

Chi Nguyen Phan, Tuan Huy Vuong, That Loc Ton, Thanh Vu Pham: Data curation, Software, Visualisation, Investigation, Writing, Methodology, Supervision, Revising the manuscript.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] P.T. Vu, V.Q. Minh, P.C. Nguyen, et al. (2020), "Estimating the criteria affected to agricultural production: Case of Chau Thanh A district, Vietnam", *Asian Journal of Agriculture and Rural Development*, **10**(1), pp.463-472, DOI: 10.18488/journal.1005/2020.10.1/1005.1.463.472.
- [2] P.C. Nguyen, L.Q. Tri, P.T. Vu, et al. (2017), "Assessment of criterion of high technology for rice and vegetable production at Thoai Son and Chau Phu - An Giang province", *Can Tho University Journal of Science*, **1**, pp.39-48, DOI: 10.22144/ctu.jsci.2017.028 (in Vietnamese).
- [3] A.T. Le (2019), "Sustainable water resource management, responding to climate change in the Mekong delta", *Vietnam Journal of Science and Technology*, <https://vjst.vn/vn/tin-tuc/1317/quan-ly-tai-nguyen-nuoc-ben-vung-ung-pho-voi-bien-doi-khi-hau-o-dong-bang-song-cuu-long.aspx>, accessed 10 July 2019 (in Vietnamese).
- [4] A.A. Yusuf, H. Francisco (2010), *Hotspots! Mapping Climate Change Vulnerability in Southeast Asia*, EEPSEA publication, 84pp.
- [5] H.Q. Nguyen, D. Korb, H.L. Ho, et al. (2019), "Farmer adoptability for livelihood transformations in the Mekong delta: A case in Ben Tre province", *Journal of Environmental Planning and Management*, **62**(9), pp.1603-1618, DOI: 10.1080/09640568.2019.1568768.
- [6] People's Committee of Ben Tre province (2011), *Report on Master Plan of SocioEconomic Development to 2020 and Vision to 2030 of Ben Tre province* (in Vietnamese).
- [7] L.A. Tuan, S. Chinvarno (2009), "Climate change in the Mekong river delta and key concerns on future climate threats", *Environmental Change and Agricultural Sustainability in The Mekong Delta*, Springer, **45**, DOI: 10.1007/978-94-007-0934-8_12.
- [8] P.T. Vu, N.N. Phuong, P.C. Nguyen (2019), "The conflicts of agricultural land use and impact factors at Hoa Binh district, Bac Lieu province", *Vietnam Soil Science Journal*, **56**, pp.70-75 (in Vietnamese).
- [9] M. Rahmah (2017), "The protection of agricultural products under geographical indication: An alternative tool for agricultural development in Indonesia", *Journal of Intellectual Property Rights*, **22**, pp.90-103.
- [10] T.T.H. Le, K.A.T. Nguyen, C.M. Jolly (2016), "Perception, preparedness and severity of climate change triggered events in Ben Tre province, Vietnam", *International Journal of Food and Agricultural Economics*, **4**(4), pp.29-44, DOI: 10.22004/ag.econ.251817.
- [11] V.K. Le, B.L. Tran (2013), *Soil Degradation and Conservation*, Can Tho University Publishing House, 128pp (in Vietnamese).
- [12] V.Q. Minh, L.V. Khoa, T.T. Du, et al. (2019), "Soil fertility capability classification (FCC) in the Mekong delta, Vietnam based on the conversion of soil map (WRB)", *International Journal of Engineering Sciences & Research Technology*, **8**(6), DOI: 10.5281/zenodo.3256145.
- [13] V.V. Binh, C.T.A. Thy, H.V. Thiet, et al. (2016), *Management of Soil Fertility and Use of Fertilizer Efficiency in The Mekong Delta*, Can Tho University Publishing House, 264pp (in Vietnamese).
- [14] R.E. Thomson, W.J. Emery (2014), *Data Analysis Methods in Physical Oceanography*, Elsevier Science, 716pp.
- [15] Food and Agriculture Organization (1976), *A Framework for Land Evaluation*, <https://edepot.wur.nl/149437>, accessed 15 June 2020.
- [16] Food and Agriculture Organization (2007), *Land Evaluation: Towards a Revised Framework*, <https://edepot.wur.nl/488269>, accessed 15 June 2020.
- [17] L.T. Linh, L.Q. Tri, V.P. Khai, et al. (2011), "Assessment of economical factor ratings as a basis for economically quantitative land suitability classification based on the practical investigation in Cang Long district, Tra Vinh province", *Can Tho University Journal of Science*, **20b**, pp.169-179 (in Vietnamese).

Solid waste generation from the hotel industry in the coastal tourism cities of Vietnam

Thi Hoa Tran¹, Boris Fabres¹, Ngoc Ha Hoang², Hoai Le Tran^{2*}

¹Centre for Supporting Green Development (GreenHub), 325 Pham Van Dong Street, Co Nhue 1 Ward, Bac Tu Liem District, Hanoi, Vietnam

²Faculty of Environmental Engineering, National University of Civil Engineering, 55 Giai Phong Street, Dong Tam Ward, Hai Ba Trung District, Hanoi, Vietnam

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Abstract:

While the tourism and hospitality sectors in Vietnam have been rapidly developing, the increasing consumption of natural resources and production of associated waste, especially in coastal and island sites, threaten these marine areas with pollution. This study provides data on solid waste from 10 hotels in three coastal provinces of Vietnam, namely, Hai Phong, Quang Nam and Phu Yen. The results indicated significant percentages and volumes of organic waste especially from large-scale hotels with in-house food services like restaurants and bars. The proportions of organic and recyclable solid waste from all three surveyed cities ranged between 35-84% and 10-27%, respectively. Plastic waste accounted for a relatively high proportion of solid waste ranging from 27 to 60% total solid waste by volume and from 7 to 30% by weight. In particular, PET plastic made up the highest proportion of solid waste in Cat Ba town (52.41%) and Tuy Hoa city (39.81%) while single-layer plastic accounted for the highest proportion (29.55%) in Hoi An city. Plastic waste from food packaging, related to the cultural habits of guests that bring instant food items during their stay, was identified as an important issue, which reflects a valuable but presently wasted potential resource. These quantities of hotel solid waste can be recovered and provide additional environmental and health benefits to the community by not being dumped, landfilled, or openly burned.

Keywords: hotels, organic waste, plastic waste, tourism, Vietnam.

Classification number: 5.3

1. Introduction

According to the World Tourism Organization (UNWTO), the total worldwide tourism revenue in July 2019 reached 1,451 billion USD with the total number of international tourists reaching 1.4 billion [1]. While tourism is an important sector and the driving force for socio-economic development in many regions, inadequately managed tourism can also lead to negative impacts such as overconsumption of resources, habitat damage, and pollution emissions from transport in addition to the generation of a substantial amount of solid and liquid waste. According to United Nations Environment Programme (UNEP) estimates, each international tourist in Europe generates at least 1 kg of solid waste per day, and up to 2 kg/person/day in the United States [2]. Tourism cities and destinations face many challenges related to waste management due to the unique nature of the tourism sector such as seasonality and concentrated areas with high tourist-associated waste generation. Research also shows that the proportion of

solid waste generated from tourism establishments and activities can account for the majority of solid waste generated in some tourist cities [3, 4]. According to the UNEP, 80% of tourism activities and destinations are in coastal cities. As such, coastal tourism cities face growing challenges to the management of solid waste from tourism and the associated environmental impacts of mismanaged waste [5]. The challenges are even more significant for tourism establishments on small islands [6]. According to International Union for Conservation of Nature (IUCN) reports, every year about 8 million tons of plastic waste leaks into the sea with 80% of that eventually being deposited on the sea bed [7]. It has been estimated that Vietnam discharges the fourth-largest amount of plastic waste into the sea, at 0.28-0.73 million tons/year, which is equivalent to 6% of the world's total plastic waste discharged into the sea [7]. Most of the plastic waste that enters the ocean each year comes from land-based activities. Plastics have a slow degradation rate, and in some cases emit toxic chemicals that pose

*Corresponding author: Email: leth@nuce.edu.vn

serious physical, public health, and environmental threats to terrestrial, coastal, and marine ecosystems [8].

Vietnam has a fast-growing tourism sector that takes advantage of more than 3,260 km of coastline, an extensive archipelago of about 3,000 islands in large and small clusters, and 125 main beaches, all of which attract tens of millions of visitors each year. Indeed, tourism directly contributes 7.02% to total national budget revenue [9]. However, the lack of uniformity in solid waste management systems, weak infrastructure, inadequate incentives and enforcement, and the lack of awareness of many tourists, coastal communities, and industries including fisheries has caused coastal tourism destinations to face serious waste pollution that threatens the country's tourism potential. Thus, an overall solid waste management program for coastal tourism cities with focus on plastic waste in particular will foster the responsible development of the tourism sector, which includes effectively enhancing the environment and further increasing Vietnam's tourism potential. This study was conducted to contribute to the scientific basis towards the development goals of the tourism sector. Therefore, this work focused on three coastal tourism cities in three provinces in Vietnam to document quantities and compositions of solid and plastic waste generated from local hotels in comparison to other regions in Vietnam. In addition, the potential for recycling the solid waste is assessed on the basis of diverting waste from dumps, landfills, open burning, or incineration.

2. Materials and methods

2.1. Study areas

This research was conducted in three coastal cities in Vietnam including Cat Ba town (Hai Phong city), Hoi An city (Quang Nam province) and Tuy Hoa city (Phu Yen province). The target cities are representative of the different urban levels and geographical regions of Vietnam. Each city has different economic and social characteristics, technical infrastructure, living standards, travel development, climate situation, consumption habits, and living habits. The locations of the study areas are shown in Fig. 1. These surveys were carried out in 2018 and 2019. Cat Ba is considered a class 4 urban area belonging to the Cat Hai island district in Hai Phong city in Northeast Vietnam. Cat Ba was recognized by UNESCO as a World Biosphere Reserve in 2004. Cat Ba has an area of 59.2 km² and a population of about 8,392 people (2019) with a population density of 141.8 people/km². According to the 2019 socio-economic report of the Cat Hai district, its total production value reached 7,973.5 billion VND in which the tourism sector accounted for 77.3%. In 2019, Cat Ba welcomed 2,180,000 visitors with an average annual growth rate of 10%. Total revenue from tourism



Fig. 1. Map of study areas.

and service activities in 2019 was 1,805 billion VND, which accounted for 77.3% of the total local budget revenue [10].

