



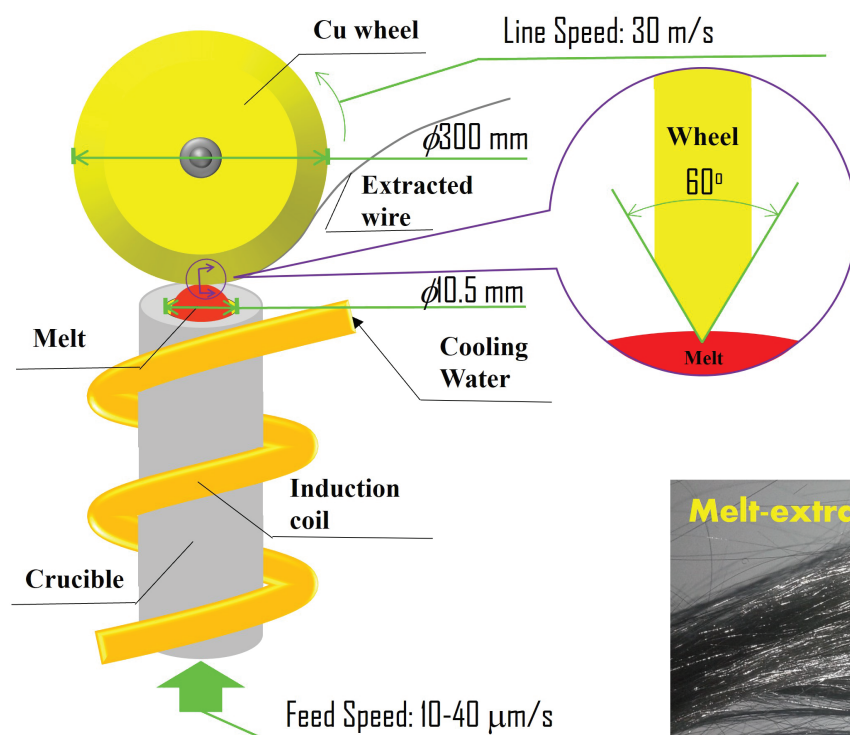
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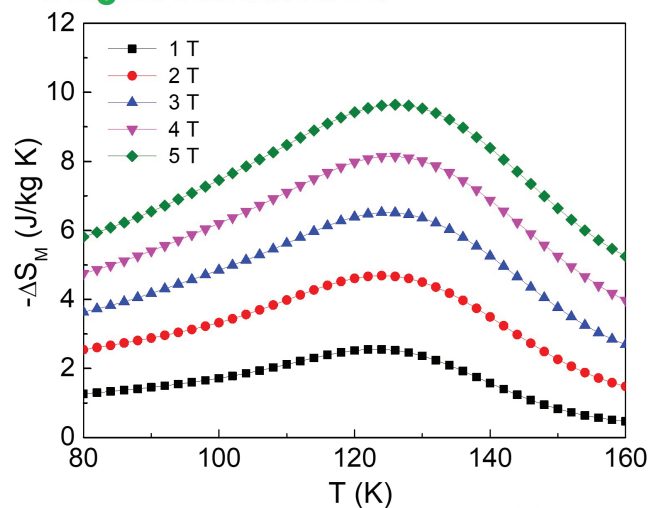
Vietnam Journal of Science, Technology and Engineering

DECEMBER 2023 • VOLUME 65 NUMBER 4

Melt-Extraction Method



Magnetocaloric Effect



Advanced magnetocaloric microwires:
What does the future hold?

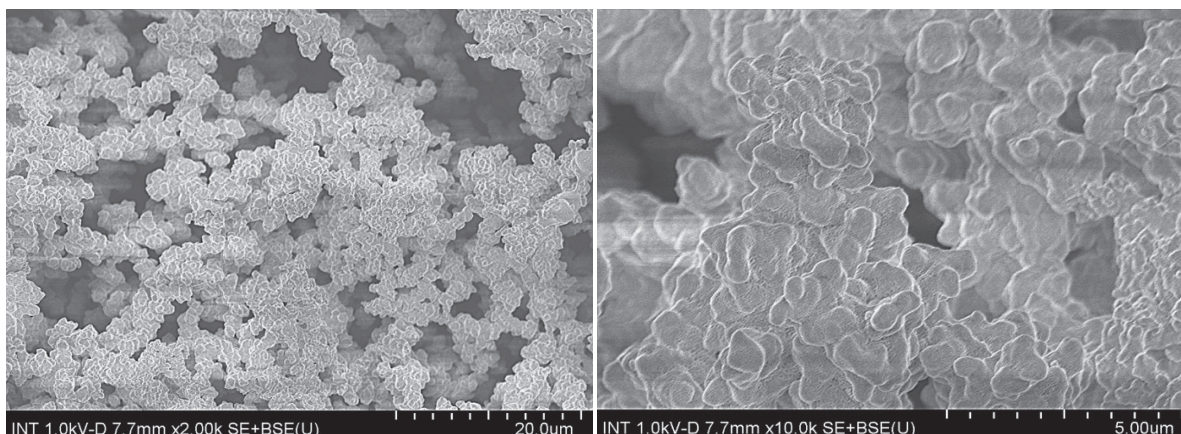
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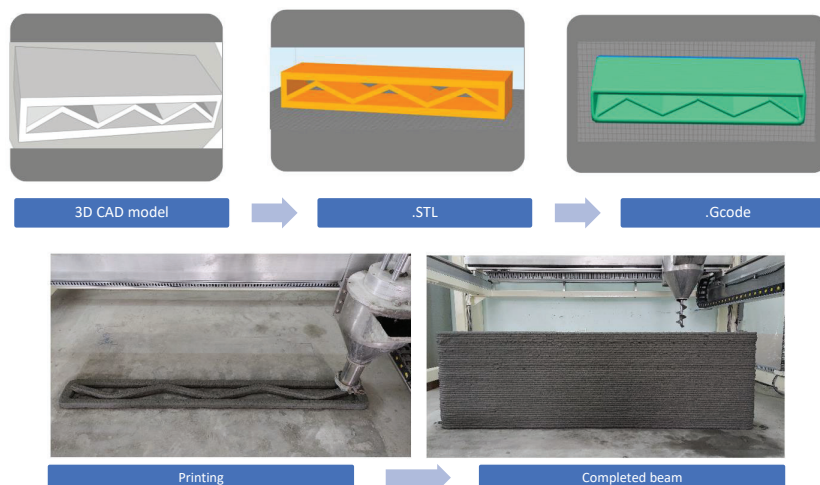
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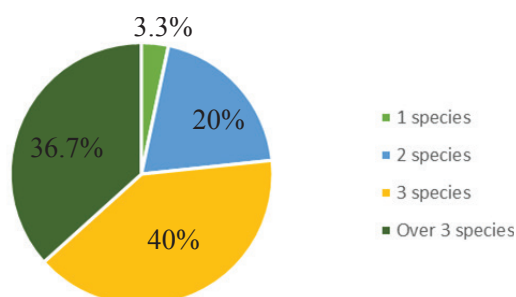
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Motion planning and control of an autonomous mobile robot

To Xuan Dinh^{1*}, Nguyen Thi Thu Huong¹, Nguyen Ngoc Tuan¹, Nguyen Thanh Tien²

¹Le Quy Don Technical University, 236 Hoang Quoc Viet Street, Co Nhue 1 Ward, Bac Tu Liem District, Hanoi, Vietnam

²Military Institute of Mechanical Engineering, 42 Dong Quan Street, Nghia Do Ward, Cau Giay District, Hanoi, Vietnam

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Abstract:

The application of autonomous mobile robots (AMRs) has gradually become crucial in smart factories due to the advantages of improving production efficiency and reducing labour costs. Motion planning has been a key part of AMR control development. This paper presents motion planning and position tracking control systems of an omnidirectional wheel AMR powered by a hybrid fuel cell and battery power source. First, the kinematical and dynamic models of the AMR are introduced. The navigation system comprises three loops, with the first loop being motor control, the second loop being position tracking control, and a motion planning layer. The position data of the AMR for feedback control is obtained through sensor fusion of data from the inertial measurement unit (IMU) sensor, encoder sensor, and ranging sensor with simultaneous localisation and mapping (SLAM) algorithm. The motion planning is then applied to obtain an optimal path with the shortest distance and collision-free movement. In addition, the tracking algorithm is designed to drive the AMR to follow the optimal path and achieve high accuracy. The experimental results show a 30% improvement in tracking accuracy compared to traditional approaches and 8 hours of continuous working, which is promising for industrial applications, and the results are satisfactory in terms of both accuracy and efficiency requirements.

Keywords: autonomous mobile robot, control algorithm, motion planning.

Classification numbers: 1.3, 2.3

1. Introduction

In recent years, autonomous ground vehicles (AGVs) have played an essential role in material handling and logistics. Additionally, AGVs are emerging in other application fields, such as hospitals, restaurants, and agricultural environments [1, 2]. Based on navigation control strategy, there are mainly two types of AGVs [3]. In the first type, AGV can only follow predefined paths and move to predefined points on the guide path. This type of AGV can use mechanical guides, magnetic guides, inductive guides, or laser navigation guides for its navigation control [4]. Since it relies solely on a fixed path, the application of such AGVs is limited. Even minor changes in factory layout would require significant time to modify the program and guidance system for AGVs, reducing factory productivity. The second type, namely AMR, uses sensing systems to detect the surrounding environment or provide positioning information, allowing it to autonomously locate itself in the environment. The sensors for AMRs include global positioning systems (GPS), depth cameras, or ranging sensors such as LiDAR. Equipped with such a smart sensor system, AMRs can naturally locate themselves and reach any accessible position in a defined area. Moreover, AMRs can rapidly adapt to changes in the working environment. Camera sensor-based localisation has been utilised in many previous

works [5, 6]. Some noteworthy algorithms for Visual SLAM include Unified point-line-plane feature fusion for RGB-D visual SLAM [7], point-line-plane features visual SLAM [8], etc. However, visual SLAM algorithms mostly utilise a sparse set of features, resulting in tracking loss and lack of robustness due to discarding most of the image information. In addition, visual SLAM faces challenges from environmental conditions such as variations in illumination and atmospheric conditions, which can result in a lack of pose estimation accuracy. Due to the high accuracy and reliability of LiDAR, and its robustness in various environmental conditions, we used it as an autonomous localisation device in this research. However, LiDAR is very sensitive to large changes in the surrounding environment.

Using high-accuracy encoders is one method to deal with environmental variations. Many commercial products employ encoders combined with camera sensors to detect QR codes and localise the AMR's position. The AMR system with omnidirectional wheels is able to perform holonomic motion, which allows it to move in all directions. This ability makes omnidirectional AMRs easily navigate even in narrow aisle spaces or crowded environments. In addition, omnidirectional wheels are a well-known mechanism that brings the advantages of a low budget and flexibility [9]. However, the slip problem of omnidirectional wheels is more serious than that of general

*Corresponding author: Email: dinhtx@lqdtu.edu.vn

wheels, leading to significant errors in localisation when using encoders alone and difficulties in navigation control [10]. To address these drawbacks, we utilised a sensor fusion algorithm that combines sensory data from LiDAR, encoders, and IMU sensors to achieve high-accuracy localisation for AMRs. On the other hand, an advanced navigation control scheme is developed to reduce slip phenomena for AMRs and achieve robust and high-accuracy performance. After a robust localisation scheme is employed to determine the AMR's position on the map, a motion planning scheme is then applied to drive the AMR from its current position to the target position.

Most previous approaches have focused on dealing with navigation and robot movement in a static environment with static obstacles. However, this is not suitable for real applications in working factories, where the environment is constantly changing with dynamic obstacles. Therefore, we propose a motion planning approach that combines a global motion planner and a local motion planner to obtain an optimal path and ensure collision avoidance for AMRs [11-13]. The global technique calculates the optimal path from the start point to the goal offline. Then, the local planner uses only a small portion of the world model to generate robot control and a local path to help the robot avoid dynamic obstacles.

In addition, this work employs a fuel cell power system as an alternative power solution for mobile robot systems [14]. The combination of a fuel cell and a battery provides a promising solution to reduce cycle time and increase productivity in the manufacturing industry. Therefore, in this research, we utilise a hybrid power source of a fuel cell and battery system to extend the continuous working time of AMRs and enhance production efficiency.

The main contributions of the article can be summarised as follows:

Firstly, to address the localisation problem with AMRs in varying environments, a combination and fusion technique to filter the AMR's position based on LiDAR sensor, encoder, and IMU sensor data is utilised to cope with localisation errors due to wheel slip phenomena, changing environmental conditions, and sensor noise.

Secondly, a motion planning scheme that combines a hybrid A-star-based global planner and a dynamic window approach-based local planner to generate an optimal path for AMRs in the presence of dynamic obstacles.

Thirdly, to increase the power efficiency of the AMR system, a hybrid power source is installed on the AMR, thereby increasing factory productivity.

Fourthly, the organisation of this research is presented as follows. Section 2 introduces an overview of the AMR system with hardware and software descriptions. The kinematics and modelling of the AMR system are presented in Section 3. The localisation algorithm is discussed in Section 4. The motion

planning scheme is investigated in Section 5. The navigation control scheme and experimental results are demonstrated in Section 6. Finally, concluding remarks are discussed in Section 7.

2. Problem statement

2.1. AMR system description

The structure of the AMR system is depicted in Fig. 1, comprising a hybrid power source, a sensing system, motor and motor control system, main CPU, DIO system, communication system, mechanical system, and AMR control system (ACS). The hybrid power source consists of a fuel cell system, battery power, and DC-DC converters. The AMR system gathers information from two ranging sensors, an IMU sensor, and four encoders to estimate its state. Additionally, laser scan data can be used for obstacle detection and avoidance algorithms. The main CPU collects data from the sensing system and processes path planning, navigation control schemes, and motor control schemes.

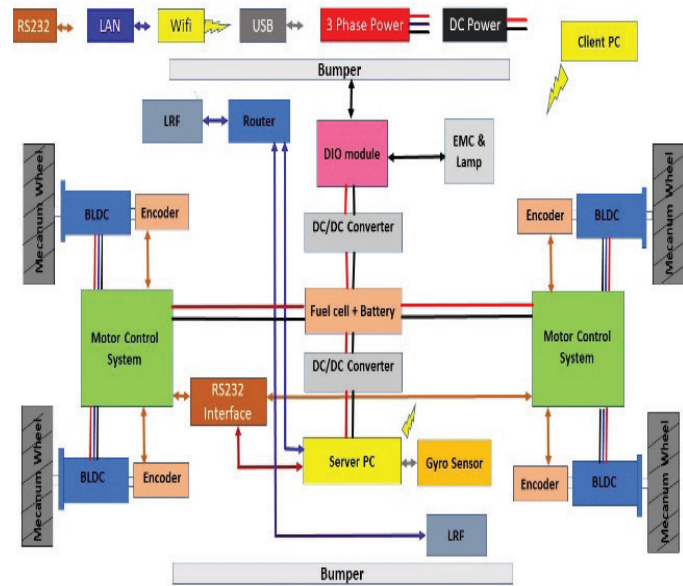


Fig. 1. Overview of developed AMR system.

2.2. Software development

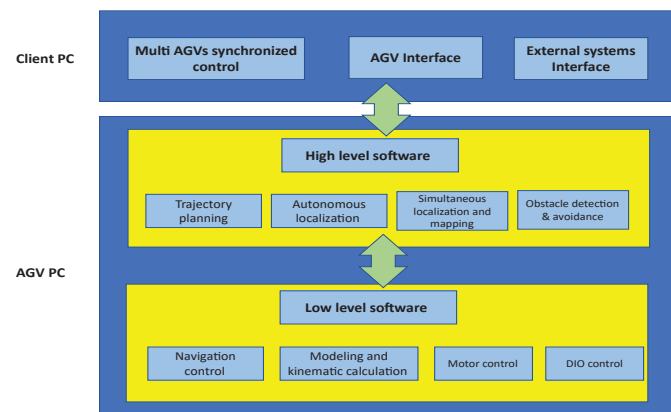


Fig. 2. Software architecture for the AMR system.

The software diagram for the AMR system, as shown in Fig. 2, consists of three levels. At the client PC level, the multi-AMR control system is developed to control multiple AMRs simultaneously. In this level, the ACS receives information and commands from a higher management system or other robots to control AMRs in performing tasks. ACS also retrieves information and status updates from AMRs and sends them to the higher management system. The tasks may include sequential movement processes and application tasks such as conveyor movement. The command is sent to AMR systems and processed at the second level. To perform movement tasks, the AMR requires information from the sensing system to autonomously estimate its location, sense the surrounding environment and obstacles, and plan its path. The third-level software calculates the kinematic and dynamic properties of the AMR and subsequently determines the desired speed for its four motors.

3. System modelling

3.1. Kinematics description

The four mecanum wheels are symmetrically positioned at the geometric centre of the AMR's body to ensure stable chassis operation. To derive the equation of motion for the AMR, we make two assumptions:

Assumption 1: The AMR moves on a horizontal and flat plane.

Assumption 2: All the parts of the AMR are rigid.

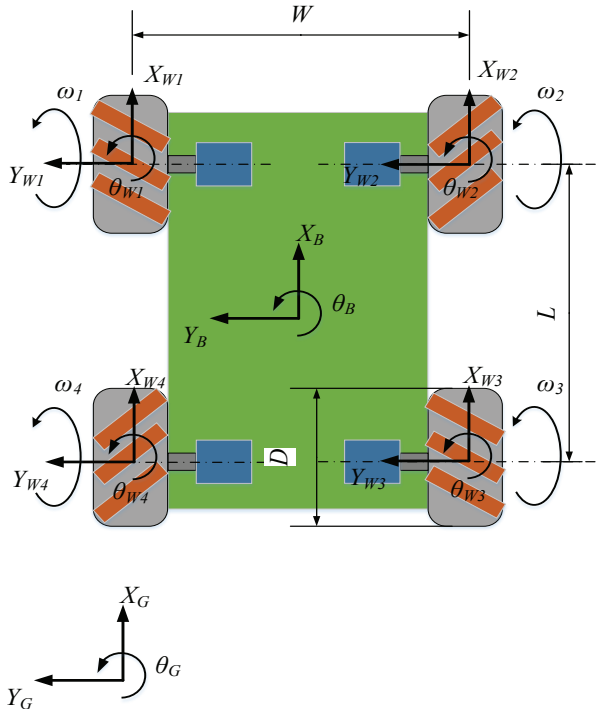


Fig. 3. Kinematics and coordinates of the AMR system.

The kinematic description of the AMR is illustrated in Fig. 3, considering three coordinate frames: the global frame OG, the body frame OB (where its centre is the gravity centre of the AMR), and the wheel coordinate frame O_{wi} (*i*=1, 2, 3, 4).

The relationship between the speeds of the mecanum wheels and the velocities of the AMR is described as follows [15]:

$$\begin{bmatrix} \omega_1 \\ \omega_2 \\ \omega_3 \\ \omega_4 \end{bmatrix} = \frac{360}{\pi D} \begin{bmatrix} 1 & 1 & -\frac{(L+W)}{720} \\ 1 & -1 & \frac{(L+W)}{720} \\ 1 & -1 & -\frac{(L+W)}{720} \\ 1 & 1 & \frac{(L+W)}{720} \end{bmatrix} \begin{bmatrix} v_x \\ v_y \\ \Omega_z \end{bmatrix} \quad (1)$$

$$\begin{bmatrix} v_x \\ v_y \\ \Omega_z \end{bmatrix} = \frac{\pi D}{1440} \begin{bmatrix} 1 & 1 & 1 & 1 \\ 1 & -1 & 1 & -1 \\ -\frac{720}{L+W} & -\frac{720}{L+W} & -\frac{720}{L+W} & -\frac{720}{L+W} \end{bmatrix} \begin{bmatrix} \omega_1 \\ \omega_2 \\ \omega_3 \\ \omega_4 \end{bmatrix} = H \begin{bmatrix} \omega_1 \\ \omega_2 \\ \omega_3 \\ \omega_4 \end{bmatrix} \quad (2)$$

where v_x , v_y , and Ω_z are the linear velocities in the x and y directions and angular velocity about z axis; ω_i (i : 1-4) is the rotation speed of i^{th} wheel in units of rotation per minute; L and W are the length and width of the AMR calculated from the centre of the wheel, respectively; D is the wheel diameter.

To regulate the motion of the AMR, the linear and angular velocities are converted into the rotational speed of each wheel using inverse kinematics. Furthermore, the AMR's velocity is determined through forward kinematics and information obtained from encoders.

3.2. Dynamic description

The dynamics of the AMR with uncertainties are derived using the Lagrange method, considering the following assumptions:

Assumption 3: The body frame O_B is located in the centre of mass of the AMR.

Assumption 4: The AMR operates on a two-dimensional floor, AMR's potential energy is assumed to be constant, with the potential energy being considered zero.

Assumption 5: The moment of inertia of the rollers in the omnidirectional wheel is ignored.

Basing on the above assumptions, the dynamics of the AMR system are presented as the following:

$$M(q)\ddot{q} + C(\dot{q}, q)\dot{q} + G(q) = \tau + \tau_{ex} \quad (3)$$

where $q=(x, y, \theta)^T$ with x and y denote the centre position and θ is the rotational angle of the AMR in the global coordinate frame xO_Gy ; τ is the applied torque input vector; $M(q)$ denotes the symmetric positive definite robot inertia matrix; $C(\dot{q}, q)$ denotes the centrifugal and Coriolis torque vectors; $G(q)$ is the gravity vector; τ_{ex} is a vector of bounded external disturbances and dynamics uncertainties.

In 2D planar movement, the vector of gravity is neglected, and the centrifugal and Coriolis torque vectors can be calculated by numerical methods and experiments. The inertial matrix is calculated as the following [16]:

$$M(q) = \begin{bmatrix} M_1 + M_2 + I_W & -M_2 & M_2 & M_1 - M_2 \\ -M_2 & M_1 + M_2 + I_W & M_1 - M_2 & M_2 \\ M_2 & M_1 - M_2 & M_1 + M_2 + I_W & -M_2 \\ M_1 - M_2 & M_2 & -M_2 & M_1 + M_2 + I_W \end{bmatrix} \quad (4)$$

$$\text{where } M_1 = \frac{m_A D^2}{32}, M_2 = \frac{I_Z D^2}{256} (W + L)^2 \quad (5)$$

with m_A being the total weight of the AMR system; I_W and I_Z being the moment of inertia of the AMR's body and wheels, respectively.

4. Robot localisation

In this section, the extended Kalman filter method is applied to fuse data from the laser-ranging finder, wheel encoder, and IMU sensor. It is assumed that the AMR system operates on an ideal flat planar surface. The AMR's pose information at the k -th sample is defined as the state vector. The position of the AMR is updated every 0.05 seconds in accordance with the update frequency of the laser-ranging finder sensor. The localisation algorithm is illustrated in Fig. 4.

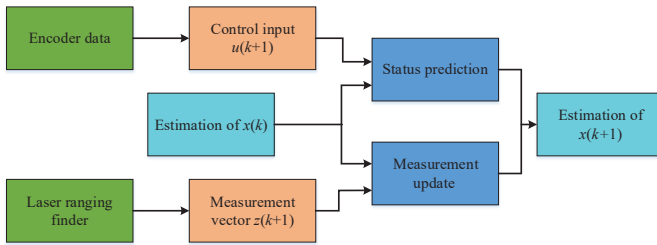


Fig. 4. Extended Kalman filter for localisation of the AMR system.

4.1. State estimation

The new position at the sampling time $k+1$ without localisation error can be given as follows:

$$x(k+1) = x(k) + f(x(k), u(k+1), \alpha(k+1)) \quad (6)$$

where $x(k)$ denotes the state variable in the sampling time k ; $u(k+1)$ denotes the control signal and is determined by the rotating angles of the wheels, measuring by wheel encoders; $\alpha(k+1)$ denotes the process noise; $f(\cdot)$ is a nonlinear function of $x(k)$, $u(k+1)$ and $\alpha(k+1)$.

The predicted value of $x(k+1)$ is given by:

$$\hat{x}^-(k+1) = \hat{x}(k) + \begin{bmatrix} \cos(\hat{\theta}_k) & -\sin(\hat{\theta}_k) & 0 \\ \sin(\hat{\theta}_k) & \cos(\hat{\theta}_k) & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} v_x \\ v_y \\ \Omega_z \end{bmatrix} dt \quad (7)$$

where the accent “ $\hat{\cdot}$ ” above the status vector indicates an approximated value and the superscript “ $-$ ” defines the estimated value of the state variable at the sampling time $k+1$; dt is the sampling time.

Combining Eqs. (2) and (7), we obtain:

$$\begin{aligned} \hat{x}^-(k+1) &= \hat{x}(k) + \begin{bmatrix} \cos(\hat{\theta}_k) & -\sin(\hat{\theta}_k) & 0 \\ \sin(\hat{\theta}_k) & \cos(\hat{\theta}_k) & 0 \\ 0 & 0 & 1 \end{bmatrix} H \begin{bmatrix} \omega_1 \\ \omega_2 \\ \omega_3 \\ \omega_4 \end{bmatrix} dt \\ &= \hat{x}(k) + \begin{bmatrix} \cos(\hat{\theta}_k) & -\sin(\hat{\theta}_k) & 0 \\ \sin(\hat{\theta}_k) & \cos(\hat{\theta}_k) & 0 \\ 0 & 0 & 1 \end{bmatrix} H u(t) \end{aligned} \quad (8)$$

The matrix $P^-(k+1)$ denotes the prior covariance matrix of $x(k+1)$ and is defined as the following:

$$P^-(k+1) = \nabla f_x P(k) \nabla f_x^T + \nabla f_\alpha Q(k+1) \nabla f_\alpha^T \quad (9)$$

where $P(k)$ denotes the covariance matrix of the state $x(k)$; $Q(k+1)$ is the covariance matrix of the process noise $\alpha(k+1)$; T denotes transpose operator of the matrix; ∇f_x and ∇f_α denote Jacobian matrices and are calculated as the following equation:

$$\nabla f_x = \begin{bmatrix} 1 & 0 & J_1 \\ 0 & 1 & J_2 \\ 0 & 0 & 1+J_3 \end{bmatrix}, \nabla f_\alpha = \begin{bmatrix} \cos(\hat{\theta}_k) & -\sin(\hat{\theta}_k) & 0 \\ \sin(\hat{\theta}_k) & \cos(\hat{\theta}_k) & 0 \\ 0 & 0 & 1 \end{bmatrix} H I_4 \quad (10)$$

where I_4 denotes identity matrix with the order of four; H is the matrix defined in Eq. (2); and J_1 , J_2 , and J_3 are derived from the following equation:

$$\begin{bmatrix} J_1 \\ J_2 \\ J_3 \end{bmatrix} = \begin{bmatrix} -\sin(\hat{\theta}_k) & -\cos(\hat{\theta}_k) & 0 \\ \cos(\hat{\theta}_k) & -\sin(\hat{\theta}_k) & 0 \\ 0 & 0 & 1 \end{bmatrix} H u(k) \quad (11)$$

4.2. Measurement update

Let $z(k+1)$ denote a new measurement vector, with its value obtained as follows:

$$z(k+1) = h(x(k+1), \beta(k+1)) \quad (12)$$

where $h(x(k+1))$ is the measurement function and $\beta(k+1)$ is the measurement noise.

The predicted measurement vector is given according to the extended Kalman filter as the following:

$$\hat{z}(k+1) = \begin{bmatrix} \cos(\hat{\theta}_k) & -\sin(\hat{\theta}_k) & 0 \\ \sin(\hat{\theta}_k) & \cos(\hat{\theta}_k) & 0 \\ 0 & 0 & 1 \end{bmatrix} [\hat{x}^-(k+1) - \hat{x}(k)] = \begin{bmatrix} v_x \\ v_y \\ \Omega_z \end{bmatrix} dt \quad (13)$$

The difference between the measurement vector and the predicted measurement value, denoted $\delta(k+1)$, and its estimated covariance matrix, denoted $\Lambda(k+1)$, are obtained by the following:

$$\delta(k+1) = z(k+1) - \hat{z}(k+1), \Lambda(k+1) \approx \nabla h_x P^-(k+1) \nabla h_x^T + S(k+1) \quad (14)$$

where $S(k+1)$ is the covariance of the measurement noise $\beta(k+1)$, $P^-(k+1)$ is derived from Eq. (9), and

$$\nabla h_x = \begin{bmatrix} \cos(\hat{\theta}_k) & -\sin(\hat{\theta}_k) & 0 \\ \sin(\hat{\theta}_k) & \cos(\hat{\theta}_k) & 0 \\ 0 & 0 & 1 \end{bmatrix} \quad (15)$$

The Kalman gain is given as the following:

$$K(k+1) = P^-(k+1) \nabla h_x^T (A(k+1))^{-1} \quad (16)$$

Thus, the posterior estimation of the AMR's position is given as:

$$\hat{x}(k+1) = \hat{x}^-(k+1) + K(k+1) \delta(k+1) \quad (17)$$

$$P(k+1) = (I_3 - K(k+1) \nabla h_x) P^-(k+1). \quad (18)$$

5. Motion planning

The motion planning scheme consists of two parts: a modified A*-based global planner and a dynamic window approach-based local planner.

5.1. Traditional A* algorithm

The A* algorithm is among the most widely used path planning algorithms for finding the optimal robot path in complex areas. It is represented by the following formula:

$$f(x) = h(x) + g(x) \quad (19)$$

where $h(x)$ represents the heuristic distance (using one of the following methods: Manhattan, Euclidean, or Chebyshev) from the current node to the target node; $g(x)$ signifies the distance from the start node to the target node through the selected sequence of nodes.

Each neighbour of the current node is evaluated using the function $f(x)$, and the next node is chosen if it has the lowest value of $f(x)$. The algorithm has been successfully applied to finding the best robot route in various applications. However, it has been observed that the algorithm requires improvement to meet the requirements of real-world applications. One of its drawbacks is the extensive computational time, which limits its real-time applicability to high-quality hardware systems. Therefore, a modification of the A* algorithm is investigated to overcome this limitation.

5.2. Modified A* algorithm

To reduce the number of examined cells, a modified version of the A* algorithm is introduced. The principle of this method involves cropping the neighbourhood cells of potential candidates.

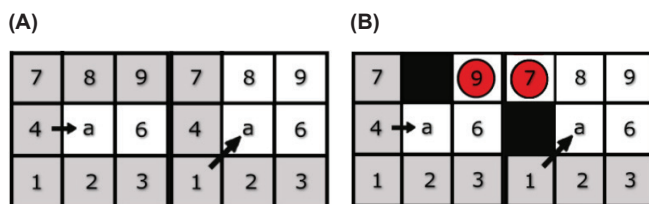


Fig. 5. The improved A* algorithm.

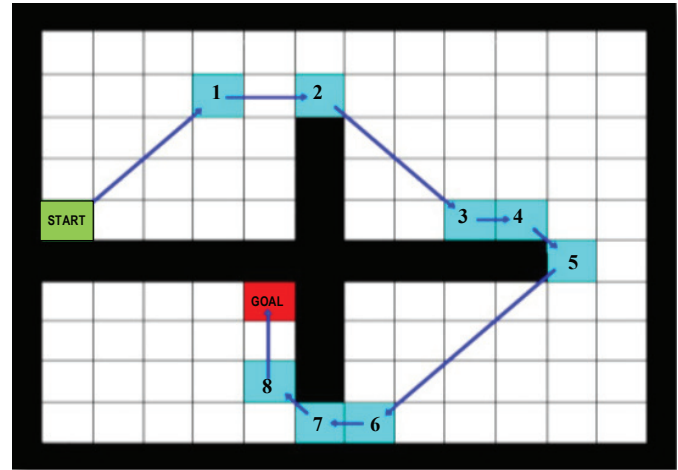


Fig. 6. Optimal path found by the modified A* algorithm.

The idea of the modified A* algorithm is illustrated in Fig. 5. We define node 'a' as the evaluated node, and the arrows represent the direction of movement from the previous node. The grey nodes are eliminated from the candidate list because they can be reached without entering node 'a'. The white nodes are considered natural neighbours, and they are the only candidates for the next step of movement. Fig. 5 depict cases without and with obstacle nodes, respectively. The general description of the modified algorithm is presented in Fig. 6. The figure demonstrates a significant reduction in the number of evaluated nodes, thereby reducing the computational load on the computer and making it suitable for real-time implementation.

5.3. Dynamic window approach

The modified A* algorithm creates a global path from the starting point to the target point without considering dynamic obstacles, which may result in collision probabilities. Therefore, a local planning scheme called the dynamic window approach is employed to ensure collision-free navigation for the AMR. The idea of the algorithm is explained as follows:

- The expected velocity towards the target is calculated based on the robot's current pose and the target.
- Suitable ranges of linear and angular velocities are computed based on the robot's dynamics.
- Velocity candidates within the calculated ranges are checked to find the nearest obstacle.
- The gap between the current position and the nearest obstacle is examined to determine if the robot can stop before a collision occurs. Velocity candidates that would lead to a collision are discarded.
- Among the velocity candidates that meet the collision avoidance requirements, the one with the best objective function is selected for the robot's next movement.

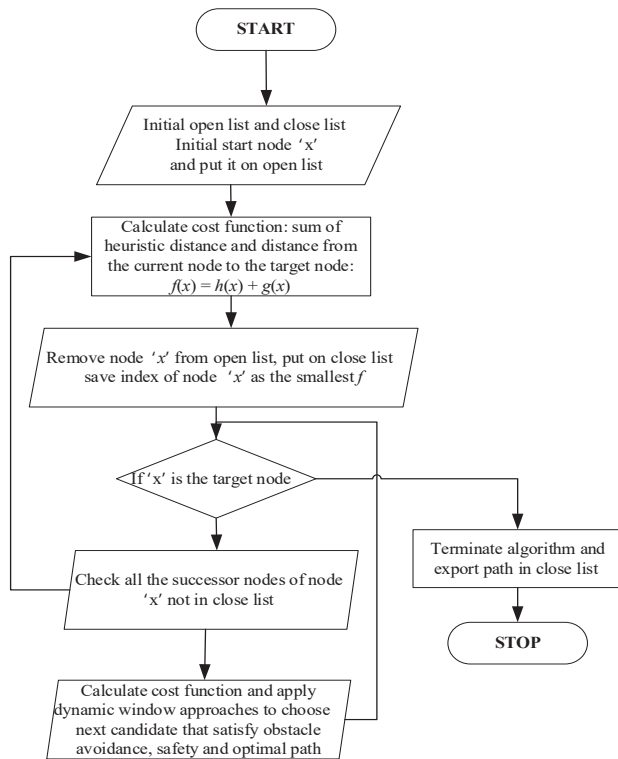


Fig. 7. Flowchart of the proposed path planning algorithm.

The flowchart of the proposed path planning algorithm is illustrated in Fig. 7. Initially, the global path planner generates a path using the modified A* algorithm to avoid static obstacles in the working environment. As the robot moves, the current area around the robot is referred to as the local area, which includes not only static obstacles but also dynamic obstacles such as changes in the environment or human movement. In such cases, the global path is no longer suitable for the local area. Therefore, dynamic window approach is employed to replan the robot's path to avoid dynamic and new obstacles. The new local path ensures optimal path planning and obstacle avoidance. As explained in the dynamic window approach algorithm, the appropriate velocity command is executed to safely navigate the robot within the local area.

6. Results

6.1. SLAM deployment

Through the sensor fusion algorithm, which combines odometry data from the laser sensor, wheel encoder, and IMU sensor, the AMR's location is updated at a frequency of 20 Hz while in motion. Subsequently, the SLAM algorithm [17-19] is applied to construct a virtual map of the warehouse, a crucial step for the navigation algorithm and path planning. The environment map was generated by the robot using a laser rangefinder and selected SLAM techniques, as illustrated in Fig. 8.



Fig. 8. Map obtained from the SLAM process.

6.2. Motion planning deployment

By implementing the motion planning scheme, the optimal path for the AMR from the starting point to the destination point is depicted in Fig. 9. The algorithm successfully finds the path between these points. Furthermore, as evident in Fig. 9, the obtained path is both the shortest and collision-free with the static obstacles in the environment. To ensure accurate path tracking for the robot, an adaptive pure pursuit controller is employed. This pure pursuit algorithm, known as lateral vehicle control, aims to follow a target point on the desired path with a look-ahead distance from the robot. The angular velocity is selected to guide the robot to the target point according to the robot's kinematics. The linear velocity is computed based on the radius of the curved path the robot follows, decelerating when the radius is small and accelerating when the radius is large. Additionally, a PID controller is applied to minimise the error between the calculated values and real measurements.

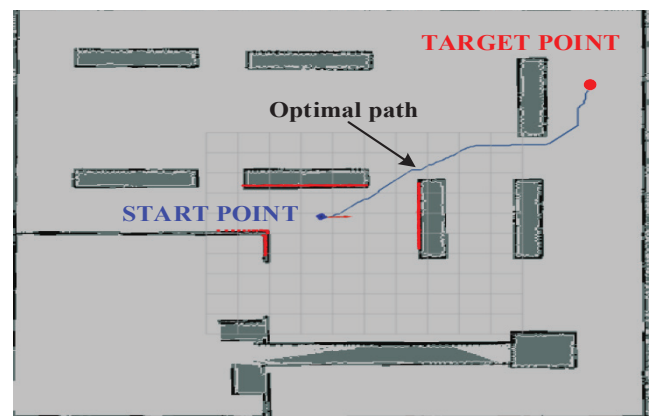


Fig. 9. Optimal path obtained by the path planning algorithm.

6.3. Experimental results

The specifications of the AMR system are detailed in Table 1. In the validation section, QR barcodes are placed at the START and TARGET positions to measure the control errors of the AMR at those positions. The tests are conducted over 15 continuous working cycles of the AMR. The tracking errors for each conveyor include linear position errors in the x and y directions and the heading angle control error of the AMR.

Table 1. Specification of the AMR system.

Component	Specification	Units
Size (L×W×H)	1500×1500×750	mm×mm×mm
Weight	230	kg
Power	15	kW
Power source	Hybrid fuel cell/battery	-
Wheel diameter	360	mm
Type of motors	BLDC	-
Type of movement	Omnidirectional	-
Laser range	30	m
Encoder resolution	3000	PPR
Maximum speed	1.2	m
Maximum working time	12	hour
Wireless device	Moxa	-

Applying the localisation method and navigation control algorithm for the AMR, the control errors in x, y direction and the angle error at START and TARGET positions are shown in Figs. 10 and 11.

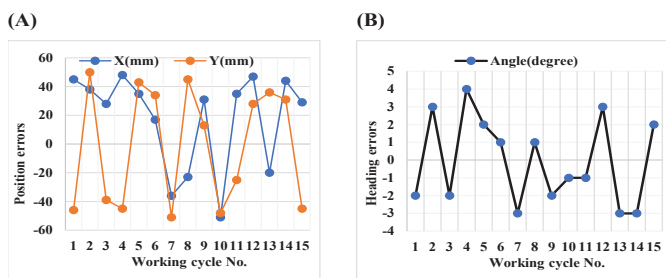


Fig. 10. Control errors at the START position. (A) Position errors; (B) Heading errors.

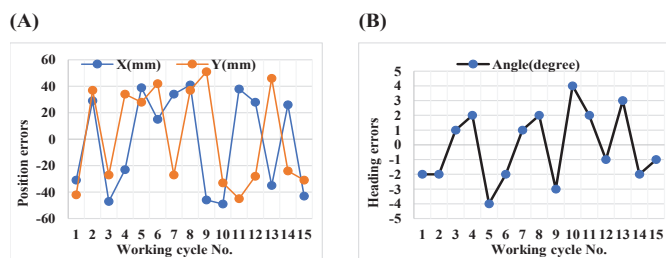


Fig. 11. Control errors at the TARGET position. (A) Position errors; (B) Heading errors.

As can be seen in these figures, the control errors of the AMR are bounded. The control errors in x and y directions are limited in the range of [-5, 5] cm while the absolute values of the heading errors are always smaller than 4 degrees. These results show the promising application of the designed AMR system for the requirement of industry. In addition, the AMR can work continuously in 8 hours before refuelling the hydrogen and the time for full refuelling is 10 minutes. These factors help reduce the working cycle time, increase the mass production ability and economy saving for industrial applications.

7. Conclusions

This article presents the development and experimental results of an omni-directional AMR system powered by a hybrid power source comprising a fuel cell and battery. A three-layered software architecture, consisting of a client interface, high-level software system, and low-level control system, ensures the flexibility of the developed AMR system. Data from wheel encoders, IMU sensors, and two laser-ranging finders were fused using an extended Kalman filter algorithm to localise the AMR. Subsequently, the modified motion planning algorithm was applied to determine the optimal path for the robot, ensuring collision-free navigation.

The preliminary experimental results indicate that the autonomous localisation and mapping method is feasible in industrial applications, resulting in a 20% reduction in travel time compared to conventional path planning methods and ensuring a safe path. Furthermore, the position errors and heading control accuracy are promising for industrial applications, with a 30% reduction compared to traditional PID controllers. The use of a hybrid power source proves to be an efficient solution for addressing cycle time issues in many manufacturing processes.

CRedit author statement

To Xuan Dinh: Algorithm, Experiment, Writing original draft preparation; Nguyen Thi Thu Huong: Algorithm, Experiment, Writing original draft preparation; Nguyen Ngoc Tuan: Writing - Reviewing and Editing; Nguyen Thanh Tien: Writing - Reviewing and Editing.