Hoi An city is a class 3 urban area and is considered the economic-political-cultural centre of Quang Nam province, which is in the central region of Vietnam. Hoi An was recognized by UNESCO as a World Cultural Heritage Site in 1999 and was also designated as part of the Cu Lao Cham - Hoi An Biosphere Reserve in 2009. The area of the city is 61.48 km² with a population of 94,579 people (2018), and a population growth rate of 0.59%/year from 2013-2017. According to the data reported in the Hoi An Waste White Book in 2018, the total production value of

the city was estimated at 10,073.7 billion VND, of which the trade-service-tourism sector accounted for 71.2%. In the tourism sector, the total visitors to Hoi An in 2018 were estimated at 4.992 million, up 31.7% over the same period (international visitors reached 3,755 million, up 90.94%). The total number of stays is 1.78 million and the average length of stay is 2.13 days [11].

Tuy Hoa city is a class 2 urban area and the political-economic centre of the Phu Yen province, which is located in the south-central region of Vietnam with an area of about 107.3 km² and a population of about 161,179 people in 2018. According to the 2019 socio-economic report of the Phu Yen province, revenue from accommodation services, catering services, travel, and tourism activities increased by 15.5% over the previous year reaching a total of 4,642.2 billion VND. From this revenue, accommodation services accounted for 232 billion VND, an increase of 20.2%, and accommodation facilities served 1,018,600 arrivals of which 15,000 were international visitors, which were up by 20.9 and 33.6%, respectively [12].

2.2. Survey method

A total of 10 hotels were sampled as described in Table 1. The surveys for Cat Ba, Hoi An, and Tuy Hoa were conducted from the 15th to the 21st of September 2018; from 1st to 7th, November 2019 and from 28th June to 4th July 2019, respectively. The surveys in each of the cities were conducted continuously and for a minimum of 7 days. Details of the sampling effort for the three study areas are also summarized in Table 1.

The sorting of hotel solid waste followed 19 sub-categories that are listed in Table 2. Each category is quantified by weight (kg) and volume (litres) and reported accordingly. The waste assessment method was adapted from the “Waste Assessment and Brand Audit (WABA)” methodology and toolkit developed by the Global Alliance for Incineration Alternatives (GAIA). This is a methodology of solid waste collection and analysis that determines the quantities and types of waste generated in a locality and identifies the brands responsible for producing this waste by calculating their contributions by percentage weight. The basic steps of the method are: (1) a waste assessment made at the source by collecting waste from each hotel and separating it into the 19 sub-categories identified in Table 2 and (2) waste auditing of 20-liter buckets for each sub-category of waste by arranging and proper labelling. Waste is sorted into the appropriate buckets and, when they are full, their weight and volume are recorded (volume is approximated based on the proportion of the bucket that is full).

Table 1. Surveyed hotels in these cities.

City/Town	Star Rating ^a (Hotels)			Total registered hotels ^b	Number of hotels sampled	Number of guests during the audit period (person)	Total weight audited (kg)
	5 and 4	3 and 2	1 and 0				
Cat Ba	0	2	1	135	3	84	62.44
Hoi An	1	1	1	120	3	1,964	876.66
Tuy Hoa	2	2	0	56	4	14,786	7,476.27
Total	3	5	2	311	10	16,834	8,363.55

Star ratings for hotels are based on TVCN 4391-2015: hotel - classification; registered hotels: total number of hotels were obtained from the local tourism department for registered hotels in the 2017-2018 period.

Table 2. List of solid waste sub - categories.

No	Sub-categories	Description	Note
1	Plastic bag	All kinds of plastic bags	Non-recyclable
2	Multi-layer plastic packaging	Package has multiple layers	Non-recyclable
3	Single-layer plastic packaging	Package has only one layer	Non-recyclable
4	Polystyrene	Polystyrene container	Non-recyclable
5	Hard Plastic	HDPE, LDPE and PP plastic	Recyclable
6	PET Plastic	PET bottles	Recyclable
7	PVC Plastic	Water pipe	Non-recyclable
8	Straws	Plastic straws	Non-recyclable
9	Diapers & Tissue	Diapers and toilet paper	Non-recyclable
10	Metal	All kinds (copper, lead, iron...)	Recyclable
11	Glass	All kinds of glass	Recyclable
12	Paper and Cardboard	Paper documents, newspaper.	Recyclable
13	Organic Waste	Leftover food, food scraps, leaves, flowers	Recyclable
14	Textiles	Tablecloths, clothes	Non-recyclable
15	Ceramics	Ceramic, minerals	Non-recyclable
16	Hazardous Waste	Paint, oils, pesticide, fertilizer.	Non-recyclable
17	Medical Waste	All medical purpose waste	Non-recyclable
18	Footwear	Shoes, flip-flops (sandals)	Non-recyclable
19	Other	Unable to categorize	Non-recyclable

HDPE: High density polyethylene; LDPE: Low density polyethylene; PET: Polyethylene terephthalate; PP: Polypropylene; PVC: Polyvinyl chloride.

3. Results and discussion

3.1. Solid waste generation rate

The results of the solid waste generated for each type of hotel in the study area are presented in Table 3 including comparisons with other hotel audit studies in Vietnam.

Table 3. Daily solid waste generation rate.

City	Method (<i>n</i> -number of surveyed hotel, methodology and surveyed time)	Solid waste generation rate (kg/guest/day)					Ref.
		5 star	4 star	3 star	2 star	1 star	
Ha Long	n=3 Sampling (collect - separate - audit), 7 consecutive days in June 2004.	-	0.38-0.98	0.3-0.58	0.34-0.74	-	[13]
Hue	n=45 Sampling (collect - separate - audit), 10 consecutive days in June 2012.	6.57	2.32	1.71	0.48	0.60	[14]
Hoi An	n=120 Sampling (collect - separate - audit) and questionnaire, 7 consecutive days in December 2016.	0.6-2.28					[15]
Hoi An	n=9 Sampling (collect - separate - audit), 14 consecutive days August 2015.	0.8-3.33					[16]
Cat Ba	n=3 Sampling (collect - separate - audit), 7 consecutive days in September 2018.	-		0.13		0.03	This study
Hoi An	n=3 Sampling (collect - separate - audit), 7 consecutive days in November 2019	0.46		0.35		0.93	This study
Tuy Hoa	n=4 Sampling (collect - separate - audit), 7 consecutive days in June 2019	0.67		0.43		-	This study

“-” indicates no data collected in the cited reference; the solid waste generation (SWG) rate is calculated by dividing the total amount of waste generated per day in the hotel by the number of guests in the hotels on the day sampled.

The amount of solid waste generated at hotels in Cat Ba ranges from 0.03 to 0.13 kg/guest/day, which is significantly lower than that of similar hotels in Hoi An and Tuy Hoa. This is because the time of the survey in Cat Ba was September 2018, which is a time of absence of tourists as the high season ranges from May to August. Similarly, the time of the survey (November 2019) in Hoi An, was also the low season of the city, and the amount of generated solid waste ranged from 0.35-0.93 kg/guest/day. This value is quite similar to the obtained results of S.T.P. Phu, et al. (2008) [17] where the sampling process was conducted over 7 consecutive days from 120 hotels. Indeed, the results showed that the average amount of solid waste released from those hotels was 2.28 kg/person/day in the rainy season and 0.6 kg/person/day in the dry season (from February to September 2018). In contrast, the amount of solid waste generated from the hotels in Phu Yen was quite high and ranged from 0.43

to 0.67 kg/guest/day. This value can be explained as the survey was conducted in Tuy Hoa in June 2019, which is the high tourist season of the city. These results are similar to the solid waste audit results reported by P. Byer et al. from 3 hotels in Ha Long with 4-star, 3-star, and 2-star hotel waste ranging from 0.38-0.98, 0.33-0.58, and 0.34-0.74 kg/guest/day, respectively [13]. However, this result is significantly lower compared to the published result of L. Son, et al. (2018) [14], which were conducted under the same conditions in June 2012 in Hue city. The amount of solid waste generated for different types of hotels from 5-star, 4-star, 3-star, 2-star, 1-star, and guest house has the average value of 6.57; 2.32; 1.71; 0.48; and 0.60 kg/guest/day, respectively. Meanwhile, the study of D.N. Trung, et al. (2005) [18] estimated the waste generation rate of 50 hotels in Vietnam. The results showed that 2-, 3-, and 4-stars hotels generated 0.7-5.6; 8.2-17.9 and 13.5-32.3 kg/person/day, respectively, of daily solid waste. Their results are significantly larger than the other published studies, and this is explained by their method of study being a questionnaire survey.

The results show that the amount of solid waste generated in hotels is mainly from tourism activities, which depend on factors related to the hotels, e.g., their size and characteristics such as the number of rooms, quality, and range of services and facilities such as food service, presence of garden or green areas, and seasonality factors like the time of year and whether it is the high or low tourist season. Besides, high standard hotels produce significantly higher quantities of solid waste than lower standard hotels, which is not dependent on the geographic area. This is explained by the many accompanying services these high-quality hotels often have to serve exceptional tourist demands such as food, spas and gyms, and expansive green spaces.

3.2. Solid waste composition and recycling potential

In terms of composition by weight, the percentage of organic waste in hotels from the three sampled areas was higher compared to inorganic waste (Fig. 2). The organic waste percentage was approximately 35, 58 and 84%, meanwhile the inorganic waste percentage was approximately 38, 25 and 6% for Cat Ba, Hoi An and Tuy Hoa, respectively. The percentage of organic waste from the hotels in Tuy Hoa were the highest with more than 80% while Cat Ba is the lowest with 35% of the total waste. In Hoi An, the percentage of organic waste was approximately 58.3%, which is similar to the result of M. Hoang, et al. (2017) [16] of 56.2%, although the percentage of recyclable waste in Hoi An was 16.9%, which is significantly different from 30.4%.

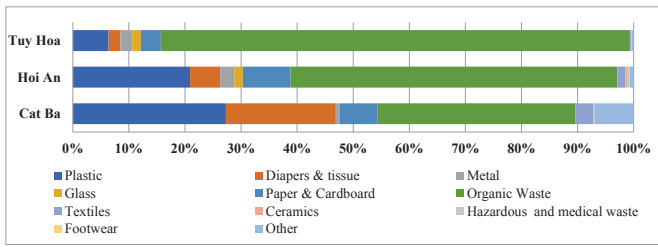


Fig. 2. Solid waste composition by weight in hotels from the 3 cities.