COMPETING INTERESTS

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

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Iron(III) promoted oxidative annulation of benzylic C-H bonds in (α -amino)arylacetic acids for synthesis of 4-aryl pyrrolo[1,2-*a*]quinoxalines

Tuan Hoang Ho^{1,2}, Tung Thanh Nguyen^{1,2,3}, Tran Hoang Bao Ngo^{2,3}, Nam Thanh Son Phan^{2,3*}

¹VNU-HCM Key Laboratory of Functional Organic Materials, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam

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

³Department of Chemical Engineering, Ho Chi Minh City University of Technology, 268 Ly Thuong Kiet Street, Ward 14, District 10, Ho Chi Minh City, Vietnam

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Abstract:

Using amino acids for synthesis of heterocycles is a synthetically promising field. However, developing the practical methods for transformations of amino acids into heterocycles is still challenging. Given that alpha amino acids are abundant or easily prepared, herein we report a method for annulation of benzylic C-H bonds in derivatives of 2-phenylglycine with 1-(2-nitroaryl) pyrroles. The reactions proceeded well in the presence of iron(III) acetylacetonate catalyst and potassium carbonate base. Scope of pyrrolo[1,2-*a*]quinoxalines was studied. Regardless of electronic properties of substituents, the pyrrolo[1,2-*a*]quinoxaline derivatives were successfully isolated, as yields varied from 42% to 52%. Pyrrolo[1,2-*a*]quinoxalines substituted with heterocycles at C4 positions as pyridine and thiophene were competent substrates. Reaction mechanism was proposed to start with a decarboxylative/deaminative sequence of 2-phenylglycine to afford benzaldehyde. The iron catalyst was presumed to facilitate the reduction of 1-(2-nitroaryl)pyrroles to furnish the corresponding aniline. Imine condensation followed by cyclisation and oxidation would yield the pyrrolo[1,2-*a*]quinoxaline. Our method would offer a convenient tactic to transform abundant alpha amino acids into synthetically useful heterocycles.

Keywords: amino acid, annulation, iron, redox, 1-(2-nitroaryl)pyrroles.

Classification numbers: 2.1, 2.2

1. Introduction

Pyrrolo[1,2-*a*]quinoxaline is a valuable scaffold ubiquitously utilised in biological studies and in synthesis of functional materials [1-3]. Thus, numerous methods have been developed, specifically those for the synthesis of 4-aryl pyrrolo[1,2-*a*]quinoxalines. For example, the synthesis of fused *N,N*-heterocycles from 1-(2-aminoaryl)pyrroles have been extensively reported. Recently, using more upstream starting materials such as 1-(2-nitroaryl)pyrroles has witnessed substantial emergence. Annulation of those nitroarenes with a benzyl synthon including benzyl amines [4], arylacetic acids [5], aromatic aldehydes [6], alcohols [7], and 1,2-diols [8] to yield substituted pyrrolo[1,2-*a*]quinoxalines is also known. Some methods feature the benefit of using metal-free tactics [4, 7]. Meanwhile, transition metal complexes have been utilised to convert arylacetic acids and 1,2-diols to the corresponding pyrrolo[1,2-*a*]quinoxalines [5, 8]. Notably, coupling of 1-(2-nitroaryl)pyrroles with amino acids such as derivatives of phenylglycine has also been reported.

The oxidation of benzylic C-H bonds in phenylglycine derivatives for the synthesis of *N*-heterocycles has been precedented. One stand-alone method was presented by M. Kumar, et al. (2015) [9], where the annulation of 2-nitrobenzonitriles and phenylglycines successfully yielded quinazolinones. In this report, the authors proposed the redox conversion of phenylglycine and

2-nitrobenzonitrile to benzaldehyde and 2-aminobenzonitrile, respectively, in the presence of an iron(III) catalyst and potassium carbonate base. We envisaged that those redox transformations could be leveraged to couple 1-(2-nitroaryl)pyrroles with phenylglycines. Herein, we report a method affording 4-aryl pyrrolo[1,2-*a*]quinoxalines from 1-(2-nitroaryl)pyrroles and phenylglycines as the benzyl equivalence. The reactions utilised an Fe(acac)₃ (acac = acetylacetonate) catalyst and K₂CO₃ base, while showing good tolerance of functional groups.

2. Experiments

Organic chemicals and metal salts were commercially obtained from Acros, Aldrich, Energy chemicals, and Bidepharm, and were used as received unless otherwise noted. The synthesis of 1-(2-nitroaryl)pyrroles followed known procedures reported in previous studies [10, 11]. A typical reaction was run in an 8-ml vial equipped with a magnetic stir bar. During the study of reaction conditions, yields were Gas chromatography (GC) yields obtained from a Shimadzu GC2010-Plus equipped with a flame ionisation detector (FID) and an SPB-5 column, with diphenyl ether as the internal standard. Nuclear magnetic resonance spectra (¹H-NMR and ¹³C-NMR) were recorded on Bruker AV 500 or 600 spectrometers.

*Corresponding author: Email: ptsnam@gmail.com

Characterization of the annulation products was as follows:

4-Phenyl pyrrolo[1,2-*a*]quinoxaline (**3aa**): ¹H NMR (500 MHz, CDCl₃, ppm) δ 8.10 (dd, *J*=7.8, 1.5 Hz, 1H), 8.02-7.99 (m, 3H), 7.88 (dd, *J*=7.9, 1.4 Hz, 1H), 7.57-7.50 (m, 4H), 7.45 (dd, *J*=7.8, 1.4 Hz, 1H), 7.00 (dd, *J*=4.2, 1.3 Hz, 1H), 6.89 (dd, *J*=4.1, 2.7 Hz, 1H). ¹³C NMR (126 MHz, CDCl₃, ppm) δ 154.4, 138.4, 136.5, 130.2, 129.8, 128.8, 127.5, 127.3, 125.5, 125.3, 114.8, 114.1, 113.9, and 108.9.

4-(3-(Trifluoromethyl)phenyl)pyrrolo[1,2-*a*]quinoxaline (**3ab**): ¹H NMR (600 MHz, CDCl₃, ppm) δ 8.30 (s, 1H), 8.21 (d, *J*=7.7 Hz, 1H), 8.05 (dd, *J*=8.0, 1.4 Hz, 1H), 8.03 (dd, *J*=2.7, 1.2 Hz, 1H), 7.90 (dd, *J*=8.2, 1.2 Hz, 1H), 7.79 (d, *J*=7.8 Hz, 1H), 7.68 (t, *J*=7.8 Hz, 1H), 7.58-7.53 (m, 1H), 7.51-7.46 (m, 1H), 6.96 (dd, *J*=4.0, 1.2 Hz, 1H), 6.93 (dd, *J*=4.0, 2.7 Hz, 1H). ¹³C NMR (126 MHz, CDCl₃, ppm) δ 152.9, 139.3, 136.2, 132.0, 131.2 (q, *J*=32.3 Hz), 130.5, 129.3, 128.1, 127.3, 126.6 (q, *J*=3.4 Hz), 125.7 (q, *J*=3.8 Hz), 125.6, 125.2, 124.2 (q, *J*=270.7 Hz), 115.1, 114.4, 113.9, and 108.5.

7-Methyl-4-(naphthalen-1-yl)pyrrolo[1,2-*a*]quinoxaline (**3bc**): ¹H NMR (600 MHz, CDCl₃, ppm) δ 8.02-7.98 (m, 3H), 7.94 (d, *J*=8.2 Hz, 1H), 7.89 (d, *J*=0.7 Hz, 1H), 7.84 (d, *J*=8.4 Hz, 1H), 7.80 (dd, *J*=7.0, 1.1 Hz, 1H), 7.61 (dd, *J*=8.2, 7.1 Hz, 1H), 7.51 (dd, *J*=8.0, 6.8, 1.0 Hz, 1H), 7.43-7.38 (m, 2H), 6.80 (dd, *J*=4.0, 2.7 Hz, 1H), 6.55 (dd, *J*=4.0, 1.3 Hz, 1H), 2.53 (s, 3H). ¹³C NMR (151 MHz, CDCl₃, ppm) δ 154.8, 136.2, 135.6, 135.3, 134.1, 131.7, 130.2, 129.6, 129.0, 128.4, 127.1, 127.0, 126.5, 126.2, 125.9, 125.3, 125.2, 114.5, 113.8, 113.6, 108.8, and 21.3.

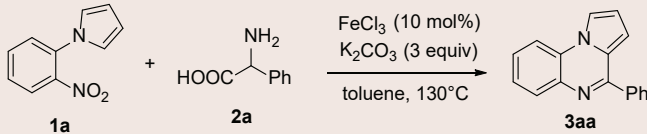
7-Methyl-4-(thiophen-2-yl)pyrrolo[1,2-*a*]quinoxaline (**3bd**): ¹H NMR (600 MHz, CDCl₃, ppm) δ 7.95 (dd, *J*=3.7, 1.1 Hz, 1H), 7.93 (dd, *J*=2.7, 1.3 Hz, 1H), 7.80 (d, *J*=0.7 Hz, 1H), 7.72 (d, *J*=8.3 Hz, 1H), 7.53 (dd, *J*=5.1, 1.1 Hz, 1H), 7.29 (ddd, *J*=8.3, 1.9, 0.5 Hz, 1H), 7.25 (dd, *J*=4.1, 1.3 Hz, 1H), 7.22 (dd, *J*=5.1, 3.7 Hz, 1H), 6.90 (dd, *J*=4.1, 2.7 Hz, 1H), 2.49 (s, 3H). ¹³C NMR (151 MHz, CDCl₃, ppm) δ 147.4, 142.7, 135.9, 135.2, 129.8, 128.7, 128.2, 127.8, 125.0, 124.1, 114.6, 113.9, 113.4, 107.7, 21.2.

7-Methyl-4-(pyridin-2-yl)pyrrolo[1,2-*a*]quinoxaline (**3be**): ¹H NMR (600 MHz, CDCl₃, ppm) δ 8.80 (ddd, *J*=4.8, 1.8, 0.9 Hz, 1H), 8.41 (dt, *J*=7.9, 1.0 Hz, 1H), 7.96 (dd, *J*=2.7, 1.3 Hz, 1H), 7.88 (td, *J*=7.8, 1.8 Hz, 1H), 7.86 (d, *J*=0.7 Hz, 1H), 7.77 (d, *J*=8.3 Hz, 1H), 7.73 (dd, *J*=4.0, 1.3 Hz, 1H), 7.40 (ddd, *J*=7.5, 4.8, 1.2 Hz, 1H), 7.34 (ddd, *J*=8.4, 1.9, 0.4 Hz, 1H), 6.93 (dd, *J*=4.0, 2.7 Hz, 1H), 2.51 (s, 3H). ¹³C NMR (151 MHz, CDCl₃, ppm) δ 156.7, 151.3, 149.0, 136.7, 135.8, 135.0, 130.3, 129.3, 125.7, 124.8, 124.3, 123.5, 114.3, 114.2, 113.5, 110.4, 21.2.

3. Results and discussion

Our study started with the annulation of 1-(2-nitrophenyl)-1*H*-pyrrole (**1a**) and 2-phenylglycine (**2a**) to yield 4-phenyl pyrrolo[1,2-*a*]quinoxaline (**3aa**). Results of the brief optimisation are presented in Table 1. The first condition was nearly identical to that reported by M. Kumar, et al. (2015) [9]. The annulation product was obtained in 45% (GC) yield (entry 1). Anions of iron salts were pivotal to successful annulation (entries 2 and 3). The results showed that Fe(acac)₃ gave the most reasonable yield of **3aa**. Potassium carbonate was superior to salts of other alkali metals (entries 4 and 5). The use of polar aprotic solvents such as dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) was not suitable for the annulation (entries 6 and 7). Those results agreed with those previously using potassium carbonate for decarboxylation and deamination of phenylglycine [9, 12]. Lastly, omitting the presence of iron salt gave trace amounts of **3aa** (entry 8), confirming the crucial role of the iron(III) salt. It should be noted that unreacting 1-(2-nitrophenyl)-1*H*-pyrrole **1a**, together with the reduced product 1-(2-aminophenyl)pyrrole could be recovered after the reactions.

Table 1. Study of reaction conditions.

		
Entry	Changes from standard conditions ^a	Yield of 3aa (%)
1	None	45
2	Fe(acac) ₃ instead of FeCl ₃	57, 52 ^b
3	Fe(NO ₃) ₃ ·9H ₂ O instead of FeCl ₃	15
4	Entry 2, Na ₂ CO ₃ instead of K ₂ CO ₃	50
5	Entry 2, Cs ₂ CO ₃ instead of K ₂ CO ₃	55
6	Entry 2, DMF instead of toluene	32
7	Entry 2, DMSO instead of toluene	28
8	No iron salt	trace

^a: standard conditions: **1a** (0.1 mmol), **2a** (0.2 mmol), FeCl₃ (0.01 mmol), K₂CO₃ (0.3 mmol), toluene (0.5 ml), 130°C, under air, 24 hours; yields are GC yields; ^b: isolated yield.

With the reaction conditions in hand, we next investigated the scope of (α-amino)arylacetic acids. The results are shown in Fig. 1. Notably, the use of electron-poor phenylglycines still afforded pyrrolo[1,2-*a*]quinoxaline in a moderate yield (**3ab**). This result somewhat indicated that the decarboxylation and deamination was slowed down by electron-poor phenylglycines [9]. Sterically hindered phenylglycine was compatible with reaction conditions (**3bc**). (α-amino)heteroarylacetic acids were competent substrates (**3bd**, **3be**).

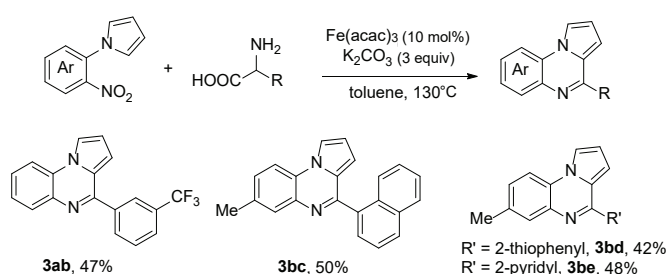


Fig. 1. Scope of (α-amino)arylacetic acids. Reaction conditions: 1-(2-nitroaryl)-1H-pyrroles (0.1 mmol), (α-amino)arylacetic acid (0.2 mmol), Fe(acac)₃ (0.01 mmol), K₂CO₃ (0.3 mmol), toluene (0.5 ml), 130°C, 24 hours. Yields are isolated yields.

At this moment, we hypothesised a plausible mechanism as that shown in Fig. 2 [9]. 2-Phenylglycine (2a) would undergo a decarboxylative/deaminative sequence to afford benzaldehyde 4 [13, 14]. Consequently, phenylglycine could be considered as the benzaldehyde equivalence in a Pictet-Spengler-type annulation. Ammonia obtained from the extrusion of 2a was likely involved in the reduction of the nitro group in 1-(2-nitrophenyl)-1H-pyrrole (1a), yielding the corresponding aniline 5. Traditional imine condensation of 4 and 5 would yield the adduct (6) followed by cyclization and hydrogen transfer to afford 7. Oxidation of 7 by 1a in the presence of FeCl₃ catalyst would furnish the desired product (3aa). Identical to the report of M. Kumar, et al. (2015) [9], we believe that iron salts were crucial for the overall condensation, although assignment of oxidation states at elementary steps (2a→4 or 1a→5) was not clear momentarily.

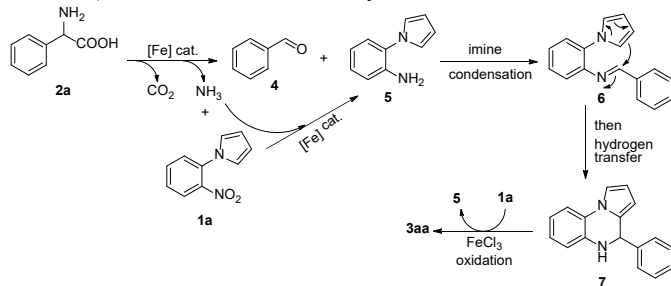


Fig. 2. Plausible mechanism.

4. Conclusions

To conclude, we developed a method for oxidative annulation of C-H bonds in (α-amino)arylacetic acids with 1-(2-nitroaryl)-1H-pyrroles. The reactions proceeded in the presence of an iron(III) acetylacetonate catalyst and a potassium carbonate base. Reaction conditions were tolerant of electron-poor and hetero (α-amino) arylacetic acids. The proposed mechanism involved two key steps: degradation of phenylglycine to afford ammonia followed by the reduction of 1-(2-nitrophenyl)-1H-pyrrole.

CRedit author statement

Tuan Hoang Ho: Methodology, Data analysis; Tung Thanh Nguyen: Conceptualisation, Data analysis, Writing, Editing; Tran Hoang Bao Ngo: Methodology; Nam Thanh Son Phan: Conceptualisation, Formal analysis, Writing, Reviewing.

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COMPETING INTERESTS

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

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Advanced magnetocaloric microwires: What does the future hold?

Hongxian Shen^{1,2}, Nguyen Thi My Duc^{1,3}, Hillary Belliveau¹, Lin Luo², Yunfei Wang⁴, Jianfei Sun², Faxiang Qin⁴, Manh-Huong Phan^{1*}

¹Department of Physics, University of South Florida, Tampa, Florida, 33620, USA

²School of Materials Science and Engineering, Harbin Institute of Technology, Harbin 150001, P.R. China

³The University of Danang, University of Science and Education,

459 Ton Duc Thang Street, Hoa Khanh Nam Ward, Lien Chieu County, Danang City, Vietnam

⁴Institute for Composites Science Innovation (InCSI), School of Materials Science and Engineering, Zhejiang University, 38 Zheda Road, Hangzhou, 310027, P.R. China

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Abstract:

Magnetic refrigeration (MR) based on the magnetocaloric effect (MCE) is a promising alternative to conventional vapor compression refrigeration techniques. The cooling efficiency of a magnetic refrigerator depends on its refrigeration capacity and operation frequency. Existing refrigerators possess limited cooling efficiency due to the low operating frequency (around tens of Hz). Theory predicts that reducing geometrical effects can increase the operation frequency by reducing the relaxation time of a magnetic material. As compared to other shapes, magnetocaloric wires transfer heat most effectively to a surrounding environment, due to their enhanced surface area. The wire shape also yields a good mechanical response, reducing the relaxation time and consequently increasing the operation frequency of the cooling device. Experiments have validated the theoretical predictions. By assembling microwires with different magnetocaloric properties and Curie temperatures into a laminate structure, a table-like magnetocaloric bed can be created and used as an active cooling device for micro-electro-mechanical system (MEMS) and nano-electro-mechanical system (NEMS). This paper assesses recent progress in the development of magnetocaloric microwires and sheds light on the important factors affecting the magnetocaloric behavior and cooling efficiency in microwire systems. Challenges, opportunities, and strategies regarding the development of advanced magnetocaloric microwires are also discussed.

Keywords: magnetic melt-extracted microwires, magnetic refrigeration, magnetocaloric effect, refrigerant capacity.

Classification numbers: 2.1, 2.3

1. Magnetic refrigeration based on the magnetocaloric effect

The MCE is a phenomenon where a magnetic material exhibits a change in temperature or magnetic entropy when subjected to an external magnetic field. MR technology based on the MCE is energy-saving, highly cooling efficient, and environmentally friendly, which makes it a promising platform to replace gas compression (GC) refrigeration techniques [1-6]. Unlike GC-based refrigerators, MR does not use harmful gases (e.g., hydrofluorocarbons) that can jeopardize the ozoneosphere and gets rid of complicated GC device components, making a simple and compacted MR device with low vibration and noise [1, 4]. Therefore, it is considered a future green solid-state cooling technology [6].

Figure 1 illustrates a full cooling cycle principle of a magnetic refrigerator based on the MCE [7]. In the absence of an applied magnetic field, the magnetic material has a temperature T_1 . As the material is adiabatically magnetised, the magnetic moments of the ferromagnetic material orient themselves in the direction of the applied field. The alignment of the magnetic moments causes a

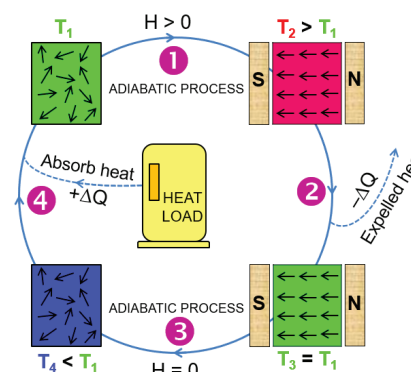


Fig. 1. The four stages of a MR cycle: (1) adiabatic magnetization, (2) heat removal, (3) adiabatic demagnetization, and (4) cool refrigerator contents.

decrease in the spin entropy and therefore the lattice entropy is increased. This increase in lattice entropy heats the material; the temperature of the material increases from T_1 to $T_2 = T_1 + \Delta T$. This excess heat (ΔT) is then removed by heat exchange with a cooler body, thus reducing the temperature of the material to $T_3 = T_1$. The

*Corresponding author: Email: phanm@usf.edu

removal of the applied field causes the magnetic moment entropy to increase and the lattice entropy and hence the material temperature to decrease to $T_4 = T_1 - \Delta T$. The increase/decrease in lattice entropy due to the decrease/increase in magnetic entropy alters temperature of the material and this can be used to refrigerate. It is worth noting that the heat transfer during the MCE process is dependent upon the entropy change in the magnetic refrigerant element [1, 4, 5]. Therefore, a material system exhibiting a large magnetic entropy change (ΔS_M) is highly valuable to MR technology [6].

2. What make magnetocaloric microwires promising for MR?

The geometric effect of refrigerants used in magnetic coolers has been overlooked by researchers looking to improve MR technology [1, 7-10]. Solid magnetocaloric materials can be fabricated in different forms: powder particles, thin films, or wires [1, 4, 5]. To improve magnetic cooling, it's very important to design them in wire form so that they can release heat better to surroundings because of their enhanced surface areas [9, 10].

M.D. Kuzmin (2007) [9] predicted that by reducing the dimensions of a magnetic refrigerant the cooling power of the device could be increased by increasing the operating frequency. His theoretical study has shown that shaping magnetic refrigerants in the form of spherical or irregular particles is inefficient, due to their high losses on viscous resistance and demagnetisation and suggests that the wire geometry could be utilized instead. Mechanical instability of the refrigerant can result in a significant loss of heat throughout due to unequal distribution of flow. In this context, the use of a bundle of magnetocaloric wires (e.g. Gd wires of $\sim 50 \mu\text{m}$ diameter) has been proposed to be more desirable because this configuration enables higher mechanical stability and lower porosity while simultaneously increasing the operating frequency of the magnetic refrigerant material [9, 10].

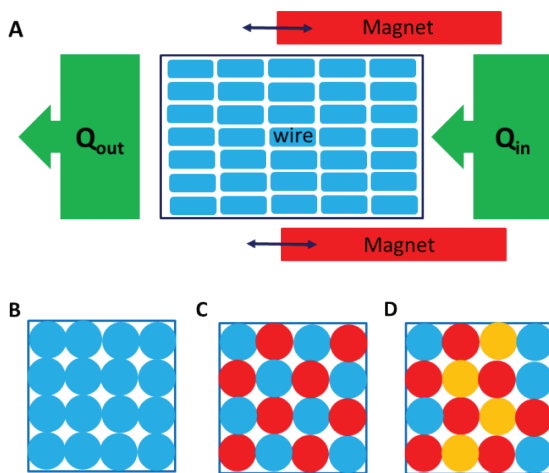


Fig. 2. (A) Illustration of a simplified active cooling system using magnetocaloric wires, similar to that proposed by D. Vuarroz, et al. (2012) [10]. Proposed magnetic beds composed of magnetocaloric microwires with same (B) and different (C, D) compositions (illustrated with different colors) possessing appropriate magnetic entropy changes and Curie temperatures, resulting in enhanced cooling efficiency.

D. Vuarroz, et al. (2012) [10] have theoretically predicted that a magnetic bed made of an array of Gd wires (Fig. 2A) produces a greater temperature difference between its ends, which results in a higher cooling load at a higher cooling efficiency, as compared to a magnetic bed made of Gd particles [10]. Unlike their bulk counterparts, the use of the wires with increased surface areas also allows for a higher heat transfer between the magnetic refrigerant and surrounding liquid [8]. These theoretical studies have opened new areas for MR devices and material design [9, 10]. Recent experiments on various magnetocaloric microwires have confirmed these predictions [11-42]. Since these magnetocaloric microwires can be easily assembled as laminate structures (Fig. 2B-D), they have potential applications as cooling devices for MEMS and NEMS. Instead of using the same type of magnetocaloric microwires (Fig. 2B) to design a magnetic bed, magnetocaloric microwires with different ΔS_M and Curie temperature values (Fig. 2C, D) can also be assembled to design a table-like magnetocaloric bed with an enhanced RC resulting from the broadened $\Delta S_M(T)$ curve.

3. Criteria for selecting magnetocaloric materials

The change in magnetic entropy ($\Delta S_M(T, \mu_0 H)$) of a magnetic material with respect to $\mu_0 H$ and T is often considered for MCE studies. The $\Delta S_M(T, \mu_0 H)$ is related to the change in magnetization (M) with respect to T through Maxwell relation [4, 5]:

$$\left(\frac{\partial S(T, H)}{\partial H} \right)_T = - \left(\frac{\partial M(T, H)}{\partial T} \right)_H \quad (1)$$

The ΔS_M caused by varying external magnetic field from $H = 0$ to $H = H_0$ is evaluated as

$$\Delta S_M(T, H_0) = S_M(T, H_0) - S_M(T, 0) = \mu_0 \int_0^{H_0} \left(\frac{\partial M(T, H)}{\partial T} \right)_H dH \quad (2)$$

The adiabatic temperature change, ΔT_{ad} , at a given temperature T_0 can be approximately calculated by

$$\Delta T_{ad}(T_0, H_0) \cong -\Delta S_M(T_0, H_0) \frac{T_0}{C(T_0, H_0)} \quad (3)$$

In these above equations, μ_0 is the permeability of vacuum and $(\partial M / \partial T)_H$ is the derivative of the magnetization with respect to temperature in a constant magnetic field. According to Eq. (2), the magnitude of ΔS_M depends on both the magnitude of M and $(\partial M / \partial T)_H$. As these values increase, so does the magnitude of ΔS_M , which is desired in the design of magnetic cooling devices. To obtain a large ΔS_M it is best to use a material with a steep change in M with respect to temperature at the transition temperature because $(\partial M / \partial T)_H$ is closely related to the magnetic order transition. From Eq. (3) a large ΔT_{ad} can be obtained by having a large ΔS_M around the magnetic ordering temperature and a small $C(T, H)$. However $C(T, H)$ varies widely between materials and as a result the larger ΔS_M does not necessarily lead to a larger ΔT_{ad} . Therefore, the parameter that characterizes the practicality of a magnetic refrigerant cannot solely be determined by the magnitude of ΔS_M .

To properly compare magnetic refrigerants, it is important to consider the temperature response of ΔS_M of the refrigerant, namely the refrigerant capacity (RC). The RC of a magnetic refrigerant material is calculated using [4]:

$$RC = \int_{T_{\text{hot}}}^{T_{\text{cold}}} -\Delta S_M(T) dT \quad (4)$$

The RC indicates how much heat can be transferred from the cold end (at T_{cold}) to the hot end (at T_{hot}) of a refrigerator in an ideal thermodynamic cycle. The relative cooling power (RCP), representing an amount of heat transfer between the hot and cold sides in an ideal refrigeration cycle, can be defined as

$$RCP = -\Delta S_M^{\text{max}} \delta T_{\text{FWHM}} \quad (5)$$

where $\delta T_{\text{FWHM}} = T_{\text{hot}} - T_{\text{cold}}$ is the temperature difference at the full width at half maximum of the magnetic entropy change curve. Both RC and RCP have been used to assess the usefulness of magnetocaloric materials for MR [1, 4, 5].

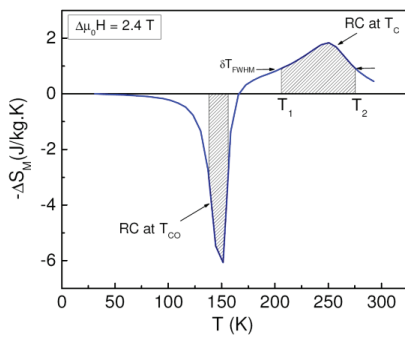


Fig. 3. The method for calculating the refrigerant capacity (RC) from the $-\Delta S_M(T)$ curve using Eq. (4) for the cases of the SOMT around the T_c and the FOMT around the T_{co} for the $\text{Pr}_{0.5}\text{Sr}_{0.5}\text{MnO}_3$ compound. Adapted from Ref. [43].

It is generally accepted that while first-order magnetic transition materials (FOMT) possess larger values of ΔS_M , they are characteristically limited to narrower temperature ranges [1, 4, 5]. This is the limiting factor of the operating temperature of this type of magnetic material. However, second-order magnetic transition materials (SOMT) have lower values of ΔS_M , which extends through a broader temperature range. Because of the broader operating temperature range as well as negligible thermal hysteretic losses, the RC of SOMT materials is generally larger than that of FOMT materials [4, 5]. Fig. 3 compares ΔS_M and RC values of the same material $\text{Pr}_{0.5}\text{Sr}_{0.5}\text{MnO}_3$ that undergoes the SOMT around the T_c (~ 250 K) and the FOMT around the T_{co} (~ 150 K) [43]. It can be seen that although the ΔS_M at the FOMT (~ 6 J/kg K) is three times greater than the ΔS_M at the SOMT (~ 2 J/kg K), the RC of the latter (~ 150 J/kg) is larger than the RC of the former (~ 120 J/kg), when the magnetic hysteresis loss is subtracted from the RC calculation according to Eq. (4).

Among the reported magnetocaloric materials, Gadolinium (Gd) is considered the benchmark material, which shows the best magnetocaloric characteristics for sub-room temperature magnetic cooling applications [1, 4, 6]. From a practical application perspective, however, Gd is too expensive, and it is therefore essential to reduce the amount of Gd used in the MR devices by doping or alloying, such as $\text{Gd}_5(\text{Si}_x\text{Ge}_{1-x})_4$ compounds [4]. Such doping or alloying also allows for the tailoring of the structural,

magnetic, and magnetocaloric properties of Gd-based materials for MR at different temperature regimes [1, 4]. The majority of the research in this field is therefore focused on the development of Gd-based microwires for MR in the nitrogen temperature regime [11-29]. Other types of magnetocaloric microwires such as Heusler and high-entropy alloy microwires have recently been designed and fabricated, and some of them exhibit excellent magnetocaloric properties in the room temperature region [30-42]. We assess below (section 5.1) the magnetocaloric properties of these microwires and their usefulness for MR applications.

4. Effects of reduced dimensionality on the magnetocaloric behavior and cooling efficiency

A clear understanding of the effects of reduced dimensionality on the magnetocaloric response of a magnetic material is essential to optimising the performance of a cooling device [8, 44]. To gain insights into these effects, Fig. 4 compares the magnetic and magnetocaloric properties of $\text{Gd}_{53}\text{Al}_{24}\text{Co}_{20}\text{Zr}_3$ in various forms of bulk alloys, a single microwire, and a multiwire array. It can be seen that the shape of the M - T curves for the single-wire and multiwire samples is rather flat, which is in sharp contrast with the sharp transition at the T_c for the bulk sample (Fig. 4A). However, this feature is only observed when the applied magnetic field is small (less than 500 Oe). As compared to the single wire, the M - T curve becomes broader near the T_c in the multiwire array, which can arise from the enhanced magnetostatic interaction between wires in the multi-wire system.

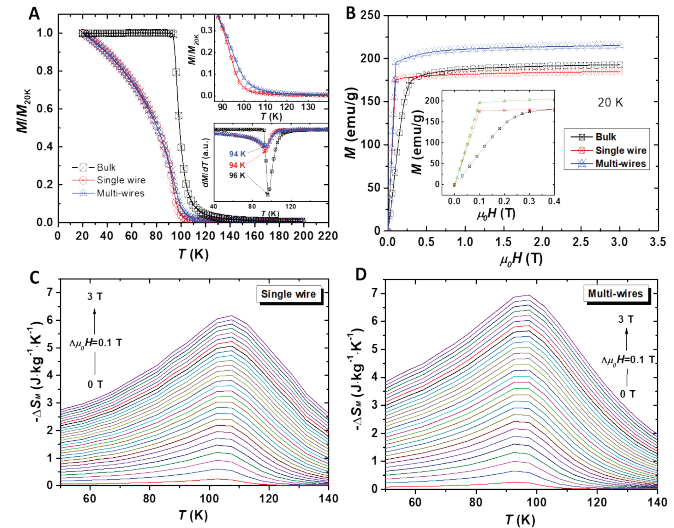


Fig. 4. (A) Temperature and (B) magnetic field dependence of the magnetization for $\text{Gd}_{53}\text{Al}_{24}\text{Co}_{20}\text{Zr}_3$ functionalized as bulk alloys, a single microwire, and a multiwire array. Temperature dependence of the magnetic entropy change $-\Delta S_M(T)$ around the T_c for the single wire and (D) the multiwire system.

The Curie temperature values of the samples are determined by the dM/dT vs. T curves, which show a slight reduction in T_c in the Gd-based materials with reduced dimensions. Interestingly, the M - H data (Fig. 4B) shows that the magnetization of the single wire is more dominant at low field range (< 0.5 T or 5000 Oe) compared

Table 1. Maximum entropy change, $|\Delta S_M^{\max}|$, Curie temperature, T_C , refrigerant capacity (RC), and relative cooling power (RCP) for the as-prepared amorphous and annealed wire samples. Values from microwires of other compositions, bulk glasses, Gd and $Gd_5Si_2Ge_{1.9}Fe_{0.1}$ are included for comparison.

Microwires	T_C (K)	$\mu_0\Delta H$ (T)	$ \Delta S_M^{\max} $ (J/kg K)	RC (J/kg)	RCP (J/kg)	Ref.
$Gd_{53}Al_{24}Co_{20}Zr_3$ (SW, Amorphous)	94	5 2	8.8 4.3	600 220	774 296	[16]
$Gd_{53}Al_{24}Co_{20}Zr_3$ (SW, at 100°C)	94	5 2	9.5 4.7	687 285	893 348	[16]
$Gd_{55}Al_{24}Co_{20}Zr_3$ (SW, at 200°C)	93	5 2	8.0 3.8	629 243	744 307	[16]
$Gd_{55}Al_{24}Co_{20}Zr_3$ (SW, at 300°C)	92	5 2	5.1 2.4	396 144	525 184	[16]
$Gd_{53}Al_{24}Co_{20}Zr_3$ (B, Amorphous)	95 95	5 3	9.6 6.2	690 340	- -	[11]
$Gd_{53}Al_{24}Co_{20}Zr_3$ (MW, Amorphous)	94 94	5 3	10.3 6.9	733 420	- -	[11]
$Gd_{50}Co_{20}Al_{30}$ (MW, A+NC)	86	5	10.1	672	896	[15]
$Gd_{55}Co_{20}Al_{25}$ (MW, A+NC)	100	5	10.1	653	870	[15]
$Gd_{60}Al_{20}Co_{20}$ (MW, A+NC)	109	5	10.1	681	908	[15]
$Gd_{55}Al_{20}Co_{25}$ (MW, Amorphous)	110	5	9.69	580	804	[44]
$Gd_{40}Co_{15}Al_{45}$ (MW, Amorphous)	100	5	9.73	732	976	[45]
$Gd_{40}Co_{25}Al_{35}$ (R, Amorphous)	125	5	10.1	645	860	[46]
$Gd_{40}Fe_{20}Al_{40}$ (MW, Amorphous)	202	5	4.8	687	900	[20]
$Gd_{45}Fe_{15}Al_{40}$ (R, Amorphous)	182	5	5.8	545	726	[47]
$Gd_{55}Fe_{15}Al_{30}$ (R, Amorphous)	158	5	5.01	555	741	[47]
$Gd_{55}Fe_{25}Al_{20}$ (R, Amorphous)	190	5	4.67	651	868	[47]
$Gd_{55}Fe_{25}Al_{20}$ (R, Amorphous)	230	5	3.77	608	811	[47]
$Gd_{55}Fe_{25}Al_{20}$ (GR+NC)	232	5	4	551	-	[48]
$Gd_{55}Co_{30}Al_{15}$	127	5	9.71	573	702	[23]
$Gd_{55}Co_{30}Ni_{10}Al_5$	140	5	8.91	532	668	[23]
$Gd_{55}Co_{30}Ni_{10}Al_5$	158	5	7.68	523	653	[23]
$Gd_{13.5}Si_{13.5}B_{13.5}/Gd_{13.5}B_{13.5}$ (MW, A+NC)	106	5	6.4	790	885	[21]
$Gd_3Ni/Gd_{65}Ni_{35}$ (MW, A+NC)	120	5	9.64	742	-	[22]
$Gd_{50}(Co_{25}Fe_{25}Si_{10}B_{10})_{50}$	170	5	6.56	625	826	[19]
$Gd_{59.4}Al_{19.8}Co_{19.8}Fe_1$ (MW, Amorphous)	113	5	10.33	748	1006	[14]
$Mn_{120}Fe_{60}P_{0.68}Si_{0.52}$ (MW, PC)	141	5	4.64	-	-	[35]
$Ni_{48}Mn_{26}Ga_{19.5}Fe_{6.5}$ (MW, PC)	361	5	4.7	-	-	[30]
$Ni_{49.4}Mn_{26.1}Ga_{20.8}Cu_{3.7}$ (MW, PC)	359	5	8.3	78	-	[31]
$Ni_{50.2}Mn_{29.5}Ga_{20}$	368	5	18.5	63	-	[32]

Microwires	T_C (K)	$\mu_0\Delta H$ (T)	$ \Delta S_M^{\max} $ (J/kg K)	RC (J/kg)	RCP (J/kg)	Ref.
$Ni_{50.6}Mn_{29}Ga_{21.4}$	340-370	5	5.2	240	-	[32]
$Ni_{45.4}Fe_{31}Mn_{38.4}Sn_{12.4}$ (MW, A+NC)	270 (F) 300 (S)	5 5	15.2 4.3	146 175	182 215	[34]
DyHoCo (MW, Amorphous)	35	5	11.2	417	530	[37]
HoErCo (MW, Amorphous)	16	5	15	527	600	[42]
HoErFe (MW, A+NC)	44	5	9.5	450	588	[36]
$Gd_{60}Al_{20}Co_{20}$ (MW, A+NC)	113	5	10.12	698.28	936.19	[25]
$(Gd_{60}Al_{20}Co_{20})_{99}Ni_1$ (MW, A+NC)	111	5	10.98	725.49	970.89	[25]
$(Gd_{60}Al_{20}Co_{20})_{99}Ni_1$ (MW, A+NC)	109	5	11.06	746.84	1000.50	[25]
$(Gd_{60}Al_{20}Co_{20})_{99}Ni_1$ (MW, A+NC)	109	5	11.57	834.14	1138.16	[25]
$(Gd_{60}Al_{20}Co_{20})_{99}Ni_1$ (MW, A+NC)	108	5	10.77	733.48	977.65	[25]
$Gd_{36}Th_{20}Co_{20}Al_{24}$ (MW, Amorphous)	91	5	12.36	731	948	[26]
$Gd_{36}Th_{20}Co_{20}Al_{24}$ (MW, A+NC)	81	5	8.8	500	625	[26]
$(Gd_{36}Th_{20}Co_{20}Al_{24})_{99}Fe_1$ (MW, A+NC)	94	5	8.5	510	635	[26]
$(Gd_{36}Th_{20}Co_{20}Al_{24})_{99}Fe_1$ (MW, A+NC)	100	5	8.0	515	660	[26]
$(Gd_{36}Th_{20}Co_{20}Al_{24})_{99}Fe_1$ (MW, A+NC)	108	5	7.6	520	680	[26]
$Gd_{19}Th_{19}Er_{19}Fe_{19}Al_{25}$ (MW, A+NC)	97	5	5.94	569	733	[24]
$Gd_{36}Th_{20}Co_{20}Al_{24}$ (MW, A+NC)	82	5	9	518	657	[40]
$Dy_{36}Th_{20}Co_{20}Al_{24}$ (MW, A+NC)	42	5	8.2	301	414	[40]
$Ho_{36}Th_{20}Co_{20}Al_{24}$ (MW, A+NC)	42	5	10.3	372	474	[40]
$LaFe_{11.9}Si_{1.4}$ (MW, Amorphous)	195	2	9.0	-	45	[49]
$Ni_{48}Mn_{26}Ga_{19.5}Fe_{6.5}$ (MW, Amorphous)	361	5	4.7	-	18	[13]
$Gd_{35}Al_{24}Co_{20}Zr_3$ (B)	93	5	9.4	509	-	[50]
$Gd_{48}Al_{25}Co_{20}Zr_3Er_4$ (B)	84	5	9.4	647	-	[51]
$Gd_{35}Co_{20}Al_{25}$ (B)	103	5	8.8	541	-	[52]
$Gd_{35}Co_{25}Ni_{20}$ (B)	78	5	8.0	640	-	[52]
Gd(B) ^b	294	5	10.2	410	-	[4]
$Gd_3Si_2Ge_{1.9}Fe_{0.1}$ (B) ^b	305	5	7.0	360	-	[4]

SW: single wire; MW: multiple wires; B: bulk; R: ribbon; A+NC: amorphous + nanocrystalline; ^b crystalline structure.

to its bulk counterpart, while an opposite trend is observed at higher magnetic fields. The multiwire system shows the largest magnetization among these samples (Table 1). This can be attributed to the enhanced multiwire interaction, where spins near the surfaces of the wires align, giving rise to an enhancement in the magnetization of the system. Owing to the reduced dimensionality and shape anisotropy effects, the single and multi-wire samples show a faster approach to saturation of magnetization with respect to an applied magnetic field, as compared to their bulk counterpart. As a result, the multi-wire sample shows the largest ΔS_M and RC values among the samples investigated (Fig. 4C, 4D), while it is found to be almost equal for the bulk and single-wire samples (Table 1). These findings are important, highlighting the superior properties of the magnetocaloric microwire arrays that can be used as magnetic beds in active magnetic refrigerators. Nonetheless, it is worth noting that the heterogeneity of wire diameters affects wire-wire interaction and therefore MCE behavior. If this can be well controlled, the heterogeneity effect is minimal, and the resulting ΔS_M and RC can be optimized in the multiwire array. As compared to the single wire, the multiwire array shows a significantly enhanced ΔS_M and RC. This feature has been mostly observed for the case of Gd-based microwires, which appears to be opposite for the cases of MnFePSi and Heusler alloy systems, due to composition inhomogeneity. Therefore, it is important to provide specific information about wire samples when reporting MCE results in journals. Also, it remains unclear how ΔS_M and RC vary with the wire diameter and/or the number of wires in an array. The case might become more complicated if different types of wires are used in the array. These important issues need to be addressed in future studies.

In the following sections, unless specified, we have assessed the magnetocaloric properties of different magnetic multiwire systems. It should be noted herewith that the comparison of ΔS_M and RC values between reported multi-wire systems is rather relative, since the total number of wires used in each multiwire array can vary.

5. Magnetocaloric microwires

While previous research focused on exploring the MCE in bulk materials (both single and polycrystalline forms) [1, 4, 5], the study of magnetocaloric materials in wire form has only drawn growing attention in the scientific community since the first work reported by F.X. Qin, et al. (2013) [12] on the excellent mechanical, magnetic and magnetocaloric properties of melt-extracted $\text{Gd}_{53}\text{Al}_{24}\text{Co}_{20}\text{Zr}_3$ amorphous microwires [12, 13]. Since that work, a wide range of Gd-alloy microwires with outstanding magnetic and magnetocaloric properties has been fabricated using the melt-extraction technique [11-29]. Other types of magnetocaloric microwires such as Heusler and high entropy alloys $\text{Ni}_{48}\text{Mn}_{26}\text{Ga}_{19.5}\text{Fe}_{6.5}$ [13], Ni-Mn-Ga-Fe [30, 33], Ni-Mn-Ga-Cu [31], Ni-Fe-Mn-Sn [34], Ni-Mn-Ga [32], HoErFe [36], DyHoCo [37], $\text{RE}_{36}\text{Tb}_{20}\text{Co}_{20}\text{Al}_{24}$ (RE= Gd, Dy or Ho), and $\text{LaFe}_{11.6}\text{Si}_{1.4}$ [49] have also been fabricated and investigated (Table 1).

5.1. Fabrication of microwires

Figures 5A, 5B illustrate a fabrication method based on the melt-extraction technique advanced by Prof. Sun's group for making a variety of high quality Gd-alloy and Heusler alloy microwires [11-26, 30-33]. The whole fabrication process is under vacuum protection, so the chamber needs to reach a high vacuum of 10^{-3} Pa first and be then filled to 5×10^3 Pa with high purity Ar (99.97%). Before melting spin, an alloy rod is placed inside a borazon crucible with an inner diameter of 10.5 mm and the Cu wheel is pre-heated to 100°C prior to melt-extraction. The line velocity of the Cu wheel is typically fixed at 30 m/s, and the edge of the wheel shows an acute angle of 60° (Fig. 5A).

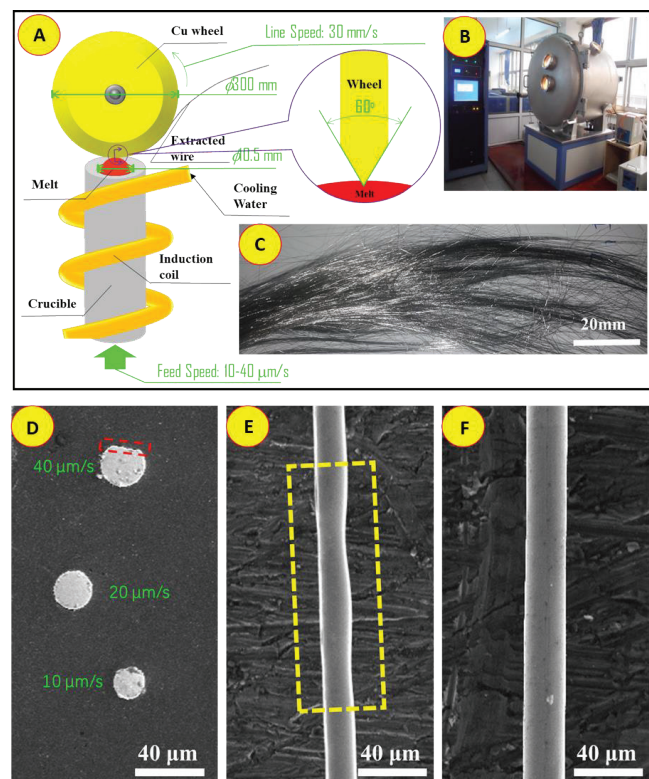


Fig. 5. (A) Detailed illustration of the melt-extraction technique; (B) the melt-extraction equipment and (C) the macro photograph of the fabricated wires. Cross-sectional (D) and SEM images of the Gd-based wires with different feed speeds of 10 $\mu\text{m/s}$ (E) and 20 $\mu\text{m/s}$ (F).

The top of the rod is re-melt by high frequency electromagnetic induction heating, the melting temperature should be kept at $T_m + 50^\circ\text{C}$. To obtain desirable microwires, the feed speed can vary between 10 and 40 $\mu\text{m/s}$. When the melt is in contact with the edge of the wheel, a thin layer of the melt is extracted and adhered to the wheel. The melt layer is quenched to a supercooled state by the wheel and then deviated from the wheel due to the centrifugal force and decreasing wettability. Finally, the melt layer is rounded as the action of the surface tension and finally solidified to form long wires during free flight stage. The SEM images of Gd alloy

melt-extracted microwires are shown in Fig. 5C. It is worth noting that an average diameter of wire can be varied by adjusting the feed speed (Figs. 5D-5F). However, the uniformity of the wire diameter varies in a more complicated way. It is important to produce wires of uniform diameter over their long length.

During melt-extraction, magnetocaloric microwires can be purely amorphous [11, 12, 20] or a mixture of amorphous and nanocrystalline phases [15, 16]. Adjusting the fabrication parameters associated with the melt-extraction process allows for the creation of either single phase (purely amorphous) wires or biphasic amorphous/nanocrystalline wires with desirable magnetocaloric properties. Biphasic amorphous/nanocrystalline wires can also be created by annealing their as-quenched amorphous counterparts [16]. The nanocrystalline to amorphous volume fraction ratio can be controlled by adjusting the annealing temperature and/or the annealing time. Based on these studies, we propose in Fig. 6 three approaches for creating nanostructures in melt-extracted magnetocaloric wires.

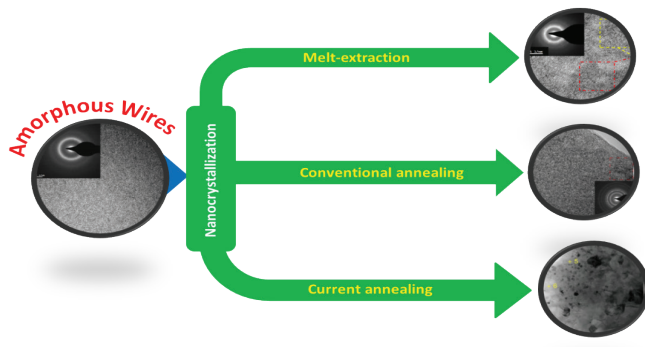


Fig. 6. Three approaches for creating nanostructures in melt-extracted amorphous wires: nanocrystallization via melt-extraction (approach #1); nanocrystallization via conventional annealing (approach #2); and nanocrystallization via current annealing (approach #3). High-resolution electron transmission microscopy (TEM) is often used to reveal the nature of nanostructures formed in melt-extracted wires.

The first method is to utilize the advantage of controlling the parameters associated with melt-extraction. Nanocrystals can be created directly within an amorphous matrix of the wire during melt-extraction process [15]. The products are typically biphasic nanocrystalline/amorphous wires. The shortcoming of this method is to sometime create unwanted minor phases in wires, which depend on alloy elements used. The second method is to create nanocrystals in amorphous wires through conventional (furnace) annealing [16]. This method also yields nanocrystalline/amorphous wires, in which nanocrystal sizes can be controlled by adjusting annealing temperature and time. This method is usually time-consuming since it takes time and effort to perform experiments to find out optimal annealing conditions. The third method is to utilize Joule heating effect by which the passage of a dc current through the wire produces heat that is sufficient to partially convert an amorphous phase (mostly on the surface of the wire) into the nanocrystalline phase [39]. We discuss below the advantages and

shortcomings of these single-phase and biphasic or multiphase magnetocaloric microwires. Table 1 lists several magnetocaloric microwires that are promising candidates for energy-efficient MR technology.

5.2. Gd-based microwires

5.2.1. Single phase materials

Several single phase Gd-alloy microwires have been investigated, such as $\text{Gd}_{53}\text{Al}_{24}\text{Co}_{20}\text{Zr}_3$ [11, 12], $\text{Gd}_{50}(\text{Co}_{69.25}\text{Fe}_{4.25}\text{Si}_{13}\text{B}_{13.5})_{50}$ [19], $\text{Gd}_{60}\text{Fe}_{20}\text{Al}_{20}$ [20], and $\text{Gd}_{55}\text{Co}_{30}\text{Ni}_{15-x}$ [23] amorphous microwires. In addition to the superior properties that the wire form promises, their amorphous nature also brings additional advantages such as broadened operating temperature ranges and hence enhanced RC values due to their magnetically disordered structure.

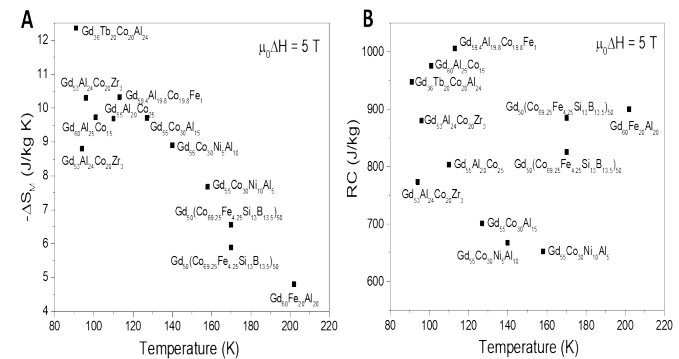


Fig. 7. (A) Magnetic entropy change $\Delta S_M(T)$ and (B) refrigerant capacity $RC(T)$ for a field change of 5 T for several single-phase Gd-alloy microwires.

Figure 7 shows the ΔS_M and RC values (at the corresponding Curie temperatures, T_C) of several single-phase Gd-based microwires for a field change of 5 T. It can be seen in this figure that both ΔS_M and T_C can be tuned by varying alloy compositions. There is a tendency that the larger values of ΔS_M are achieved in materials with lower T_C values. This tendency is rather different from that observed for biphasic systems (Fig. 8). In the temperature range of 80-200 K, the $\text{Gd}_{36}\text{Tb}_{20}\text{Co}_{20}\text{Al}_{24}$ microwires show the largest ΔS_M among samples compared (Fig. 7A), while the largest RC is achieved in the $\text{Gd}_{59.4}\text{Al}_{19.8}\text{Co}_{19.8}\text{Fe}_1$ microwires (Fig. 7B). The $\text{Gd}_{60}\text{Fe}_{20}\text{Al}_{20}$ microwires possess the largest RC around 200 K, due to the broadened $\Delta S_M(T)$ distribution [20]. The RC values of the single-phase magnetocaloric microwires are also reported and compared in Fig. 9 along with the biphasic magnetocaloric microwires.

5.2.2. Biphasic materials

While single phase magnetocaloric microwires have been shown to exhibit superior magnetocaloric properties, there is an opportunity for further improvement in their magnetocaloric figures of merit. This can be achieved by creating magnetic nanocrystals embedded in an amorphous magnetic matrix [15, 16]. Magnetic coupling between the crystalline and amorphous phases

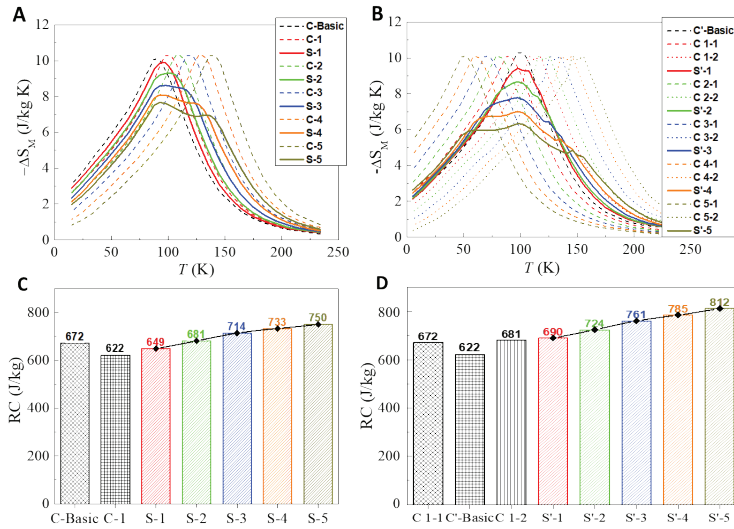


Fig. 11. Simulated $-\Delta S_M$ vs. T curves at $\mu_0\Delta H=5$ T of the designed systems and their components for (A) two-component systems and (B) three-component systems. RC values of (C) Sample C-Basic and (D) Sample C'-Basic and their components. Adapted from Ref. [29].

By selecting ΔS_M and RC values for the designed structures with different T_C intervals, it is possible to design different samples named S-1, S-2, S-3, S-4, and S-5 by two components with different T_C intervals. Herein, the $\Delta S_M(T)$ data of $\text{Gd}_{50}\text{Al}_{30}\text{Co}_{20}$ microwires was used as one basic composition (named C-Basic) and $\text{Gd}_{50}\text{Al}_{25}\text{Co}_{25}$ microwires as other varying compositions (C-1, C-2, C-3, C-4 and C-5). The T_C intervals between varying compositions (C-1, C-2, C-3, C-4, C-5) and C-Basic are ~ 10 K, 20 K, 30 K, 40 K and 50 K, respectively. The $-\Delta S_M$ vs. T curves at $\mu_0\Delta H=5$ T for the designed structures are calculated using Eq. (6) are displayed in Figs. 11A, 11B, which shows that the designed samples (S-1, S-2, S-3, S-4, and S-5) exhibit more table-like MCE with the increasing T_C intervals. Additionally, the values of $-\Delta S_M^{\max}$ decreases when the T_C intervals increase. The RC values of the designed samples and their components (C-1, C-2, C-3, C-4, and C-5) at $\mu_0\Delta H=5$ T are plotted in Figs. 11C, 11D for comparison. The RC value of S-1 is lower than that of C-Basic when T_C interval is ~ 10 K, while RC values of other samples are higher than those of both components. Remarkably, the RC values of the designed samples increase with increasing the T_C interval, which means that the enhanced RC values for the designed systems can be obtained by using the Gd-based wires with large T_C intervals in a range of temperature. The designed systems with three components show the same tendency as those with two components, which exhibits a table-like MCE and the values of $-\Delta S_M^{\max}$ decreases when the T_C intervals increase (Fig. 11D).

7. Concluding remarks and outlook

Refrigerants in form of microwires can yield higher cooling efficiencies compared to their bulk or other shape counterparts. The use of these microwires promises high applicability in active MR due to their outstanding soft magnetic, magnetocaloric and mechanical properties. The micro-size of the microwires enhances the surface area for the designed structures, which improves the

efficiency of heat transfer significantly in cooling systems. These excellent magnetocaloric and mechanical properties can be harnessed by creating novel biphasic nanocrystalline/amorphous structures via thermal annealing or during melt-extraction. These functional magnetocaloric microwires can be assembled as laminate structures, rendering them applicable in active cooling devices for MEMS and NEMS.

A variety of magnetocaloric microwires have been designed, fabricated, and investigated (Table 1). It has been shown that the Gd-alloy microwires are promising candidates for active MR in the liquid nitrogen temperature regime. Due to their SOMT, these microwires show no hysteresis losses, along with large MCEs and RCs. While alloying or doping has been found to tune the Curie temperature of the Gd-alloy microwires between 80 and 200 K, it appears that achieving these superior properties at ambient temperature is challenging in Gd-based microwire systems. Heusler alloy microwires such as Ni-Mn-Ga [32], Ni-Mn-Ga-Fe [30, 33], Ni-Mn-Ga-Cu [31], and Ni-Fe-Mn-Sn [34] are interesting candidates for cooling applications in the room temperature regime, since they order ferromagnetically at or above room temperature. While these systems show large MCEs around the FOMT transition, the RCs are found to be much smaller compared to those around the SOMT transition, the MCEs of the latter are much smaller though. The disadvantage of these microwires also lies in the large hysteretic losses associated with the FOMT nature. Refining chemical compositions/nanostructures in these systems may offer an approach for reducing the hysteretic losses and improving the RCs while preserving the large MCEs [61]. High entropy alloy microwires are also perspective candidates for cooling applications, but their MCEs and RCs are relatively smaller compared to the Gd-alloy and Heusler alloy microwires.

Moving forward, further research should be undertaken to examine the applicability of the currently available magnetocaloric microwires in cooling devices. It is essential to quantify how high the cooling energy efficiency can be achieved in a cooler using the microwires compared to their other shape counterparts. Several challenges need to be overcome. For instance, it is important to improve the existing melt-extraction techniques to create magnetocaloric microwires with uniform diameters. It is also challenging to control the fabrication parameters associated with the melt-extraction process for achieving pure amorphous phase microwires or biphasic microwires with controlled amorphous/nanocrystalline volume fraction ratios. The design of magnetocaloric microwires with controlled large, broadened magnetic entropy changes around room temperature is a very important but challenging task. The RCs of these microwires can be enhanced by coating the microwires with soft ferromagnetic layers such as FeNi, creating core/shell wire structures. Interfacial magnetic coupling and interaction between the core and shell is expected to broaden the magnetic entropy change, giving rise to the RC enhancement. Combining the melt-extraction and electrodeposition methods is considered most appropriate for making these novel core/shell wire systems. In an

array of magnetic microwires, magnetostatic interactions can play an important role in determining the magnetocaloric response of the system. In this context, tuning magnetostatic interactions through incorporating short magnetocaloric microwires into a thermally conductive polymer matrix appears to be an interesting prospective approach for creating novel magnetic refrigerants. Combined theoretical and experimental studies are needed to validate these hypotheses. Bringing the fundamental studies to the point of making prototype refrigeration devices will require the concerted effort of interdisciplinary scientists and engineers.

Since the Heusler alloy microwires [30-34] exhibit ferromagnetic order above room temperature, they may also be promising candidates for magnetic/temperature sensing [62, 63], magnetic hyperthermia [64], and electromagnetic microwave applications [65, 66]. Harnessing these novel functionalities might make them truly multifunctional for modern device applications, opening new opportunities for research in this interdisciplinary area.

CRedit author statement

Hongxian Shen, Nguyen Thi My Duc, Hillary Belliveau, Lin Luo, Yunfei Wang: Performed experiments and analyzed the data; Hongxian Shen, Nguyen Thi My Duc, Hillary Belliveau, and Manh-Huong Phan: Wrote the initial manuscript with inputs from Jianfei Sun, Faxiang Qin; Manh-Huong Phan: Led the project.

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COMPETING INTERESTS

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

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Tensile behaviour of the dissimilar friction stir welding between pure copper and aluminium 1050

Hoai Nam Quach¹, Dinh Hao Duong¹, Thanh Nhut Pham², Huu Huy Ho¹,
Hong Chuong Pham³, Van Hao Pham⁴, Hung Tra Tran^{1*}

¹Mechanics Department, Nha Trang University, 2 Nguyen Dinh Chieu Street, Vinh Tho Ward, Nha Trang City, Khanh Hoa Province, Vietnam

²Department of Naval Architecture, Nha Trang University, 2 Nguyen Dinh Chieu Street, Vinh Tho Ward, Nha Trang City, Khanh Hoa Province, Vietnam

³Quang Nam College, 224 Huynh Thuc Khang Street, An Xuan Ward, Tam Ky City, Quang Nam Province, Vietnam

⁴Institute of Scientific Research Application, Hanoi Pedagogical University, 136 Xuan Thuy Street, Dich Vong Hau Ward, Cau Giay District, Hanoi, Vietnam

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Abstract:

Copper-aluminum hybrid connectors play a crucial role in electric vehicles, but welding these metals together poses a significant challenge due to their distinct physical and mechanical properties. Friction stir welding, a solid-state welding technique, proves to be suitable for joining dissimilar metals. In this study, friction stir welding was employed to create a joint between pure copper 1100 and aluminum 1050 plates, each with a thickness of 5 mm. The fabricated results showed that a significant amount of copper was dispersed into the aluminum side to establish the bonding. The bonding strength was evaluated through tensile testing, while the macrostructure and fracture paths of the joints were monitored using an optical microscope. Despite efforts, eliminating defects in the joint proved challenging, with most defects attributed to vacancies. Increasing the tool rotation speed resulted in greater mixing intensity between the aluminum and copper. The interface between the copper and aluminum was found to be prone to crack propagation. The tensile strength of the joint improved with higher tool rotation, reaching up to 88 MPa. For further analysis, stress within the joint under tensile loading was simulated.

Keywords: dissimilar Cu/Al joint, friction stir welding, interface stress, tensile failure.

Classification numbers: 2.1, 2.3

1. Introduction

To reduce emissions of greenhouse gases, electric vehicles have been the most interesting option in recent years. By increasing the number of electric vehicles on the road, the dependency on fossil fuels will reduce. Regarding hybrid connectors, some studies have addressed dissimilar welding of copper to aluminium as one of the main electrical connections in electric vehicles [1, 2]. It is well known that copper and aluminium are key materials in the field of electronics. In comparison with aluminium, copper possesses excellent electric conductivity, but this metal is remarkably more expensive and heavier. Thus, both copper and aluminium are used to optimize the cost. Unfortunately, since there is a significant difference in the potential energy between copper and aluminium, contact between them leads to galvanic phenomena in the joints, resulting in a short lifespan. Thus, several efforts have been made toward welding copper to aluminium. However, the large difference in properties of the two alloys is a big challenge in the

joining process. Traditional fusion welding methods seem to be an ineffective solution for this dissimilar joint. So, some methods of solid-state welding such as cold rolling, brazing, explosive welding, among others, have been used to join them.

Recently, FSW technology has emerged as a key method to join copper to aluminium with high strength and long life [3-9]. However, upon mixing copper and aluminium with their remarkable differences in physical and mechanical properties, a non-uniform flow can form during the FSW technique and an unstable welding process seems to be inevitable. Thus, welding parameters sensitively affected the microstructure and mechanical properties of this type of joint. The high demand on the welding machine and instability in the welding process are the challenges in FSW of dissimilar joints. In this study, a joint between aluminium 1050 and pure copper C1100 was manufactured by FSW technology in the Friction Welding Laboratory of Nha Trang University. The tensile behaviour of the joints fabricated with various tool rotation speeds was

*Corresponding author: Email: tra@ntu.edu.vn

investigated. Attention was placed on defect morphology and crack paths. The results in this work provide a view to enhance the possibility of fabrication of hybrid Cu/Al connectors by the FSW technique in Vietnam.

2. Materials and methods

A dissimilar joint between 5.0-mm-thick pure copper 1100 and aluminium 1050 plates was friction stir welded in Nha Trang University's Friction Welding Laboratory as demonstrated in Fig. 1. The properties of the two metal alloys aluminium 1050 and copper 1100 are presented in Tables 1 and 2 [10]. Before welding, the base metals were cut to 150×60 mm (length × width) and then cleaned with air pressure. A pin tool used for fabricating the joints was 8.0 mm in diameter and 4.8 mm in length. The tilt angle was set to 2.0 degrees off the perpendicular direction to the work pieces. Regarding the welding parameters, the welding speed was a constant value of 100 mm/min while the rotational speed was varied from 600 to 1000 rpm. The cross sections of the welded joints were sliced by a specimen cutting machine (MA-CU250M) to examine the macro- and microstructure. The specimens' surfaces were polished by a polishing machine (MA-PO250M). An Olympus microscope was used to examine the macrostructure and microstructure. The geometry of the tensile specimens was fabricated via ASTM E08 standard as seen in Fig. 2. The loading direction of the tensile specimen was perpendicular to the welding line. The tensile test was carried out by employing an Instron machine 3366 with a speed of 2 mm/min.

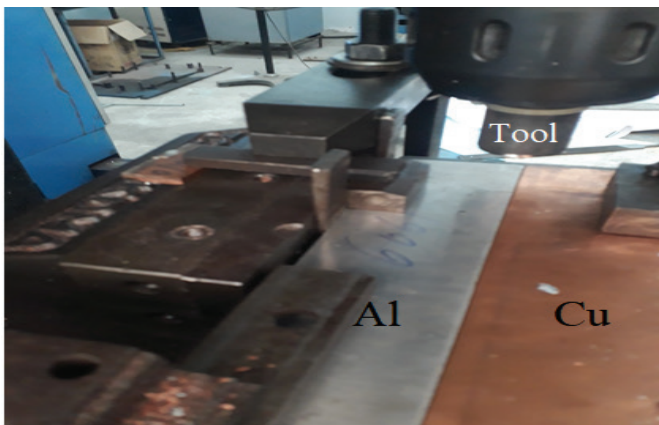


Fig. 1. Welding process setup.

Table 1. The chemical composition of aluminium 1050 and copper 1100 (% wt).

Element	Al	Cu	Fe	Mg	Mn	Si	Ti	V	Zn	Other
Aluminium 1050	≥99.50%	≤0.05%	≤0.40%	≤0.05%	≤0.05%	≤0.25%	≤0.03%	≤0.05%	≤0.05%	≤0.03%
Pure copper 1100		≥99.9%								<0.1%

Table 2. The mechanical properties of aluminium 1050 and copper 1100.

Mechanical properties	Yield strength (MPa)	Tensile strength (MPa)	Elongation (%)	Hardness (HV)	Elastic module (GPa)	Poisson ratio
Aluminium 1050	118.0	127.0	9.1	38.0	69.0	0.33
Copper 1100	230.0	245.0	26.0	87.0	115.0	0.33

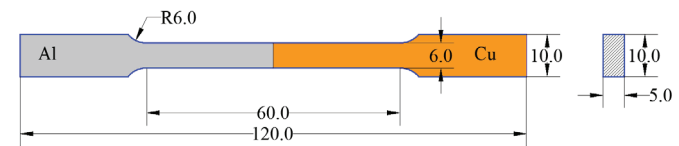


Fig. 2. The geometry of the tensile specimens.

3. Results and discussion

The tensile strengths of the joints produced by various rotational speeds are shown in Fig. 3. It seems that the joint strength improved owing to an increase in tool rotational speed. Here, the tensile stress of the joint gradually increased with increasing the rotational speed. The joint's strength reaches about 88 MPa corresponding to 1000 rpm.

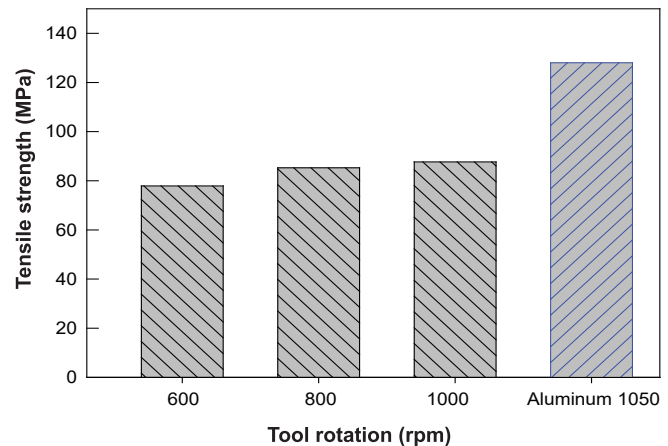
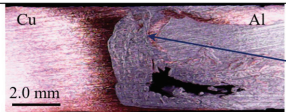
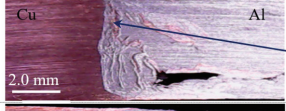
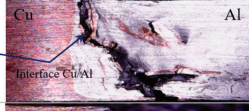
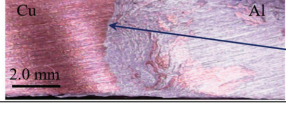
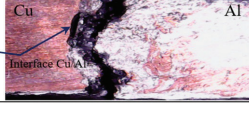


Fig. 3. The tensile strengths of the joints at various rotational speeds.

The macrostructure and the tensile fracture of the joints were monitored. Table 3 summarises the cross sections of the dissimilar joints between copper 1100 and aluminium 1050 at various welding regimes. The macrostructures of the joints are shown in the second column of Table 3. The third column represents the bonding's fracture location under tensile loadings. The FSW process could be used to achieve an advantageous bond between copper 1100 and aluminium 1050. The welded zone was formed in the inhomogeneous features. The copper metal was stirred and dispersed dramatically into the aluminium in all welding regimes, as shown in Table 3 and Fig. 4. Tunnel defects formed on the bottom side of the joints and appeared to

be dominant at low rotation speeds (600 and 800 rpm) as shown in Table 3. The tunnel seems to be eliminated when the rotation speed reaches 1000 rpm.

Table 3. The cross sectional macrostructure of the joint at three welding regimes (copper on the left and aluminium on the right).

Tool rotation speed, rpm.	Macrostructure	Fracture location
600		
800		
1000		

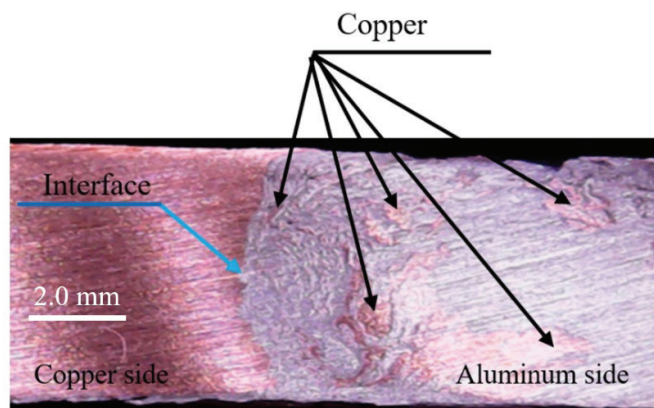


Fig. 4. The cross section of the joint fabricated at a tool rotation speed of 1000 rpm.

The cracks initiated from the tunnels and propagated to the copper/aluminium interface when the joints were subjected to tensile loading. However, in the case without a tunnel defect (at 1000 rpm welding regime), the crack also initiated and propagated mostly at the interface (Table 3).

Focusing on the failure of the joint without defects fabricated at 1000 rpm, various copper particles were mixed into aluminium in the welded zone, as seen in Fig. 4. This means that the tensile strength of the welded zone (on the aluminium side) might be enhanced by copper particles since the tensile strength of the base copper C1100 is twice as high as that of base aluminium 1050. This view also implies that the tensile strength of the stirred zone might be higher than that of the heat-affected zone on the aluminium side. From this point of view, the joint fractured near the interface in the aluminium side as seen in the 4th row of

Table 3 could not be explained by the microstructure view. Instead, the failure might be associated with the intermetallic compounds (IMCs) mentioned in [10].

One of the factors suspected to enhance failure at the interface zone is the stress concentration at the interface induced by the dissimilar properties between the two metals. To clarify the role of the dissimilar effect in the stress state at the interface, the stress and strain in the copper/aluminium joint were numerically simulated using Ansys software. The stress state in the joint under tensile loading was simulated using an elastic analysis model. Copper has a Young's modulus of 115 GPa and a Poisson's ratio of 0.33. Aluminium 1050 has a Young's modulus of 69 GPa and a Poisson's ratio of 0.33, respectively. The welded zone's Young's modulus and Poisson's ratio are assumed to be the same as the base aluminium 1050. Fig. 5 depicts a finite element model of the joint. Figs. 6 and 7 show the equivalent stresses in the joints. There was a significant stress concentration at the interface and on the copper side. Because of the dissimilar effect, the stress at the interface on the aluminium side was significantly reduced. From this point of view, it is obvious that the stress induced by the dissimilar base metals has no role in the failure of the joint where the fracture took place, which is near the interface and on the aluminium side (as seen in the 4th row of Table 3).

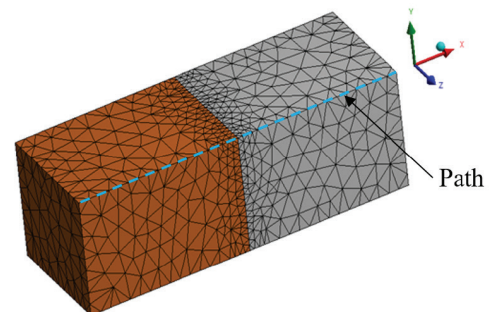


Fig. 5. FEM model of the dissimilar copper/aluminium joint. Cu on the left side, Al on the right side.

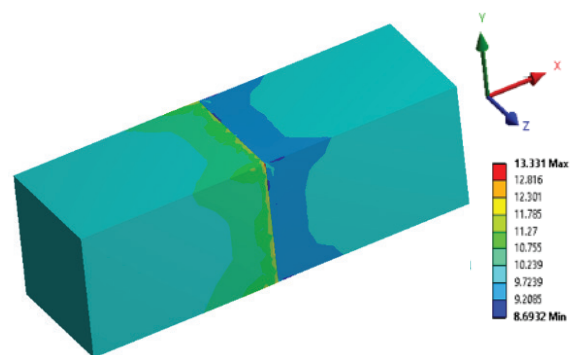


Fig. 6. Von Mises equivalent stress distribution in the joint with 10 MPa axial loading. In the direction of the x-axis, Cu is on the left side and Al on the right side.

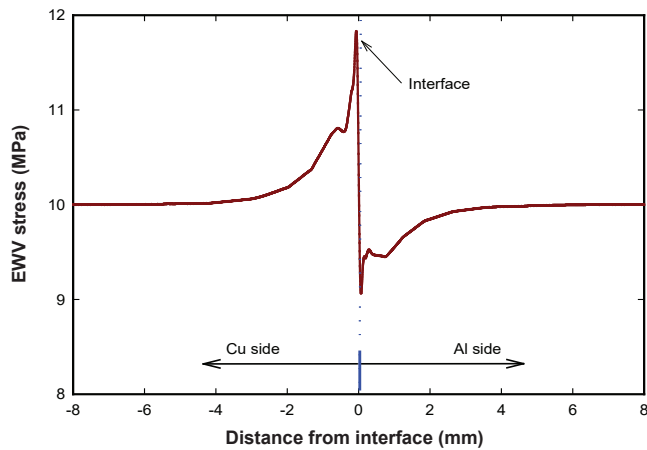


Fig. 7. Von Mises equivalent stress distribution along the path shown in Fig. 4 (with axial loading of 10 MPa).

In summary, from the above-mentioned facts, joints were established by mixing copper with aluminium. The interfaces between copper and aluminium were believed to be suitable places for cracking and intermetallic compounds at the interface may be the prevailing mechanism behind the failure behaviour of the joints.

4. Conclusions

A joint between copper 1100 and aluminium 1050 was produced by FSW at Nha Trang University. A joint free of defects and with a tensile strength of about 88 MPa was reached. At low rotation speeds (below 1000 rpm), tunnel defects formed on the joint's bottom side. The copper/aluminium interface appeared to be the preferred place for cracks to propagate and the IMCs formed at the interface were believed to be an important factor in the failure path in the joints. The interface stress induced by the dissimilar properties between copper and aluminium has a minor role in the failure of the joint. The results of this work encourage the possibility of fabricating dissimilar copper and aluminium connections for advanced performance in electronic applications.

CRedit author statement

Hoai Nam Quach: Writing original draft preparation; Dinh Hao Duong: Methodology and Formal analysis; Thanh Nhut Pham: Methodology; Huu Huy Ho: Formal analysis; Hong Chuong Pham: Formal analysis; Van Hao Pham: Editing; Hung Tra Tran: Conceptualisation and Methodology.

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COMPETING INTERESTS

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

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Electrochemical synthesis of conjugated copolymer poly(3-hexylthiophene-co-dithienopyrrole): Stepwise approach to enhance electro-optical properties

Thao Phuong Le Nguyen¹, Cam Hong Thi Nguyen¹, Tam Hoang Luu²,
Thuy Thu Truong¹, Bao Kim Doan¹, Thiet Quoc Nguyen³, Ha Tran Nguyen^{1, 2*}

¹National Key Laboratory of Polymer and Composite Materials, Ho Chi Minh City University of Technology, Vietnam National University - Ho Chi Minh City,
268 Ly Thuong Kiet Street, Ward 14, District 10, Ho Chi Minh City, Vietnam

²Faculty of Materials Technology, Ho Chi Minh City University of Technology, Vietnam National University - Ho Chi Minh City,
268 Ly Thuong Kiet Street, Ward 14, District 10, Ho Chi Minh City, Vietnam

³Institute of Applied Materials Science, Vietnam Academy of Science and Technology,
1B TL29 Street, Thanh Loc Ward, District 12, Ho Chi Minh City, Vietnam

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Abstract:

Electrochemical polymerisation of 3-hexylthiophene (3HT) and electrochemical copolymerisation of 3HT and *N*-benzoyl dithienopyrrole (BDP) were performed to prepare polymer and copolymer films using a linear sweep voltammetry potentiostatic technique with a voltage range from 1.7-1.8 V and a scan rate of 0.1 mV/s. Cyclic voltammetry (CV) curves, Fourier-transform infrared (FTIR) spectra, scanning electron microscope (SEM) images, and UV-Vis absorption spectra of the polymer and copolymer were recorded for analysis and comparison of the electropolymerisation process and properties of the resultant films. The SEM photographs of the copolymer base on 3HT and BDP exhibited a rigid structure with large spherical aggregates with an average size around 200 nm. The copolymer of 3HT and BDP exhibited the band gaps of 2.25 eV which was determined via electrochemical measurement that is higher than of band gaps of homopolymer poly(3-hexylthiophene) (P3HT). The copolymer's energy bandgap HOMO-LUMO was enhanced compared to polymer P3HT. Moreover, the increased conjugation length significantly improved the morphology structure as well as the electrochemical stability of the conjugated copolymer.

Keywords: conjugated polymer, copolymer, electropolymerisation, optoelectronic application, P3HT, thiophene.

Classification numbers: 2.1, 2.3

1. Introduction

Conductive polymers, such as polypyrrole, polythiophene, and polyaniline, have garnered significant attention recently due to their potential applications in various technologies, including electrochemical displays, smart windows, solar cells, sensors, antistatic coatings, catalysis, and redox capacitors [1-4]. Among organic conductive polymers, poly(3-alkylthiophene) and its derivatives, particularly P3HT, are intriguing materials known for their ease of synthesis, good charge carrier mobility, and chemical stability. P3HT has also served as model systems for fundamental research due to its characteristic hair-rod structure, featuring a rigid

conjugated backbone and flexible alkyl side chains, which are favourable for solubilisation [5, 6].

Copolymerisation has emerged as a promising process for creating high-performance conjugated polymers in recent years. Copolymerisation is frequently used to generate a new conjugated polymer with physical and chemical characteristics distinct from and intermediate between those of the constituent homopolymers. However, it is crucial to note that a copolymer significantly differs from a composite and a blend. For example, electropolymerisation (EP) of styrene and pyrrole monomers was performed and found that the product polymer was a copolymer of pyrrole and styrene instead of a composite or blend of polypyrrole and

*Corresponding author: Email: nguyentranha@hcmut.edu.vn

polystyrene. The copolymerisation approach has recently been shown to be capable of tuning the wavelength range of light absorption, frontier energy levels, and solubility of conjugated polymers [7, 8]. Notably, P. Acker, et al. (2022) [9] designed the novel N-anisylphenothiazine-bithiophene conjugated copolymer p(APT-T2), which could be a promising material for designing an electrode for energy storage applications due to its intrinsic conductivity combined with the presence of defined redox centers.

Due to the low cost of thiophene monomers and the improved redox potential compared to polypyrrole, polythiophene holds promise for optoelectronic applications [10]. However, the regio-random chemical structure of polythiophene and its derivatives has been shown to provide limited charge transfer in the solid state due to their reduced backbone planarity and disordered molecule packing, which limits their applicability [11]. In contrast, dithiopyrrole has long been recognised for its unique properties, such as excellent thermal and electrical stability, owing to its wide HOMO-LUMO energy gap. Therefore, we attempted to incorporate thiophene with dithienopyrrole units through copolymerisation, aiming to improve polythiophene properties through this specific combination.

Strategies for modifying the main conjugation length of optoelectronic materials, such as organic solar cells, dye-sensitised solar cells, organic field-effect transistors, and electrochromism, have recently received considerable attention to enhance their physical and chemical properties. Additionally, electrochemical polymerisation is well-known as a simple, fast, efficient, and cost-effective method to prepare polymer films by employing an electric field. This method also offers several advantages: (1) It is highly effective for synthesising polymer films because the reaction may be completed in several seconds or minutes, whereas the conventional method requires several hours or days, and the prepared films only form on the electrodes without any visible signs of a reaction in the solution; (2) The electrochemical reaction may be carried out at ambient conditions, in contrast to the severe conditions, high temperatures, and inert environments required for conventional chemical synthesis; (3) Instead of requiring specialised catalysts and expensive complexants for solution-phase synthesis, the electropolymerisation approach employs inexpensive supporting electrolytes [12-14]. Therefore, extending the main chain's conjugation length via electrochemical polymerisation is effective for optoelectronic applications.