The 2020 law of environmental protection (law No72/2020/QH14) stipulates that domestic waste has to be classified into 3 categories: (1) recyclable waste, (2) food waste and (3) other waste. Food waste is considered as any food or inedible parts of food that is removed from the food supply chain to be recovered or disposed. In the organic waste generated from accommodation facilities, the organic component from fruits and food scraps often accounts for the largest proportion. They can be used for composting or food for animals. In our study, the percentage of food waste in hotels in the three sampled areas was higher compared to other waste items (30.5% for Cat Ba, 44.6% for Hoi An and 70.1% for Tuy Hoa). The high percentage of food waste from hotels in Tuy Hoa can be linked to the hotel type; all were larger hotels (upper 3-star) with large restaurants and bars for food services, while the Cat Ba hotels that were audited were mainly without food services.

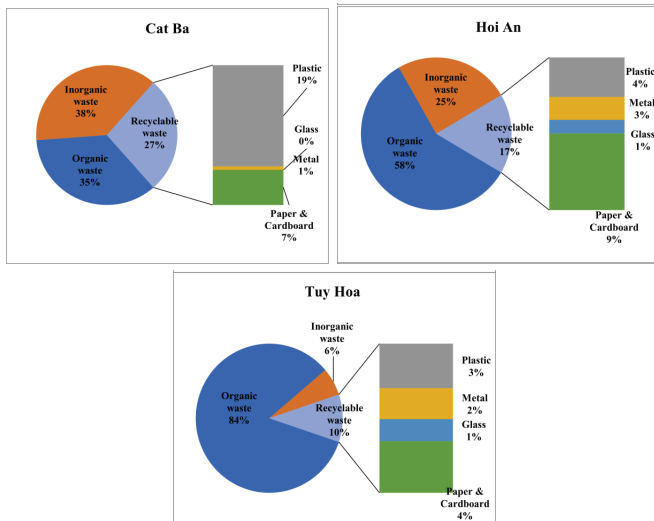


Fig. 3. Composition of waste from hotels in 3 cities.

The highest amount of recyclable waste was recorded in Cat Ba with 27% total solid waste. Meanwhile, this value was only approximately 10% in Tuy Hoa City. For the recyclable waste composition, plastic and paper accounted for the highest proportion (19 and 7% for Cat Ba, 4 and 9% for Hoi An and 3 and 4% for Tuy Hoa,

respectively). The proportion of recyclable waste such as paperboard, metal, and plastic accounted for quite a large proportion ranging from 10-30%, and these materials can be generally recycled and reused for other purposes.

The total amount of waste that can be recycled and reused (including food waste and recyclable waste) accounts for up to 50-80%. This waste is presently delivered to dumps, landfills, open burning sites, or incineration facilities and is currently being lost as a significant and valuable resource from hotel waste. If source segregation can be implemented with a collection and treatment system connected to the type of waste sorted, this can redirect up to 80% of the waste to become new, usable, and potentially valuable economic resources.

The results of the solid waste composition of this study in comparison to some coastal tourism cities in Vietnam are shown in table 4. For Da Nang city, the average amount of solid waste generated at hotels was 89.72 kg/hotel/day or 0.95 kg/room/day that includes 62.8% organic, 13.5% inorganic and 23.7% recyclable waste [19]. The percentage of organic, inorganic and recyclable waste in Ha Long and Hue city were 64.33; 30.07; 5.6 and 45.2%; 21.20; 33.6%, respectively. The difference in the solid waste composition among the hotels can be explained by the different types of hotels, the survey time (high or low season), the methodology of the survey, and the characteristics of the studied city. In addition, since the volume and composition of solid waste generated by the hotel industry in Vietnam are still quite limited, this comparison will be more accurate when these data are complete and detailed.

Table 4. Comparison of solid waste composition in coastal Vietnamese hotels.

City (n - number of the surveyed hotel)	Average composition (%)			References
	Organic	Inorganic	Recyclable	
Da Nang (n=10)	62.8	13.5	23.7	[19]
Ha Long (n=3)	64.3	30.1	5.6	[13]
Hue (n=45)	45.2	21.2	33.6	[14]
Hoi An (n=9)	56.2	10.4	33.4	[16]
Cat Ba (n=3)	35.3	37.7	27.0	This study
Hoi An (n=3)	58.3	24.8	16.9	This study
Tuy Hoa (n=4)	83.6	6.1	10.3	This study

3.3. Plastic waste from the hotel

The plastic waste generation rate from hotels in Hoi An is the highest (0.09 kg/guest/day), while in Tuy Hoa and Cat Ba they are the same (0.03 kg/guest/day). Recyclable plastic waste included PET plastic and hard plastics such as HDPE, LDPE, and PP plastic. Non-recyclable plastic waste consisted of plastic bags, PVC,

polystyrene plastic, single and multi-layer plastic bags, and straws. The proportion of plastic waste by weight and volume in the total waste generated from the studied hotels is shown in Figs. 4 and 5. The plastic waste from the hotels by weight and volume accounts for a relatively high proportion ranging from 27 to 60% total solid waste by volume, and from 7 to 30% by weight. The high proportion of plastic waste by volume suggests handling and management challenges in utilizing plastic waste for storage or transport facilities for each city.

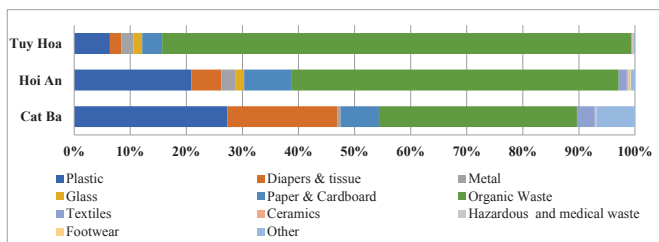


Fig. 4 Proportion of plastic waste from the hotels in 3 cities by weight.

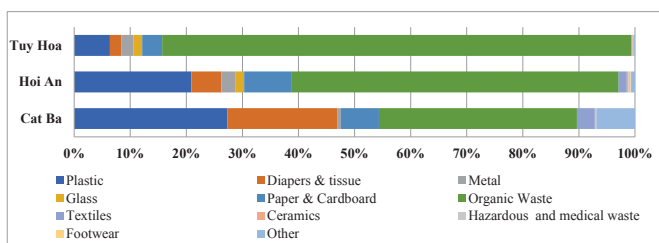


Fig. 5 Proportion of plastic waste from the hotels in 3 cities by volume.

This study also evaluated the composition of plastic waste in each studied city and the results are shown in Fig. 6. Among the types of plastic waste, PET plastic accounts for the highest proportion in Cat Ba (52.41%) and Tuy Hoa (39.81%), meanwhile, single-layer plastic accounts for the highest proportion (29.55%) in Hoi An. The generation rate of the multi-layer plastic packaging per guest day in Hoi An (0.0153 kg/guest) is approximately five times higher than in Tuy Hoa (0.0028 kg/guest) and eight times higher than in Cat Ba (0.0019 kg/guest). Survey interviews suggest that this may be related to cultural factors as most hotel visitors during the survey period were Korean, with a reported habit of bringing ready-to-eat food packages for their stay. Further, the amount of plastic waste (multi-layer plastic packaging) related to food packaging with the label origin from Korea is very high. This result suggests that the characteristics of waste, in general, and plastic waste, in particular, depend on the characteristics and consumption habits of the guests.

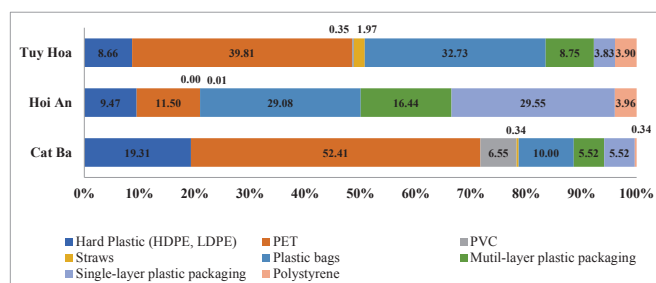


Fig. 6. Composition of plastic waste by weight from the hotels in 3 cities.

The percentage of recyclable plastic waste by weight was highest in Cat Ba (more than 71%), followed by Tuy Hoa is 48.47%, and Hoi An's recyclable plastic waste was the lowest in both weight and volume (21 and 23%, respectively). Comparing across large and small hotels, although the total waste generation rate is higher in larger hotels due to greater quantities of food waste (leftovers), there was no significant difference in plastic waste composition between the two hotel types. Thus, the rate of non-recyclable plastic waste from hotels in the study cities are still at a high level, especially in Hoi An. It is these significant challenges to the management of plastic waste in coastal cities. Audit results also show the need to address non-recyclable plastic in hotels. For example, the number of used plastic straws found was quite large, up to 0.8 kg of plastic straws/day (hundreds per day) from a 5-star hotel in Tuy Hoa city. However, most of these hotels are willing to stop providing plastic straws and convert to other types like bamboo, almond grass, or paper. Indeed, changes like these will require coordination with suppliers, the adoption of technically accurate advice, and awareness-raising with guests for modification or replacement, including the use of authentic biodegradable products. This should extend to other plastic items available for guests e.g., brushes, combs, and other toiletries, including their packaging.

3.4. Proposing the solutions in solid waste management from the hotel industry

Along with the study on generated solid waste, this work also conducted surveys of the current status of solid waste collection, its classification, and transportation from the hotels in the study areas. The results show that all hotels have contracted the collection and transportation of waste with a collection company, with a frequency of two times per day (morning and afternoon)

to ensure that all of generated wastes are collected the same day. For large-scale hotels, especially those with restaurants and bars, the daily amount of waste is very large. Some of the hotels sorted food waste and then sold or gifted it to livestock facilities, which has significantly reduced the amount of waste that has to be transported to landfills. The recyclable wastes are collected and sold to informal collection systems by the hotel's staff, and profits are transferred to the union fund of the hotel. However, most single-use plastics such as plastic bags, spoons, combs, toothbrushes, and straws can not be sold to a junk buyer due to low profit, thus, it transported to landfills. In addition, hotels in coastal areas often contain seafood shells in their waste composition that are heavy, fast-degrading, and smelly, along with coconut shells that are heavy and bulky. These types of waste are still being collected and treated through the municipal waste without being reused or recycled, and these conditions are placing even more pressure on landfills.