In this work, the electrochemical polymerisation of 3HT and the electrochemical copolymerisation of 3HT and BDP were prepared using linear sweep voltammetry potentiostatic technique. FTIR spectroscopy was used to characterise and study the chemical structure of the obtained polymer and copolymer. Most importantly, the resultant polymer and copolymer were further examined for their morphological, optical, and electrical properties to assess the efficiency of the copolymerisation approach.

2. Materials and methods

2.1. Materials

3HT (99%), N-benzoyl dithieno[3,2-b:2'3'-d]pyrrole (98%), and Lithi perchlorate (LiClO_4 , 98%) were purchased from Acros Organic and used as received.

Chloroform (CHCl_3 , 99.5%) was purchased from Fisher/Acros and dried using molecular sieves under N_2 . Acetonitrile (CH_3CN , 99%) and methanone (99%) were purchased from Fisher/Acros and used as received.

2.2. Characterisation

FTIR spectra, collected as the average of 64 scans with a resolution of 4 cm^{-1} , were recorded from the KBr disk on the FTIR Bruker Tensor 27. UV-vis absorption spectra of polymers in solution were recorded on an Ocean Optics USB2000 spectrometer over a wavelength range of 200-1100 nm. Surface morphology analysis of polymers coated specimen surfaces was performed by SEM (model Jeol JSM-6480 LV). The AUTOLAB equipment (Utrecht, Netherlands) was used to measure the electrochemical properties of molecules. The AUTOLAB software is NOVA 1.11. The electrochemical experiments used an ITO glass as a working electrode, Pt as a counter electrode, and an Ag/Ag^+ reference electrode. The solutions were prepared with 0.1 M TBAPF₆ (tetrabutylammonium hexafluorophosphate) in acetonitrile. Ag/Ag^+ reference electrode (0.1 M AgNO_3 /0.1 M TBAPF₆ in CH_3CN ; 0.320 V and SCE) was used as a reference potential.

2.3. Investigation the onset oxidation potentials (E_{onset}) of monomers 3HT and N-benzoyl dithieno[3,2-b:2'3'-d]pyrrole (BDP)

First, the electrochemical experiment of the 3HT was conducted by adding 3HT (0.27 ml, 1.5 mmol) and LiClO_4 (319.2 mg, 3.0 mmol) to acetonitrile (30 ml) in

an electrochemical cell, using an indium tin oxide (ITO) glass as a working electrode and platinum disk as a counter electrode. Then the mixture was subjected to CV at a voltage range of 0-2 V, with a scan rate of 0.1 mV/s.

The same experiment was applied to the monomer BDP, in the electrochemical cell BDP (16.98 mg, 0.06 mmol), LiClO₄ (319.2 mg, 3 mmol), and acetonitrile (30 ml). The electrochemical experiment was conducted using ITO glass and Pt as working and counter electrodes, respectively, with an applied voltage of 0-2 V and a scan rate of 0.1 mV/s.

2.4. Electrochemical polymerisation of 3HT

For electrochemical polymerisation of 3HT, the electrolyte consisted of 3HT monomer (0.27 ml, 1.5 mmol) and LiClO₄ (319.2 mg, 3 mmol) dispersed in acetonitrile (30 ml). The ITO glass and Pt were used as working and counter electrodes, respectively. The P3HT was electrochemically polymerised at the voltage of 1.7-1.8 V and a scan rate of 0.1 mV/s for about 15 min. After that, the thin-film on ITO glass was soaked in acetonitrile for 15 min to remove small molecules and dried in a vacuum oven at 50°C for 24 h.

2.5. Electrochemical copolymerisation of 3HT and BDP

For electrochemical polymerisation of the copolymer from 3HT and BDP, the electrolyte consisted of monomer 3HT (0.27 ml, 1.5 mmol), BDP (16.98 mg, 0.06 mmol) and LiClO₄ (319.2 mg, 3 mmol) dispersed in acetonitrile (30 ml). The ITO glass and Pt were used as working and counter electrodes, respectively. The copolymer was electrochemically polymerised at the voltage of 1.7-1.8 V, with a scan rate of 0.1 mV/s for about 15 min. After that, the thin-film on ITO glass was soaked in acetonitrile for 15 min to remove small molecules and dried in a vacuum oven at 50°C for 24 h.

3. Results and discussion

3.1. Electrochemical polymerisation of 3HT and electrochemical copolymerisation of 3HT and BDP

Before conducting the electropolymerisation to synthesise P3HT and copolymer, the onset oxidation potentials (E_{onset}) of monomers 3HT and BDP were investigated from their CV (Fig. 1). The figure shows that the E_{onset} of 3HT and BDP was initiated at 1.4 and 1 V, respectively, and BDP

also exhibits oxidation peaks at 1.16 and 1.7 V. Based on the results, the voltage for the electropolymerisation should be above the E_{onset} of both monomers. Therefore, the polymer P3HT and copolymer were prepared by linear sweep voltammetry potentiostatic technique with a voltage range from 1.7-1.8 V and a scan rate of 0.1 mV/s.

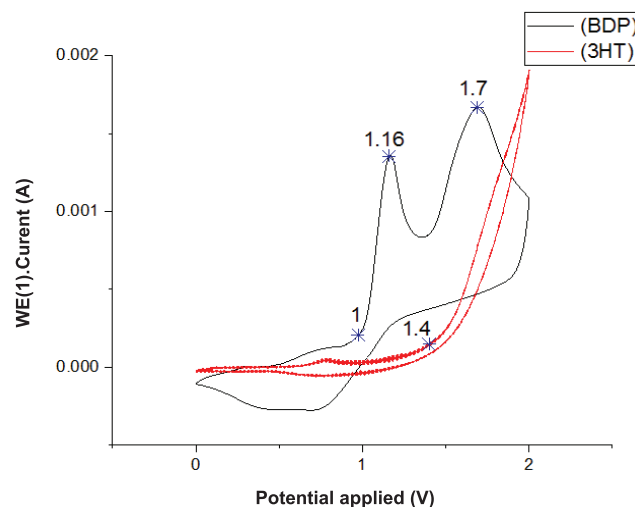


Fig. 1. CV of monomers 3HT and BDP with the range of voltage from 0-2 V and a scan rate of 0.1 mV/s.

The acquired current-voltage (I-V) characteristic curves for the electropolymerisation to synthesise polymer P3HT and copolymer are shown in Fig. 2. The current intensity increases gradually with the increase in applied voltage for both electropolymerisations. The prepared conductive polymers exhibited good electrochemical redox activity, as evidenced by the significant growth of the corresponding conductive polymers on the ITO glass.

After the successful electropolymerisations of both polymers, P3HT and copolymer, the chemical structure was characterised using FTIR spectroscopy. FTIR spectroscopy was scanned in the frequency region of 400-4000 cm⁻¹ using an infrared spectrometer by employing the KBr pellet technique. In the case of P3HT, Fig. 3 and Table 1 exhibit bands from 2927-2854 cm⁻¹ attributed to the C-H stretching vibrations of the ethyl-hexyl group and C-H linkage in the aromatic structure. Aromatic C=C stretching, and C-H deformation vibrations correspond to the characteristic band between 1700 and 1610 cm⁻¹. The band from 1245 to 970 cm⁻¹ is ascribed to the C-S stretching vibrations of thiophene structures. The FTIR result clearly indicated that the electropolymerisation has been performed successfully to obtain the conjugated polymer P3HT.

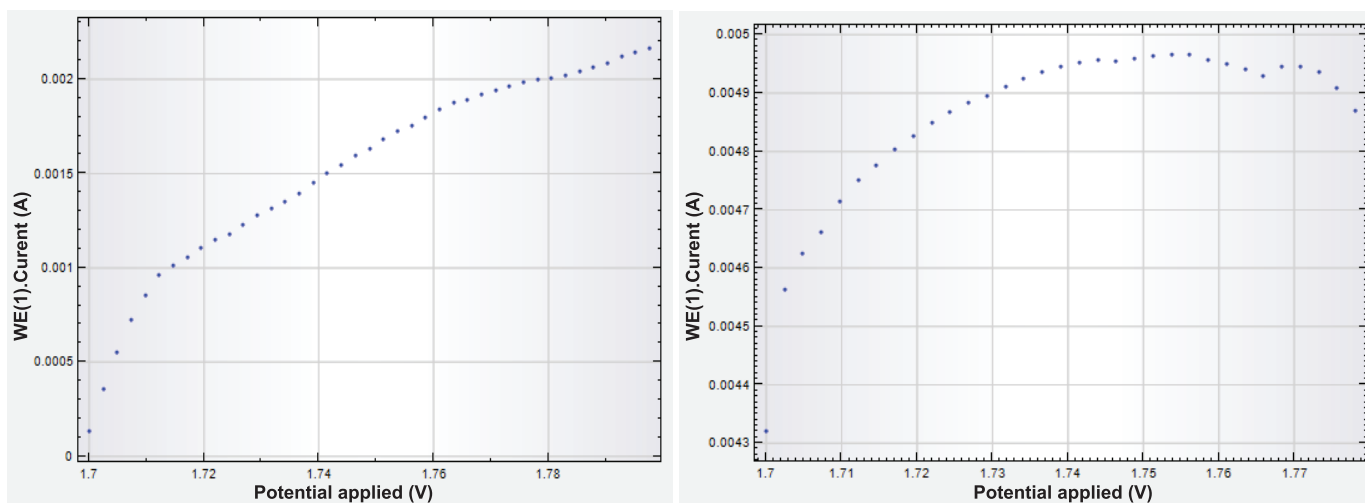


Fig. 2. Current-voltage (I-V) graphs of the electropolymerisations to synthesise P3HT (left) and copolymer (right).

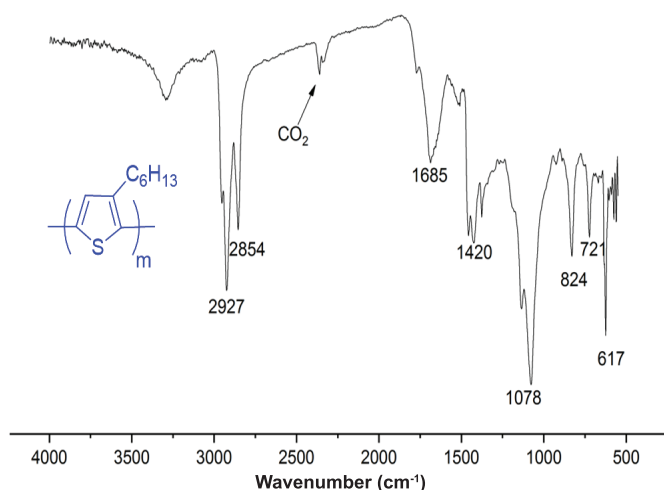


Fig. 3. FTIR spectrum of polymer P3HT.

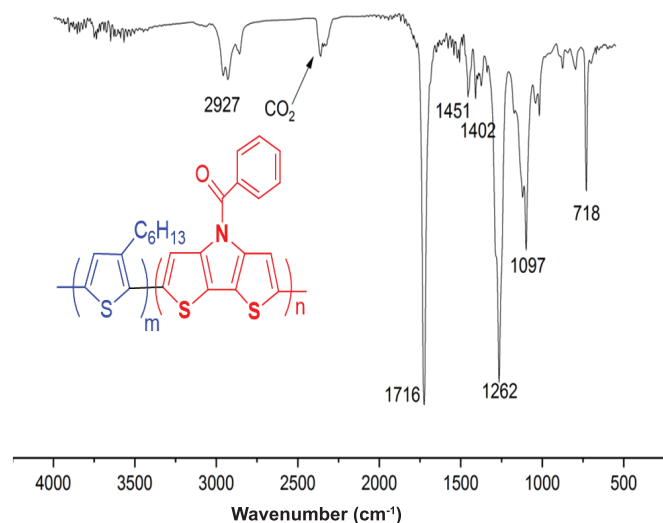


Fig. 4. FTIR spectrum of copolymer from 3HT and BDP.

Table 1. IR absorption wavenumbers (cm^{-1}) and their functional group of P3HT and copolymer.

P3HT	Copolymer	Functional group
Wavenumbers (cm^{-1})		
2927	2927	Methylene C-H stretch
2854	2854	Methyl C-H stretch
1685	1716	C=C aromatic ring stretch
1420	1451, 1402	Methyl and methylene C-H bend
-	1262	C-N aromatic stretch
1078	1097	C-S stretch
824	718	Aromatic C-H bend

Similarly, the presence of chemical functional groups in the copolymer has been identified by the FTIR spectrum, as shown in Fig. 4. The spectrum indicates bands in the range of 2927-2854 cm^{-1} relating to the $-\text{CH}_2$ and $-\text{CH}_3$ stretching and peak at 1451-1402 cm^{-1} relating to the $-\text{CH}_3$ and $-\text{CH}_2$ bending, while the C=C stretching mode of the aromatic ring is exhibited in the peak at 1716 cm^{-1} . Moreover, the peaks at 1262 cm^{-1} indicate the existence of C-N of pyrrole structures. This investigation has demonstrated that the electropolymerisation of monomers 3HT and BDP has been performed successfully to generate the copolymer.

3.2. Structure analysed of the P3HT and copolymer using SEM

The morphology of P3HT and copolymer films formed on the electrodes was investigated using SEM. SEM

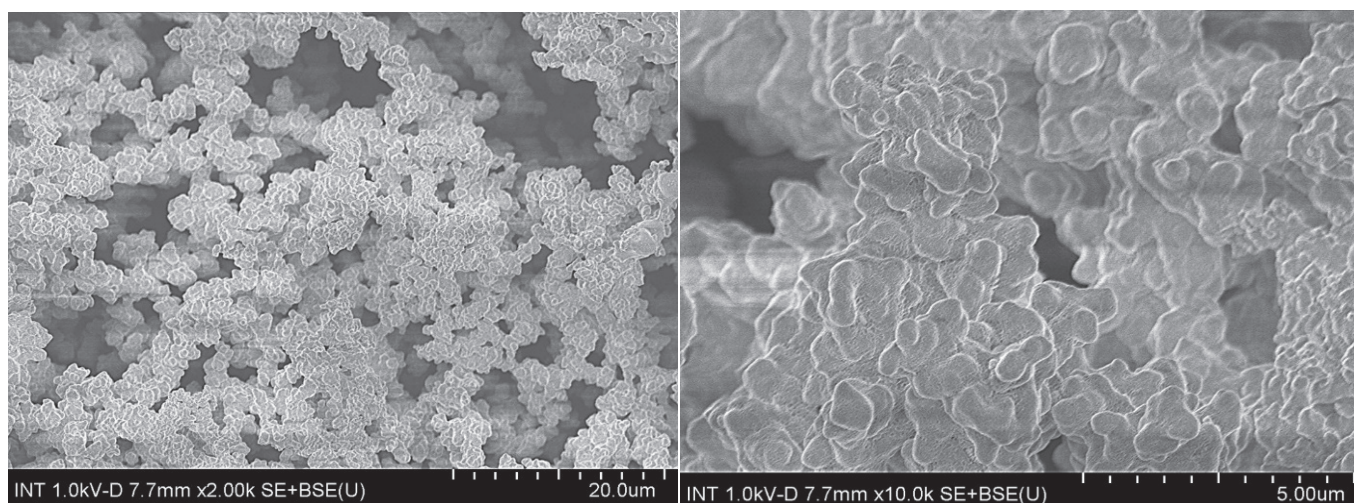


Fig. 5. SEM images of polymer P3HT.

micrographs of the P3HT film were shown in Fig. 5. A low magnification (2,000x, Fig. 5, left) image of the P3HT sample revealed a “coral-like” morphology, whereas a high magnification (10,000x, Fig. 5, right) image revealed large amounts of small spherical particles separated by empty pores. In fact, the decrease in the polymer’s regularity, which is evident in the globular forms, might be caused by structural defects such as reticulation, cross-linking, and α,β - coupling instead of α,α' -coupling between thiophene units [15].

On the other hand, SEM photographs of the copolymer prepared from 3HT and BDP exhibited a more rigid structure (2,000x, Fig. 6, left; 10,000x, Fig. 6, right) with large spherical aggregates (5-7 mm) of small-sized particles

with an average size of around 200 nm. In general, the macroscopic structure of P3HT is relatively loose compared to the copolymer. This comparison also confirms that the electrochemical copolymerisation was performed successfully to generate the 3HT-BDP copolymer.

3.3. Optical properties of the P3HT and copolymer

The optical properties of P3HT and the copolymer were characterised and compared using UV-Vis absorption. According to Fig. 7, the maximum absorption peak of the copolymer is at 430 nm, which is red-shifted compared to that of P3HT, which is at 390 nm. This effect results from the fact that larger molecules absorb light at longer wavelengths than smaller ones because they are more polarisable as they grow in size.

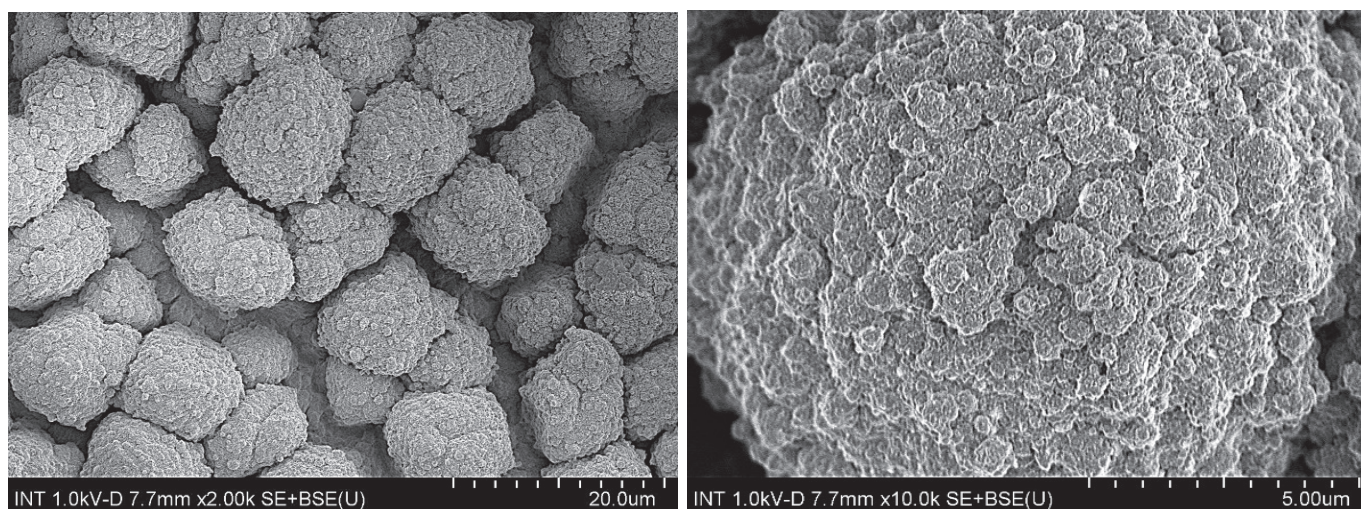


Fig. 6. SEM images of the copolymer.

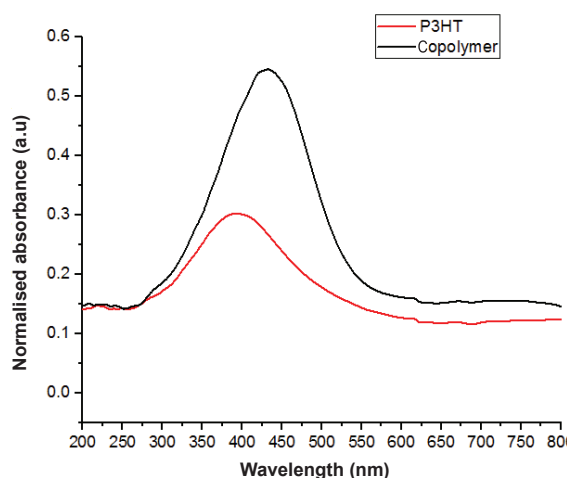


Fig. 7. UV-Vis absorption spectrum of P3HT (red line) and copolymer (black line) at 0.5 mg.ml⁻¹ in chloroform.

Another important parameter of the optical properties is the energy bandgap of the polymer, which can be determined using the UV-Vis absorption spectrum. Using the Planck equation ($E_g = 1240/\lambda_{\text{onset}}$), the calculated energy bandgap of P3HT and the copolymer are 2.16 and 2.25 eV, respectively. The results indicate that both polymers have a high value

of optical bandgap (above 2 eV), making them useful for high-temperature optical devices such as LEDs, lasers, or solar cells. However, the copolymer has a higher value due to the increased number of conjugated bonds in its structure.

3.4. Electrochemical properties of P3HT and copolymer

The electrochemical behaviours of P3HT and the copolymer were examined using the CV method to gain a better understanding of their electrochemical activity. Both the polymer and copolymer exhibited distinct redox peaks with hysteresis (potential drift) between the anodic and cathodic peak potentials. Slow heterogeneous electron transfer, the mutual transformation of various electronic species, local polymer chain rearrangement, and electronic charging of the interfacial exchange at the metal/polymer and polymer/solution interfaces were attributed to the potential shifts of the redox peaks among the CVs [4].

The stability of both polymers was also investigated, with a total of four cycles performed, as shown in Figs. 8A, 8B. P3HT's redox activity was sustained at around 70% after four cycles of scanning, exhibiting generally good redox activity. However, the increased conjugation length significantly improved the electrochemical stability of the copolymer, achieving stability above 90% after four

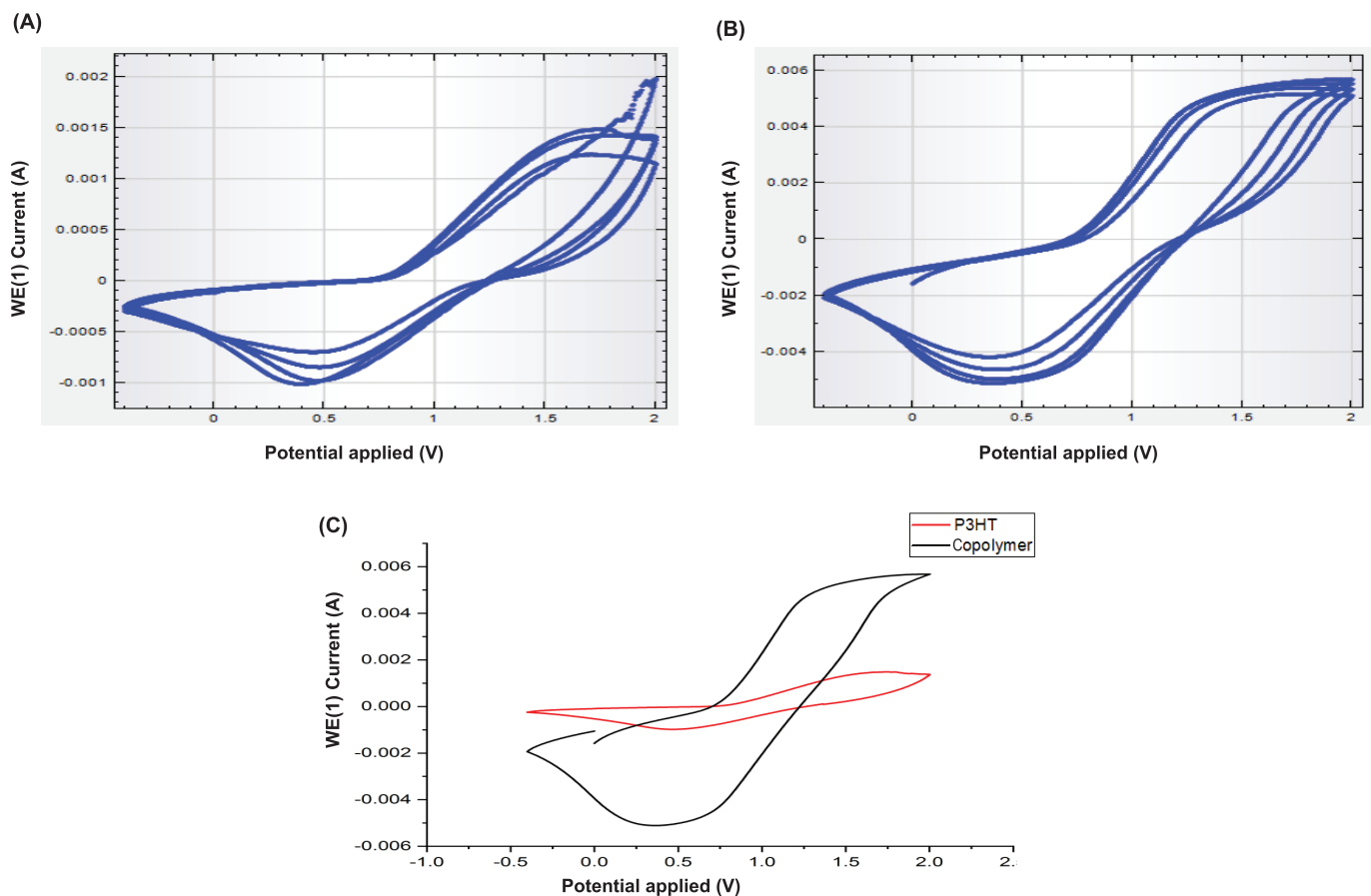


Fig. 8. CVs of (A) P3HT, (B) copolymer, and (C) first cycle of P3HT and copolymer.

scanning cycles. Furthermore, CV was employed to evaluate the HOMO and LUMO energy levels of the polymer using an empirical equation. The results are shown in Table 2. The experimental HOMO of the copolymer exhibited an elevated value, attributed to the increase in the conjugation length in the structure.

Table 2. Electrochemical properties and calculation parameters of P3HT and copolymer.

Compound	E_{pa} (V)	i_{pa} (mA)	E_{pc} (V)	i_{pc} (mA)	E_g (eV)	(V)	HOMO* (eV)	LUMO* (eV)
P3HT	1.53	1.23	0.50	1.3	2.16	0.82	-5.22	-3.06
Copolymer	1.28	3.8	0.65	5	2.25	0.74	-5.14	-2.89
HOMO* = $-(4.4+) \text{ eV (Ag/Ag}^+)$					LUMO* = $E_g + \text{HOMO}$			

HOMO: highest occupied molecular orbital; LOMO: least unoccupied molecular orbital; *: excited energy state.

4. Conclusions

3HT and BDP were used as precursor monomers to construct a conjugated copolymer through electrochemical copolymerisation using a linear sweep voltammetry potentiostatic technique. The structure, optical, and electrical properties of the copolymer were analysed and compared with the polymer P3HT, which was also synthesised using the same method. The comparison highlighted the improvement in the morphology and HOMO-LUMO energy gap of the copolymer, which significantly enhances the electrochemical stability of the material. This study has demonstrated that electropolymerisation is a highly applicable, straightforward, and efficient approach for preparing conductive polymers and copolymers for diverse optoelectronic applications.

CRedit author statement

Thao Phuong Le Nguyen: Conceptualisation, Methodology, Formal analysis; Cam Hong Thi Nguyen, Tam Hoang Luu: Software, Data curation, Validation; Thuy Thu Truong, Bao Kim Doan, Thiet Quoc Nguyen: Visualisation, Investigation; Ha Tran Nguyen: Conceptualisation, Methodology, Formal analysis, Supervision, Writing original draft preparation, Writing - Reviewing and Editing, Project administration.

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COMPETING INTERESTS

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

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Methyl orange-doped thiourea barium chloride crystals: Natural bond orbital analysis of the fock matrix for laser applications

S. Panimalar¹, P. Kumaresan¹, S. Nithiyanantham^{2*}, K. Selva Sambath Kumar³, R. Mohan⁴

¹PG & Research Department of Physics, Thiru A. Govindasamy Government Arts College, Tindivanam - 604 307, Tamil Nadu, India

²PG & Research Department of Physics, Thiru Vi. Ka. Govt. Arts College, Thiruvavur, Tamilnadu - 610 003, India

³PG & Research Department of Physics, Arignar Anna Government Arts College, Villupuram - 605 603, Tamil Nadu, India

⁴Department of Chemistry, Presidency University, Bangalore -560 064, India

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Abstract:

Pure thiourea barium chloride (TBC) crystals were synthesised using a slow cooling rate (0.05°C/day) and gradual evaporation from an aqueous solution. The growth process occurred over more than 19 days at a temperature of 39°C. Natural bond orbital (NBO) analysis has proven beneficial for studying charge transfer and conjugative interactions in molecular systems. Moreover, it sheds light on intermolecular bonding, and the interactions between these bonds have implications for optical applications. Additionally, the charge transfer interactions within the molecule that influence its catalytic activity can be elucidated by a lower HOMO and LUMO energy gap. This energy difference can be an indicator of a compound's excitability; the more readily a compound can be excited, the narrower the energy gap. The energy gap might be significantly affected by the influence of thermal energy. The non-linear optical (NLO) efficiency of methyl orange-doped (MOD) TBC crystals was contrasted with that of the pure form, and the doped version exhibited greater efficiency, particularly for high-frequency applications.

Keywords: HOMO and LUMO, methyl orange, nonlinear optical studies, thermodynamic parameters, thiourea barium chloride crystal.

Classification numbers: 2.1, 2.3

1. Introduction

TBC is a crystal that has been extensively researched and is frequently used as a frequency doubler in laser applications. The performance of this crystal-based technology, as well as the quality of TBC crystals, can be enhanced with appropriate dopants. Dye was doped into TBC crystals to investigate the effects of dye-based dopants on the NLO properties of TBC crystals. The influence of impurity atoms on crystal quality and performance was explored. Solution growth was employed to produce bulk crystals of dye-doped TBC [1-3].

In recent decades, various NLO materials with strong non-linear susceptibilities have been identified as potential candidates for frequency conversion. Nonetheless, in actual device applications, they often exhibit inadequate transparency, poor optical quality, a lack of resilience, a low laser damage threshold, and challenges in cultivating organic materials [4-6]. Additionally, whilst the mechanical and thermal properties of large single crystals are exceptional, their non-linearity is relatively low. Hence, substantial research has been undertaken on semi-organic materials, which amalgamate the attributes of both organic and inorganic compounds [7].

Given that the majority of organic molecules are constituted by weak van der Waals and hydrogen bonds with conjugated electrons, organic materials tend to possess high SHG efficiency but are less

stable. Conversely, inorganic compounds are notably stable but manifest a diminished SHG efficiency. This piques interest in semi-organic compounds, which represent a nexus between stability and efficiency. Thiourea is a compelling compound that possesses centrosymmetry but becomes non-centrosymmetric when coordinated with metal complexes [8-10].

The linear and non-linear behaviours in organic and semi-organic crystals largely hinge on their chemical properties. The chemical reactivity, stability, chemical hardness, electrophilicity index, and donor-acceptor interactions through NBO analysis in strontium chloride, palladium chloride, antimony triborate, and zinc selenate with thiourea have been extensively probed [11-14].

Numerous researchers have endeavoured to cultivate both organic and inorganic crystalline materials, which were then subjected to various analyses for catalytic, sensory purposes, pharmaceutical applications, and COVID-19 virus detection, among other biomedical applications. Novel fused triazine, thiourea derivatives, bis-thiazoles, and certain hydroxyquinolines with their metal complexes were produced and studied using various experimental and theoretical methods. Density functional theory (DFT) was employed, using the 6-311G** and LANL2DZ basis set for selected atoms. Furthermore, NBO, energy gap using HOMO and LUMO, first-order hyperpolarizability, and anisotropic polarizability were assessed for optical applications [15-18].

*Corresponding author: Email: prof.s.nithiyanantham@tvkgac.co.in

In the current work, the analysis of TBC crystals, encompassing various thermodynamic and related parameters, is discussed in relation to their energies, bonding, and other models.

2. Materials and methods

All Analar grade chemicals are purchased from S.D Fine Chemicals, Mumbai, India. The purity of the chemical is 99.5% and they are used directly without further purification. The simplest and convenient method to grow crystals is adopting the Slow evaporation technique. The good quality crystals free from strain and dislocation from an aqueous solution and slow chilling (0.5°C/day) were used to generate pure TBC crystals. For Sudan doped TBC crystals, the same process is used (0.1 mol percent of Sudan dye). The solubility of the doped TBC in the solvent was determined for each dopant, and Sudan was found to have a solubility of 16 g/100 ml at 410°C. Here, the substances do not react with each other while growing. For growth tests, seed crystals with the correct shape and no macro flaws were employed. In order to maintain the temperature, the Constant Temperature Bath (CTB) was used to generate large single crystals of TBC and Sudan doped TBC with an accuracy of 0.10°C. The initial pH value, 4.5 for Sudan dye, was saturated in the mother solution. The growth was carried out for more than 18 days at a temperature of 40°C in the bath. Throughout whole experiment double distilled water can be used [19].

3. Results and discussion

3.1. HOMO-LUMO analysis

An enhancement in the gap between the bonding and anti-bonding orbitals in the molecule under study results in hypsochromic shifts. The energy disparity between the highest and lowest occupied molecular orbitals is pivotal in ascertaining the molecular electronic transport properties, as it offers an insight into electron conductivity. Based on the wave function analysis, the transition associated with the initial state of excitation, or electron absorption, is predominantly characterised by one-electron excitation from HOMO to LUMO. The energy difference between the HOMO and LUMO, indicative of electron-donating and electron-accepting capacities respectively, conveys molecular chemical stability. The other attributes of molecules are largely governed by the energy gap between the ground state and the first excited state. The frontier electron density utilises this to pinpoint the most reactive location in p-electron systems and to elucidate various reactions in conjugated systems.

Table 1. Calculated molecular properties of MOD TBC from orbital energies.

Parameter	Value (a.u.)
HOMO	-0.201642
LUMO	-0.087242
Energy gap	0.1144
SCF energy	-538.6770
η	-0.0572
μ	0.144442
χ	-0.144442
S	-17.482517
ω	-0.182373

Table 1 presents the HOMO and LUMO energies of TBC determined at the DFT level. Fig. 1 illustrates several frontier orbitals of the title compound, accompanied by their respective energies [20]. Both HOMO and LUMO in TBC are predominantly derived from the p orbital of all the compound's atoms. As depicted in Fig. 1, the HOMO is characterised by a charge distribution across the entire structure, whereas the LUMO has a charge distribution on certain carbons of the ring. For TBC (Fig. 1), the HOMO possesses a nonbonding character for some ring atoms and two oxygen atoms, whilst the LUMO displays an anti-bonding character for some pairs, including two oxygen atoms. Red and green denote positive and negative phases, respectively. The HOMO has a calculated energy of -0.201642 a.u., whereas the LUMO's calculated energy stands at -0.087242 a.u.

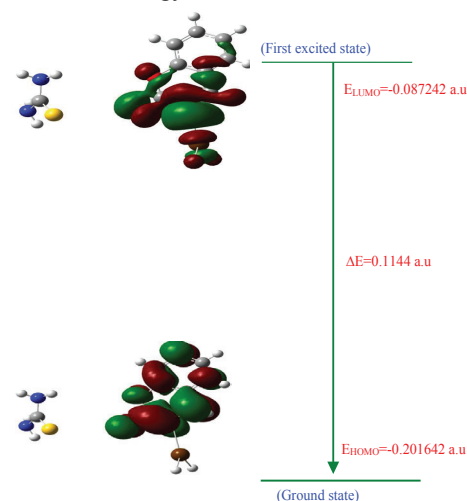


Fig. 1. The frontier molecular orbitals for MOD TBC.

Consequently, the reduction in the HOMO-LUMO band gap primarily stems from the notable stability of the LUMO due to the strong electron-accepting capacity of the electron-acceptor group. The chemical hardness (η) and chemical potential (μ) have been derived using HOMO and LUMO energies. Chemical hardness, a reactivity index, is calculated using the formula:

$$\eta = (I - H)/2 \quad (1)$$

The chemical potential, which assesses the tendency of electron clouds to escape, is given by

$$\mu = -(I + H)/2 \quad (2)$$

In a molecular system, I and H denote ionisation potential and electron affinities respectively. The inverse of hardness alludes to the flexibility of the bonding [21]. Table 1 displays the computed values of chemical properties. Recent studies have highlighted the significance of these values in ascertaining the toxicity of diverse contaminants.

3.2. Natural bond orbital analysis

The grown crystals were subjected to NBO analysis to gain insights into bond bending, rehybridisation, intramolecular charge transfer (ICT), and electron density delocalisation within the molecule. NBO calculations were conducted at the HF/6-

311++G(d,p) level using the NBO 5.0 programme, as incorporated in the Gaussian 09 package. This was done to comprehend different second-order interactions between the occupied orbitals of one subsystem and the vacant orbitals of another, reflecting delocalisation or hyperconjugation [22]. The hyperconjugative interaction energy was deduced using the second-order perturbation method:

$$E^{(2)} = \Delta E_{ij} = q_i \frac{F(i,j)^2}{\epsilon_j - \epsilon_i} \quad (3)$$

where $E^{(2)}$ and E_{ij} are the populations of the donor orbital, are the energies of ϵ_j and ϵ_i NBO'S, and $F(i,j)^2$ is the Fock matrix element between i and j NBO orbitals. Examination of the Fock matrix in the NBO basis through second-order perturbation theory uncovers significant intermolecular hyperconjugative interactions of π -electrons, as detailed in Table 2.

Table 2. Fock matrix in NBO basis corresponding to the intramolecular bonds of MOD TBC (second order).

Donor(i)	ED(i)(e)	Acceptor(j)	ED(j)(e)	^a E ⁽²⁾ (KJ/mol)	^b E(j)-E(i)(au)	^c F(i,j)(a.u.)
σ (C1-C2)	1.97406	σ^* (C1-C6)	0.02283	3.82	1.27	0.062
σ (C1-C2)	1.97406	σ^* (C2-C3)	0.01618	3.32	1.26	0.058
σ (C1-C6)	1.96974	σ^* (C5-O19)	0.03239	4.31	0.97	0.058
π (C1-C6)	1.67968	π^* (C2-C3)	0.37156	20.29	0.27	0.067
π (C1-C6)	1.67968	π^* (C4-C5)	0.42853	22.96	0.26	0.071
σ (C2-C3)	1.97178	π^* (C1-C2)	0.02340	3.63	1.27	0.061
σ (C2-C3)	1.97178	σ^* (C1-C7)	0.02441	6.87	1.08	0.058
σ (C2-C3)	1.97178	σ^* (C4-O17)	0.02939	4.49	0.94	0.058
π (C2-C3)	1.70600	π^* (C1-C6)	0.35696	20.83	0.29	0.071
π (C2-C3)	1.70600	π^* (C4-C5)	0.42853	19.87	0.27	0.068
σ (C2-H15)	1.97795	σ^* (C1-C6)	0.02283	4.89	1.09	0.065
σ (C4-C5)	1.97578	σ^* (C3-C4)	0.02345	4.44	1.28	0.067
π (C4-C5)	1.65314	π^* (C1-C6)	0.35696	17.48	0.31	0.066
π (C4-C5)	1.65314	π^* (C2-C3)	0.37156	20.85	0.30	0.071
σ (C5-C6)	1.96939	σ^* (C1-C6)	0.02283	3.87	1.28	0.063
σ (C5-C6)	1.96939	σ^* (C1-C7)	0.02441	3.58	1.09	0.056
σ (C5-C6)	1.96939	σ^* (C4-C5)	0.03487	4.63	1.24	0.068
σ (C5-C6)	1.96939	σ^* (C4-O17)	0.02939	4.38	0.95	0.058
σ (C6-H24)	1.97065	σ^* (C41C2)	0.02340	4.60	1.09	0.063
σ (C6-H24)	1.97065	σ^* (C4-C5)	0.03487	4.38	1.05	0.061
π (C10-H12)	1.98037	π^* (C7-H9)	0.01806	3.69	0.67	0.044
σ (O17-H18)	1.99047	σ^* (C4-C5)	0.03487	3.78	1.29	0.063
σ (O19-C20)	1.99209	σ^* (C5-C6)	0.02109	3.38	1.35	0.061
σ (C20-H23)	1.98953	σ^* (C5-O19)	0.03239	3.94	0.78	0.050
LP2(O19)	1.86933	π^* (C4-C5)	0.42853	19.61	0.34	0.080
LP2(O19)	1.86933	σ^* (C20-H21)	0.01652	4.93	0.72	0.055
LP2(O19)	1.86933	σ^* (C20-H22)	0.01650	4.93	0.72	0.055

Owing to the pronounced interaction between the lone pair O19 and the antibonding C20-H22, the stabilisation energy of 4.93 kJ/mol indicates heightened delocalisation. Noteworthy interactions between LP2O19 and antibonding C4-C5, C20-H21 in the title

molecule yield stabilisation values of 19.61 and 4.93 kJ/mol, respectively. Such interactions around the ring might augment the molecule's bioactivity. The π^* (C4-C5), π^* (C2-C3), and π^* (C1-C6) bond orbitals facilitate an intra-molecular interaction culminating in significant intra-molecular charge transfers with stabilisation values of 227 and 145 kJ/mol, respectively. Such charge transfers can render the molecule highly nonlinear.

The second-order perturbation theory delineates various electron donor orbitals, acceptor orbitals, and the corresponding stabilisation energy. The more intense the interaction between electron donors and electron acceptors, or the higher the proclivity of donors to bequeath to acceptors, the elevated the $E(2)$ value becomes, indicating an increased degree of conjugation within the system. A stabilising donor-acceptor interaction is manifested when electron density delocalises between occupied Lewis-type (bond or lone pair) and formally vacant non-Lewis NBO orbitals.