Based on the experimental data and survey results, our study proposes several solutions to effectively manage solid waste arising from the hotel systems including:

Internal communication within the enterprise to all employees and tourists to bring awareness and understanding to the meaning of waste management.

Most hotel management units want to change because they have a good understanding and awareness of environmental responsibility, however, there are still hotels that say they only change solid waste management activities upon request from local authorities. Some hotels are willing to change solid waste management practices such as reducing single-use plastics (water bottles, toiletries) or practicing plastic reduction activities, but are still afraid of the reaction of customers. The results show that communication programs to update knowledge and behaviour are very necessary for the tourism industry. Besides, tourists are an extremely important factor, so they will play a role in guiding the changing trends of hotels. If they are fully informed, after the change, they will become supporters and continue spreading the change to the community.

Development of a set of guiding criteria for waste management, waste classification, criteria for green hotels in the field of waste reduction, and honouring typical elements such as green labelling and corporate

image promotion. The local agencies of tourism and environmental management need to issue specific guidelines and routes to the hotels. In addition, it is necessary to cooperate with solid waste collection and treatment entities, the media, NGOs, etc.

Synchronizing the classification, collection and treatment system in the city. Waste segregation and waste minimization activities at hotels are only really effective when there is synchronization of the stages of solid waste management system such as classification at the source, collection, transportation, recycling, and treatment. This needs consensus from central and local management agencies and each citizen.

4. Conclusions

This study focused on the solid waste generation and composition of the hotel industry from three coastal cities in Vietnam. The results showed that the percentage of organic waste in hotels from lowest to highest was 35% for Cat Ba, 58% for Hoi An and 84% for Tuy Hoa. The percentage of food waste is similar with 30.5, 44.6, and 70.1% for Cat Ba, Hoi An, and Tuy Hoa, respectively. For large-scale hotels with in-house restaurants and bars, more attention must be paid to food waste separation, which can be linked to supporting the development of an associated value chain of using food scraps for livestock as well as compost. Presently, these hotels have initiated bio-product development from organic waste, e.g., by using fruit peels to develop organic detergents, thus, reducing some direct waste and improving the image of hotels as environmentally-friendly businesses. However, many other bio-products can also be developed from organic food waste from hotels and hospitality [20] and should be commercially explored. The percentage of recyclable plastic waste by weight is highest in Cat Ba (more than 71%), while, by volume, Tuy Hoa has the highest (52%). Hoi An's recyclable plastic waste is the lowest in both weight and volume (21 and 23%, respectively). To reduce plastic waste, the hotels also need to obtain technical guidance and work with their suppliers to request authentic environmentally-friendly packaging modifications or replacements based on the life cycle and other analyses.

At the waste collection level, it is necessary to both promote and connect the informal waste collection chain to hotel waste management systems and work with hotel

management to conduct training and increase awareness of the hotel staff so they too understand the benefits and importance of waste recovery [21]. Organic and plastic waste recovery can not only increase income but also improve the local environment on which their tourism businesses depend. This study contributes to raising awareness of the potential of waste recovery to guide waste management in coastal tourist cities in Vietnam. This work can also be used as a basis to encourage tourism service establishments to limit single-use plastic products and, in particular, actively classify, reuse, and recycle waste, as well as increase the use of environmentally-friendly hotel products.

CRediT author statement

Thi Hoa Tran, Boris Fabres, Ngoc Ha Hoang, Hoai Le Tran: Data curation, Software, Visualisation, Investigation, Writing, Methodology, Supervision, Revising the manuscript.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] UNWTO (2019), *International Tourism Highlights 2019 Edition*, 24pp.
- [2] UNEP (2003), *A Manual for Water and Waste Management: What The Tourism Industry Can Do to Improve Its Performance*, 68pp.
- [3] S. Bashir, S. Goswami (2016), "Tourism induced challenges in municipal solid waste management in hill towns: Case of Pahalgam", *Procedia Environmental Sciences*, **35**, pp.77-89, DOI: 10.1016/j.proenv.2016.07.048.
- [4] M.R. Dileep (2007), "Tourism and waste management: A review of implementation of 'zero waste' at Kovalam", *Asia Pacific Journal of Tourism Research*, **12(4)**, pp.377-392, DOI: 10.1080/10941660701823314.
- [5] C. Beretta, S. Hellweg (2019), "Potential environmental benefits from food waste prevention in the food service sector", *Resources, Conservation and Recycling*, **147**, pp.169-178, DOI: 10.1016/j.resconrec.2019.03.023.
- [6] K. Sealey, J. Smith (2014), "Recycling for small island tourism developments: Food waste composting at Sandals Emerald Bay, Exuma, Bahamas", *Resources, Conservation and Recycling*, **92**, pp.25-37, DOI: 10.1016/j.resconrec.2014.08.008.
- [7] IUCN (2005), *Marine News*, <https://www.iucn.org/our-work/oceans-and-coasts>, accessed 20 February 2021.
- [8] B. Worm, H.K. Lotze, I. Jubinville, et al. (2017), "Plastic as a persistent marine pollutant", *Annual Review of Environment and Resources*, **42(1)**, DOI: 10.1146/annurev-environ-102016-060700.
- [9] General Statistics Office (2019), *Statistical Yearbook of Vietnam 2019*, Statistical Publisher House (in Vietnamese).
- [10] Cat Hai District People Committee (2019), *Report on National Defense Security and Social-economic Situation 2019* (in Vietnamese).
- [11] Hoi An People Committee (2018), *White Book on Hoi An Solid Waste* (in Vietnamese).
- [12] General Statistics Office (2018), *Phu Yen Statistic Year Book 2018* (in Vietnamese).
- [13] P.H. Byer, C.P. Hoang, T. Nguyen, et al. (2006), "Household, hotel and market waste audits for composting in Vietnam and Laos", *Waste Manag. Res.*, **24(5)**, pp.465-472, DOI: 10.1177/0734242X06068067.
- [14] L. Son, Y. Matsui, D.T.T. Trang, et al. (2018), "Estimation of the solid waste generation and recycling potential of the hotel sector: A case study in Hue city, Vietnam", *Journal of Environmental Protection*, **9(7)**, pp.751-769, DOI: 10.4236/jep.2018.97047.
- [15] S. Phu, M. Hoang, T. Fujiwara (2018), "Analyzing solid waste management practices for the hotel industry", *Global J. Environ. Sci. Manage.*, **4(4)**, pp.19-30, DOI: 10.22034/gjesm.2018.04.01.003.
- [16] M. Hoang, T. Fujiwara, S. Phu (2017), "Municipal waste generation and composition in a tourist city - Hoi An, Vietnam", *Journal of Japan Society of Civil Engineers*, **5(1)**, pp.123-132, DOI: 10.2208/journalofjsce.5.1_123.
- [17] S.T.P. Phu, T. Fujiwara, M.G. Hoang, et al. (2018), "Waste separation at source and recycling potential of the hotel industry in Hoi An city, Vietnam", *Journal of Material Cycles and Waste Management*, **21(1)**, pp.23-34, DOI: 10.1007/s10163-018-0807-5.
- [18] D.N. Trung, S. Kumar (2005), "Resource use and waste management in Vietnam hotel industry", *Journal of Cleaner Production*, **13(2)**, pp.109-116, DOI: 10.1016/j.jclepro.2003.12.014.
- [19] S. Otoma, I. Miyazaki, H. Hoang, et al. (2013), "A survey on municipal solid waste and residents awareness in Da Nang city, Vietnam", *Journal of Material Cycles and Waste Management*, **15(2)**, pp.187-194, DOI: 10.1007/s10163-012-0109-2.
- [20] P.F. Albizzati, D. Tonini, T.F. Astrup (2021), "High- value products from food waste: An environmental and socioeconomic assessment", *Sci. Total Environ.*, **755(1)**, DOI: 10.1016/j.scitotenv.2020.14.2466.
- [21] Global Alliance for Incinerator Alternatives (2019), "Cities waste assessment and brand audit (WABA) methodology and toolkit", https://www.no-burn.org/wp-content/uploads/Waste-Assessment-and-Brand-Audit-Methodology-Vol-1_-Households.pdf, accessed 20 February 2021.

Effects of bentonite and zeolite minerals on mobility of lead in paddy soil in Chi Dao commune, Van Lam district, Hung Yen province, Vietnam

Tu Ngoc Nguyen*, Huy Quang Trinh, Cong Huy Vo

Vietnam National University of Agriculture, Trau Quy Town, Gia Lam District, Hanoi, Vietnam

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Abstract:

Used lead-acid battery recycling activities in Minh Khai handicraft village, Chi Dao commune, Van Lam district, Hung Yen province, Vietnam has markedly increased the lead (Pb) content in paddy soil. Reducing the mobility of lead and lead accumulation in rice plants/plain rice are major priorities to reduce the impacts of lead in paddy soil. Application of the minerals zeolite (4A and Faujasite) and bentonite (natural and modified) to lead-contaminated soil has been carried out in lab scale for three years. The results showed the efficiencies in reducing accumulated lead in rice were 58 and 56% after adding the artificial additives zeolite 4A and zeolite Faujasite, respectively. These results were better than those of modified bentonite and natural bentonite, which were only 44 and 24%, respectively. The control efficiency of Pb accumulated in rice plants between the supplemented samples of zeolite Faujasite, zeolite 4A, modified bentonite, and natural bentonite were 69, 56, 42, and 40%, respectively, compared with the control samples. The addition of minerals to the soils has also resulted in decreases of the growth and yield of the experimental rice plants compared with the control samples. In this research, 0.1 to 0.2% of zeolite Faujasite showed the best results in terms of reducing Pb content in soil as well as low effect on plant growth. This research opens up on-site pollution control solutions for lead-contaminated agricultural soils.

Keywords: heavy metals, lead immobilizing, minerals, rice uptake.

Classification number: 5.3

1. Introduction

Lead content in natural soil ranges from 10 to 50 ppm [1]. Due to biogeochemical cycling changes and imbalances from manmade activities such as use of fertilizer [2, 3], manure [4], sludge disposals [5], or polluted irrigation water [6, 7] result in the accumulation of lead in soil and create risks to human health and ecology [8]. Lead in soil may be in a soluble form, or found as lead inorganic compounds PbS , $PbSO_4$, $PbSO_4 \cdot PbO$, α - PbO [9], or be associated with organic compounds such as amino acids, fulvic acids, and humic acids [10]. The mobility of lead in soil is largely controlled by pH [11, 12], the presence of organic matter [13], and clay mineral content [14]. Lead phytoavailability and toxicity are dependent on their speciation.

Zeolite is the general name for aluminosilicate minerals called tectosilicates, which have three-dimensional frameworks [15]. Zeolite has high cation exchange capacity and selective absorption, so it is widely used in environmental treatment especially for heavy metal absorption in contaminated soils [16-19]. Chemical stabilization of heavy metals by adding artificial additives has been evaluated as one of the most cost effective in situ remediation techniques for metal contaminated sites [16, 20]. Chemical stabilization may lead to a decrease in extractable metal content in soil [21] and metal phytoavailability in plants [16, 22].

Used lead-acid battery recycling activities in Minh Khai handicraft village, Chi Dao commune, Van Lam district, Hung Yen province, Vietnam discharges copious amounts of acidic wastewater and causes soil and water pollution. Some studies reported that Pb concentration in soils in the handicraft village exceeded the allowable value [23, 24] and causes major health issues in the local community [25, 26]. Therefore the agricultural soil surrounding the handicraft village is not safe enough for cultivation.

This study was implemented to evaluate and determine a suitable in situ remediation for Pb-contaminated sites by adding artificial minerals into soils to immobilize lead and decrease its phytoavailability in rice plants. Some effects of additives on the rice growth (such as plant height, number of panicles, length, and weight of plain rice) in this study were also determined.

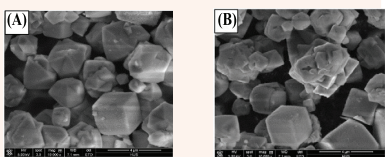
2. Materials and methods

2.1. Materials

Zeolite minerals: In this study, minerals of zeolite 4A and zeolite Faujasite were synthesized from silica particles of rice straw. The hydrothermal crystallization method was used to synthesize zeolite minerals and the products, shown in Table 1, were characterized using x-ray powder diffraction (XRD) and observed by scanning electron microscope (SEM).

*Corresponding author: Email: nguyennngoctu@vnua.edu.vn

Table 1. Properties of zeolite minerals.

No.	Element	Zeolite 4A	Zeolite Faujasite
1	Chemical formula	$\text{Na}_{12}\text{Al}_{12}\text{Si}_{12}\text{O}_{48} \cdot 27\text{H}_2\text{O}$	$\text{Na}_2\text{Al}_2\text{Si}_{25}\text{O}_9 \cdot 6\text{H}_2\text{O}$
2	Mineral compositions	Na_2O ; Al_2O_3 ; SiO_2	Na_2O ; Al_2O_3 ; SiO_2
3	Crystalline size, μm	2.5	4
4	CEC, meq 100 g^{-1}	341	432
5	Pb absorption efficiency, %	82.67	96.56
6	SEM captured off (A) zeolite 4A and (B) zeolite Faujasite		

Natural bentonite and modified bentonite minerals: The natural bentonite in this research was collected from the Tam Bo bentonite mines, Di Linh district, Lam Dong province, Vietnam. The mineral was compounded by high Montmorillonite content (about 64%) while the remains were Kaolinite (9.5%), Illite (6.0%), Quartz (5.0%), Feldspar (3.5%), Goethite (3.0%), Canxit (little), and other minerals. Chemical compositions of the natural bentonite were mainly composed of SiO_2 (50.5%), Al_2O_3 (17.67%), and Fe_2O_3 (7.0%). The mineral had a CEC of $19.5\text{ meq } 100\text{ g}^{-1}$ and the basal spacing of $15.49\text{ \AA}$.

Al-pillared bentonite was created by activating the natural bentonite with polyoxymetal cations of Al solution. The activated mineral had CEC of $58.6\text{ meq } 100\text{ g}^{-1}$ and the basal spacing of $16.81\text{ \AA}$.

Contaminated-Pb soil samples: Soil samples used in the research were collected from the 0-20 cm surface layers of 10 small scale paddy fields surrounding the used lead-acid battery recycling facilities in Minh Khai handicraft village, Chi Dao commune, Van Lam district, Hung Yen province, Vietnam (Fig. 1).

**Fig. 1. Soil sampling locations.**

Rice plants: Greenhouse pot experiments were conducted at the Vietnam National University of Agriculture (VNUA) and used to evaluate the effects of zeolite and bentonite minerals on the immobility of lead in soil and the growth and grain yield of rice plants. The Bac Thom No.7 resistance leaf blight variety was used in the pot experiments. Rice plants of age 10-13 days after sowing were planted in the experimental pots. Three rice plants with the same height were planted in each experimental pot.

Rice grain samples: The rice grains were used in this research to determine the effectivity of lead cumulative control after adding mineral additives to the soil. These rice grains were collected from the experimental pots.

2.2. Methods

Soil analysis: Soil samples were examined by the PIXE method (particle-induced x-ray emission) to determine its chemical composition. Other physio-chemical properties of the soil samples such as pH, electro-conductivity (EC), texture, and organic matter (OM) content were also determined.

Plant-available Pb analysis: Pb phytoavailability was extracted from soil by the diethylenetriamine pentaacetic acid (DTPA) method at a pH of 7.3. Each 10 g portion of air-dried soil was passed through a 2.0-mm sieve to which 20 ml DTPA extractant was added. The suspensions were shaken at 175 rpm for 2 h. The experiments were terminated by filtration of the suspension by a cellulose acetate filter, then determining the soluble ion of Pb using ICP-OES (PE 7300 V-ICP, Perkin Elmer).

Determination of Pb content in rice plant and grain: Pb content in rice plants and grains was determined by using aqua regia (3:1 HCl/HNO_3). Briefly, 50 mg of dried sample was drilled and digested in 50 ml of the aqua regia solution. The solution was then gently shaken and filtered by a cellulose acetate filter. The soluble ion of Pb was determined using ICP-OES (PE 7300 V-ICP, Perkin Elmer).

Greenhouse pot experimental design method: After assessing the composition and properties of the soil, the soil samples were mixed together and then NPK fertilizer was added with an amount of 25 kg per 360 m^2 (corresponding 1.1 g per experimental pot). This soil was then filled into the experimental pots (30 cm diameter x 20 cm height). The experiment was conducted over three seasons. Four types of minerals (natural and modified bentonite, zeolite 4A, and zeolite Faujasite) with six treatments (5 levels of additives from 0.1 to 0.5% and the control) were replicated three times in one season resulting in 72 pots ($4 \times 6 \times 3$) in total (Table 2). The weight of both soil and added mineral was 5 kg in total. The Bac Thom No.7 resistance leaf blight variety was used in the pot experiment, which was submerged in 5 cm of water over the entire growth period. Three seedlings 13 days in age

were planted in each pot. NPK 17-12-5 fertilizer with a dosage of 1.1 g per pot was supplemented three times during the experiment beginning at basal fertilizing (before experiment operation), then the first application of fertilizer (10 days after plantation), and second application addition fertilizer (49 days after plantation) (Table 3).

Table 2. Lab-scale experimental design.

Element	Control	Level 1	Level 2	Level 3	Level 4	Level 5
Ration (w/w)	0%	0.1%	0.2%	0.3%	0.4%	0.5%
Mineral amount, g	0	5	10	15	20	25
Soil amount, g	5,000	4,995	4,990	4,985	4,980	4,975
Total, g	5,000	5,000	5,000	5,000	5,000	5,000

Table 3. Growth stages of rice plant and evaluated elements.

No	Stages	Time, day	Element
1	Seeding	13	Pb total, extractable Pb in soil, pH _{KCl}
2	Transplanting	26	- Extractable Pb in soils - pH _{KCl}
3	Tillering	36	
4	Panicle formation	49	
5	Flowering	61	
6	Harvest	109	- pH _{KCl} , extractable Pb in soil, Pb content in rice plant and polished rice, height of rice plant, number of panicles, length of rice grain and weight of 1000 grains.

3. Results and discussion

3.1. Compositions and properties of soil samples

The results show that soil pH_{KCl} values ranged from 3.4 to 5.2 with an average of 4.1. The soil in the study area is acidic. Although the sampling locations were within a narrow area, the variations in pH_{KCl} value were relatively large. This can be explained by external effects such as the use of wastewater containing high H⁺ ions discharged from the village for irrigation. The low pH_{KCl} values in soil may lead to increase in the risk of pollution by mobilizing heavy metals and thus increasing its bioavailability for plants [27] (Table 4).

The OM contents of soil samples ranged from 1.33 to 2.44%. According to the Ministry of Natural Resources and Environment (2015) [28], the soil samples from this area were from the low-to-medium organic matter content groups (from 2.60-3.36%). Soil texture analysis showed that the proportion of clay ranged from 3.7 to 8.0%, limon from 50.0 to 63.1%, and sand from 32.1 to 46.3%. The average CEC value was about 12.8 meq 100g⁻¹. These low CEC and organic matter values contribute to conditions that make the exchange of Pb content in the soil high. Total Pb content in the 10 soil samples ranged from 403.8 to 1766 mg kg⁻¹ with an average of 999±322.28 mg kg⁻¹.

Table 4. Soil compositions and properties in the study.