Intramolecular hyperconjugative interactions emerge from the orbital overlap of the (C-C) and π^* (C-C) bond orbitals, facilitating ICT and system stabilisation. As a consequence of these interactions, which augment the electron density (ED) in the C-C antibonding orbital, the bonds become attenuated [23]. A pronounced intramolecular hyperconjugative interaction transfers electrons from the C1-C2, C2-C3, and C4-C5 bonds to the benzene ring's π^* (C1-C6) bond, enhancing and stabilising the electron density at 50 kJ/mol.

3.3. Thermodynamic properties

A range of thermodynamic properties are delineated in Table 3. For precise prediction of the zero-point vibrational energies (ZPVE), rotational constants, thermal energy, and entropy, scaling factors have been proposed.

Table 3. Theoretically computed parameters-I.

Parameter	Value	
	LSDA	HF
Zero-point vibrational energy (kcal/mol)	121.44639	132.94025
Rotational constant (GHz)		
A	1.67529	1.67633
B	0.56727	0.54977
C	0.43809	0.43691

The total energy appears to diminish as the basis set dimension grows. The total capacity and entropy of the title chemical at ambient temperature exhibit minor variations across different basis sets [24] (Table 4).

Table 4. Theoretically computed parameters-II.

Parameter	Thermal energy (kcal/mol)		Entropy (cal/mol)		Enthalpy (cal/mol K)	
Total	129.082	140.180	109.196	107.822	45.588	41.838
Translational	0.889	0.889	41.194	41.194	2.981	2.981
Rotational	0.889	0.889	31.024	31.057	2.981	2.981
Vibrational	127.304	138.403	36.978	35.570	39.626	35.877

Table 5 catalogues the constant pressure values for heat capacities (C_p), entropies (S), and enthalpy changes ($\Delta H_{0 \rightarrow T}$) for zero (monomer) and applied electric fields. These metrics derive from vibrational analysis at the HF/6-311++G(d,p) level and statistical thermodynamics. As molecular vibration intensities amplify, conventional thermodynamic functions escalate with temperature, with entropy increasing whilst enthalpy and heat capacity remain stable, as demonstrated in Table 5.

Table 5. MOD TBC determined by DFT/HF/6-311++G(d,p) method (temperature-dependent parameters).

T(K)	S ($J \square mol^{-1} K^{-1}$)	C_p ($J \square mol^{-1} K^{-1}$)	$\Delta H_{0 \rightarrow T}$ ($kJ mol^{-1}$)
100	379.02	30.1	8.52
200	490.88	199.95	25.05
298.15	583.95	272.06	48.6
300	585.68	273.46	48.67
400	674.66	348.35	79.79
500	759.78	415.29	118.06
600	840.61	471.26	162.48
700	916.83	517.27	211.98
800	988.45	555.24	265.67
900	1055.73	586.85	322.82
1000	1118.98	613.39	382.87

Compression at constant temperature results in a molecule's volume reduction [25-27]. The potential positions a molecule's particles can occupy become restricted with a decrease in molecular volume. Consequently, at a constant temperature, entropy tends to diminish as pressure ascends. Fig. 2 portrays the correlation graph for zero applied EFs. The fitting equations are:

$$S_m^0 = 311.40643 + 1.13227T - 3.22688 \times 10^{-4} T^2 \quad (R^2=0.99981) \quad (4)$$

$$C_{p,m}^0 = 6.48149 + 5.36266T - 0.0397 \times 10^{-4} T^2 \quad (R^2=0.99866) \quad (5)$$

$$H_m^0 = -5.87053 + 2.49217T - 0.0397 \times 10^{-4} T^2 \quad (R^2=0.99960) \quad (6)$$

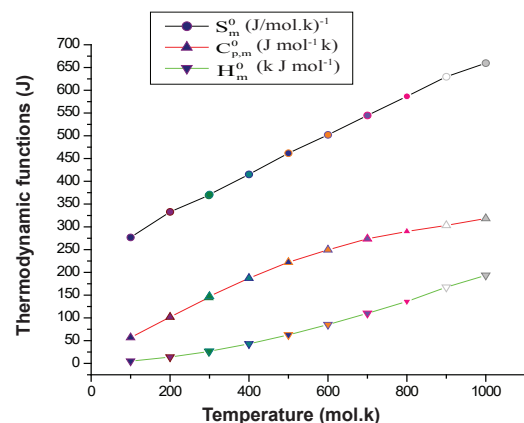


Fig. 2. Correlation of MOD TBC at various temperatures.

3.4. Electrostatic potentials

Constructing a three-dimensional ESP map over molecular surfaces using experimental charge densities markedly accentuates the electrostatic character of the three configurations (Figs. 3-5).

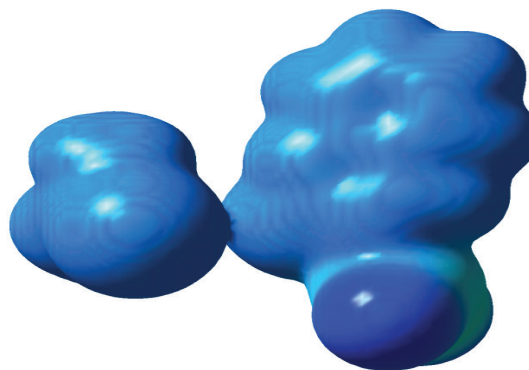


Fig. 3. MOD TBC (electron density).

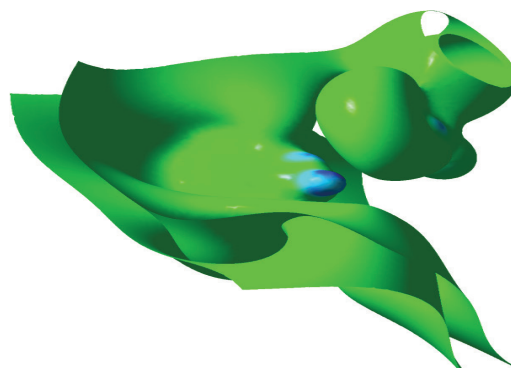


Fig. 4. Electrostatic potential surface of MOD TBC (contour map).

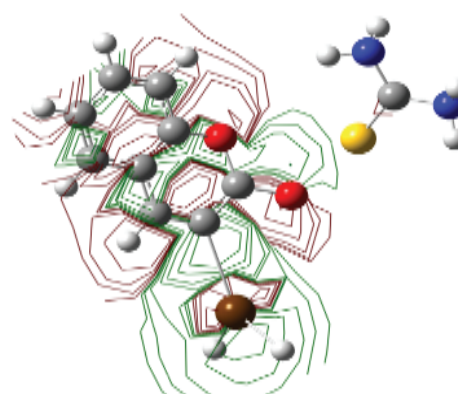


Fig. 5. Electron density maps of MOD TBC.

The electropositive and electronegative surfaces are clearly differentiated in Fig. 4. ESP mapping reveals a broader electronegative surface, attributed to the conformational difference of the C-O side chain, the involvement of electrons of the triad C1-C2-H12 in C-H... type interactions, and the presence of π , π ... contacts.

The electronegative surface predominantly surrounds oxygen atoms in C-H...O type intermolecular interactions. However, in contrast to other Ba atoms in the structures, atom O11 exhibits a less pronounced electronegative surface. This stems from atom O11's lack of engagement in intermolecular interactions, whilst a comparable atom in S is seen to interact with the neighbouring molecule (Fig. 5). The theoretical analyses' affiliated maps display analogous traits. The ESP maps lucidly highlight the optimal binding sites to construct interaction networks and the variances in interaction modalities [28].

3.5. NLO studies

The crystallinity of MOD TBC, pertaining to its suitability for NLO applications, and its frequency, were examined employing a solid-state laser [29] (Table 6).

Table 6. NLO efficiency of TBC.

No.	Compound	NLO efficiency
1	Pure TBC crystals	1.79
2	MOD TBC crystals	1.89

Determined using the S.K. Kurtz, et al.'s powder method (1968) [30], the crystal was placed and the optical beam input realigned. Its significance was subsequently analysed in conjunction with green light. Comparing the NLO efficiency of the doped crystal to pure TBC reveals that the former boasts superior efficiency and broader applications [31-34]. Both TBC variants, including the doped version, underwent NLO measurement. The NLO efficiencies of pure TBC and MOD TBC crystals are presented in Table 6.

4. Conclusions

Single crystals of pure and dye-doped TBC with well-defined optical quality were synthesised through slow solvent evaporation. Furthermore, optically superior quality crystals of TBC with enhanced dye-doped TBC have been examined. The inherent electronic parameters affiliated with the orbitals manifest in the notable electronic energy gaps of molecules. These orbitals not only delineate how a molecule interacts within its species but also juxtapose the efficacy of the doped TBC against its pure crystalline counterpart. The correlation in thermal properties leans favourably towards the dopant rather than the pure crystal. A heightened electronegativity is discernible in the doped crystal. The doped crystal was observed to amplify the NLO attribute of TBC crystals. The MOD TBC crystal demonstrated 1.89 times the SHG performance of KDP and 1.05 times that of pure TBC crystal. The electron distribution in metallic elements significantly influences the chemical bond.

CRedit author statement

S. Panimalar: Conceptualisation, Methodology, Formal analysis; P. Kumaresan, K.S. Kumar, R. Mohan: Software, Data curation, Validation, Visualisation, Investigation; S. Nithiyanantham: Conceptualisation, Methodology, Formal analysis, Supervision, Writing original draft preparation, Writing - Review and Editing, Project administration.

COMPETING INTERESTS

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

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Effects of the wheel rotation speed during the melt-spinning process on the structural morphology and the thermoelectric properties of $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$ alloy

Nguyen Van Du^{1*}, Nguyen Tu¹, Do Quang Trung¹, Noy Sysavanh², Manh Trung Tran³, Tong Thi Hao Tam⁴

¹Faculty of Fundamental Sciences, Phenikaa University, Yen Nghia Ward, Ha Dong District, Hanoi, Vietnam

²Thai Nguyen University of Education, Tan Thinh Ward, Thai Nguyen City, Thai Nguyen Province, Vietnam

³Faculty of Materials Science and Engineering, Phenikaa University, Yen Nghia Ward, Ha Dong District, Hanoi, Vietnam

⁴School of Information Technology and Digital Economic, National Economics University,
207 Giai Phong Street, Dong Tam Ward, Hai Ba Trung District, Hanoi, Vietnam

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Abstract:

Half-Heusler alloys based on (Ti, Hf, Zr)NiSn possess excellent mechanical and chemical properties as well as high thermal stability, making them promising materials for high-performance thermoelectric (TE) power generators. However, the high cost of Hf limits its use in these alloys. Therefore, Hf-free (Ti, Zr)NiSn alloys synthesised using a rapid process offer a cost-effective approach to reducing product costs. In this study, we employed the fast synthesis melt-spinning (MS) method to prepare an Hf-free $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$ alloy while systematically optimising MS conditions. We found that by varying the rotation speed of the copper wheel in the MS system from 1000 to 4000 rpm, a marked change in the structural morphology of the melt-spun ribbons occurred. Grain growth during the spark plasma sintering process significantly affected TE properties of the bulk $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$ alloy depending on the structural morphology. As a result, a maximum ZT of ~1.1 at 823 K was achieved for a wheel rotation speed of 3000 rpm, which is comparable to ZT values obtained from the (Ti, Hf, Zr)NiSn-based half-Heusler alloys prepared by other methods.

Keywords: arc melting, half-Heusler, melt-spinning, spark plasma sintering, TEs.

Classification numbers: 2.1, 2.3

1. Introduction

TE materials are essential components in the production of devices used to convert thermal energy into electricity and vice versa. The energy conversion efficiency in TE devices depends largely on the dimensionless figure-of-merit (ZT) of the material. ZT is determined by the relationship between the Seebeck coefficient (S), electrical conductivity (σ), thermal conductivity (K), and absolute temperature T, and is calculated as $\text{ZT} = S^2\sigma T/K$ [1, 2]. Various materials have been developed with high TE performance, including Bi_2Te_3 -based alloys [3, 4] and skutterudite-based materials [5, 6]. XNiSn (X=Ti, Zr, Hf)-based alloys are interesting research subjects because they possess good mechanical and chemical properties, high thermal stability, and a large power factor (PF=power factor, $\text{PF} = S^2\sigma$). These alloys are suitable for applications that harvest waste heat in the medium-to-high temperature range (600 to 900 K) [1, 2, 7, 8]. However, the relatively high thermal conductivity of XNiSn materials compared with other potential materials remains a limitation for improved performance [7, 9, 10].

Efforts to improve the TE efficiency of XNiSn (X=Ti, Zr, Hf) alloys have been undertaken with various approaches. The common approach is to optimise the carrier concentration through donor doping to enhance power factor. At the same time, point defect

phonon scattering induced by mass and strain field fluctuations can be achieved by isoelectronic substitutions between Ti, Zr, and Hf at the X site to suppress phonon thermal conductivity [1, 11-15]. To implement these approaches, various synthesis methods such as arc melting combined with the long-term annealing [16-19], the MS method [1, 20], solid phase reactions [21], mechanical alloying [22, 23], self-propagating high temperature [24], levitation melting [7, 25-27], and microwave methods [28] can be used. Among these techniques, MS is known as a fast synthesis process, which has an added advantage of fine-tuning the nanostructure to improve both electrical and thermal properties, thereby enhancing the TE efficiency of the material [1, 20, 29].

Studies on enhancing TE efficiency for a specific material is always of interest. However, for the product to be commercialised, cheap and readily available raw materials are also required. Besides, rapid synthesis methods save time and energy, reducing product costs. In the present work, we selected the Hf-free $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$ alloy along with a fast preparation method like MS for a cost-effective approach. To improve the TE performance of the $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$ alloy, we focused on optimising technology conditions by investigating the effect of the cooling rate induced by varying the copper wheel rotation speed from 1000 to 4000 rpm in the MS system. We found that the structural morphology

*Corresponding author: Email: du.nguyenvan@phenikaa-uni.edu.vn

of $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$ alloy was strongly affected by wheel rotation speed. Nano-grains with uniform shape and size of about 160 nm distributed over the ribbons were observed for wheel rotation speeds of 3000 rpm and higher. The grain growth during the SPS process directly affects the thermal and charge transport properties of the $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$ alloy. As a result, a high ZT value of ~ 1.1 at ~ 800 K was achieved for the sample with the optimised wheel rotation speed of 3000 rpm. The results suggest that optimising preparation conditions is vital to improving the ZT of $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$ alloy besides other approaches.

2. Materials and methods

The experimental procedure is shown in Fig. 1. Accordingly, four $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$ alloy ingots, approximately 8 grams each, were prepared using Ti (99.4%, powder), Zr (slug, 99.5%, slug), Ni (99.8%, powder), and Sn (99.99+%, shot) from Alfa Aesar by arc melting method under high purity argon gas. The ingots were then placed into graphite crucibles of inner diameter 14 mm with a 4-mm circular nozzle at the bottom for melt spinning. In the MS process, the ingot is heated to a liquid state with a copper induction coil, then injected directly onto the outer surface of the steadily rotating copper wheel (25 cm in diameter) under an Ar gas pressure of 0.2 MPa. The wheel rotation speed was set to 1000, 2000, and 4000 rpm, corresponding to wheel surface speeds of 13.1, 26.2, 39.3, and 52.4 m/s for each melted ingot. The melt-spun ribbons were pulverised into fine powders using an agate mortar and pestle to investigate their TE properties. The ground powders were simultaneously pressed and sintered with a pressure of 65 MPa and temperature of 1,373 K for 1 hour inside the high vacuum chamber of the SPS system. The spark plasma sintered (SPSed) bulk samples were then cut into suitable shapes using precision diamond saws for measurements and analysis.

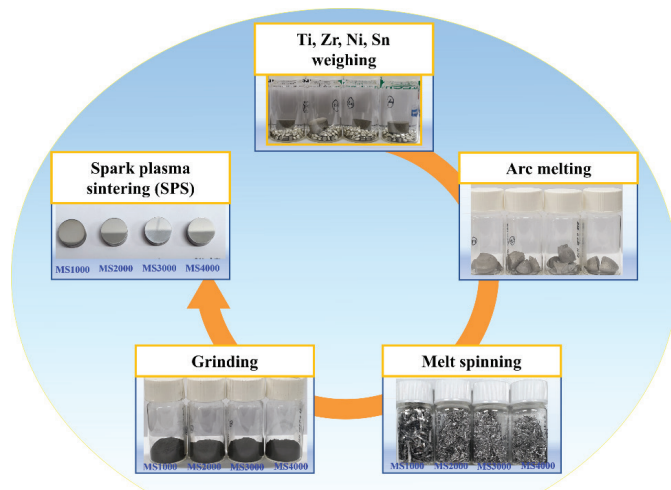


Fig. 1. Experimental procedure for the $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$ alloy.

The morphology, elemental distribution, and chemical composition were investigated using a field emission-scanning electron microscope (FE-SEM) (JSM-7600F, JEOL) with integrated energy-dispersive X-ray spectrometry. The phase purity

of the SPSed samples was examined by X-ray diffraction (XRD) using a Rigaku D/max 2500 instrument with $\text{Cu K}\alpha$ ($\lambda=1.5418 \text{ \AA}$) radiation operating at 40 kV. The temperature-dependent electrical conductivity and the Seebeck coefficient were simultaneously measured using a ZEM-3 (ULVAC-RIKO) system under low pressure He gas. Thermal conductivity was determined from the thermal diffusivity, measured density, and specific heat. The temperature-dependent thermal diffusivity was measured using NETZSCH LFA 457 equipment. For convenience, the samples examined with wheel rotation speeds of 1000, 2000, 3000, and 4000 rpm were named MS1000, MS2000, MS3000, and MS4000, respectively.

3. Results and discussion

To study the effect of wheel rotation speed on the structural morphology of $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$ alloys, FE-SEM images were taken on the freshly fractured cross-sectional surface of the melt-spun ribbons, as shown in Fig. 2. As the wheel speed increased, the thickness of the ribbons decreased monotonically from 26.3 to 10.2 μm , as shown in Figs. 2A-2D. Due to the difference in cooling rate between the contact and free surfaces of the ribbon, the structural morphology on either side of the ribbons was significantly different. In particular, at a wheel rotation speed of 1000 rpm, grain sizes of 500 nm were observed as well as agglomeration to form larger grains of several μm as shown in Fig. 2A-1, with an enlarged view of the selected area marked by the orange square in Fig. 2A. When the wheel speed increased to 2000 rpm, the obtained ribbons displayed precise layering with two layers formed with different structural morphologies, as shown in Fig. 2B-1. The contact surface of the ribbon exhibited grains with high uniformity in size and shape. Grain sizes less than 200 nm were observed, and the grain boundaries appeared clearer. In contrast, the free side exhibited a similar structure to that obtained in the MS1000 ribbon. For samples with higher wheel speeds of 3000 and 4000 rpm, uniform rapid heat absorption occurred over the entire ribbon thickness resulting in grain formation of a smaller size, roughly 160 nm. Note that nano-scale grain sizes are desired in the nanostructure approach for lowering the thermal conductivity of TE materials, which can only be prepared through long-term ball milling when using other synthesis methods.

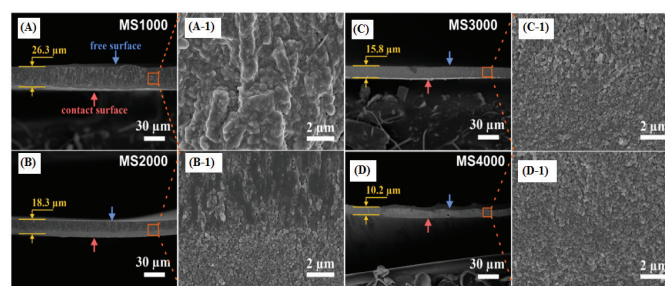


Fig. 2. Cross-section FE-SEM images of the melt-spun ribbons with varying copper wheel speed during MS: (A) MS1000, (B) MS2000, (C) MS3000, (D) MS4000 and (A-1)-(D-1) are enlarged views of a selected area marked by orange squares in Figs. 2A-2D, respectively.

To estimate the composition and elemental distribution of the ribbons, EDS analysis was carried out on the cross-section surface of the MS3000 melt-spun ribbons, and the results are shown in Fig. 3. The EDS layered map (Fig. 3A) reveals the elements of the $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$ alloy with a homogenous distribution over the entire observed area. The existence and concentration of Ti (16.36%), Zr (16.83%), Ni (31.88%), and Sn (34.93%), shown in Fig. 3B, suggests that the elemental composition is very close to the stoichiometric composition $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$, indicating that arc melting followed by MS did not change the sample's composition. A uniform distribution of elements over the entire observed area is more clearly confirmed with individual elemental maps, as shown in Figs. 3C-3F.

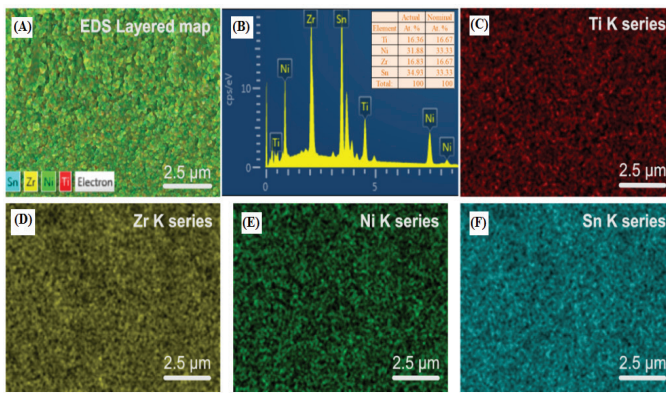


Fig. 3. EDS analysis results for the MS3000 melt-spun ribbon. (A) EDS layered map images; (B) EDS spectrum with inset table of elemental concentration (actual composition at at.% compared to nominal composition); (C-F) Elemental map images of individual Ti, Zr, Ni, and Sn elements, respectively.

After MS, the ribbons were ground into fine powders and consolidated into bulk via SPS. The evolution of the microstructure during the SPS process was examined by FE-SEM on the fractured cross-sectional surface of the MS1000, MS2000, MS3000, and MS4000 samples. The resulting images are given in Fig. 4. Despite the short sintering time, within 10 minutes, a temperature of 1100°C and applied pressure of 65 MPa showed that large grains formed quickly during the SPS process. Grain sizes greater than 20 μm were observed for the MS1000 sample, while the MS2000 sample demonstrated a grain size distribution in the range of a few μm to 20 μm . MS at 3000 and 4000 rpm produced more uniform grain sizes and shapes with grain sizes of approximately 5 μm as a result of grain growth from smaller nanostructures of melt-spun ribbons, as shown in Figs. 2C-1, 2D-1. In addition, high-density compaction, which favours charge-transport, was performed during the SPS process as shown in the high-resolution FE-SEM images of the MS2000 and MS4000 samples (see Figs. 4 B-1, 4D-1). These results suggested that maintaining nanostructures from melt-spun ribbons for enhanced phonon scattering at grain boundaries requires an ultra-fast sintering process at lower temperatures.

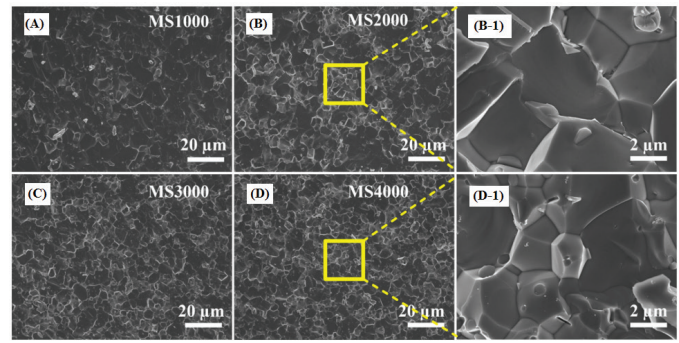


Fig. 4. FE-SEM images taken on the fresh fracture of a cross-section of SPSed bulk (A) MS1000, (B) MS2000, (C) MS3000, and (D) MS4000 samples. (B-1) and (D-1) are enlarged views of the selected areas marked by yellow squares in (B) and (D), respectively.

In addition to the work conducted, EDS analysis of the bulk SPSed MS3000 was also performed, and the results are presented in Fig. 5. The EDS layered map (Fig. 5A) and elemental maps of Ti, Zr, Ni, and Sn elements (Figs. 5C-5F) confirmed the highly homogeneous distribution of elements over the entire observed area. The chemical composition of the bulk SPSed MS3000 was determined through EDS analysis, and the results are presented in the inset table in Fig. 5B. The analysis identified the amount of Ti 15.82, Ni at 33.13, Zr at 17.11, and Sn at 33.94 at.%. These results indicate that the elemental percentages were consistent with the observed results on the ribbon and did not differ significantly from the stoichiometric composition of $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$.

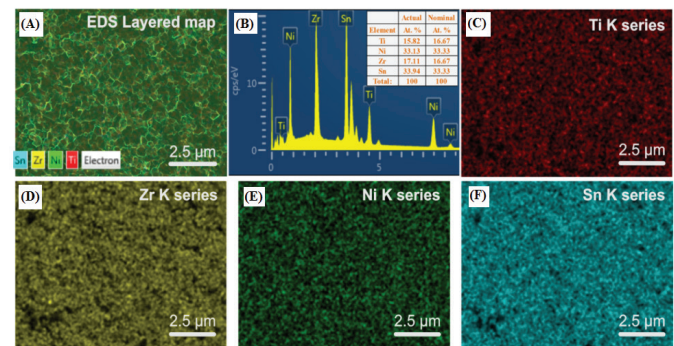


Fig. 5. EDS analysis results for the SPSed MS3000 sample. (A) EDS layered map images; (B) EDS spectrum with inset table of elemental concentration (actual composition (at.%) compared to nominal composition); (C-F) Elemental map images of individual Ti, Zr, Ni, and Sn elements, respectively.

The XRD analysis results of the SPSed bulk samples melt-spun at different wheel speeds are presented in Fig. 6. All diffraction peaks observed at the crystal planes (111), (200), (220), (311), (222), (400), (311), (420), and (422) in the 2θ angle range from 20 to 80° exhibit a single phase that can be indexed to the cubic half-Heusler crystal structure, consistent with all observed XRD patterns for (Ti, Zr, Hf)NiSn-based alloys in the literature [25, 30-32]. By extending the observation to the main diffraction peaks at the (220) plane, an asymmetrical distribution of those diffraction

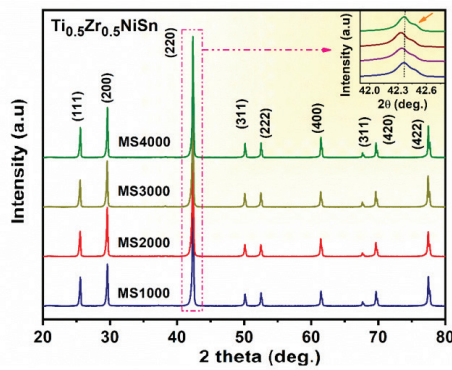


Fig. 6. XRD patterns of bulk $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$ alloys prepared by MS-SPS.

peaks with a high-angle shoulder was observed (Fig. 6). This could be attributed to phase separation into two Ti-rich and Zr-rich phases induced by alloying between Ti and Zr in the crystal. The combined effects of phase separation and alloying between Ti and Zr in the structure could enhance phonon scattering, thus effectively reducing the thermal conductivity of the $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$ alloy.

The temperature-dependent electrical conductivity and Seebeck coefficient are shown in Fig. 7. The electrical conductivity for all samples (Fig. 7A) increases with increasing temperature from room temperature to the maximum measured temperature of 873 K, caused by the thermal excitation of electrons across the energy gap, exhibiting intrinsic semiconductor-like behaviour. With an increase in wheel rotation speed, the electrical conductivity increased from 167 S/cm for MS1000 to the highest value of 194 S/cm for the MS3000 sample. Then, it decreased to 139 S/cm for the MS4000 sample, which was compared at room temperature. The electrical conductivity trend and variation showed an insignificant effect of the rotating wheel speed during the MS process. The rapid grain growth during the SPS process may be responsible for the small changes in microstructure and thus influence the observed variation in the electrical properties of those samples. Besides, decreasing absolute values of the Seebeck coefficients were obtained with increasing temperature for all

samples and were negative over the entire measured temperature range, indicating electrons as the major charge carrier. Among the SPSed samples, the highest Seebeck coefficient was achieved for the MS3000 sample at temperatures above 400 K. Large absolute Seebeck coefficients of about 200-280 $\mu\text{V/K}$ achieved for the samples confirmed the high Seebeck coefficient of half-Heusler (Ti, Zr, Hf)NiSn-based alloys as reported from the literature [10, 33]. The high Seebeck coefficient along with a relatively low electrical conductivity could be suitable for doping or substitution donor impurities for optimising carrier concentration to further improve the power factor for this material.

Figure 8A presents the temperature-dependent power factor ($\text{PF} = S^2\sigma$) calculated from the measured electrical conductivity and Seebeck coefficient for the $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$ alloys. Due to low electrical conductivity at room temperature, the PF for all SPSed samples exhibited values of around $14 \times 10^{-4} \text{ W/mK}^2$. With increased temperature, PF increases rapidly to a peak value at temperatures above 600 K caused by the predominance of the electrical conductivity and then decreases due to the weakening of the Seebeck coefficient at high temperature. The highest PF over the entire temperature range was obtained for the MS3000 sample with a peak of $37.5 \times 10^{-4} \text{ W/mK}^2$ at 774 K due to a significant increase in the Seebeck coefficient even though the electrical

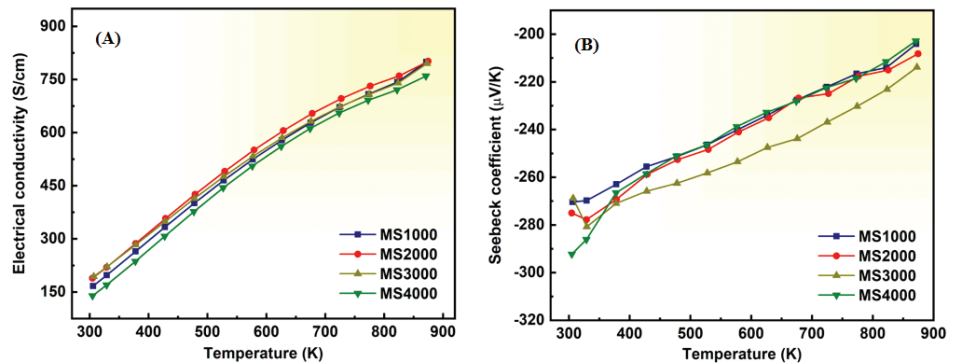


Fig. 7. Temperature dependence of (A) electrical conductivity and (B) Seebeck coefficient of the MS1000, MS2000, MS3000, and MS4000 samples.

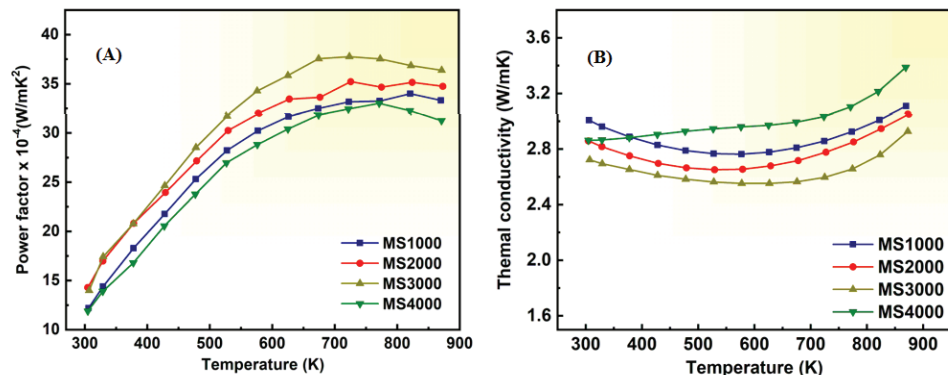


Fig. 8. The temperature dependence of (A) power factor and (B) thermal conductivity of the MS1000, MS2000, MS3000, and MS4000 samples.

conductivity was somewhat worse than that of the MS2000 sample. In addition, obtained relatively high PF obtained in this work suggests that the optimization of preparation conditions can improve the charge transport properties for effective power factor enhancement, besides the approaches to optimising the carrier concentration through doping and/or substitution have been performed for the (Ti, Zr, Hf)NiSn-based alloys [11, 26, 34].

The temperature-dependent thermal conductivity for all samples is presented in Fig. 8B. The thermal conductivity values of the MS1000-MS3000 samples exhibit a similar trend: decreasing at low temperatures, up to 523 K, and increasing with temperatures above 523 K, suggesting bipolar thermal conduction. Thermal conductivity was also found to decrease with wheel rotation speed up to 3000 rpm over all temperatures. This observation could be explained in terms of the combined phonon scattering effects that exist in $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$ alloys. Accordingly, grain growth from a smaller grain structure at the same sintering temperature could lead to an increase in structural disorder, thereby enhancing point defect phonon scattering. Furthermore, the grain boundary density of the MS3000 sample is larger than that of the MS samples with lower wheel rotational speed (see FE-SEM images in Figs. 4A-4C, further contributing to the enhanced phonon scattering at the grain boundaries. As a result, the lowest thermal conductivity of 2.55 W/mK at around 600 K was obtained for the MS3000 sample among investigated samples, which is comparable to the lowest reported values for (Ti, Zr, Hf)NiSn-based half-Heusler alloys [1, 12, 35].

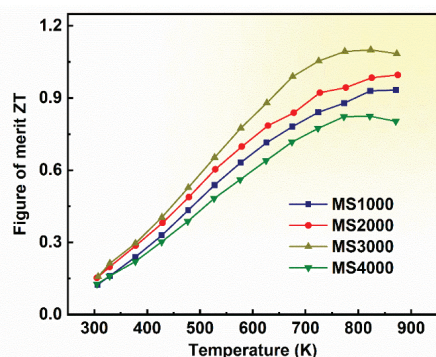


Fig. 9. The temperature dependence of the dimensionless ZT of the MS1000, MS2000, MS3000, and MS4000 samples.

The temperature dependence of the dimensionless ZT is calculated from PF and thermal conductivity and is shown in Fig. 9. With an increase in temperature, the ZT values exhibited a similar trend as the PF obtained for all samples when varying the copper wheel rotation speed from 1000 rpm to 4000 rpm. The combination of high PF and the low thermal conductivity led to a significant enhancement in ZT over the entire measured temperature range for the MS3000 sample of which the highest ZT value of ~ 1.1 at 823 K was achieved. The obtained ZT of ~ 1.1 is higher than most reported values for Hf-free (Ti, Zr)NiSn-based half-Heusler alloys prepared by other methods and/or other approaches [12, 18, 19, 36-39].

4. Conclusions

We studied the effects of wheel speed during the melt spinning process on the structural morphology of melt-spun ribbons. Specifically, the morphology, phase formation, and temperature-dependent TE properties of SPSed $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$ bulk samples from melt-spun ribbons. It was found that with an increase in wheel speed, the obtained alloy ribbons had a reduced thickness, more uniform nanograin structure, and the observed grain size of the ribbons decreased to a minimum of 160 nm due to increase in cooling rate induced by high wheel speed. Grain growth occurred quickly when sintering at high temperatures with observed grain sizes of several micrometres on the SPSed bulk samples. A single-phase structure with slight phase separation was observed in all SPSed samples. Both electrical and thermal properties were also observed with speed-dependent changes during MS due to the influence of grain growth starting from different morphologies. As a result, the highest ZT value of 1.1 at 823 K was achieved for the optimised wheel rotation speed of 3000 rpm. The results suggest that the optimization of experimental parameters plays a vital role in the strategic enhancement of TE efficiency of melt-spun $\text{Ti}_{0.5}\text{Zr}_{0.5}\text{NiSn}$ alloys and other TE materials.

CRedit author statement

Nguyen Van Du: Conceptualisation, Methodology, Validation, Formal analysis, Data curation, Visualisation, Supervision, Writing - Reviewing and Editing; Nguyen Tu: Data curation, Formal analysis, Writing - Reviewing and Editing; Do Quang Trung: Data curation, Formal analysis, Writing - Reviewing and Editing; Noy Sysavanh: Investigation, Data curation, Writing original draft preparation; Manh Trung Tran: Data curation, Formal analysis, Writing - Reviewing and Editing; Tong Thi Hao Tam: Data curation, Formal analysis, 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.

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Study on flexural behaviour of printed concrete wide beams using polypropylene fibres

Thi Loan Pham*, Duy Thanh Trinh, Thi Hoai Thu Nguyen, Trong Quang Do, Phan Anh Nguyen

Department of Civil Engineering, Haiphong University, 171 Phan Dang Luu Street, Ngoc Son Ward, Kien An District, Hai Phong City, Vietnam

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Abstract:

Sustainability in building construction is an inevitable aspect of future construction projects. The justification of sustainability is highly appreciated by comparing 3D concrete printing technology with conventional construction. However, the 3D printing concrete system has limitations and challenges in industrial applications. The reason is that this process was initially used in small non-structural applications and is now being adopted for large-scale structures. Thanks to concrete printing machines, a wide variety of web frames are also available - something completely impossible to achieve using traditional formwork for pouring concrete. In this study, the girder web is designed in the style of truss beams. Three wide beams with different amounts of polypropylene (PP) fibre were printed, and 3-point loading tests were conducted. The failure mode, load-bearing capacity, and deflection were reported in this study. According to the results, applying concrete printing technology in civil and industrial construction is entirely feasible. The printing process successfully produced models with a nozzle diameter of 22 mm and layer height of 10 mm, ensuring sufficient adhesion force between the printing layers. Using a PP fibre content of about 0.25% yielded the best results in terms of concrete compressive strength and beams' flexural strength, while a PP fibre content of about 1.00% tended to increase the ductility of the member. Although the failure mode is brittle, the beams exhibited deflection before fracture far beyond the allowable deflection value of a flexural member. Therefore, the application of printed components to construction is feasible.

Keywords: deflection, failure mode, flexural behaviour, load-bearing capacity printed concrete, polypropylene fibre, wide beam.

Classification numbers: 2.1, 2.3

1. Introduction

Sustainability in building construction is an essential aspect of future construction projects, making 3D concrete printing construction a standout advantage over traditional construction practices. The justification for sustainability becomes highly appreciated when comparing 3D concrete printing technology with conventional construction techniques, particularly due to the reduced labour requirement, absence of formwork, and decreased waste production. For example, the Dubai project (the United Arab Emirates National Committee) has reported labour cost reductions of 50 to 80% and a decrease in construction waste of 30 to 60% [1, 2].

In addition to the advantages mentioned above, a 3D printing concrete system carries some limitations and challenges in industrial applications. There is a lack of knowledge regarding the effects of different environmental factors on 3D concrete printed structures. Manipulating large-sized 3D printers at construction sites can be challenging. Higher capital investment is required for creating and developing digital models, which also requires skilled personnel and is especially limited in terms of design. Additionally, there is a lack of understanding about the behaviour of 3D concrete printed structures under different loading conditions. This knowledge gap stems from the initial use of this process in small non-structural

applications and its subsequent adoption for large-scale structures. Therefore, research on the structural behaviours of printed components [3] or whole systems has been limited. By examining the progress of the development stages for concrete printing technology applications in the construction field, as shown in Fig. 1, we can reflect on past advancements and anticipate future ones.

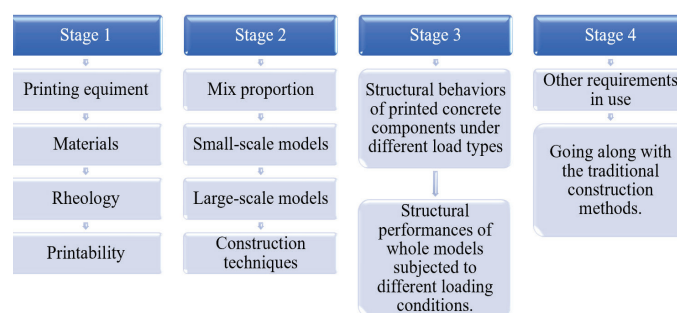


Fig. 1. Development stages of concrete printing technology applications in the construction field.

Stages 1 and 2 have been thoroughly researched and successfully published in recent years. Extensive literature reviews have been conducted worldwide [4-7]. Many researchers have investigated and designed effective printing

*Corresponding author: Email: loanpt297@gmail.com

approaches for cement-based materials based on their experiments with printers [8-12]. One of the disadvantages of concrete printing technology is the use of rebars. To overcome this obstacle, reinforced concrete with random fibres such as PP fibres, glass fibres, steel fibre, palm fibre, and others have been explored [13-18].

Both small and larger-scaled models have been constructed using revealed construction techniques [19-22]. These achievements have significantly enhanced the application of concrete printing technology in the construction field.

Stage 3 is still in its initial steps, with some studies focusing on the structural behaviours of components such as panels [3], walls [23], and printed concrete specimens [24, 25]. From the authors' perspective and the experimental conditions at Haiphong University, research on the flexural behaviour of wide beams, including failure modes, moment resistance, and deflection of printed beams, has been carried out and analysed. The results obtained from this research will contribute to the success of Stage 3 and become an essential part of Stage 4 in the future.

Beams play a crucial role in the structural system of buildings, especially in frame structures. They support floors, walls, and connect columns to form the frame system. The most common cross-section for beams is rectangular, with simple construction and design processes that do not require complex or unique tools. Beams can be classified into high beams, wide beams, and typical beams based on the beam height and span ratio. Typical beams have a width-to-height ratio of about 0.5 to 0.75, while wide and typical beams have a span-to-height ratio greater than 5. The rest are considered high beams [26]. Typical and wide beams are widely used in all structural systems and are designed to withstand bending, shear, and deformation to ensure the work of the building. High beams are less commonly used as transfer beams to support additional columns. These act as girders in high-rise building systems, helping to increase the rigidity of the structural system and reduce the impact of lateral loads.

However, the presence of beams in the structural system of buildings often reduces the clearance height of floors. Therefore, there is a trend towards designing smaller height beams to optimise space utilisation, although this compromises the bearing capacity and deformation compared to conventional beam members with the same cross-sectional area. Nevertheless, the trend of choosing wide beams over traditional beams is increasingly popular as it serves two simultaneous goals: reducing building height and enhancing spatial aesthetics.

To meet the requirements of workability and the growing trend of using wide beams, studying the behaviour of this type of beam is indispensable. While wide beams are typically studied for shear strength in conventional reinforced concrete structures, beams produced by concrete printers exhibit a different failure mode. As wide beams made by concrete printers are designed without reinforcement, the development of web ribs becomes necessary. For wide beams fabricated by concrete printing machines, a wide variety of web frames are available depending on their requirements, thanks to the printability of the material - something that is completely impossible to achieve by pouring concrete using formwork. In this study, the girder web is designed in the style of the truss beams. Failure due to shear in inclined sections is avoided, and instead, failure on vertical sections caused by the concrete reaching its tensile strength due to moments induced by unreinforced beams is proposed. Therefore, this study focuses wide beams with flexural behaviour, rather than shear behaviour like conventional reinforced concrete beams, including analyses of failure modes and load-bearing capacity.

2. Materials and methods

2.1. Details of wide beams

The dimensions of length, width, and height of the beams are 1450, 450, and 150 mm, respectively. The thickness of the perimeter line is 40 mm, and the thickness of the girder web is 30 mm. Details of the printed concrete wide beams are shown in Fig. 2.

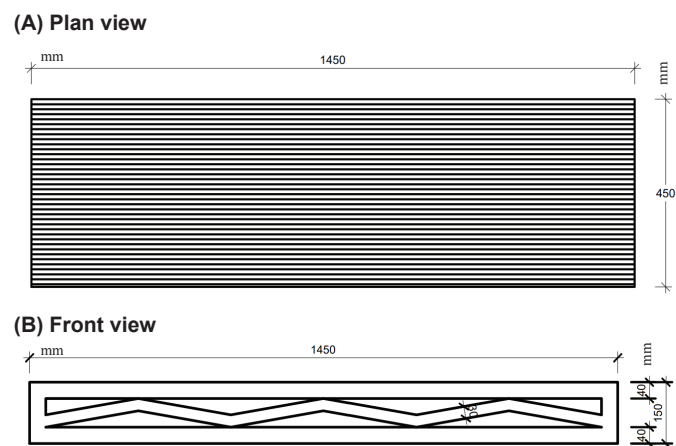


Fig. 2. Details of the printed wide beam. (A) Plan view; (B) Front view.

2.2. Materials and mix proportions

In this research, ordinary portland cement (OPC) by Chiffon PC40 and fly ash from Haiphong Thermal Power Plant were used to form the binder component, thanks to prior research on successfully applied cement-based materials

[9-12, 27, 28]. Table 1 illustrates the chemical composition of the OPC and fly ash. According to ASTM C618, fly ash from the Hai Phong Thermal Power Plant is class F.

Table 1. Chemical composition of fly ash and OPC [29].

Chemical composition	Weight percentage (%)	
	Fly ash	Cement
SiO ₂	45.56	22.45
Al ₂ O ₃	23.04	5.42
Fe ₂ O ₃	5.34	3.6
TiO ₂	0.1	-
K ₂ O + Na ₂ O	7.44	1.1
CaO	1.08	61.75
MgO	1.29	1.15
P ₂ O ₅	0.2	-
SO ₃	-	2.18
MnO	0.1	-

Commercially available manufactured natural sand with a nominal maximum aggregate size of 1.5 mm was used. PP fibres were used to decrease the shrinkage of the printed concrete - the main properties of PP fibre are listed in Table 2.

Table 2. Properties of the PP fibre.

Tensile strength	500 MPa
Modulus	6000 MPa
Diameter	35+/-5 µm
Length	12 mm
Specific density	0.910 kg/l

Superplasticiser, namely Visconcrete 3000-200M, was used to adjust the workability of the fresh concrete. Materials used for mixing concrete in this research are presented in Fig. 3.



Fig. 3. Materials used for mixing concrete.

Based on the approaches and achievements of previous researchers, the authors highly value practical methods conducted in both laboratory and on-site settings. As a result, the authors propose the following mix proportion design process. In this method, the mix design incorporates coefficients and slump value approach, as shown in Fig. 4. To maximise the replacement of fly ash for cement but while minimising the impact on the compressive strength of the concrete, a fly ash to cement ratio of 1.0 was adopted. Additionally, the water-to-cement ratio ranged from 0.27 to 0.32. The workability of the concrete was adjusted using a superplasticizer. The fine sand used in this study was natural sand passed through a 1.25-mm square hole sieve. The mix proportions were designed based on the procedure proposed in Fig. 4 and are listed in Table 3.

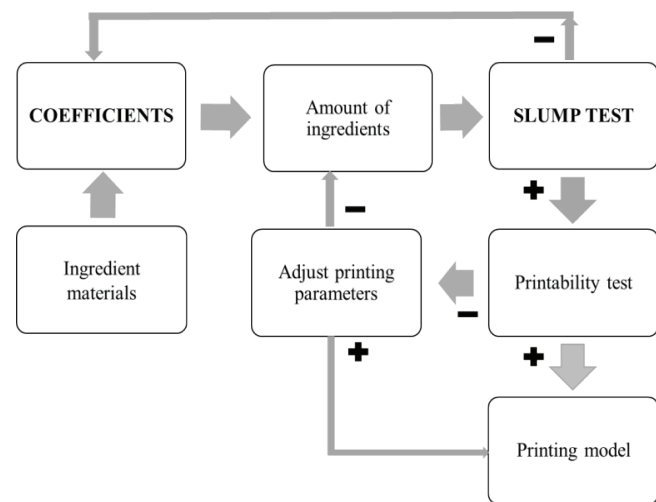


Fig. 4. Mix proportions design process [30].

Table 3. Mix proportions.

Beam label	Fly ash to cement	Water to binder	Natural sand to binder	PP fibre to binder (%)	SP to binder (%)
PB01				0.25	
PB02	1.00	0.27	1.00	1.00	0.40
PB03				1.50	

2.3. Printing process and fabrication

The process begins with a 3D CAD model of the object. Subsequently, slicing software is employed to create cross-sectional layers of the model, which are saved as a computer file and sent to the 3D printer. The 3D printer then fabricates the object by sequentially depositing each layer of material through the selective material placement. In this study, round-shaped nozzle with a diameter of 22 mm was used, and the model was sliced with a layer height of 10 mm. Fig. 5 visually depicts this process.

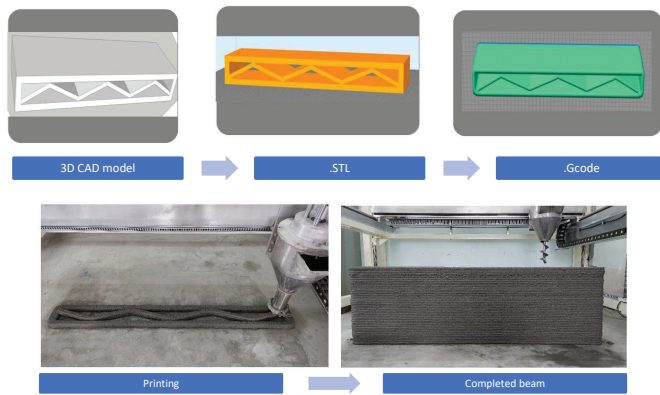


Fig. 5. Steps of the printing process.

2.4. Experimental program

2.4.1. Flexural test setup

A 3-point bending test was conducted on the experimental beams, which had a nominal length of 1350 mm. Each specimen was positioned on roller assemblies to accurately locate the supporting points. Linear variable differential transformers (LVDTs) were used to record the deflection based on the applied load. An LVDT was affixed at the mid-section of the beam specimen, precisely beneath the loading point. The test setup and instrumentation for tested specimens are shown in Fig. 6.

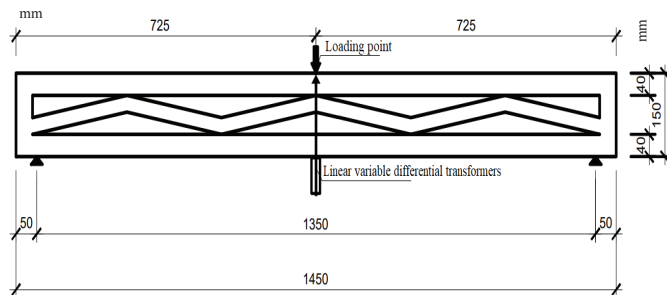


Fig. 6. Flexural test setup.

2.4.2. Compression test

Following the casting of the 10x10x10 concrete cubes, the concrete samples were cured under controlled humidity conditions. As a result, the testing procedure and loading protocol comply with TCVN 3118-1993 [24], as shown in Fig. 7.



Fig. 7. Compression test of concrete cubes.

3. Results and discussion

3.1. Failure modes

The failure patterns of the beams, as shown in Figs. 8-10 indicate that sudden failure of the concrete printed beams is characteristic of a brittle failure mode due to the absence of reinforcement. The inclusion of dispersed PP fibres did not result in a significant increase in the plasticity of the concrete. However, the failure mode of beam PB02 demonstrates a more extensive development of cracks in the concrete's tensile zone compared to the other two beams, PB01 and PB03.

Another noteworthy observation from these figures and the examination of the beam surfaces after fracture is the absence of delamination between the printed layers. This indicates that the adhesion force between the printed layers ensures the structural integrity of the entire system. Furthermore, the examination of the beam surfaces after failure reveals the solidity of the printed layers.

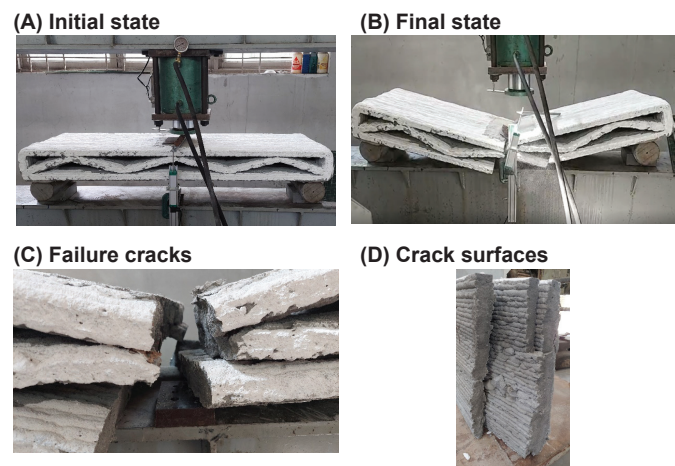


Fig. 8. The failure mode of PB01. (A) Initial state; (B) Final state; (C) Failure cracks; (D) Crack surfaces.

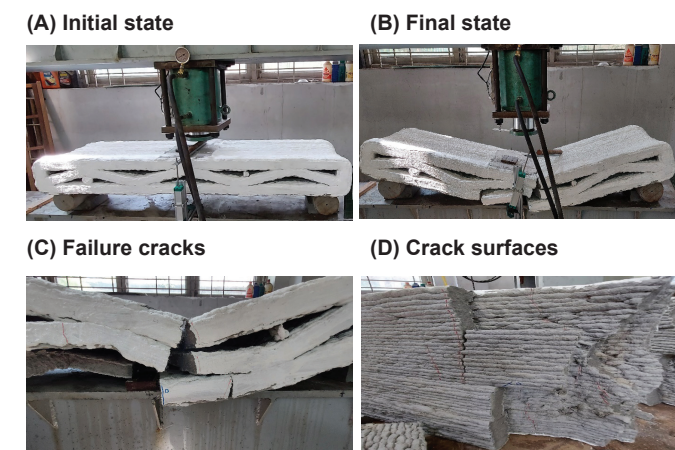


Fig. 9. The failure mode of PB02. (A) Initial state; (B) Final state; (C) Failure cracks; (D) Crack surfaces.

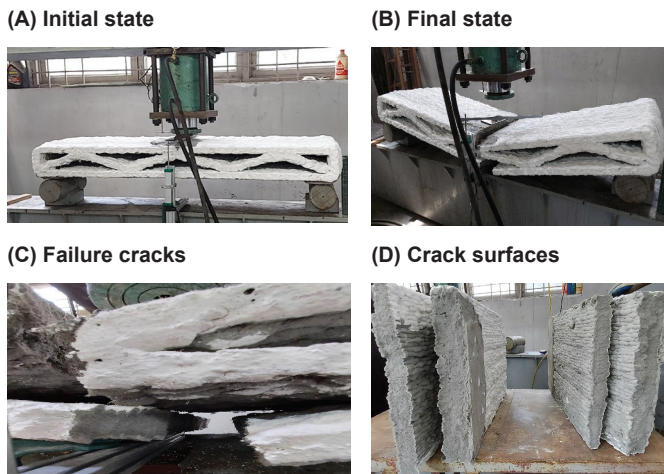


Fig. 10. The failure mode of PB03. (A) Initial state; (B) Final state; (C) Failure cracks; (D) Crack surfaces.

3.2. Loading capacity

The results of the force measurement and displacement meter on the bearing capacity of the beams are shown in Table 4.

Table 4. Load bearing capacity.

Beam label	Ultimate load (kN)	Deflection
PB01	24.74	16.17
PB02	19.84	24.73
PB03	20.87	17.78

The results indicate that the PB01 beam, corresponding to a PP fibre content of 0.25%, exhibits the highest bearing capacity among the tested beams. Additionally, an increase in PP fibre content tends to reduce both the flexural and compressive strength of the concrete, as shown in Table 5. This finding is consistent with the research results of other authors [16, 31, 32].

Table 5. The compression strength of printed concrete.

Beam label	Ultimate load (kN)	Dimensions (mm)	Coefficient α	Compression strength R_a (MPa)
PB01	525.64	100x100x100	0.91	47.8
PB02	256.46	100x100x100	0.91	23.3
PB03	285.15	100x100x100	0.91	25.9

Furthermore, the results table reveals that the PB02 beam, with a PP fibre content of 1.00%, exhibits the highest beam deflection. Notably, beam PB02 demonstrates a significant improvement in member ductility, as evidenced by a deflection value of 24.73 mm. This value is 35% higher than that of PB01 and PB03, respectively, by 35 and 39%, which is similar to results reached by other authors [33]. In addition, the failure of PB02, characterised by crack propagation as depicted in Fig. 9, shows a good agreement with the observed results. Although the failure mode is brittle, the deflection values prior to beam fracture far

exceed the allowable deflection value of a flexural member of 9.00 mm (corresponding to 1/150 of the girder span).

4. Conclusions

Based on the combination of theoretical study and experimental research within the scope of this topic, the following conclusions can be drawn: 1) Concrete printing technology is feasible and can be successfully applied in the construction of civil and industrial works; 2) The adhesion force between the printing layers ensures the structural integrity of the printed components; 3) The flexural failure of all beams is characterised as brittle.

Furthermore, it has been observed that using a PP fibre content of approximately 0.25% yields the best results in terms of concrete compressive strength and flexural strength of the beams. On the other hand, a PP fibre content of around 1.00% tends to enhance the ductility of the member but leads to a reduction in the compressive strength of the concrete and the flexural strength of the beam. To further enhance the research and increase the reliability of the conclusions, the following points should be developed: It is recommended to use similar increments when adjusting the PP fibre content and conduct experiments with a larger number of beams. This will help determine an optimal value of PP fibre reinforcement for structural plasticity. Additional research should be carried out to investigate the influence of geometrical errors in the printed layers on beam workability. Further study is needed to explore the effects of different geometries of girder webs on the overall performance of the structures. By addressing these points, the research can be expanded, and the reliability of the conclusions can be improved.

CRedit author statement

Thi Loan Pham: Conceptualisation, Methodology, Data analysis; Duy Thanh Trinh: Laboratory bending test; Thi Hoai Thu Nguyen: Writing original draft preparation; Trong Quang Do: Reviewing and Editing; Phan Anh Nguyen: Laboratory printing test.

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COMPETING INTERESTS

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

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Investigation of residual stress in epoxy-based coatings using X-ray and FEM-ANN techniques

Jasim Haider Hadi*

University of Basrah, Basra City, Iraq

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Abstract:

Residual stresses play a significant role in the properties and performance of epoxy-based coatings, with their origins rooted in various factors encountered during production and application. This study focuses on quantifying residual stresses in three distinct epoxy-based coatings, commonly used as linings for crude oil storage tanks, namely, pure epoxy, Novolac epoxy, and glass-flake-reinforced epoxy. We employ X-ray diffraction to measure these residual stresses and compare them against predicted values obtained through finite element and artificial neural network methods. Our findings reveal notable differences in residual stresses among the three types of epoxy coatings. Specifically, pure epoxy coatings exhibit higher residual stresses, Novolac epoxy coatings display the lowest, and those reinforced with glass flakes fall in between. Utilising the FEM-ANN model for simulations yields results that closely align with experimental measurements obtained via the X-ray method. Test results demonstrate that the coatings cured at high temperatures have high residual stresses compared to those cured at lower temperatures. Increasing the curing temperature from 10 to 50°C will increase residual stresses by 40.81, 11.085, and 56.98% for coatings reinforced with glass-flake, Novolac, and pure epoxy-based coating, respectively.

Keywords: curing temperature, glass-flakes, residual stress, volumetric shrinkage, X-ray method.

Classification numbers: 2.2, 2.3

1. Introduction

Epoxy coatings consisting of two components, a base and a hardener, are commonly employed for safeguarding crude oil storage tanks, ensuring their continued operational performance. However, a range of factors, including intrinsic characteristics, thermal influences, volumetric changes, and lattice disparities, can introduce residual stress into these epoxy coatings, potentially compromising their performance [1]. Throughout the crosslinking and curing process, volumetric transformations occur, driven by the interaction between the base and the hardener [2]. The presence of fillers, fibres, or glass flakes can exacerbate this issue as these materials often shrink at different rates compared to the resin and hardener, leading to the formation of residual stresses. Furthermore, parameters such as curing temperature and coating thickness significantly impact the initiation of residual stresses during the curing process of epoxy-based coatings [3]. Intrinsic residual stresses may also be present, stemming from defects like air bubbles and grain boundaries within the epoxy coating [4].

Various methods are employed to evaluate residual stresses, encompassing both experimental and numerical techniques [5]. The X-ray diffraction (XRD) technique is widely utilised for

measuring residual stresses in diverse materials. XRD assesses strain within the crystal lattice, allowing for the calculation of residual stress based on elastic constants [6]. In contrast, numerical methods such as finite element method (FEM) and artificial neural networks (ANN) have gained popularity for predicting residual stresses. FEM entails complex non-linear analyses, influenced by boundary conditions and modelling procedures [7, 8], while ANN, as a computational model, employs artificial neurons to simulate structural organisation, predict relationships between input parameters, and model the system [9, 10].

Despite the critical implications of residual stresses, their evolution within epoxy coatings has received limited research attention. P. Kamarchik, et al. (1980) [11] reviewed the application of the X-ray technique for measuring and estimating residual stresses in coating and coating-related systems. The results indicated that less adhesion and misleading cross-linking between coating molecules can result from the residual stresses. I. Kishore (2003) [12] used finite element analysis to simulate the formation of residual stress in epoxy resins created due to the influence of dimensional constraints. The simulation results indicate that there are possible areas where delamination and cracking in epoxy

*Email: ashaqu73@gmail.com

can occur, and the residual stress in epoxy resins is generated from volumetric changes that occur throughout the curing and crosslinking process. S.B. Aboubakar (2009) [13] computed and analysed residual stresses at fibre-epoxy interfaces using an axisymmetric model. Two cases are considered, an epoxy matrix with glass and carbon fibres respectively with different fibre volume. The results show that the stresses at the interface are affected by residual thermal stresses, particularly in the free edges. J. Cheng, et al. (2009) [14] synthesised and studied the curing temperature and thermal stability of novel epoxy coatings containing 4,4-diaminodiphenyl sulfone as a hardener and 4,4-diaminodiphenylmethane as a base using differential scanning calorimetry. They found that the thermal decomposition temperature of epoxy coatings increased with the increase in curing time and the rise in curing temperature. M. Frantisek, et al. (2014) [15] developed a new approach using a neural network method to analyse residual stresses and compared their results with that obtained from the experimental C-Ring measurement method. It has been shown that the neural network method is a good procedure for the analysis of residual stresses. D. Taisei, et al. (2015) [16] measured the residual stress in polyamide film polymer using the X-ray diffraction technique. The diffractometer with a transmission method was used in experimental measurement. They discussed the problems related to measuring residual stresses in polymer coatings and showed that the X-ray technique is a good method to measure residual stresses in polymer coatings. Q. Li, et al. (2022) [17] studied the internal stresses in epoxy coatings using both analytical and experimental methods, including the attenuated total reflection-fourier transform infrared technique and dynamic mechanical thermal analysis. They investigated the effect of various parameters, including the molecular structures of binders and hardeners, filler type and concentration, and initial solvent concentration on internal stress values.

In our study, we focus on measuring the residual stress in three types of Hample epoxy coatings, namely pure epoxy, Novolac epoxy, and epoxy reinforced with glass fibres. These coatings find application in crude oil storage tank linings. We employ X-ray diffraction to measure residual stresses and compare the results with those calculated using the FEM-ANN method. Our investigation delves into the impact of coating thickness, volumetric shrinkage, coating viscosity, and curing temperatures, simulating these variables using FEM-ANN techniques.

2. Materials and methods

2.1. Experimental procedure

2.1.1. Types of epoxy coatings

Three types of Hample epoxy coatings have been used for studying. These types are commonly used for covering crude oil storage tanks in South Iraq. These coatings consist of two parts (base and hardener) which are in the liquid state and mixed together before being applied to the steel surface.

These coatings are: (1) HEMPADUR 15600, amine adducts cured pure epoxy coats. The mixed volume ratio is 1:1 of base to hardener. Thickness of coatings ranges from 160-200 μm . Refer to this coat as model A; (2) HEMPADUR 85671, this is a two-component solvent-free amine-cured phenol Novolac epoxy coating. The volume ratio of mixing is 1:2 of base epoxy to hardener. The thickness of this coating is in the range of 250-600 μm . Refer to this type of coats as model B; (3) HEMPADUR multi-strength GF-35870 is an amine-adduct cured epoxy coating, the coats are reinforced with glass-flakes. The mixing volume ratio is 3:1 of base to hardener. The maximum thickness of coatings is 350 μm . Refer to this type of coats as model C.

These types of Hample epoxy-based coatings are manufactured by Sharjah Coating Company, U.A.E., and they are used as manufactured for plain coating of tanks without external additives except model C is reinforced by glass fibre as produced by the company.

2.1.2. Specimen preparation and coats

The 3×3 cm samples with a thickness of 6 mm were used of ASTM A537 C1 carbon steel which was widely applied in the building of crude oil storage tanks of South Iraq. The specimen dimensions are considered according to ASTM-E0915-96R02 [18]. The chemical compositions of ASTM A537 carbon steel as a percentage are Fe: 97.58, C: 0.24, Mn: 1.6, P: 0.035, S: 0.035, Cr: 0.025, Si: 0.5, Mo: 0.8, Ni: 0.25, Cu: 0.35 [19]. Fig. 1 shows specimens before and after coatings in addition to SEM image (100 times magnified) of the cross-section to show the thickness of epoxy coatings. All Hample coatings were applied by airless spray to steel substrates at nominally standard film thicknesses as given by the manufacturing company. The PosiTector 6000 coating thickness gauges manufactured by LABOMAT (France) were used to check the coating thickness.

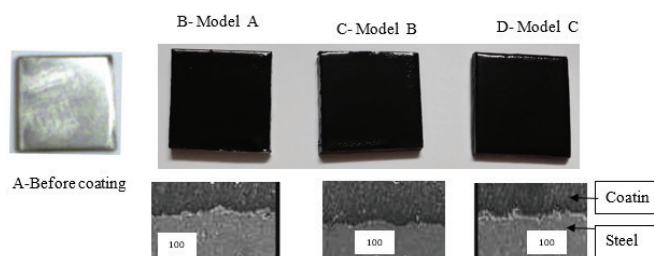


Fig. 1. Specimens before and after coatings with SEM image.

To study the effect of curing temperature on the residual stresses induced during solidification, the samples of the three models of coatings were left to cure at nine temperatures (10, 15, 20, 25, 30, 35, 40, 45 and 50°C), respectively. Based on information provided in the manufacturer's data sheets for various types of epoxy-based coatings, the curing time for model A is up to 72 hours, the curing time for model B is 7 days, and the curing time for model C typically takes 10 days. A total of 100 specimens were used for X-ray measurement.

2.1.3. Residual stress measured by X-ray

In X-ray diffraction (using the $\sin^2 \psi$ method), the strains in the crystal lattice of the coatings were measured along the tangential direction, and the associated stress is computed from the elastic constants (modulus of elasticity and Poisson's ratio) assuming a biaxial state of stresses [20]. The basic assumption throughout strain measurement by X-ray diffraction is that the plane of the crystal lattice in epoxy-based coating has a uniform linear elastic distortion. The d-spacing was measured by a diffractometer for different values of ψ , and the residual stress is calculated using the following relationship [21]:

$$\sigma_{\text{res.}} = \frac{E}{(1+\nu)\sin^2(\psi)} \frac{d_{\psi} - d_n}{d_n} \quad (1)$$

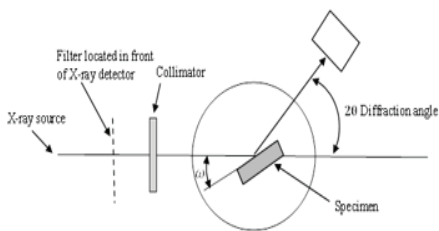
where d_{ψ} is the inter-planar spacing of planes at an angle ψ to the surface (μm); d_n is the inter-planar spacing of planes normal to the surface (μm); E is the elastic modulus of the coatings (GPa), ν is Poisson's ratio; ψ are the angles through which the sample is rotated.

Table 1. List of specification of X-ray diffraction.

Specification	Values
Chart speed	15 mm/min
Incident angle of X-ray	0,18,36,45
Gonio scan speed	1 deg/min
Divergent angle	0.35 deg
Irradiation area	3×3 mm ²
Time constant	5 sec
Tube current	15 mA



(A) X-Ray diffraction machine



(B) Method of measured residual stresses

Fig. 2. X-Ray diffraction machine and method of measured residual stresses using X-ray diffraction.

The X-ray measurements were performed on an ADX-8000 Mini θ - θ Powder X-ray diffraction instrument manufactured by Angstrom Advanced Inc. The X-ray source is a Cu tube, which is used for all specimens tested. Since the thickness of epoxy coats is relatively small, and only the surface strain can be achieved. The goniometer was used to read and record the angle (2θ). The collimator was used to control the irradiated area. Table 1 lists the specifications used by X-ray diffraction. X-ray diffraction experiments were conducted using the machine illustrated in Fig 2A at the Ministry of Science and Technology. A voltage of (10 kV) and a current of 15 mA were supplied to the target, which is copper with a wavelength of 1.5406 Å, and nickel as the filter material. Fig 2B shows the method of measured residual stresses.

Table 2. Epoxy coating elastic modulus and Poisson's ratio obtained from tensile test.

Cure temperature °C	Elastic modulus (GPa.)			Poisson's ratio		
	Model A	Model B	Model C	Model A	Model B	Model C
10	2.24	1.65	0.96	0.42	0.31	0.27
15	2.20	1.61	0.95	0.42	0.31	0.27
20	2.18	1.60	0.94	0.43	0.34	0.28
25	2.15	1.59	0.91	0.43	0.34	0.28
30	2.14	1.57	0.89	0.40	0.34	0.28
35	2.12	1.55	0.88	0.40	0.35	0.28
40	2.11	1.52	0.85	0.45	0.35	0.28
45	2.10	1.51	0.84	0.45	0.35	0.29
50	2.10	1.50	0.83	0.45	0.35	0.29

To compute the residual stress from Eq. (1), the unstressed lattice spacing d_n , the stressed lattice spacing d_{ψ} , the elastic modulus and Poisson's ratio of epoxy-based coatings must be determined. Different methods for obtaining the unstressed and stressed lattice spacing are found. Due to simplicity, the $\sin^2 \psi$ method is used for determining d_n and d_{ψ} lattice spacing. A number of X-ray measurements are performed to determine the interplanar spacing (d_n) in μm for various values of ψ . Then, a linear curve of d vs. $\sin^2 \psi$ is plotted, and the value of the lattice spacing is intercepted on the y-axis at $\sin^2 \psi = 0$, which is considered d_n [22]. The two common causes of error that affect XRD results are specimen- and instrument-sensitive errors. The best precision attainable for the machine used is about +0.4%, and the probable error does not exceed +1% according to the manufacturing data sheet. The samples were sent for measuring the residual stresses immediately after curing at each temperature.



Model A



Model B



Model C



Fig. 3. Epoxy coating specimens for tensile testing and tensile testing machine.

The measurement of mechanical properties (e.g., elastic modulus and Poisson's ratio) of the epoxy coating has some difficulties because the structure of epoxy coatings is complex and contains lamellae, pores, micro-cracks, and so on. The Hounsfield hand-operated universal tensile testing machine SM1002, Hounsfield Company, UK), in accordance with ASTM E8M-01 [23] and ASTM D638-14 [24], shown in Fig. 3, is used for achieving tensile tests to measure the elastic modulus and Poisson's ratio of the three types of epoxy coatings. Two strain gauges were used, one to measure longitudinal deformation and the other to measure contraction in the median of the specimen during the test.

Figure 3 shows specimens after moulding and solidification. Two glass plates with dimensions and shape similar to the tensile test specimen given by ASTM E8M-01 and ASTM D638-10 [23, 24] were used as moulds for casting the three Hample epoxy coatings to prepare the tensile specimens. Rubber binding around the glass plates was used to fix them. The specimens were left to cure at temperatures (10, 15, 20, 25, 30, 35, 40, 45, and 50°C). The specimen has a 50 mm gauge length and 5 mm diameter. The specimens are treated as elastic materials for tensile tests. A total of 54 specimens were prepared for tensile testing.

2.2. Numerical methods

2.2.1 FEM

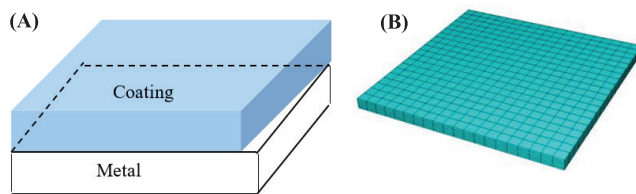


Fig. 4. The meshes used in the models. (A) Metal-coating system; (B) Finite element mesh of coating.

FEM was carried out using the commercial software ANSYS V.7 for both thermal and displacement analysis. The coatings were modelled in 3D with 8-node quadratic quadrilateral elements. The meshes used in the models are shown in Fig. 4. The boundary conditions for the models state that all nodes on the top surface are subjected to temperature (10, 15, 20, 25, 30, 35, 40, 45, and 50°C), while bottom surface nodes are constrained in the X-Y directions.

The finite element analyses were carried out on 3D models using temperature-dependent material properties: elastic modulus and Poisson's ratio. The elastic modulus and Poisson's ratio are determined from tensile test specimens cured at various temperatures. The reference coefficient of thermal expansion (CTE) is $70 \times 10^{-6}/^\circ\text{C}$ for amine-cured phenol Novolac epoxy, while for amine-adduct cured pure epoxy paint, it is about $121.4 \times 10^{-6}/^\circ\text{C}$, and for amine-adduct cured epoxy coating reinforced with glass flakes, it is about $30 \times 10^{-6}/^\circ\text{C}$ at room temperature according to company manufacturing data sheet information. For other temperatures, the coefficient of thermal expansion used in ANSYS V.7 software program is calculated using the Eq. [25]:

$$\alpha_T = \frac{\ln(\alpha_{\text{ref}}*(T-T_{\text{ref}})+1)}{(T-T_{\text{ref}})} \quad (2)$$

where α_T is the coefficient of thermal expansion at temperature T , T is the temperature ($^\circ\text{C}$), α_{ref} is the reference coefficient of thermal expansion, a value of 0.01 in this analysis, and T_{ref} is the reference temperature for thermal expansion, usually taken as 22°C .

There are 2000 elements in the FEM analysis, each with a size of 0.0065 mm.

2.2.2. Artificial neural networks

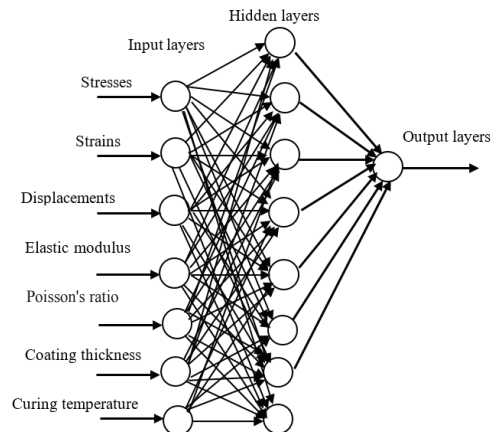


Fig. 5. Schematic diagram of ANN method for predicting residual stresses.

For the predictive analysis using ANN, the ANN model consists of three layers: the input layer, the hidden layer, and the output layer [26]. The input parameters for the ANN model consist of the strains and displacements obtained from the numerical simulations using FEM, with mechanical properties obtained from tensile tests, and the outputs are the corresponding predicted residual stress. The learning function is the gradient descent algorithm with momentum weight and bias learning functions. The number of hidden layers and neurons are determined through a trial-and-error method to accommodate the convergence error. The structure of the proposed ANN model is 7 neurons in the input layer, 1 neuron in the output layer, and the number of neurons in the hidden layer was obtained by trial testing from 10 to 20 neurons using the Levenberg-Marquardt (LM) learning algorithm. The learning rate is 0.55, with a momentum term of 0.9, and the network training consists of 10,000 iterations. The error between the desired and actual output is less than 0.001 at the end of the training process. Fig. 5 shows the basic architecture of the artificial neural network model. MATLAB R2010b with the neural network toolbox was used during the studies for training and testing data obtained from FEM and tensile tests. The root mean square error (RMSE) and correlation coefficient (R^2) values were used for comparison [9]:

$$RMSE = \left[\left(\frac{1}{n} \sum_j |t_j - o_j|^2 \right) \right]^{1/2} \quad (3)$$

$$R^2 = 1 - \left(\frac{\sum_j (t_j - o_j)^2}{\sum_j (o_j^2)} \right) \quad (4)$$

where t is the goal value; o is the output value, j is the sampling point, and n is the number of samples. The standard error (uncertainty) can be determined by calculating the mean stress using the following equation [9]:

$$\sigma_m = \frac{(\sigma_1 + \sigma_2 + \dots)}{N} \quad (5)$$

where N is the number of data points, m is the mean of stresses.

The standard deviation can be given by:

$$\sigma_{standard} = \sqrt{\frac{(\sigma_i - \sigma_m)^2}{N-1}} \quad (6)$$

and the standard error is evaluated by:

$$\sigma_{error} = \frac{\sigma_{standard}}{\sqrt{N}} \quad (7)$$

3. Results and discussion

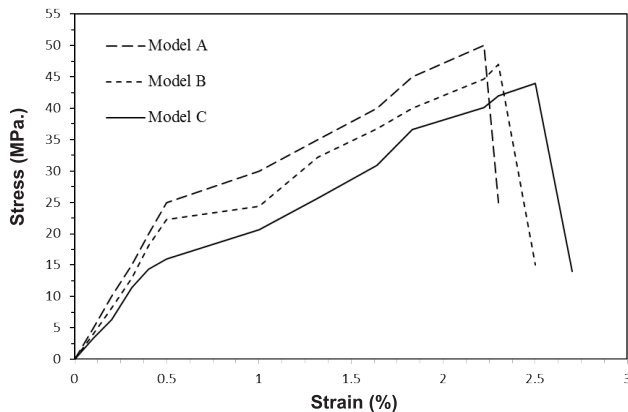


Fig. 6. The stress-strain curves of epoxy coatings cured at 10°C.

Figure 6 shows stress-strain curves of the three Hample epoxy coatings cured at 10°C obtained from tensile tests of specimens given in Fig. 3. The thickness of each sample differs from others but is within the range of thickness given by the manufacturer's data sheets. As illustrated in Fig. 6, there is a linearly elastic response of the stress-strain curves until the point of maximum stress, followed by abrupt failure at strains larger than 2.28, 2.47, and 2.68% for models A, B, and C, respectively. Elastic modulus was extracted from the stress-strain curves of Hample epoxy specimens cured at various temperatures, while Poisson's ratio is determined from longitudinal and lateral strain measured by strain gauges during the test. The behaviour of the three epoxy coatings is similar to metallic materials, as indicated in all stress-strain curves, i.e., the tensile strength increased with increasing load until failure. The tensile test is conducted for the similar tensile test specimens cured at temperatures (15, 20, 25, 30, 35, 40, 45, and 50°C). Similar curves are obtained and used for determining the elastic modulus and Poisson's ratio at these curing temperatures. Table 2 summarises the elastic modulus and Poisson's ratio for the three models of epoxy coatings at different curing temperatures. The yield strength is obtained by the 0.2% offset yield strength. This is used as points to obtain elastic modulus and Poisson's ratio. When taking 30 points arbitrarily from curves in Fig. 6, the uncertainty error analysis using Eqs. 5-7 show that the error in data obtained from tests is less than 0.1%.

As indicated in Table 2, model C has lower values of elastic modulus and Poisson's ratio due to its rigidity because of reinforcement by glass flakes compared to others. Also, the effect of increasing curing temperature on elastic modulus values is less compared to other types of coatings. For model A and B, the values of elastic modulus have a high effect on curing temperature, and

the modulus reduces as curing temperature increases. The effect of curing temperature on values of Poisson's ratio is very small compared to the effect on the values of elastic modulus.

All data obtained from the tensile test in Table 2 are parameters input to FEM programs. The displacements and strains in each element of the coating surface system are obtained by solving the n independent equations resulting from FEM meshes in the three-dimensional case. In the finite element analysis, the boundary temperatures are set at curing temperature 10°C and then increase by step increments of 5°C for each case of calculating displacement and strains. The FEM results, in addition to cure temperature and coating thickness, are inputs to the neural network toolbox of MATLAB R2010b and used to train the ANN model that is shown in Fig. 5.

By training several networks and estimating the error, the optimal number of neurons in the hidden layers is determined. Trials were conducted using two learning algorithms, the Levenberg-Marquardt learning algorithm (LM) and resilient backpropagation (RP). The trials conducted that the LM learning algorithm to be the best one for the residual stresses. Table 3 presents the optimal number of neurons in hidden layers for residual stresses. According to Table 3, the best input layers, hidden layers, and output layers are 7-13-1. The Log-sigmoid transfer function is used for training the input data in the hidden layer with backpropagation algorithm. The predicted residual stresses were validated with that obtained from the X-ray method.

Table 3. Statistical data of residual stresses for 10 learning algorithms.

Learning algorithm	Number of neurons	Training data		Testing data	
		RMSE	R ²	RMSE	R ²
LM	7-10-1	0.1043	0.9632	0.0930	0.9540
LM	7-11-1	0.1047	0.9678	0.0962	0.9483
LM	7-12-1	0.0944	0.9555	0.0931	0.9644
LM	7-13-1	0.0845	0.9110	0.0882	0.9514
LM	7-14-1	0.0946	0.9473	0.0931	0.9453
RP	7-15-1	0.0959	0.9337	0.0945	0.9588
RP	7-16-1	0.0924	0.9550	0.0950	0.9633
RP	7-17-1	0.0980	0.9588	0.0828	0.9623
RP	7-18-1	0.1075	0.9578	0.0910	0.9468
RP	7-19-1	0.1081	0.9589	0.0942	0.9394

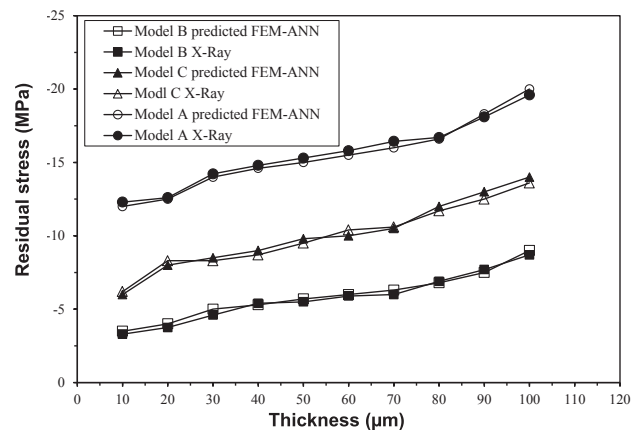


Fig. 7. Variation of residual stresses with thickness at 10°C.

Figure 7 compares the residual stresses of the three types of epoxy coatings cured at 10°C as a function of coating thickness. It also compares those measured using the X-ray method at the centre of coated specimens and those calculated by the FEM-ANN method at room temperature. As indicated, the results appear to be in excellent agreement. The residual stresses in the radial direction are very small, so they are neglected, and the stresses in the tangential direction are considered only.