Parameters	Soil sample No.									
	S ₀₁	S ₀₂	S ₀₃	S ₀₄	S ₀₅	S ₀₆	S ₀₇	S ₀₈	S ₀₉	S ₁₀
pH _{KCl}	3.40 (±0.01)	3.59 (±0.01)	3.41 (±0.01)	3.90 (±0.01)	4.14 (±0.01)	3.92 (±0.01)	4.65 (±0.02)	4.59 (±0.01)	3.69 (±0.01)	5.20 (±0.03)
OM (%)	2.44 (±0.02)	2.24 (±0.02)	2.39 (±0.05)	2.09 (±0.02)	1.89 (±0.02)	2.90 (±0.05)	2.04 (±0.02)	2.12 (±0.02)	2.60 (±0.05)	1.33 (±0.04)
EC (μS cm ⁻¹)	305.3 (±5.51)	339.3 (±9.71)	234.3 (±17.6)	163.3 (±2.46)	178.7 (±11.3)	165.2 (±4.07)	129.5 (±4.90)	156.2 (±4.16)	163.5 (±4.15)	92.6 (±2.20)
CEC meq 100g ⁻¹	13.0 (± 3.7)	15.4 (± 1.9)	12.2 (±4.6)	11.4 (± 3.1)	11.6 (± 4.7)	13.2 (± 1.5)	12.7 (±0.8)	11.9 (±3.4)	13.5 (± 2.4)	12.8 (± 4.6)
Pb, mg kg ⁻¹	1,116	921	1,014	1,090	1,766	972	816	827	1,064	404
Cu, mg kg ⁻¹	218.3	208.3	184.2	240.0	297.0	178.4	189.5	189.3	209.8	183.5
Zn, mg kg ⁻¹	219.5	228.7	271.7	289.7	264.5	263.0	202.7	200.1	220.3	174.3
Ni, mg kg ⁻¹	44.09	38.98	46.20	46.99	39.72	40.61	47.18	40.05	39.99	44.37

3.2. Effects of minerals on plant-available Pb in soil and Pb-uptake by rice plant

Effects on plant-available Pb content in soils: Analysis results after three consecutive experiments showed that there was a signification decrease in the concentration of Pb extracted by the DTPA solution when the four types of adsorbents at different levels were added. The average mobile Pb content in the control sample was 53.94 mg kg⁻¹. After the experiment (over 3 crops, 109 days for summer-autumn crop or 137 days for winter-spring crop), the average content over 3 crops of mobile Pb was about 32.26 mg kg⁻¹ achieving an efficiency of about 40.14% reduction in soluble Pb content in the soil (Fig. 2). One-way ANOVA analysis showed that the difference in Pb content values between the control and mineral added samples was significant (p<0.05).

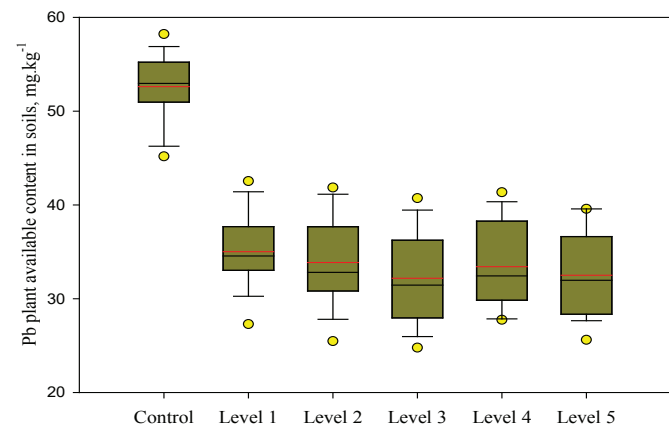


Fig. 2. Plant-available Pb content in soils in relation to minerals added. Amount of added minerals, level 1: 0.1%; level 2: 0.2%, level 3: 0.3%, level 4: 0.4%; level 5: 0.5%. The above Pb contents were calculated by the average value of 4 minerals within 3 crops.

Although the immobility of Pb in soil by two minerals of zeolite and bentonite was relatively good during the whole experiment, there was a significant difference between the growth stages of the rice plants. The effect of minerals on Pb mobility was shown immediately after two growth stages of sowing (day 13) and transplanting (day 26). In the subsequent stages, the degree of reduction in mobile Pb content was lower. The impact of minerals reached an equilibrium state after the first two growth stages of the rice plants (Fig. 3).

Effects on Pb content uptake by rice plant: The results from three experimental crops showed a significant difference in Pb content in rice plants depending on the type and level of minerals added to the soil. All of the mineral-supplemented pots showed a lower Pb accumulated content in rice compared to the control pots. The ability of the minerals to control Pb accumulation in rice plants ranged from 24 to 58% compared to the control samples. The efficiency of reducing Pb accumulation in rice plants treated with the minerals zeolite 4A and zeolite Faujasite was 58 and 56%, respectively, which was better than the modified and natural bentonite minerals (44 and 24%,

respectively). The largest content of Pb intake in the rice plants reached 78.76 mg kg^{-1} (dry biomass) at the pot with natural bentonite added, meanwhile, the lowest Pb content was only 52.41 mg kg^{-1} with the supplementation of zeolite 4A. In the two experiments that added zeolite Faujasite and modified bentonite, the amount of Pb accumulated in rice was 57.07 mg kg^{-1} and 70.82 mg kg^{-1} , respectively (Fig. 4).

There is a relatively strong correlation between the Pb content accumulation in rice plants and the additional mineral levels as seen from R^2 ranging from 0.66 to 0.94 ($n=15$). Although the capacity to control Pb content in rice varied between the types of mineral supplementation, the general trend for all four materials was that Pb concentration in rice decreased with the increase of mineral amount added to the soil.

Effects on Pb contents in rice grain: There was a significant difference in the extracted Pb content between the control and mineral-added samples for all four types of adsorbent. The general trend was that the Pb content of rice was much lower than that of the control treatment. The more mineral

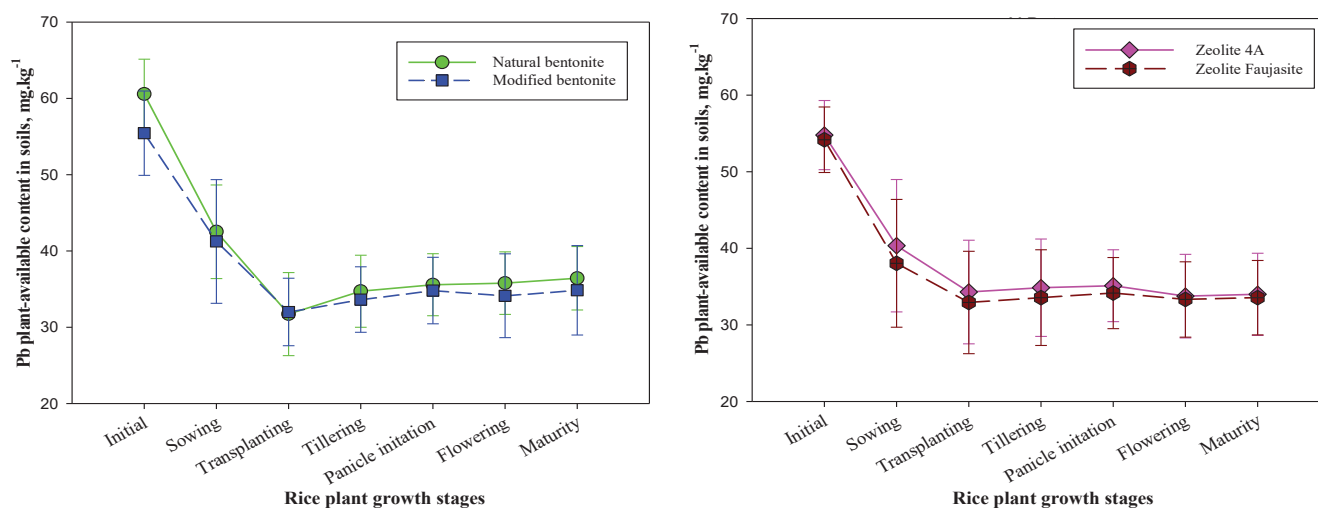


Fig. 3. Ranges of soluble Pb content in soil during growth stages of rice plants.

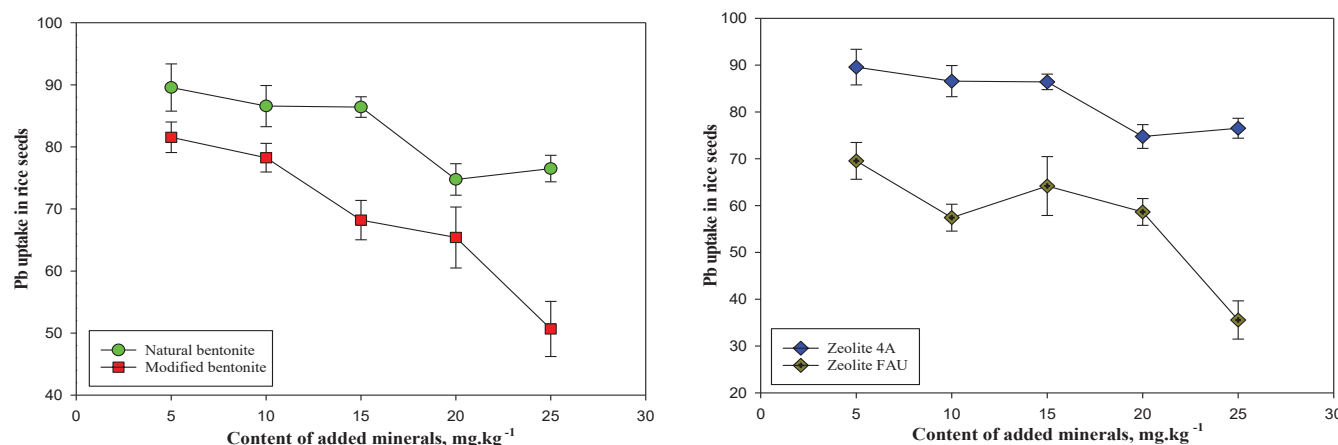


Fig. 4. Pb content accumulated in rice plants with amount of added minerals.

supplementation, the lower the Pb content in rice. The mean impact of controlling Pb content in rice when comparing the supplement levels of zeolite Faujasite, zeolite 4A, modified bentonite, and natural bentonite was 69, 56, 42, and 40%, respectively (Table 5).

Table 5. Intake Pb contents in rice grain.

Type of minerals	Pb contents in rice grain, ($\mu\text{g}\cdot\text{g}^{-1}$)					
	Control	Level 1	Level 2	Level 3	Level 4	Level 5
Natural bentonite	0.81 ± 0.01	0.42 ± 0.02	0.57 ± 0.06	0.65 ± 0.05	0.39 ± 0.03	0.42 ± 0.04
Modified bentonite	0.78 ± 0.05	0.40 ± 0.03	0.54 ± 0.11	0.56 ± 0.09	0.38 ± 0.06	0.38 ± 0.03
Zeolite 4A	0.75 ± 0.07	0.58 ± 0.06	0.37 ± 0.09	0.26 ± 0.08	0.20 ± 0.01	0.24 ± 0.02
Zeolite Faujasite	0.67 ± 0.05	0.35 ± 0.16	0.23 ± 0.04	0.23 ± 0.06	0.12 ± 0.03	0.11 ± 0.03
Std. threshold [†]	$0.2\ \mu\text{g}\cdot\text{g}^{-1}$					

Amount of added minerals, level 1: 0.1%; level 2: 0.2%, level 3: 0.3%, level 4: 0.4%; level 5: 0.5%; [†]: QCVN 8-2:2011 of the Ministry of Health.