The maximum residual stresses for epoxy coatings obtained from the X-ray measurements are -20.9, -9.11, and -14.04 MPa for coatings A, B, and C, respectively. While those obtained from the FEM-ANN method are -19.57, -8.72, and -13.61 MPa for coatings A, B, and C, respectively. The magnitude of the residual stresses is lower at the interface region of samples and begins to increase with coating thickness increases. The increase in coating thickness increases the time needed to cure the coatings, and this leads to an increase in reactions between base and hardener to form the crosslink density of epoxy coatings. This leads to an increase in the values induced by stresses. Also, it can be noted that the residual stress distribution induced in a coating layer is not uniform through thickness and varies due to different types of compositions and reinforcement. Also, it can be noted that all residual stresses are compressive stresses. As seen, the compressive residual stress would be produced because the reaction between base and hardener during mixing to produce epoxy coating was completely occurs after coating applied. Also, the residual stress in the epoxy coating is compressive due to the smaller coefficient of thermal expansion of the epoxy coat than that of the substrate of steel coated.

Model A consists of amine adduct cured pure epoxy paint which has a high content of residual solvents, monomers, and low molecular weight extractables that induce high residual stress compared to models B and C. model C is reinforced by glass flakes, and this reduces the internally induced stresses.

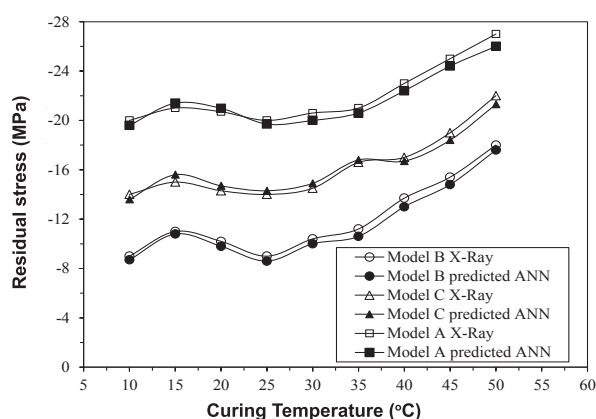


Fig. 8. Variation of residual stresses with curing temperatures.

Figure 8 shows a comparison of residual stresses obtained from X-ray measurement and the FEM-ANN method as a function of curing temperatures at a thickness of 100 μm . The temperatures selected for study range from 10 to 50°C, increasing by steps of 5°C. From Fig. 8, the residual stress value has a major influence

on the solidification temperature of the epoxy-based coating. The residual stresses acquired from X-ray diffraction at 10°C and a thickness of 100 μm are -19.60, -9.11, and -14.04 MPa for models A, B, and C, respectively, while values obtained from FEM-ANN methods are -19.57, -8.72, and -13.61 MPa for models A, B, and C, respectively. The residual stresses acquired from X-ray diffraction at 50°C and a thickness of 100 μm are -27.11, -18.10, and -22.04 MPa for models A, B, and C, respectively, while those obtained from FEM-ANN methods are -26.05, -17.61, and -21.30 MPa for models A, B, and C, respectively. This shows that there is a significant increase in residual stress values with an increase in curing temperatures of coatings, whereas these values are lower at lower temperatures. It is found from Fig. 8 that the best curing temperature is 25°C, which provides a lower value of residual stress. From these results, it is demonstrated that the residual stresses generated will increase by percentage values of 40.81, 11.085, and 56.98% for models A, B, and C, respectively, when the curing temperature rises from 10 to 50°C.

In the case of amine adduct cured pure epoxy coating, if the curing temperature is low, it leads to low reactivity of the amine group when mixing with the epoxy. This causes an excess of epoxy rings in the backbone and results in fluctuations in the curing rate or solidification rate of coatings, making the cure mechanism difficult. This process induces high residual stress compared to other types. However, a high curing temperature may accelerate the reactivity of amine hardeners and reduce the time of solidification. In other words, increasing cure temperatures increase the evaporation of hardener (exothermic reactions or increase activation energy between base and hardener), which leads to an increase in the values of residual stresses induced. Also, if the thickness of the coating increases and the curing temperature rises, this makes the coatings soft and more flexible due to the heat not escaping or dissipating as easily. Thus, increases in residual stresses with the thickness of coatings.

One of the most important properties of epoxy coatings influenced by curing temperature is the cross-linking density of the epoxy coats and its process, and this directly affects the values of initiated residual stress. A very dense cross-linking between coating components forms a very strong network through the epoxy coating and enhances its mechanical properties. In the case of model B, which contains Novolac coats, the Novolac epoxy coating results from the reaction of phenol functional groups (hardener) and formaldehyde resin (base). After the mixing process, the curing process results in a very cross-linked material. During cure, the cross-link density of Novolac epoxy coating increases gradually, transforming the liquid reactants into a solid to yield an amorphous solid. As the physical state of the Novolac coating changes during cure, it experiences residual stresses due to volume shrinkage and the effect of the curing temperature.

For model C, as the curing temperature rises, thermal expansion causes a decrease in tension on the glass flakes and an increase in compression of the epoxy matrix. Due to the difference in the coefficient of expansion between the glass flakes and

the epoxy matrix, the induced residual stresses are rather small compared to model A. For coating C, the coatings have medium values of compressive residual stresses, but they have high densities compared to the other types of coatings. On the other hand, in the glass flake/epoxy systems, residual stress develops during thermal cure due to the shrinkage of the epoxy. The cure shrinkage between the glass flakes and epoxy base is less at lower curing temperatures compared to elevated ones, which also leads to an increase in crosslink density and reduces initiated stresses. However, the shrinkage due to chemical curing and crosslinking of the Novolac epoxy is larger than that of model C coatings.

To make a comparison between the volumetric shrinkage of the tested epoxy coatings, the volumetric shrinkage of the three epoxy coats was calculated using the following equation [27, 28]:

$$\text{Volumetric shrinkage (\%)} = -\frac{\rho_u}{\rho_c} \times 100\% \quad (8)$$

where ρ_u is the density of the uncured epoxy coatings (kg/m^3) and ρ_c is the density of the curing epoxy coatings (kg/m^3).

The density of curing epoxy coats was determined experimentally using a simple method on a cylindrical specimen. A cavity is made on the specimen, following which the epoxy coats are sprayed on it. After spraying, the coating is ground to make this specimen cylindrical. Given the known size of the substrate, the volume of the coating can then be calculated. After the specimen is ground and weighed, the coats are ground to obtain the mass of the coating [29]. Table 4 shows the density of uncured epoxy coats which is given by data sheets of the manufacturer's company [30-32] and those calculated by using a direct method for curing epoxy coats.

The values of volumetric shrinkage calculated for the three models are 5.10, 3.73, and 4.55% for models A, B, and C, respectively. Model A, containing amine adduct pure epoxy coatings, shows a large volume shrinkage upon curing, while model B shows low shrinkage during the curing process, and model C shows a moderate value.

The reinforcement occupies a small position in epoxy coats and is formed from local shrinkage during the curing of the epoxy and hardener reactions, acting as a bridge interconnecting coating molecules. This results in reduced volume shrinkage in addition to an increase in cross-linking density [33]. The formation of voids in the cured amine adduct pure epoxy coats occurs due to the volume shrinkage during the curing reaction, resulting in an increase in the inducing internal stresses in these coats [34]. The volume shrinkage has conventionally been reduced by the addition of organic and inorganic reinforcement. However, the addition of reinforcement to curable compositions frequently results in an increase in viscosity and thus reduces fluidity [35, 36].

At low temperatures, the base-hardener epoxy coating cure reaction proceeds very slowly compared to that at high temperatures, affecting the values of initiated stresses. At 20°C, the reaction becomes stable compared to other temperatures, leading to smaller initiated residual stresses compared to other curing

temperatures. The results show that the tangential residual stress at high curing temperatures is higher for the three models of epoxy coat when compared to lower curing temperatures.

Compared to the amine adduct curing epoxy, the Novolac coating has greater viscosity. The amine adduct curing epoxy has lower reactivity when reacting the epoxy resin with hardeners, which reduces the curing process of this type of coating [37]. The chemical backbones of Novolac epoxies have more reactive groups (functionality) which can result in higher curing temperature and a highly cross-linking density compared to amine cure pure epoxy coat. This highly chemical cross-linking enhances the thermal and chemical resistance of the coating [38]. As the curing process is slow with time, the residual stress created is increased due to the increased cure time. Adding glass flakes to model C increases the crosslink density but has also been found to increase the residual stress compared to model B.

4. Conclusions

This study has investigated the influence of curing conditions and epoxy coating thickness on residual stress values. The discussion has highlighted a strong agreement between X-ray diffraction and the FEM-ANN model in assessing residual stresses. Curing temperature, in particular, has emerged as a key factor, with lower curing temperatures resulting in reduced residual thermal stresses compared to higher temperatures. Specifically, the results indicate that at 10°C, the residual stresses measured by X-ray diffraction are -19.60, -9.11, and -14.04 MPa for models A, B, and C, respectively. Correspondingly, the values obtained from the FEM-ANN methods are -19.57, -8.72, and -13.61 MPa for models A, B, and C, respectively. Similarly, at 50°C, the residual stresses recorded by X-ray diffraction are -27.11, -18.10, and -22.04 MPa for models A, B, and C, while the FEM-ANN methods yield -26.05, -17.61, and -21.30 MPa for the same models. Importantly, regardless of the assessment method, all residual stresses are found to be compressive.

Furthermore, the investigation reveals that residual stresses exhibit variation across the coating thickness. For instance, at a thickness of 10 μm , the residual stresses determined by X-ray diffraction and FEM-ANN methods are -11.97, -3.52, and -6.25 MPa for coatings A, B, and C, respectively. When the thickness is increased to 100 μm , the corresponding values are -20.9, -9.11, and -14.04 MPa for coatings A, B, and C using X-ray diffraction, and -19.57, -8.72, and -13.61 MPa for the same coatings using the FEM-ANN method.

In summary, model B, consisting of free amine-cured phenol Novolac epoxy, displays lower residual stress values. Conversely, model A, featuring amine-adduct cured pure epoxy, exhibits higher values. Model C, which incorporates glass flakes for reinforcement, demonstrates moderate levels of residual stress. These findings underscore the significance of curing conditions and coating thickness in determining residual stress profiles in epoxy coatings.

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COMPETING INTERESTS

The author declares that there is no conflict of interest regarding the publication of this article.

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Chitosan oligomer/silver nanoparticles/PCL composite electrospun membrane for severe burn wound healing

Anh Mai Que^{1,2}, Tuan Ngan Tang^{1,2}, Tam Phan Thi Thanh^{1,2}, Thanh Tu Duong^{1,2},
Thanh Binh Vu^{1,2}, Van Khiem Nguyen^{1,2}, Hoan Ngoc Doan^{1,2}, Hiep Thi Nguyen^{1,2*}

¹Department of Tissue Engineering and Regenerative Medicine, 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

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

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Abstract:

In the management of burn wounds, antimicrobial and wound healing properties are crucial. In this study, we prepared a three-layer electrospun poly(ϵ -caprolactone) (PCL) membrane for burn wound dressing. The hydrophobic electrospun PCL layer, embedded with silver nanoparticles (SNPs) (PCL-Ag), was coated with another layer of electrospun plasma-treated PCL-Ag to enhance the wettability of the membrane. This enhancement was to facilitate the absorption of the hydrophilic chitosan oligomer (COS) - the third layer of the dressing. The resultant membranes were characterised and tested for different properties to demonstrate their applicability as wound dressing materials. The combination of COS and SNPs of the fabricated membrane supports the healing process and reduces burn severity due to the healing capacity of COS and the antibacterial activity of SNPs, without compromising mechanical strength. We conducted several *in vitro* and *in vivo* experiments to evaluate its applicability for burn wound healing. The PCL-Ag/COS sample demonstrated outstanding *in vitro* biocompatibility and excellent antibacterial activity against *Staphylococcus aureus* strains. We used the membrane to treat burns on rabbits in the laboratory over 30 days, and consistently observed positive outcomes. This research offers insights into the development of bioactive dressings for wound healing and opens up opportunities for practical applications of COS-incorporated materials.

Keywords: antibacterial wound dressing, electrospun membrane, oligomer chitosan, polycaprolactone, SNPs.

Classification numbers: 2.2, 3.2

1. Introduction

Burns are classified as physical, thermal, electrical, radiation, laser, or chemical, depending on the causative agent. Each type of burn wound differs in local and systemic management. Heat not only damages the skin locally but also causes many generalised effects on the body. The increase in capillary permeability caused by heat and damage leads to local reaction (persistent progressive vasodilatation and oedema) as a part of the inflammatory response.

Wound management involves cell proliferation, tissue remodelling, and matrix deposition. Among these, regenerating wound tissue is considered a complex process that involves blood clotting, epithelialisation, wound contraction, and synthesis of collagen and blood vessels. Throughout all these stages, a wound dressing containing antimicrobial agents is required to prevent pathogens from infecting wounds via microbicidal or microbiostatic mechanisms. Dressing materials are typically designed to inhibit bleeding and protect the wound from environmental discomforts. In this context, skin tissues play a critical role in preventing infections caused by microorganisms after

trauma. As a result, it is crucial to cover damaged skin with a dressing as soon as possible. Moisture, electrolyte balance, haemostatic, analgesic, healing, and antimicrobial properties must all be maintained. An ideal wound dressing should also be inexpensive, easily accessible, non-allergic, and painless to remove.

Electrospinning (Es) is a versatile, viable, and potentially the most effective and advanced electrohydrodynamic technique [1, 2] for generating continuous ultrathin fibres (down to a few nanometres) from various types of materials, enabling for various applications [2-5]. For tissue engineering and wound dressing, the flexibility to tune the structure, composition, and performance of Es is a great advantage. With a uniform diameter, strong interconnectivity of porosity, high specific surface area, and small pore size, dressings created by this technique can be loaded with bioactive molecules or suitable agents that provide extra functions to meet treatment needs [3, 6-9]. Furthermore, in terms of scale and morphology, these electrospun dressings absorb exudates, inhibit wound drying, and enable gas permeability more efficiency [9].

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

In recent years, advances in burn wound care have significantly reduced the incidence of bacterial burn wound infection, altering the epidemiology of infection in burn patients. It has been found that besides bacteria, other microorganisms such as viruses and fungi are also implicated in burn-related sepsis [10]. A study by Pruitt et al. points out that *S. aureus* and *Pseudomonas* are common pathogens responsible for 48 and 16% of lung infections, respectively. Apart from the increased virulence of organisms invading the wound, there has been no significant change in the statistics of bacterial burn wound infections in the years following B.A. Pruitt, et al.'s study (1992) [10].

The denatured protein in burn-injured tissue provides an ideal environment for microbial growth and proliferation [11, 12]. The ultimate goals of burn management and therapy are to prevent infection, minimise functional impairment, and facilitate wound healing and epithelisation. Wound dressings are created to aid in wound coverage, prevent wound infection, inhibit skin desiccation, and protect against further skin damage. Antimicrobial dressings aim to minimise bacterial colonisation to prevent wound infection. SNPs exhibit broad-spectrum and robust antimicrobial properties against a wide range of microorganisms [13-15]. Despite significant efforts devoted to understanding the origin of the antimicrobial activity of SNPs, the precise mechanism remains elusive [13, 15]. Antimicrobial agents are reported to disrupt the outer membrane of target cells. An important aspect of the antimicrobial activity of SNPs is the synergistic effect that occurs when these particles are combined with other natural and synthetic compounds. Cellular toxicity exhibited by metal nanoparticles slow oxidation through the release of reactive oxygen species and liberate small Ag^+ ions that penetrate through cell membranes, affecting intracellular processes and causing death of the organism [14]. The beneficial effects of silver on wound biology, based on this antimicrobial property, have re-emerged as a viable treatment option for infection-prone wounds such as burns. Some common commercial wound dressings that use ionic silver include Aquacel Ag® (Conva Tec), UrgoClean Ag® (Laboratories URGO), and Silversorb (Medline).

Materials for treating burns and infectious wounds should be biodegradable and environmentally friendly, while also possessing wound healing properties. Chitosan is a polysaccharide macromolecule formed by partial deacetylation of chitin, primarily derived from arthropod exoskeletons and crustaceans. Dressing materials based on chitosan and its derivatives have high durability, good biocompatibility, non-toxicity, and good water uptake capacity [16-19]. Aside from its biocompatibility, biodegradability, and low toxicity, chitosan increases serum protein absorption during wound leaching [16, 20,

21]. Its haemostatic properties, adhesive nature, antifungal and antibacterial effects, and oxygen permeability benefit late-stage wound healing by inhibiting scar formation and promoting epithelial regeneration [16, 22, 23]. However, because of its low molecular weight, COS decreases the overall mechanical strength of the membrane. To compensate for this limitation, polyvinylpyrrolidone (PVP) - a synthetic polymer known for its mechanical stability, swelling behaviour, and low cytotoxicity - is added.

In this study, we present a gamma-assisted one-pot method for preparing SNPs in a non-aqueous mixture of PCL and acetone under varying gamma irradiation doses for the fabrication of electrospun PCL-embedded SNP (PCL-Ag) membranes. An auxiliary solvent, dimethyl sulfoxide (DMSO), was used for the dissolution of silver nitrate in acetone. Prepared solutions were electrospun to form PCL-Ag membranes that undergo plasma treatment (PT) and a coating of COS/PVP solution to create the final antibacterial wound dressing. The obtained membranes were characterised and tested for membrane morphology, mechanical strength, silver release profile, bacterial inhibition, and biocompatibility to demonstrate their suitability as wound dressing materials. The combination of COS and SNPs supports healing in infected wounds and reduces the severity of burns based on the healing capabilities of COS and antibacterial activity of SNPs.

We conducted comprehensive research on the incorporation COS-PCL electrospun membranes and their benefits for wound dressing purpose. The concentrated COS solution created the coating layer with ideal thicknesses and COS densities. To coat the hydrophilic COS onto EsPCL, PT was employed to improve the wettability of the EsPCL membrane. It is expected that this combination possesses synergistic effects between the electrospun PCL and the COS coating layer to improve antibacterial characteristics and biocompatibility of the membranes without compromising mechanical strength. This research provides insights into the development of bioactive dressings for wound healing and paves the way for practical applications of COS-incorporated materials (Fig. 1).

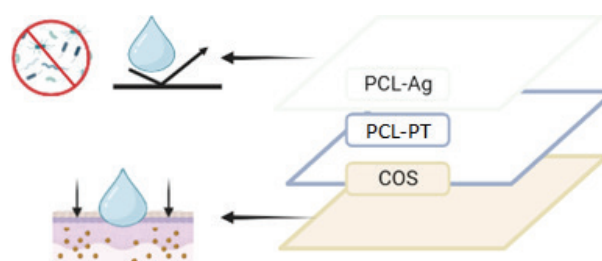


Fig. 1. The ideal design of the PCL-Ag/COS membrane with 3 layers (hydrophobic-adhesive-hydrophilic) using PT.

2. Materials and methods

2.1. Materials

PCL, Mn 80.000, dimethyl sulfoxide (DMSO), acetone (99%), silver nitrate (AgNO_3), and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were purchased from Sigma-Aldrich Co. Ltd., Dorset Gillingham. Mueller Hinton Broth (M391-500G) was purchased from Hi-Media (India). The pathogen, *S. aureus* ATCC 25913, was provided by the Marine Laboratory, International University, Vietnam National University - Ho Chi Minh City. Mouse fibroblast L929 cell line was thawed and cultured in Dulbecco's Modified Eagle Media (DMEM, Gibco, USA) supplemented with 10% foetal bovine serum (Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA).

2.2. Methods

2.2.1. Fabrication of PCL-Ag solution

The SNPs dissolved in PCL solution were synthesised by gamma irradiation following a previously reported procedure [24]. The PCL solution (12 %w/v) was first prepared by dissolving PCL pellets in acetone with stirring at 500 rpm at 60°C for 4 h. After that, a solution of AgNO_3 (500 ppm) was added and stirred at room temperature for 10 min. Then, the final solution of PCL-Ag (500 ppm) was obtained by gamma irradiation at 15 kGy. The PCL-Ag (50 ppm) solution was obtained by diluting the PCL-Ag (500 ppm) solution.

2.2.2. Fabrication of membranes: PCL-Ag/PT

EsPCL membranes were fabricated by Es. Parameters were set as following: feed rate of 2.5 ml/h, voltage of 15 kV, rotating speed of 150 rpm/min, a membrane collection area of 60 cm², syringe of 10 ml, and 18G needle. The bilayer membranes were electro-spun with three syringes in parallel. The total volume of PCL-Ag solution was 45 ml. The PCL-Ag membrane (10×20 cm) was centred in the plasma chamber (GaLa Instrumented, Germany) to which air plasma was applied for 2 min with 30 W RF power at 13.56 MHz.

2.2.3. Preparation of COS/PVP solution

COS was prepared by diluting the H_2O_2 stock solution (30%) to 6%, then immersing the chitosan powder to obtain 3% (w/v) concentration. The mixture was then magnetically stirred for 24 h at 50°C before microwave irradiation at 400 W (medium) for 2 min to dissolve the residuals. By dissolving PVP powder in the filtered COS solution for 30 min at room temperature, a COS/PVP solution containing 6% (w/v) PVP was obtained.

2.2.4. Coating of COS on the membrane

The EsPCL-Ag/PT membrane was cut into 10×20 cm² pieces and sandwiched between two polypropylene frames using double-sided tape and rubber bands to form the mould. A total of 18 ml of COS/PVP solution was evenly spread onto the PCL-Ag/PT membrane 3 times at 6 ml volume each time using a micropipette. Between each coating section, the moulds were placed into a 50°C oven to dry the membrane before more solution was added. The COS-coated sample was denoted as PCL-Ag/COS.

2.2.5. Scanning electron microscope analysis

Membranes were cut into 1×1 cm² samples and sputter-coated with Au before microstructures were observed under a scanning electron microscope (SEM, JSM-IT100, Jeol, Japan) at an acceleration voltage of 10 kV. From the SEM images, fibres and pores chosen from random locations were measured using ImageJ software (NIH, USA) to obtain average fibre diameter and pore size. The diameter and pore size data were presented in a histogram to assess the uniformity of the electrospun mats.

2.2.6. SNPs and COS analysis

XRD patterns of the fabricated mats were investigated using D8 Advance Eco, Bruker AXS (Germany) operated at a voltage of 45 kV and a current of 30 mA with Cu K α radiation ($\lambda=1.54$ nm). The 2 θ diffraction angles ranging from 30 to 80° (0.2°/step per 0.5 s) were recorded to verify the crystalline structure of the SNPs. Fourier transform-infrared spectra (FT-IR) was employed to observe the interaction between the PCL membrane and chitosan oligomers. The FT-IR spectra were recorded using a Spectrum GX (PerkinElmer Inc., USA) over a wavenumber range of 400-4000 cm⁻¹.

2.2.7. Moisture permeability

The moisture permeability of the electrospun mats was evaluated by moisture vapor transmission rate (MVTR) testing based on BS EN 13726-2:2002 guidelines. Briefly, circular specimens were fixed onto and completely covered by the rims of containers with an area of 10 cm². The containers were filled with an appropriate amount of distilled water to ensure that the membranes were 5 mm from the liquid level. Then, the systems were placed in the oven at 37°C for 24 h. The initial (W_1) and final (W_2) weight of water was measured and the MVTR values were calculated using the equation:

$$\text{MVTR} = (W_1 - W_2) \times 1000 \text{ (g/m}^2\text{/24 h)} \quad (1)$$

2.2.8. Investigating of controlled release of SNPs

The release testing method (Franz diffusion cells) was used to investigate the *in vitro* release of the SNPs. Release testing was performed in release medium of pH 7.4 (phosphate buffer - PBS) at 37°C. Cellulose acetate membranes (Sartorius®, 0.45 μm average pore size) were used as the artificial

membrane and were maintained in distilled water for 30 min prior to sample membrane loading. At predetermined time intervals (1, 3, 6, 12, and 24 h), a certain volume of sample was withdrawn and replenished with fresh media. Vertical Franz diffusion cells with a volume of 20 ml (contact area: 2 cm²) were used. After the addition of the release media, cellulose acetate membranes were applied on top of the cell receptor chambers. The donor chambers then were placed on the membranes and tightly clamped. Samples 10 mm in diameter were loaded into the donor chambers and 250 µl of the release media were added to the top of the membrane. The stirring speed of the Franz diffusion cells was set at 600 rpm. At the pre-determined time intervals, 0.15 ml of the media were withdrawn from the receptor chambers and replenished with fresh media. The solution at various times (1, 3, 6, 12, and 24 h) was then taken for ICP-MS analysis to quantify the release of silver content. The experiment was performed in triplicate.

2.2.9. Cytotoxicity

The PCL membrane (control) and PCL-Ag/COS membranes (1×1 cm²) were soaked in DMEM cell culture (ratio 0.1 g/ml) with 10% bovine serum (Foetal Bovine Serum, FBS) and 1% antibiotic (Penicillin, PS) for 24 h to collect the extract solution. The extract solution was used to culture L929 mouse fibroblasts in a 96-well culture dish at a concentration of 10⁴ cells/100 µl/well for 24 h. MTT was added to each well at a concentration of 2 µg/100 µl and incubated for another 4 h. The fluorescence signal was measured at 550 nm. The experiments were triplicated.

2.2.10. In vitro antibacterial experiment

The antibacterial activity of the membranes was evaluated using a strain of Gram-positive bacteria, *S. aureus*, using the antimicrobial fabric zone of inhibition test (AATCC 147). Prior to the experiments, a colony of *S. aureus* strain was extracted from the stock agar plate, placed into a 5-ml Mueller-Hinton broth (MHB) tube, and cultured at 37°C for 24 h. Then bacterial suspension of this strain was prepared by dilution to reach an optical density at 620 nm OD₆₂₀=0.08-0.1 (equal to 0.5 McFarland standards, approximately 1-2×10⁸ CFU/ml).

The microbial susceptibility of each sample against specific bacterial strain was investigated and spread onto a petri dish containing Mueller-Hinton agar (MHA). Briefly, 150 µl of the diluted bacterial suspension was introduced and swabbed evenly on the MHA surface. Then, square membranes of 1×1 cm² each were placed on the MHA plate and incubated for 24 h. The bacterial inhibitory zone around the samples was measured.

2.2.11. In vivo animal burn experiment

The *in vivo* wound healing was evaluated by using nine adult male rabbits with an average weight of 2-2.5

kg. Animals were kept at a suitable temperature (27°C) and humidity (40%) for one week before testing. At first, the rabbits were anesthetised by the injection of 50 mg/kg body weight ketamine (10%). For the preparation of the experiment, the hair of the back area of the animals was shaved. The skin area was disinfected with alcohol and the 2nd-degree burn wounds (four full-thickness burn wounds per rabbit) were induced in the surgical area by using a temperature-controlled modified heating instrument. The burn wound (3×3 cm² and full thickness) was produced on the back (lumbar dorsal) of each rabbit. The heated template (3 cm diameter) was applied perpendicularly to the designated area with 15 N of pressure and a temperature of 100°C for 15 s [25]. The standard cotton, Betaplast silver, and PCL-Ag/PT/COS dressing were directly applied once a day for 30 d. The animals were randomly divided into three groups with three rabbits in each group. In the control group, the wounds were treated with cotton dressing. The second group was treated with a wound dressing containing PCL-Ag/COS and the third group was treated with the commercial wound dressing Betaplast silver. Animals were caged individually and provided food and water ad libitum. After 30 days, all three groups were euthanised, and the burned tissue was collected.

2.2.12. Burns curing experiment

For 30 days, all three groups of rabbits received dressing changes once per day. The burn wounds were depilated and washed with a solution of povidone-iodine. Each burn wound site was photographed with a camera (Canon, Japan) after 0, 7, 14, 21, and 30 days to measure wound contraction. The photographs were taken directly above the wound. An image analyser (Image J 2.0, NIH, USA) was used to detect wound surface areas during healing. The wound closure percentage (%) was detected as follows:

$$\text{Wound contraction (\%)} = \frac{\text{Wound area at measured day}}{\text{Wound area at the beginning}} \times 100\% \quad (2)$$

2.2.13. Histological examination

The tissue was placed in haematoxylin dye solution for 1 min and rinsed with distilled water. Afterward, the sample was immersed in eosin staining solution for 1 min. Then, dehydration and transparency were performed. Finally, the transparent sample was sealed with neutral gum, observed with an inverted fluorescence microscope, and photographed for analysis.

2.2.14. Statistical analysis

All the data were analysed with statistical software (SPSS 19.0); the measurement data were shown by mean ± standard deviation. By using one-way analysis of variance (ANOVA) with the Turkey post hoc test, with *p*<0.05 indicating the difference is significant and *p*<0.01 indicating the difference is of great significance.

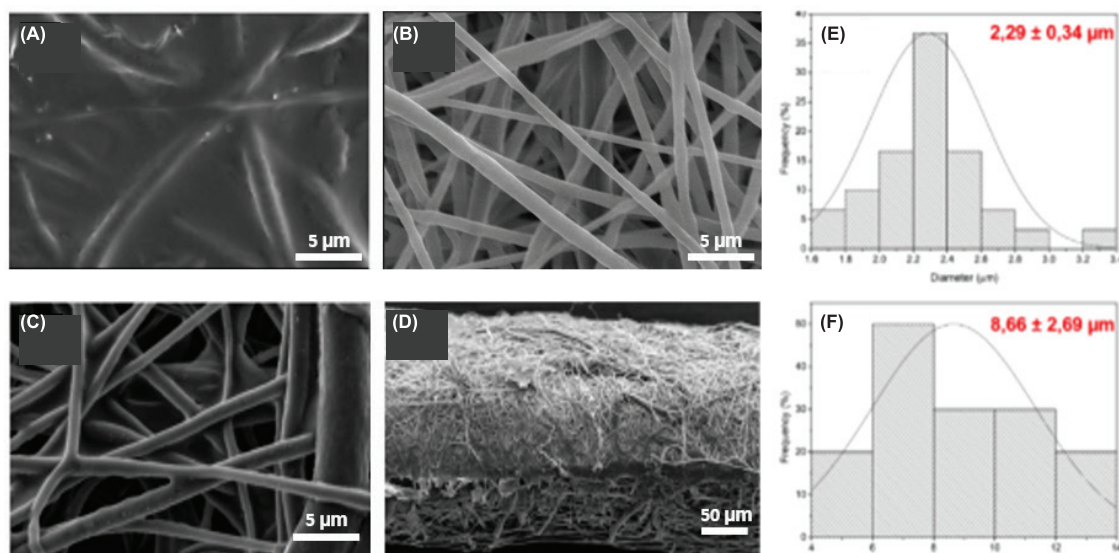


Fig. 2. Morphology of PCL-Ag/COS (A), PCL-Ag (B), PCL-Ag/PT (C), wide view of PCL-Ag/COS (D), distribution of fibre diameter (E), and distribution of pore size (F).

3. Results and discussion

3.1. Membrane characterisation

SEM images of the morphology of the fabricated membrane with different layers are shown in Fig. 2. Under SEM observation, all sample surfaces were macroscopically smooth and microscopically bead-free. The evenly-coated COS on the fibre is shown in Fig. 2A, demonstrated by a thin film of COS covering the fibrous membrane. Three layers of the fabricated membrane are shown in the cross-sectional SEM image (Fig. 2E). Two electrospun outer layers are distinctive while the COS layer, as discussed, is the thin layer at the top. The fabricated PCL-Ag/COS membrane is uniform in fibre diameter ($2.29 \pm 0.34 \mu\text{m}$) with a small pore size ($8.66 \pm 2.69 \mu\text{m}$).

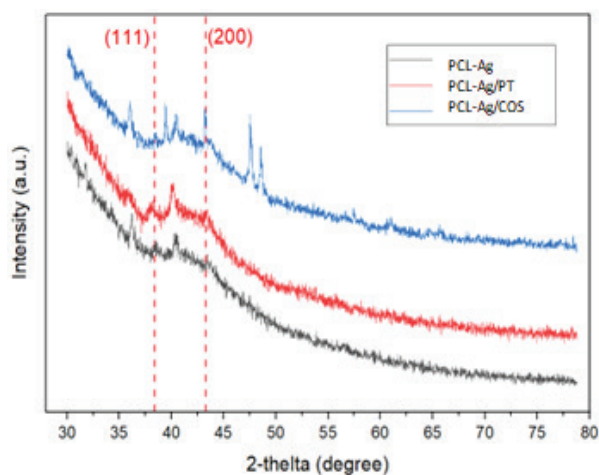


Fig. 3. X-ray diffraction (XRD) patterns of the prepared membranes.

XRD was used to investigate the structure of the SPNs in the membrane (Fig. 3). According to previous studies [26–28], the two peaks at 38.13° and 43.32° correspond to the (111) and (200) planes of the face-centred cubic crystal of silver. The results indicate that the SNPs were successfully incorporated into the PCL membrane.

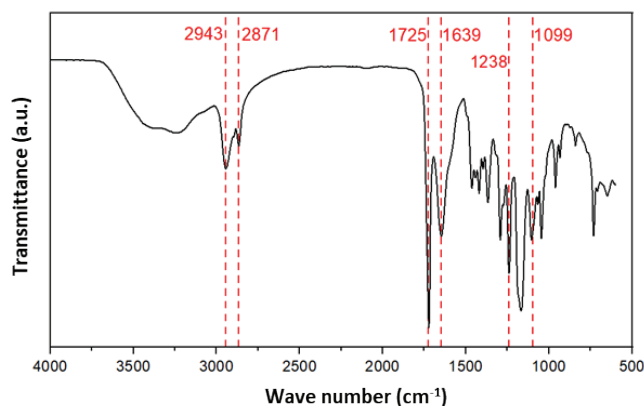


Fig. 4. FT-IR spectra of the PCL-Ag/COS membrane.

FT-IR analysis was carried out to determine the functional groups of COS, as shown in Fig. 4. The broad peak at $3700\text{--}3100 \text{ cm}^{-1}$ illustrates the stretching motions of the hydroxyl (-OH) and amino (-NH) groups from COS. The vibrations at 2943 and 2871 cm^{-1} are ascribed to the asymmetric and symmetrical CH_2 stretching bands of the material, respectively. Another characteristic peak of PCL is observed at 1725 cm^{-1} , which is from the carbonyl ($\text{C}=\text{O}$) groups. Furthermore, the dominant peaks of the base polymer, PVP, are also shown in the FT-IR spectrum at 1639 and 1238 cm^{-1} .

3.2. Controlled release of SNPs

The measurement of SNP release via ICP-MS was also carried out. The result confirmed the presence of SNPs in the membrane (Fig. 5) and also revealed the release behaviour of the silver components from the three membranes. The overall trend of the three samples was the same. Compared to the release profile of PCL-Ag, which was described above, the accumulative concentrations were higher for PCL-Ag/PT and PCL-Ag/COS. In the first 6 h, the Ag concentration of PCL-Ag/PT was 0.72 mg/l while that of PCL-Ag/COS was lower. After 24 h, the release rate of PCL-Ag accelerated, and the concentration reached at 8.40 mg/l. The Ag concentration reached 7.47 and 7.04 mg/l after 24 h for PCL-Ag/PT and PCL-Ag/COS, respectively.

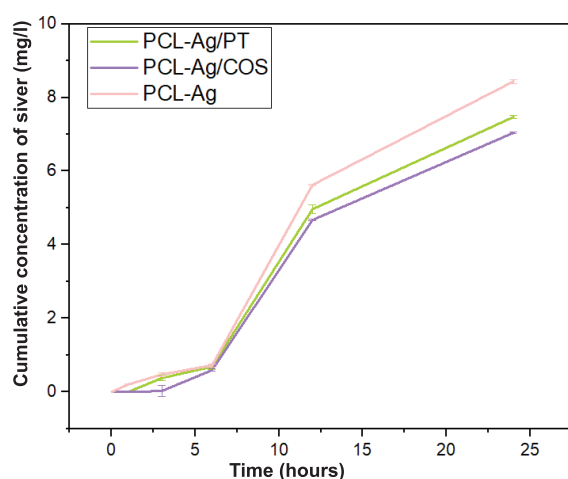


Fig. 5. Quantification of the in vitro release of silver. Cumulative Ag release profiles from the PCL-Ag, PCL-Ag/PT, and PCL-Ag/COS in PBS solution (pH 5.5). Aliquots were taken after 1, 3, 6, 12, and 24 h, then treated with concentrated HNO_3 to convert SNPs into Ag^+ and quantified by ICP-MS (data=mean $\pm$ SD, n=3).

3.3. Moisture permeability

The moisture permeability is an essential feature of wound dressing, which is to provide sufficient moisture conditions, alleviate the pain, and promote the healing process without dehydration. In numerous studies, MVTR is the standard to evaluate the moisture modulation ability of healing mats [29-31]. The more permeable or breathable the dressing, the higher the MVTR. However, high moisture vapor can lead to wound dehydration, whereas low can lead to wound maceration.

Normal skin has an average MVTR of 204 $\text{g/m}^2/24\text{ h}$, while wounds exhibit an MVTR ranging from 279 $\text{g/m}^2/24\text{ h}$ (first-degree burn) to 5138 $\text{g/m}^2/24\text{ h}$ (granulating tissue) [32-35]. Although, a suitable MVTR value for moist wound healing was previously defined as 840 $\text{g/m}^2/24\text{ h}$ [36], recent research has shown that the vapour rate up to 2000-2500 $\text{g/m}^2/24\text{ h}$ could still provide optimal moisture

content for epidermal cells and fibroblasts proliferation [29, 35-38]. As shown in Fig. 6, high MVTRs were observed in all samples, which could be due to the swelling capacities of the fibrous dressings [39]. The cotton dressing had the highest permeability rate ($3029\pm133\text{ g/m}^2/24\text{ h}$), exceeding the recommended value. The thickness of the fabricated membrane impacts its transmission rate, increased layers limit water evaporation through the membrane. When compared to the commercialised product Betaplast silver ($1210\pm56\text{ g/m}^2/24\text{ h}$), our PCL-Ag/COS membrane exhibited a higher MVTR value ($1755\pm84\text{ g/m}^2/24\text{ h}$). Nevertheless, it still maintains a moisture condition conducive to the healing process.

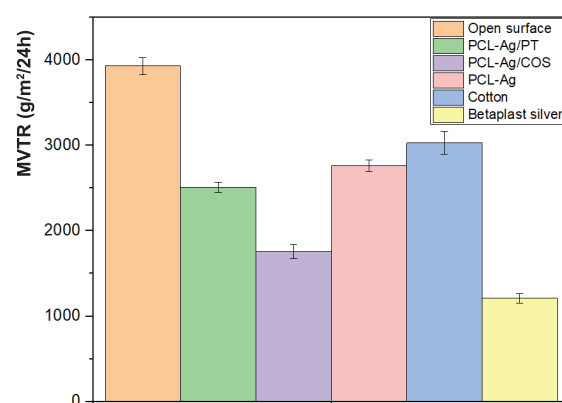


Fig. 6. Moisture vapor transmission rate ($\text{g/m}^2/24\text{ h}$) of PCL-Ag/PT, PCL-Ag/COS, PCL-Ag, cotton, and Betaplast silver (data=mean $\pm$ SD, n=6).

3.4. Cytotoxicity

The MTT assay was conducted to assess cell viability after being cultured in the extract solution for 24 h, providing a measure of the cytotoxicity of the materials. As Fig. 7 illustrates, the 100% extract solution significantly reduced the cell survival rate to a mere 10% compared to the untreated well. This outcome could be attributed to the synergetic effect of the silver and COS from the membranes, aligning with their antimicrobial properties. In fact, a similar reduction in cell viability has also been observed in several commercially available silver dressings such as Aquacel® Ag or Algisite™ Ag [40]. However, it is important to note that when a dressing is applied to a wound, the tissue does not come into contact with the entire extract simultaneously, as in the case with the cultured cells in this *in vitro* test. Therefore, the cytotoxicity level of the 100% extract does not necessarily reflect the actual behaviour of the membrane when applied to tissue. Moreover, cell viability levels in 12.5, 25, and 50% extract solutions were nearly 100% compared to the control, suggesting that the cells can tolerate the membrane components when released kinetically, rather than being exposed to all components simultaneously.

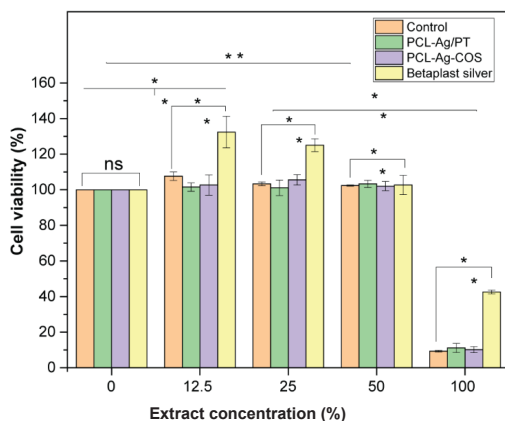


Fig. 7. Viability (%) of fibroblasts after 24 h of incubation in different concentrations of extracted solution of PCL-Ag/PT and PCL-A/COS membranes. Data=mean±SD, n=3, ns: p>0.05, *: p<0.05.

3.5. *In vitro* antibacterial experiment and related to wound inflammation

The antimicrobial activity of the PCL-Ag/COS membranes was assessed by monitoring their inhibitory zone through agar diffusion tests against Gram-positive strains. Fig. 8 presents images suggesting that SNPs effectively inhibited the growth of the *SA* strain. Regarding *S. aureus*, the PCL-Ag/COS

membrane with a low Ag concentration (50 ppm) was sufficient enough to inhibit bacterial growth with a diameter of inhibitory zone of 8.00 ± 0.45 mm.