The content of Pb accumulated in rice varied considerably (0.11 to $0.65\ \mu\text{g}\cdot\text{g}^{-1}$) between mineral added levels, and these values were lower than that of the control samples (from 0.67 to $0.81\ \mu\text{g}\cdot\text{g}^{-1}$). The mean Pb intake by rice among the mineral supplements was significantly different when compared with the control sample ($p<0.05$, $n=9$). The most general trend was that Pb intake in rice decreased in accordance with an increase in added mineral amount. Artificial minerals of the zeolite group have better control on Pb accumulation in rice than minerals of the bentonite group. The content of Pb in different parts of the plant tended to decrease in the order of root > stem > leaf > flower > seed. J. Liu, et al. (2003) [29] showed that the ratio of Pb content in root:stem:leaf of the rice plants was 60:5:1 at the flowering stage and 19.4:2.9:1 at the mature stage. In this study, Pb concentrations in rice plants were 155 to 274 times greater than that in polished rice. This is because the fixation of Pb to the root cell wall is greater than that of other plant parts [30]. The addition of minerals to the soil reduced the Pb accumulation in rice plants and this led to a decrease in the accumulation of Pb in rice grains. Due to the higher CEC of the artificial minerals in the zeolite group (zeolite 4A and Faujasite zeolite are 341 and 432 meq.100 g^{-1} , respectively) compared to the bentonite group (natural bentonite and modified bentonite are 19.5 and 58.6 meq.100 g^{-1} , respectively), a difference in the Pb concentration in the rice grain is understood.

3.3. Effects of minerals on rice plant grow

Effects on rice plant's height: At the mature stage, the height of the rice plants in all experimental treatments reached an average value of 67.65 cm. However, the growth heights of these plants varied between different types of added minerals. While the average height of the rice plants in the control sample was 84.25 cm, the maximum growth height in the formula with natural bentonite was only 74.33 cm.

The lowest height occurred with the Faujasite zeolite mineral supplement experiment at only 66.64 cm. The average heights of the rice plants in the two treatments supplemented with zeolite 4A and modified bentonite was 73.38 cm and 70.77 cm, respectively (Fig. 5). The research by N. Hung, et al. (2019) [31] showed that less than 10 $\text{mg}\cdot\text{kg}^{-1}$ Pb content in soil can promote rice growth and increase tillering ability and root length. However, concentrations greater than 10 $\text{mg}\cdot\text{kg}^{-1}$ will inhibit the tillering stage and plant height ($R^2=0.8-0.9$, $p<0.05$).

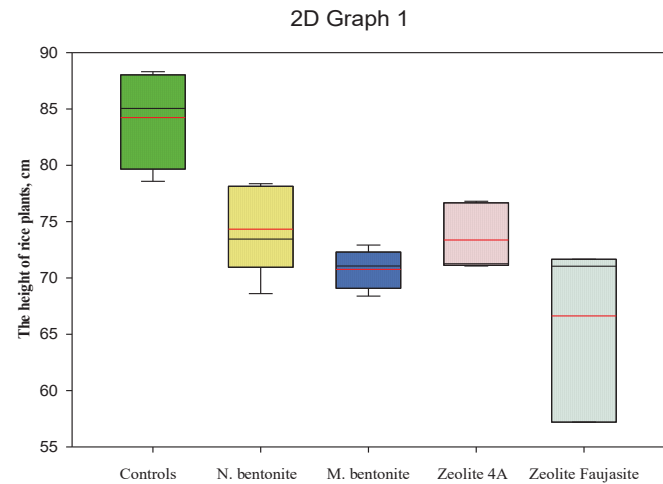


Fig. 5. The height of rice plants in different rate of added minerals.

Effects on the number of rice panicle: The average number of rice panicles obtained in the experiments of adding natural bentonite, modified bentonite, and zeolite 4A was 11.2, 12.4, and 12.8 panicles per crop, respectively. For zeolite Faujasite, the average number of panicles was only 9.0 per crop. Thus, the rice cultivation efficiency of the soil amended with Faujasite zeolite was much lower than that of other minerals. In addition, the average number of rice panicles of the experimental treatments was also 14.17 panicles lower than that of the control samples. These results show that the amount of rice panicles

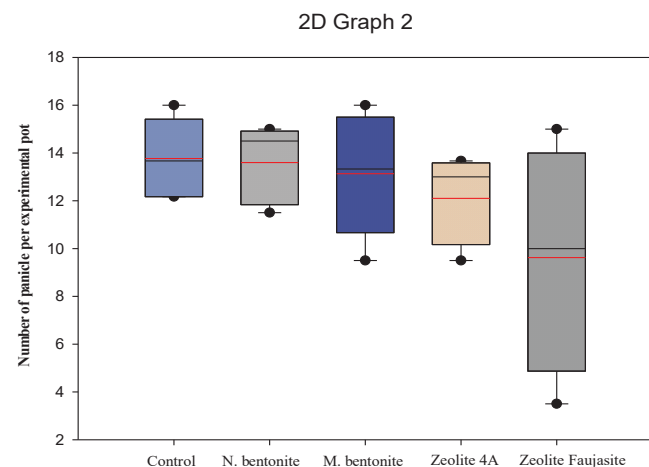


Fig. 6. Number of rice panicles based on the type of mineral added.

was significantly affected by the amount of minerals added to the cultivated soil environment, especially for Faujasite zeolite at the levels of 0.4-0.5% by weight (Fig. 6).

Effects on the length and weight of rice grain: The obtained average rice grain length ranged from 5.88 to 6.25 mm, meanwhile, this value in the control sample was 6.27 mm. With the average grain length of the blight-resistant Bac Thom No. 7 cultivated under the standard conditions of 6.2 - 6.3 mm, all four experiments in this study yielded lower mean grain lengths than those in the control sample (Table 6). The weight of 1,000 grains of rice listed from 4 experimental formulas in this study ranged from 18.47 to 18.85 grams/1,000 seeds (Fig. 7). Both agronomic indicators of rice yield (the length and weight of 1,000 grains) showed that these values in the mineral-added formulas were lower than in the control ($p < 0.05$, $n = 12$) and lower than in the normal growth conditions of the rice varieties, which are 6.2-6.3 cm and 19.0, respectively [32].

Table 6. The length and weight of 1000 rice grains ($n = 3$).

Additives	Parameters	Control	Level 1	Level 2	Level 3	Level 4	Level 5
Natural bentonite	Length of seed (mm)	6.29±0.01	6.14±0.02	6.13±0.01	6.18±0.05	6.01±0.13	6.25±0.04
	Weight of 1000 grains (g)	19.46±0.05	18.61±0.28	18.86±0.13	18.72±0.16	18.59±0.10	18.71±0.21
Modified bentonite	Length of seed (mm)	6.22±0.04	6.11±0.09	6.07±0.15	5.88±0.15	6.02±0.17	5.88±0.18
	Weight of 1000 grains (g)	19.20±0.22	18.55±0.26	18.52±0.12	18.46±0.04	18.33±0.26	18.51±0.19
Zeolite 4A	Length of seed (mm)	6.30±0.02	6.23±0.05	6.17±0.13	6.16±0.08	6.09±0.18	6.01±0.21
	Weight of 1000 grains (g)	19.29±0.34	18.92±0.28	18.93±0.18	18.97±0.17	18.86±0.06	18.55±0.21
Zeolite Faujasite	Length of seed (mm)	6.26±0.03	5.99±0.15	5.99±0.10	6.21±0.04	6.01±0.04	6.25±0.02
	Weight of 1000 grains (g)	19.24±0.20	18.51±0.09	18.89±0.10	18.80±0.28	18.35±0.24	18.69±0.35

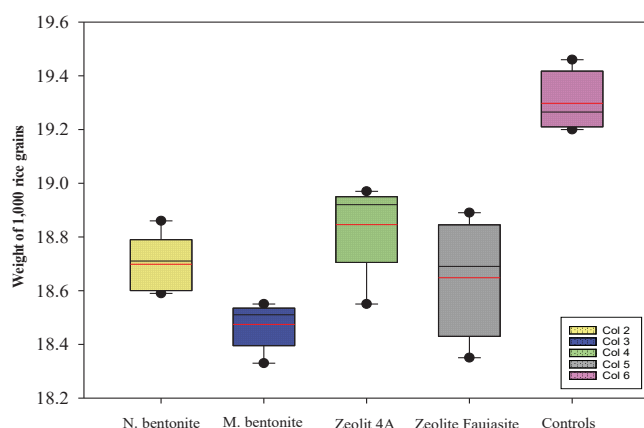


Fig. 7. Weight of 1,000 rice grains under different amount of added minerals.

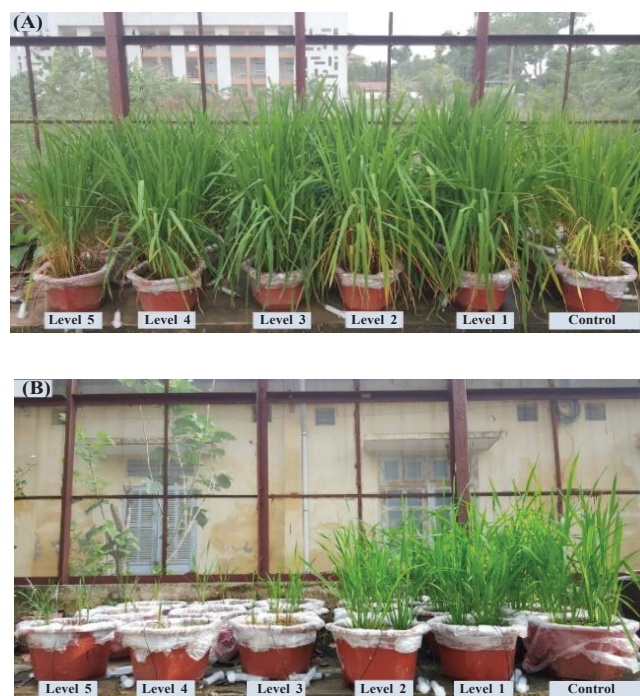


Fig. 8. (A) The relatively uniform growth and (B) the phenomenon of dead of plants with the addition of zeolite Faujasite in the first crop.

The decline in the growth and yield of rice plants can be explained by the fact that materials with a very high cation-exchange capacity (CEC) have claim on the nutrient minerals in the soil and reduce the plant's access to these nutrients thus affecting some agricultural agronomic indicators of the rice plants.

4. Conclusions

The agricultural soil for rice cultivation in Minh Khai village, Chi Dao commune, Van Lam district, Hung Yen province is acidic (pH_{KCl} ranges from 3.4 to 5.2), has low cation exchange capacity (about $13.2 \text{ meq.}100 \text{ g}^{-1}$), and is classified from silty to sandy silt. Pb content in soil in the area surrounding the craft village is at a high level, which is 17 times higher than the National Technical Regulation on the allowable limits of heavy metals in soils. The addition of minerals of zeolite and bentonite groups at the rate of 0.1 to 0.5% significantly reduced the mobility of Pb metal in the soil solution extracted by DTPA. The efficiency of reducing the mobile Pb content in soil decreased from 24 to 58% compared with the control sample. The ability to control Pb flexibility in the soil of the zeolite mineral group was higher than that of the bentonite group. The effect of reducing the flexible Pb content in the soil led to a decrease in Pb accumulation in rice and rice plants ($R^2 = 0.66-0.93$; $n = 15$). The addition of minerals of the zeolite and bentonite groups had a deterrent effect on the growth and yield of experimental rice plants. The most appropriate mineral

supplementation ratio in this study was found to be between 0.1 and 0.2%. From the research results, it is possible to use artificial zeolite minerals from agricultural by-products or modified bentonite minerals to limit the mobility of Pb^{2+} metal in the soil and reduce its accumulation in plants.

CRediT author statement

Tu Ngoc Nguyen, Huy Quang Trinh, Cong Huu Vo: Data curation, Software, Visualisation, Investigation, Writing, Methodology, Supervision, Revising the manuscript.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article

REFERENCES

- [1] M.J. McLaughlin, D.R. Parker, J.M. Clarke (1999), "Metals and micronutrients-food safety issues", *Field Crops Research*, **60**(1-2), pp.143-163, DOI: 10.1016/S0378-4290(98)00137-3.
- [2] M.M. Lasat (2000), "Phytoextraction of metals from contaminated soil: A review of plant/soil/metal interaction and assessment of pertinent agronomic issues", *Journal of Hazardous Substances Research*, **2**, DOI: 10.4148/1090-7025.1015.
- [3] S.R. Tariq, M. Shafiq, G.A. Chotana (2016), "Distribution of heavy metals in the soils associated with the commonly used pesticides in cotton fields", *Scientifica*, **2016**, DOI: 10.1155/2016/7575239.
- [4] N.T. Basta, J.A. Ryan, R.L. Chaney (2005), "Trace element chemistry in residual-treated soil: Key concepts and metal bioavailability", *Journal of Environmental Quality*, **34**(1), pp.49-63, DOI: 10.2134/jeq2005.0049dup.
- [5] K. Weggler, M.J. McLaughlin, R.D. Graham (2004), "Effect of chloride in soil solution on the plant availability of biosolid-borne cadmium", *Journal of Environmental Quality*, **33**(2), pp.496-504, DOI: 10.2134/jeq2004.4960.
- [6] S.D. Pam, S.B. Sally, H. Dean, et al. (2003), "Metal bioavailability and speciation in a wetland tailings repository amended with biosolids compost, wood ash, and sulfate", *Journal of Environmental Quality*, **32**(3), pp.851-864, DOI: 10.2134/jeq2003.8510.
- [7] J. Bjuhr (2007), *Trace Metals in Soils Irrigated with Waste Water in a Periurban Area Downstream Hanoi City, Vietnam*, Sveriges lantbruksuniversitet, 50pp.
- [8] J.J. D'Amore, S.R. Al-Abed, K.G. Scheckel, et al. (2005), "Methods for speciation of metals in soils: A review", *Journal of Environmental Quality*, **34**, pp.1707-1745, DOI: 10.2134/jeq2004.0014.
- [9] G. Uzu, S. Sobanska, Y. Aliouane, et al. (2009), "Study of lead phytoavailability for atmospheric industrial micronic and sub-micronic particles in relation with lead speciation", *Environmental Pollution*, **157**(4), pp.1178-1185, DOI: 10.1016/j.envpol.2008.09.053.
- [10] C.B. Tabelin, T. Igarashi (2009), "Mechanisms of arsenic and lead release from hydrothermally altered rock", *Journal of Hazardous Materials*, **169**(1-3), pp.980-990, DOI: 10.1016/j.jhazmat.2009.04.049.
- [11] H.A. Elliott, M.R. Liberati, C.P. Huang (1986), "Competitive adsorption of heavy metals by soils", *Journal of Environmental Quality*, **15**, pp.214-219, DOI: 10.2134/jeq1986.00472425001500030002x.
- [12] S. Sauve, M. McBride, W. Hendershot (1998), "Soil solution speciation of lead (II): Effects of organic matter and pH", *Soil Science Society of America Journal*, **62**(3), pp.618-621, DOI: 10.2136/sssaj1998.03615995006200030010x.
- [13] F. Margaret, T. Yiping, Z. Ping, et al. (2013), "Extractability and bioavailability of Pb and As in historically contaminated orchard soil: Effects of compost amendments", *Environmental Pollution*, **177**, pp.90-97, DOI: 10.1016/j.envpol.2013.02.013.
- [14] E.E. Hildebrand, W.E. Blum (1974), "Lead fixation by iron oxides", *Naturwissenschaften*, **61**(4), pp.169-170, DOI: 10.1007/BF00602595.
- [15] E. Erdem, N. Karapinar, R. Donat (2004), "The removal of heavy metal cations by natural zeolites", *Journal of Colloid and Interface Science*, **280**(2), pp.309-314, DOI: 10.1016/j.jcis.2004.08.028.
- [16] S.F. Cheng, Z.Y. Hseu (2002), "In-situ immobilization of cadmium and lead by different amendments in two contaminated soils", *Water, Air, and Soil Pollution*, **140**(1-4), pp.73-84, DOI: 10.1023/A:1020132106541.
- [17] Z. Ioannou, T. Papapostolou, N. Georgoulas, et al. (2012), "Use of modified zeolites for the remediation of waters and cultivated soils from Cu(II)", *Water, Air & Soil Pollution*, **223**(9), pp.5841-5854, DOI: 10.1007/s11270-012-1320-4.
- [18] S.R. Mallampati, Y. Mitoma, T. Okuda, et al. (2012), "Enhanced heavy metal immobilization in soil by grinding with addition of nanometallic Ca/CaO dispersion mixture", *Chemosphere*, **89**(6), pp.717-723, DOI: 10.1016/j.chemosphere.2012.06.030.
- [19] A. Argiri, Z. Ioannou, A. Dimirku (2013), "Impact of new soil amendments on the uptake of lead by crops", *Communications in Soil Science and Plant Analysis*, **44**(1-4), pp.566-573, DOI: 10.1080/00103624.2013.745214.
- [20] B. Gworek (1992), "Lead inactivation in soils by zeolites", *Plant and Soil*, **143**(1), pp.71-74, DOI: 10.1007/BF00009130.
- [21] A. Moirou, A. Xenidis, I. Paspaliaris (2001), "Stabilization Pb, Zn, and Cd-contaminated soil by means of natural zeolite", *Soil and Sediment Contamination*, **10**(3), pp.251-267, DOI: 10.1080/20015891109248.
- [22] X. Xian (1989), "Effect of pH on chemical forms and plant availability of cadmium, zinc and lead in polluted soils", *Water, Air and Soil Pollution*, **45**, pp.265-273, DOI: 10.1007/BF00283457.
- [23] Q.H. Phan (2011), *Study on Removal of Lead (Pb), Copper (Cu) and Zinc (Zn) in Paddy Soil by Biological Technology*, Doctoral Thesis, Hanoi University of Agriculture (in Vietnamese).
- [24] V.H. Cao (2012), "Evaluation for lead and copper pollution in agricultural land in Van Lam district, Hung Yen province", *Journal of Science and Development*, **10**(4), pp.648-653 (in Vietnamese).
- [25] T. Noguchi, T. Itai, N.M. Tue, et al. (2014), "Exposure assessment of lead to workers and children in the battery recycling craft village, Dong Mai, Vietnam", *Journal of Material Cycles and Waste Management*, **16**(1), pp.46-51, DOI: 10.1007/s10163-013-0159-0.
- [26] F. Takashi, E. Akifumi, A. Tetsuro, et al. (2016), "Lead contamination in surface soil on roads from used lead-acid battery recycling in Dong Mai, Northern Vietnam", *Journal of Material Cycles and Waste Management*, **18**(4), pp.599-607, DOI: 10.1007/s10163-016-0527-7.
- [27] D. Houben, L. Evrard, P. Sonnet (2013), "Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar", *Chemosphere*, **92**(11), pp.1450-1457, DOI: 10.1016/j.chemosphere.2013.03.055.
- [28] Ministry of Natural Resources and Environment (2015), *Circular No.60/2015/TT-BTNMT on Promulgation of National Technical Regulation on Soil Evaluation* (in Vietnamese).
- [29] J. Liu, K. Li, J. Xu, et al. (2003), "Lead toxicity, uptake, and translocation in different rice cultivars", *Plant Science*, **165**(4), pp.793-802, DOI: 10.1016/S0168-9452(03)00273-5.
- [30] X. Bi, L. Ren, M. Gong, et al. (2010), "Transfer of cadmium and lead from soil to mangoes in an uncontaminated area, Hainan Island, China", *Geoderma*, **155**, pp.115-120, DOI: 10.1016/j.geoderma.2009.12.004.
- [31] N. Hung, I. Kosinova (2019), "Environmental toxicology of cadmium and lead (in the case of Long Khang, Vietnam)", *Russian Agricultural Sciences*, **45**(3), pp.271-275, DOI: 10.3103/S106836741903008X.
- [32] T.L. Nguyen, H.Q. Vu, T.T. Nguyen, et al. (2014), "Result of breeding Bac Thom No.7 resistance leaf blight variety", *Journal of Science and Development*, **12**(2), pp.131-138 (in Vietnamese).

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