3.6. *In vivo* studies

As shown in Figs. 9 and 10, a gradual reduction in wound area can be seen in both the treatment groups (PCL-Ag/COS and Betaplast silver membrane) and the control group (cotton) over a 30-day period. Throughout the observation, no significant difference was observed between the wound sizes of the control and treatment groups. Starting from day 7, the wound area began to change colour and the burn site appeared red, blistered, and potentially swollen. By day 14, the skin of the burn areas had completely receded compared to its original extent. After a month, the wounds in both groups achieved an average wound contraction of 84%. Throughout the 30 days, no signs of infection were seen in the wounds from the treatment and control groups. Minor differences were observed in the extent of granulation and epithelialisation across all wounds. We found the Betaplast silver membrane more difficult to handle during application because of its thickness. Displacement of the dressing from the wound site was more pronounced in the treatment group. Dressings from both groups were only loosely attached to the wounds, causing little pain when they were changed. Compared to the control

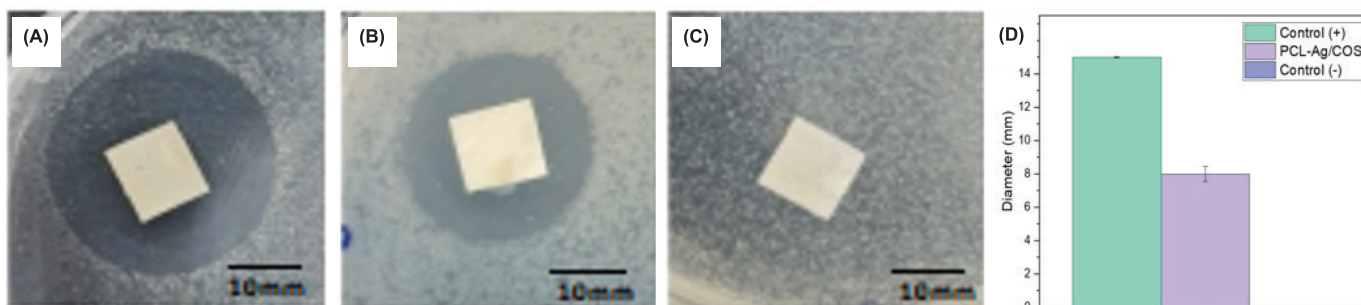


Fig. 8. Images of the inhibition zones formed by ampicillin - positive control (A), PCL-Ag/COS (B), and raw PCL-negative control (C); chart of inhibition diameter of the membranes against *S. aureus* (D).

Macroscopic burn healing

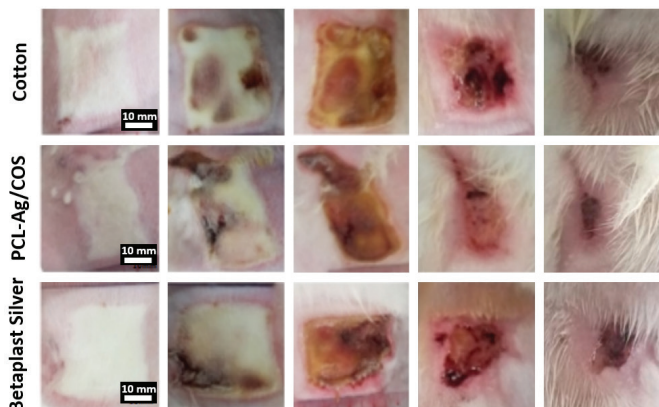


Fig. 9. Representative images of wounds treated with cotton, Betaplast silver, and the PCL-Ag/COS membranes.

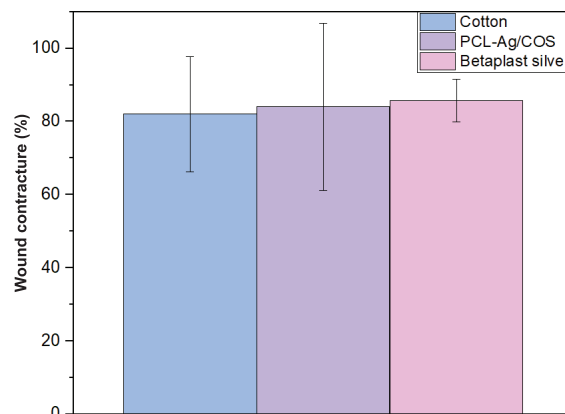


Fig. 10. Wound contracture (%) of wounds treated with cotton, Betaplast silver, and PCL-Ag/COS membranes.

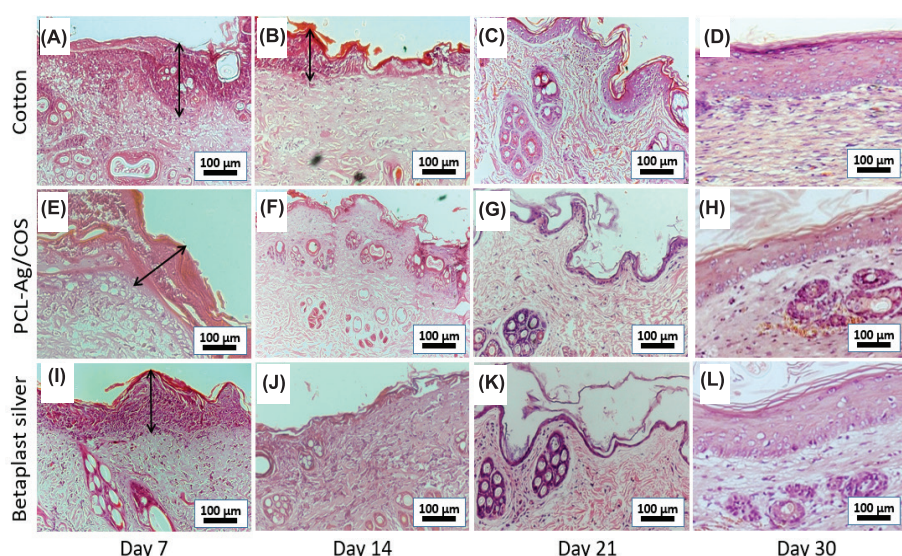


Fig. 11. Histopathology of wound tissue at days 7, 14, 21, and 30 after surgery: wound treated with cotton (A-D), PCL-Ag/COS (E-H), and Betaplast silver membranes (I-L). The arrow indicates the inflammatory area due to the burn wound.

group, dressings from the treatment group were easier to mould to the wound surfaces. Wounds from the treatment group also displayed a larger degree of exudate accumulation beneath the dressings, covering the entire wound surface, while exudate collection from the control group only covered a portion of the wound's surface.

3.7. Histological examination

Histological analyses of the wounds were conducted on days 7, 14, 21, and 30 post-wound creation, as shown in Fig. 11. The H&E staining images successfully demonstrated the creation of secondary degree burn wounds on a rabbit model. By day 7, regenerated granulation tissue had formed in both the dermis and hypodermis, making it difficult to distinguish these layers from normal tissue samples. The thickness of the epidermal layer was inconsistent, and no hair, sweat glands, or fat were observed in the wound area. Wounds dressed with cotton (control) exhibited a thicker inflammatory area compared to those treated PCL-Ag/COS and Betaplast silver dressings, and this inflammation persisted until day 30 of the experiment. The healing process in the treatment groups appeared to progress more quickly, with matrix fibres beginning to synthesise and align in parallel and neatly on day 14 - signals of the granulation phase. In the PCL-Ag/COS group, distinct layers of the epidermis, dermis, and hypodermis could be observed separately, and the thickness of the epidermal layer appeared uniform.

By day 21, tissue re-epithelialisation, fibroblastic proliferation, the formation of a dense collagen mesh, and moderate fibrosis were all observable. By day 30, both treatment groups had transitioned to the inflammation-repair

phase more rapidly than the control group. The wounds were covered with healthy epithelial tissue and a new epidermis. The fabricated dressings were found to be compatible with the injured tissue, with no evidence of toxicity or irritation that could potentially prolong the inflammatory process.

4. Conclusions

This research outlines the effects of applying a burn wound dressing made of a COS-coated PCL-Ag membrane. The antibacterial properties of both SNPs and COS enhanced the speed of tissue regeneration and healing. Additionally, COS was able to foster optimal conditions for the intricate process of wound healing. In conclusion, the PCL-Ag/COS membrane was successfully fabricated and is suggested as a protective antimicrobial dressing that can aid in the treatment of burn injuries.

CRedit author statement

Anh Mai Que: Conceptualisation, Methodology, Formal analysis, Writing, Editing; Tuan Ngan Tang: Methodology, Editing; Tam Phan Thi Thanh: Conceptualisation, Data analysis; Thanh Tu Duong: Methodology; Thanh Binh Vu: Methodology; Van Khiem Nguyen: Reviewing; Hoan Ngoc Doan: Reviewing, Data analysis; Nguyen Thi Hiep: Conceptualisation, Reviewing.

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COMPETING INTERESTS

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

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Biodegradable containers from rice husk as substitutes for single-use plastic products

Nhung Thi-Tuyet Hoang*, Anh Thi-Kim Tran

*Faculty of Chemical and Food Technology, Ho Chi Minh City University of Technology and Education,
1 Vo Van Ngan Street, Linh Chieu Ward, Thu Duc City, Ho Chi Minh City, Vietnam*

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Abstract:

Vietnam ranks among the top four nations for single-use plastic (SUP) waste, trailing only China, Indonesia, and Philippines. The abundant rice husk (RH) by-product of rice cultivation presents an opportunity to fabricate biodegradable containers, potentially supplanting single-use disposables. This study focuses on creating biodegradable containers from RH via thermo-pressing. Utilising the Taguchi orthogonal array method, we examined the impact of the RH to modified starch (MS) ratio (w/w %), pressing temperature (°C), and time (minutes) on the trays' hardness, colour variation, and density, aiming to refine the manufacturing process. Additionally, variations in adhesive concentrations and glycerol were assessed for their influence on the aforementioned properties. The identified optimal conditions for fabricating viable biodegradable containers were a RH and MS composition of 80% (w/w), with a pressing duration of 3 minutes at 150°C. The resulting product exhibited a hardness of 5.26 ± 0.22 kgF. Moreover, the thickness, density, and colour on the front and back sides were measured at 2.05 ± 0.01 mm, 0.97 ± 0.004 g/cm³, 50.87 ± 0.96 , and 48.05 ± 0.87 , respectively. Consequently, RH-based materials have emerged as promising candidates for crafting consumer-safe, environmentally benign biodegradable containers.

Keywords: biodegradable containers, hardness, rice husk, thermo-pressing.

Classification numbers: 2.3, 5.3

1. Introduction

SUP based on expanded polystyrene (EPS) are prevalent in food packaging due to their low density, superior thermal insulation, high tensile strength, and affordability. However, SUPs derived from petroleum are chemically inert and non-biodegradable, posing significant challenges in recycling and potentially generating toxic byproducts during decomposition [1]. With global plastic production reaching 390.7 million tonnes in 2021 [2], SUPs not only exacerbate environmental issues but also deplete petroleum resources. Commonly, SUP waste is either buried or incinerated, compromising the quality of land, air, water, oceans, and human health [3].

In response, biodegradable composites are being developed as alternatives to SUPs. Research on biodegradable products has largely focused on natural materials. Proteins such as soy protein [4], whey protein [5] and polysaccharides (chitosan, cellulose, starch) [6] have been utilised as raw materials for biodegradable products. Amongst these, starch-based materials are extensively studied due to starch's abundance, biocompatibility, non-toxicity, renewability, and cost-effectiveness. Moreover, the polyhydroxy structure of starch facilitates the modification

of its functional and structural properties through chemical or enzymatic treatments [3]. To enable the use of these materials in container applications, reinforcement with cellulose is necessary to enhance tensile strength and load-bearing capacity. Cellulose fibres can form a dense network structure with starch molecules, thanks to the numerous hydroxyl groups on their surfaces [7]. The addition of cellulose fibres is shown to improve thermal properties, tensile strength, and hydrophobicity [8]. Additionally, cellulose is recognised for its excellent biocompatibility, biodegradability, non-toxicity, abundance, and affordability [9]. Agricultural waste, such as banana trunk [10], bagasse [11], RH [12], peanut skin [13], has been repurposed to create green products to supplant SUPs.

In Vietnam, according to the Ministry of Agriculture and Rural Development, the rice yield exceeded 43.86 million tonnes in 2021. Consequently, about 20% of this harvest, equating to 8.772 million tonnes, becomes RH agricultural residue [14]. Typically utilised for animal feed, biomass, and organic fertiliser production, RH with its high cellulose, silica, lignin, and moisture content, and a bulk density ranging from 90-150 kg/m³ presents an opportunity for creating environmentally friendly materials [14].

*Corresponding author: Email: nhunghtt@hcmute.edu.vn

To streamline the experimental process, various design and optimisation methods have been explored. The Taguchi method, a well-known approach, utilises statistical analysis to assess the impact of different parameters on the means and variance of a production or operational process [15]. The Taguchi method employs statistical analysis to determine how various parameters influence the means and variance of the production or operation process [16]. The system design procedure aims to establish optimal test levels for design parameters, while the factor design procedure identifies parameter levels that enhance the performance of the product in question.

This study focuses on producing biodegradable trays from RH and starch via a thermo-pressing process. Using the Taguchi method, we investigated how operational factors such as the RH to starch ratio, pressing time, and pressing temperature affect the mechanical properties of the container. Additionally, the influence of additives such as glycerol and adhesive on the colour difference, hardness, and density of the trays was examined.

2. Materials and methods

2.1. Preparation of RH trays

RH was sourced from rice milling operations in Dong Thap province, Vietnam. The husks were ground to a 0.25 mm granularity. Cationic MS VN1605, with a moisture content of 14%, was supplied by Thuan Phat Hung Company. Varied ratios of RH to modified starch (MS) were employed: 20/80, 35/65, and 50/50. Polyvinyl acetate (PVA) from China (87%) and glycerol from Merck (99.5%) were added prior to the pressing process and thoroughly blended using a mixer. For the process, a stainless-steel mould of 150 mm diameter and 2 mm thickness was utilised (Fig. 1) [10]. The thermo-pressing was performed at 130, 150, and 170°C, for durations of 3, 4, and 5 minutes, respectively. Post thermo-pressing, the trays were cut into shape. The process followed a prescribed flowchart (Fig. 2).

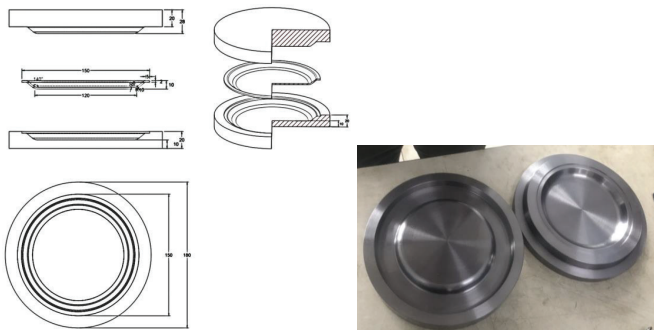


Fig. 1. Mould for the thermo-pressing process.

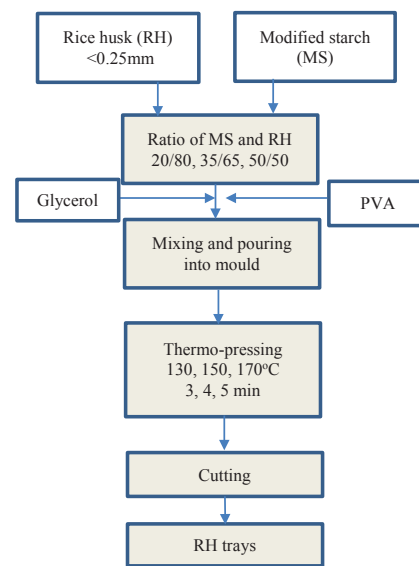


Fig. 2. Flowchart of the RH trays production process.

2.2. Design of experiments for biodegradable RH trays

A preliminary study identified factors impacting product quality, focusing on hardness, colour difference, thickness, and density. Factors examined included concentrations of glycerol, PVA, pressing temperature, time, and the RH to MS ratio. Glycerol and PVA were found to have a negligible effect on the RH tray quality, leading to the selection of three influential factors for the Taguchi method: the ratio of MS to RH (A), pressing temperature (B), and pressing time (C). An orthogonal array was designed for the experiment, requiring nine tests ($3^2=9$) to economise on time and resources. Each test was conducted thrice (Table 1).

Table 1. The Taguchi method design for RH tray experimentation.

Independent factors	Level 1	Level 2	Level 3
The ratio of MS and RH (A) (w/w)	20/80	35/65	50/50
Pressing temperature (B) (°C)	130	150	170
Pressing time (C), (min)	3	4	5

2.3. Samples analysis

2.3.1. Hardness test

Hardness is defined as a product's resistance to permanent deformation such as indentation, abrasion, wear, and scratch. In this study, a hardness tester (IC-FR5105) was used to determine the load of the container.

2.3.2. Thickness and density

A manual micrometre (Mitutoyo, Japan) was used to measure the thickness of the RH container. The density was calculated using the weight-thickness relationship. The reported value is the mean of three calculations.

2.3.3. Colorimetry

A colour spectrophotometer (CR-400/CR 410) was used and the 0% colour was calibrated with white and 100% with black standards. The colour parameters L^* , a^* , and b^* were recorded for each product. Each product was analysed at six different points on the upper and lower surfaces of the product. The comprehensive colour difference, ΔE , was calculated using the formula:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

where ΔL represents the brightness difference (lightness and darkness); Δa denotes the red-green difference; Δb the yellow-blue difference.

3. Results and discussion

3.1. Effect of additives in RH container process

The role of additives in product processing is crucial and cannot be overstated. Additives may take various forms, contingent upon the desired properties of the product. They can enhance fibre hardness, bonding strength, and resistance to moisture and oil. Additionally, additives can influence fibre and production costs, whiteness, brightness, colour stability, and energy consumption [17]. While additives can augment the natural bonding between fibres, their effect is secondary to that achieved through fibre refining and beating [18]. In this study, the impact of additives was examined using a fixed MS/RH ratio of 50/50 (w/w), a pressing temperature of 170°C, and a pressing time of 3 minutes. We analysed all formulations to optimise tray quality without the presence of cracks

3.1.1. Effect of PVA

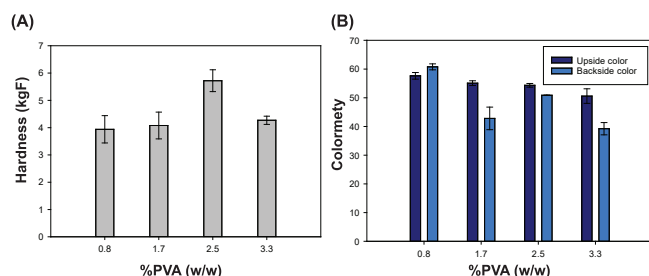


Fig. 3. Effect of poly(vinyl alcohol) on the hardness (A) and colour (B) of biodegradable trays.

Poly(vinyl alcohol) (PVA) is a biodegradable, biocompatible, and hydrophilic synthetic polymer [19]. The trays were hot-pressed for 3 minutes at 170°C and 1 MPa, with PVA concentrations varying from 0.8% to 3.3% (w/w) (Fig. 3). PVA served as an adhesive, bonding the MS and RH fibres. The tray with 2.5% PVA (w/w) exhibited the highest hardness. Beyond this concentration, an increase in PVA reduced the hardness of the product and caused it to adhere more to the mould. The colorimetric differences (ΔE) were measured on both the front and back

of the products, revealing a slight fading of colour with increasing PVA concentration. The thickness and density of the products remained consistent at 2.05 ± 0.01 mm and 0.93 ± 0.03 g/cm³, respectively, indicating that the variation in PVA concentration did not significantly impact these parameters. Additionally, the high lignin content within RH fibres contributed to the adhesion of product components and reduced porosity within the trays when subjected to elevated temperatures and pressing force [20].

3.1.2. Effect of glycerol

Glycerol, a prevalent plasticiser in starch and fibre-based biodegradable material production, significantly affects mechanical properties. Its presence can enhance the flexibility of biodegradable containers by reducing their brittleness and permeability to water vapour and oxygen [21]. In this study, glycerol concentrations varied from 3% to 6% (w/w). The hardness of the product increased with glycerol concentrations up to 5% (w/w) but decreased when the glycerol level exceeded 5% (Fig. 4). E. Basiak, et al. (2018) [21] observed that higher glycerol content in starch structures diminishes tensile strength and elastic modulus.

The colour difference values on both sides of the products changed with glycerol concentration. At 3% (w/w), the colour difference value was low and remained stable at higher concentrations. However, the colour did not change markedly with increasing glycerol levels. Glycerol concentration had no significant effect on product thickness or density, with thickness around 2.05 ± 0.01 mm and density approximately 0.9 ± 0.07 g/cm³.

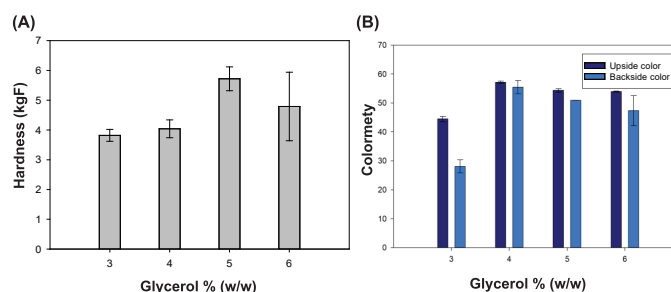


Fig. 4. Effect of glycerol on the hardness (A) and colour (B) difference of biodegradable trays.

3.2. Optimum analysis by Taguchi approach

Given that the additives did not impact the product's quality significantly, the main factors influencing the tray production process were the MS/RH ratio, pressing temperature, and pressing time (Table 2). The Taguchi method was employed to assess the effects of these variables, recommending a signal-to-noise (S/N) ratio to optimise the quality of the biodegradable container. Maximising the S/N ratio implies minimising the variability and effects of noise factors. In this context, biodegradable trays with higher hardness, lower density, and lighter colour were preferred.

Thus, a ‘larger-is-better’ approach was used for hardness analysis, while a ‘smaller-is-better’ approach was adopted for analysing tray density and colour (Tables 3-5).

Table 2. L9 orthogonal array design for three factors at three levels.

No.	A	B	C	The ratio of MS/RH (w/w)	Pressing temperature (°C)	Pressing time (min)	Hardness (kgF)	Density (g/cm ³)
1	1	1	1	20/80	130	3	4.92	0.954
2	1	2	2	20/80	150	4	4.02	0.980
3	1	3	3	20/80	170	5	5.68	1.062
4	2	1	2	35/65	130	4	4.49	0.926
5	2	2	3	35/65	150	5	4.44	0.951
6	2	3	1	35/65	170	3	4.55	0.971
7	3	1	3	50/50	130	5	3.58	0.879
8	3	2	1	50/50	150	3	5.72	0.923
9	3	3	2	50/50	170	4	3.55	0.926

Table 3. Response table for signal-to noise ratios and means of the hardness of the product.

Level	The ratio of starch and RH	Pressing temperature (°C)	Pressing time (mins)
<i>Response table for signal to noise ratios larger-is-better</i>			
1	13.67	12.65	14.05
2	13.05	13.40	12.04
3	12.41	13.09	13.04
Delta	1.26	0.74	2.01
Rank	2	3	1
<i>Response table for means</i>			
1	4.874	4.329	5.064
2	4.492	4.728	4.019
3	4.284	4.594	4.568
Delta	0.590	0.399	1.046
Rank	2	3	1

Density is a crucial physical property for the tray’s practical use. In the response table for product density, the MS/RH ratio is the most influential factor. As the MS/RH ratio increases, the tray’s density decreases. The pressing temperature and time have lesser effects on product density. The best results, combining low density with good incorporation of RH fibre in the polymeric matrix, were achieved with a 50/50 MS/RH ratio, a pressing temperature of 130°C, and a pressing time of 4 minutes. These conditions, however, are in contrast to those for optimal hardness. The better the tray in terms of lower density, the lower its hardness. An increased MS/RH ratio results in softer products (Fig. 5). The addition of more RH fibres makes the tray harder due to the dense network structure created by the hydroxyl groups on the fibres’ surfaces [7]. It has been demonstrated that adding RH fibres can improve the thermal properties, tensile strength, and hydrophobicity of biodegradable trays [22]. Nonetheless, the density variations were small, ranging from 0.87 to 1.06 g/cm³, and the product’s hardness was prioritised when selecting the optimum starch-to-rice-husk ratio of 20/80 (w/w). A higher proportion of RH implies a longer biodegradation time since cellulose degrades more slowly than starch [8].

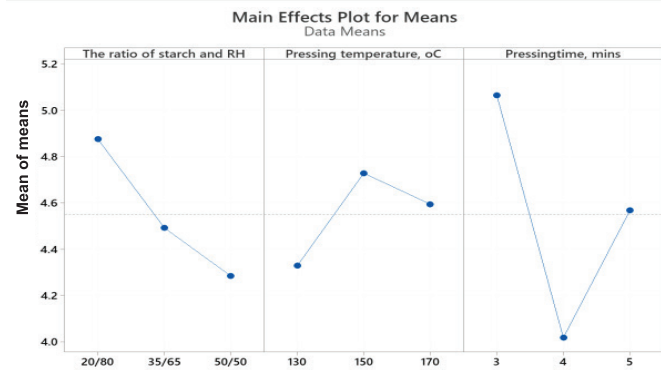


Fig. 5. Effect plots for the hardness of biodegradable trays.

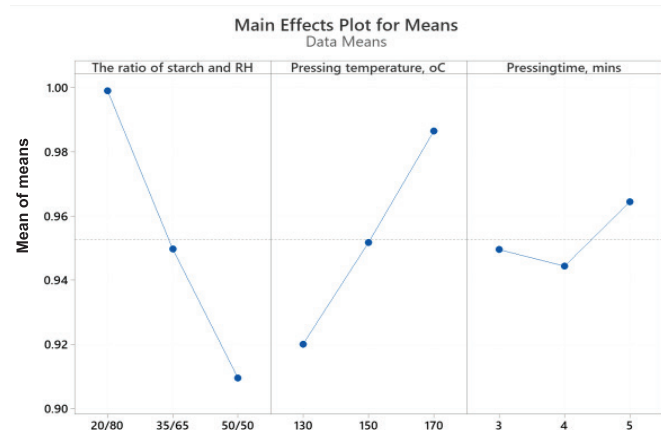
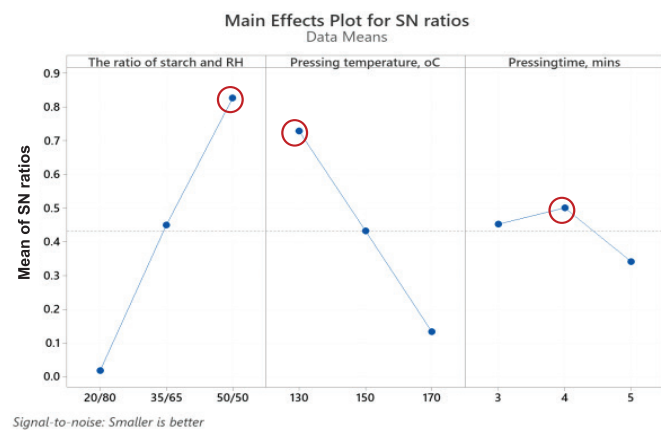


Fig. 6. Effect plots for the density of biodegradable trays.

The results for the biodegradable trays indicate that a pressing temperature of 150°C and a pressing time of 3 minutes yield the highest hardness (Fig. 6 and Table 4). Conversely, the most effective density is achieved at a pressing temperature of 130°C with a pressing time of 4 minutes. Nonetheless, this condition also results in the lowest hardness S/N values and means. While pressing temperature marginally affects hardness, it significantly impacts product density. Higher moulding temperatures, such as 170°C, enhance the crystallinity index of cellulose, reduce its hygroscopic nature, and create a stronger, less porous network resistant to moisture absorption. This is corroborated by N. Suderman, et al. (2016) [23], who noted the impact of drying temperature and time on the mechanical properties of biodegradable containers.

Table 4. Response table for signal-to-noise ratios and means for the density of the product.

Level	The ratio of MS and RH	Pressing temperature (°C)	Pressing time (mins)
<i>Response table for signal to noise ratios (smaller is better)</i>			
1	0.01796	0.72831	0.45216
2	0.44994	0.43263	0.50051
3	0.82599	0.13296	0.34123
Delta	0.80803	0.59535	0.15928
Rank	1	2	3
<i>Response table for means</i>			
1	0.9990	0.9201	0.9495
2	0.9497	0.9517	0.9443
3	0.9095	0.9864	0.9644
Delta	0.0894	0.0663	0.0200
Rank	1	2	3

Table 5. Response table for signal-to-noise ratios and means for the colour differences.

Level	The ratio of MS and RH	Pressing temperature (°C)	Pressing time (mins)
<i>Response table for signal to noise ratios (smaller is better)</i>			
1	-33.76	-33.57	-33.81
2	-33.78	-33.92	-32.73
3	-33.04	-33.10	-34.05
Delta	0.74	0.82	1.32
Rank	3	2	1
<i>Response table for means</i>			
1	48.70	47.46	48.83
2	48.62	49.68	43.06
3	44.99	45.18	50.43
Delta	3.71	4.50	7.37
Rank	3	2	1

The colour difference (ΔE) of a biodegradable tray, detailed in Table 5, shows that trays with the highest MS/RH ratio (20/80) exhibit the highest ΔE values. Conversely, a decrease in the MS/RH ratio leads to a significant reduction in ΔE as the RH content increases (Fig. 7). The addition of RH fibres to the starch matrix results in an increased

ΔE value, giving the trays a brownish and yellowish hue, likely influenced by the natural colour of the RH fibres and their high lignin content [24]. Moreover, the high-temperature and pressure conditions cause the degradation of lignocellulosic materials, releasing compounds such as furfural and glycolaldehyde, which contribute to the brown colouration of the product.

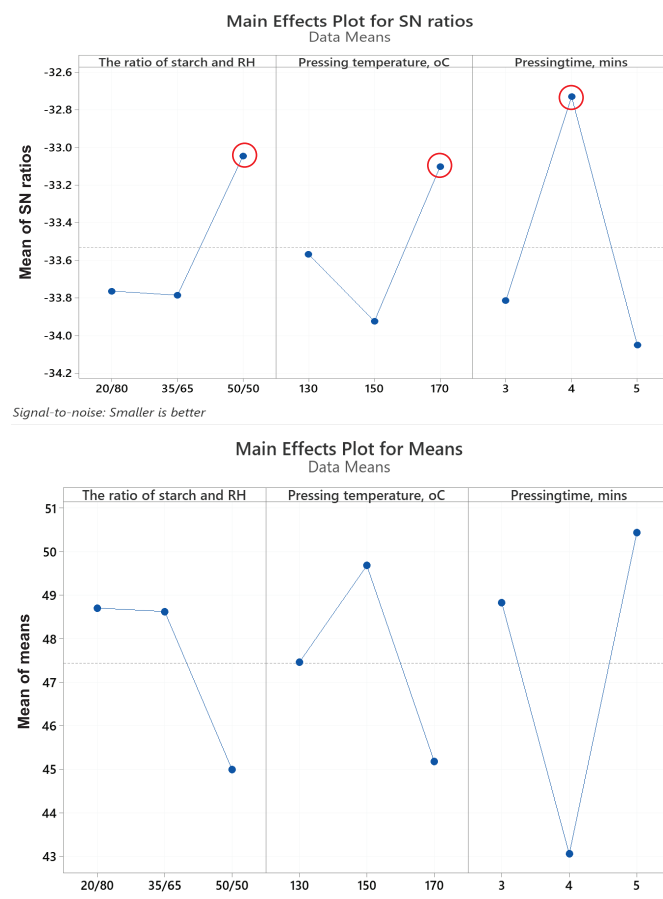


Fig. 7. Effect plots for the colour difference of biodegradable trays.

For optimal product quality, biodegradable trays were produced with an MS/RH ratio of 20/80, a pressing temperature of 150°C, and a pressing time of 3 minutes (Fig. 8). The colourimetry, hardness, and density were evaluated, with results showing hardness (5.26 ± 0.22 kgF), frontside colour difference (50.87 ± 0.96), backside colour difference (48.05 ± 0.87), and density (0.97 ± 0.004 g/cm³).



Fig. 8. Biodegradable trays based on RH and starch. (A) Frontside; (B) Backside.

4. Conclusions

The thermo-pressing process has been successful in manufacturing biodegradable trays from MS and RH fibre. The optimal trays are characterised by an appealing off-white brown colour, robust hardness, and low density. The ideal starch to RH ratio is 20/80, with a pressing temperature of 150°C and a pressing time of 3 minutes. The mechanical properties of the trays include hardness (5.26 ± 0.22 kgF), frontside colour difference (50.87 ± 0.96), backside colour difference (48.05 ± 0.87), the thickness (2.05 ± 0.01 mm) and density (0.97 ± 0.004 g/cm³). The Taguchi method identifies pressing time as the most significant factor affecting the mechanical properties (hardness and colour) of the product, with the starch/RH ratio being paramount for density criteria.

CRedit author statement

Nhung Thi-Tuyet Hoang: Conceptualisation, Resources and Data curation, Writing - Reviewing and Editing; Anh Thi-Kim Tran: Writing original draft preparation, Writing - Reviewing and Editing.

COMPETING INTERESTS

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

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Assessing the risk and morphological characteristics of weedy rice outbreak in Thap Muoi district, Dong Thap province, Vietnam

Cong Vinh Kieu, Ho Le Thi*

Department of Plant Protection, College of Agriculture, Can Tho University,
Area II, 3/2 Street, Xuan Khanh Ward, Ninh Kieu District, Can Tho City, Vietnam

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Abstract:

The Thap Muoi district in Dong Thap province, Vietnam, is renowned as the largest rice producer, cultivating 112,762 hectares and specialising in three rice crops per year, with some areas achieving 3.5 annual crops. However, changing farming practices, low-quality rice seeds, and mechanisation have led to the emergence and spread of weedy rice (*Oryza* spp.) in Thap Muoi and the broader Mekong delta. This comprehensive assessment of weedy rice outbreak risks in Thap Muoi provides a vital foundation for effective management strategies to prevent its spread in the Mekong delta. Sixty questionnaires were collected from rice-farming households. Approximately 80% of farmers reported the appearance of weedy rice approximately five years ago. Alarming, 95% of surveyed farmers (57 individuals) reported weedy rice infestations, with 40% of them encountering infection levels ranging from 11 to 20 plants/m². To distinguish weedy rice from cultivated varieties, seven distinct types were identified based on various morphological characteristics, including growth time, leaf blade structure, plant height, leaf/stem colour and shape, grain beard/tail, seed/husk colour, grain length, ripening time, and seed dispersal potential. However, all seven weedy rice varieties exhibited short growth cycles and the capacity to produce seeds, posing significant challenges to rice production management in Thap Muoi. This research underscores the critical importance of assessing weedy rice outbreak risks in Thap Muoi and understanding its morphology and prevalence. This understanding is essential for developing effective preventive measures to mitigate the threat of weedy rice outbreaks across the entire Mekong delta in Vietnam.

Keywords: assessment, morphological characteristics, rice pests, weedy rice.

Classification number: 3.1, 3.4

1. Introduction

Weedy rice (*Oryza sativa* f. *spontanea*) is a troublesome weed species that adversely affects rice production globally. With its widespread distribution and adaptability, weedy rice poses a significant threat to agricultural systems in various rice-growing regions. Weedy rice has been reported in several countries across different continents, with Asia being one of the most affected regions. Countries such as China, India, Bangladesh, Indonesia, the Philippines, and Thailand have faced persistent challenges from weedy rice infestations [1-3]. In Africa, weedy rice has been documented in countries like Nigeria and Madagascar, where it poses a considerable threat to rice production [4, 5]. Furthermore, the Americas and Oceania have also experienced weedy rice outbreaks, with the United States, Brazil, and Australia being among the affected countries [6-8]. The genetic diversity of weedy rice has been extensively studied, revealing its complex origin resulting from hybridisation events with cultivated rice and other wild relatives [9]. This genetic diversity contributes to its adaptability to various ecological and

environmental conditions. Weedy rice exhibits traits such as seed shattering, dormancy, and competitive ability, which enhance its survival and spread in agricultural fields [10]. The impact of weedy rice on rice production is significant, causing yield losses ranging from 20 to over 80%, depending on the severity of infestation and management practices [11]. Additionally, weedy rice can hybridise with cultivated rice varieties, leading to the transfer of undesirable traits into the cultivated gene pool [12]. Researchers worldwide have been actively investigating effective management strategies to control weedy rice outbreaks. Integrated weed management approaches that combine cultural, mechanical, and chemical control methods have shown promising results [13]. However, the development of herbicide resistance in weedy rice populations has emerged as a concerning issue, emphasising the importance of sustainable weed control practices [14].

In the Mekong delta region of Vietnam, rice (*Oryza sativa*) constitutes the predominant crop, covering an extensive cultivation area of 1.418,2 thousand hectares,

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

which represents 79,28% of the total rice cultivation area in the Summer-Autumn crop of 2023 (General Statistics Office, 2023). The escalating encroachment and detrimental effects caused by weedy rice are intricately linked to the continuous adoption of straight-line rice cultivation in recent years, with over 90% of the rice cultivation area in the Mekong delta region employing this method. Thap Muoi district, situated in Dong Thap province within the Mekong delta, is an area where three rice crops are cultivated annually, with certain locations even experiencing three and a half rice crops per year. The sustained and expanded implementation of straight-line rice cultivation, combined with specific agricultural practices and intensified cropping, has led to the widespread and vigorous proliferation of weedy rice. Consequently, this situation exerts adverse effects on the quality, productivity, and income of local farmers, as well as on the overall rice export of Vietnam.

The primary objective of this research is to address the challenges associated with weedy rice in straight-line rice fields in Thap Muoi district, Dong Thap province. To achieve this, the study employed diagnostic surveys to ascertain and document the cultural practices of farmers engaged in rice cultivation. Furthermore, it involved morphological observations and agronomic characteristics of the prevalent weedy rice within the rice fields of Thap Muoi district, Dong Thap province, Vietnam. Additionally, the research aims to assess the production constraints, evaluate the current extent of weed invasion, determine the magnitude of damage inflicted, and assess the potential risk of weedy rice evolving into pests within the rice fields of Thap Muoi district. In particular, the study examined the farmers' familiarity with weedy rice, with a focus on its distribution, occurrence, and impact within the area. Moreover, it identifies the morphological and agronomic traits that aid in the identification of weedy rice species present in the fields. Based on these findings, appropriate and effective weed management strategies can be proposed and recommended to the farmers in the region.

2. Materials and methods

Surveys were conducted in Thap Muoi district, Dong Thap province during the Summer-Autumn rice crop of 2022. A random selection of 60 households of farmers was made, with each household located at least 200 m apart and having a cultivated area of 1 ha or more.

The primary data collection method involved structured interviews, where all participating farmers were asked the same set of questions. Information was gathered through direct interviews using pre-prepared survey questionnaires. The collected data covered the following main aspects: i) General information about the farmers, ii) Farmers'

knowledge and understanding of weedy rice, iii) Extent of weedy rice infestation in the rice fields (infection rate), iv) Yield losses caused by weedy rice, v) Farmers' experiences and weed control measures applied during the Summer-Autumn rice crop of 2022.

Weedy rice samples were collected in Thap Muoi district during the Summer-Autumn rice crop of 2022. Seven weedy rice samples were selected for this study and designated as WR1, WR2, WR3, WR4, WR5, WR6, and WR7. The commonly cultivated rice variety in Thap Muoi district is OM18.

Seeds of the seven weedy rice lines were soaked and placed on Petri dishes for germination in a controlled temperature incubator (Daichi warm germination cabinet). After approximately 7 days, healthy seedlings were transplanted into experimental plots with an area of 2,2x3 m and spaced 20-25 cm apart. The experiment was arranged sequentially (10 seedlings per line) without replication. The cultivation process for the weedy rice lines was similar to that of the OM18 rice variety, with a fertiliser formula of 80:60:30 kg NPK/ha.

The growth and development of the weedy rice lines and the OM18 rice variety were observed and recorded from the seedling stage to maturity using the Standard Evaluation System for Rice of the International Rice Research Institute [15, 16]. Morphological and agronomic characteristics were visually observed, measured, and recorded between 60 to 80 days after transplanting (DAT). Fifteen morphological traits were assessed, including plant height, growth duration, stem angle, tiller number per plant, panicle number per plant, flag leaf length, panicle type, panicle length, seed shattering trait, hull colour, grain colour, grain length, grain width, awn length, and 1000-grain weight. The data obtained from the weedy rice lines were averaged and comparatively analysed with the OM18 rice variety.

3. Results

Results from the investigation involving 60 farming households revealed a total surveyed area of 161,50 ha, with an average of 2,69 ha per household. The educational attainment of rice farmers was relatively low, with 61,7% (37 households) having received only primary education. The survey indicated that 100% of the farmers were familiar with weedy rice and perceived its presence in their fields for over a decade. However, 80% of them reported weedy rice emergence around 5 years ago, while 16,7% mentioned its presence for more than 10 years. Moreover, 3,3% of the farmers believed that weedy rice appeared between 5 to 10 years ago (Fig. 1). These findings align with historical records indicating the initial occurrence of weedy rice in Vietnam in 1994 [17].

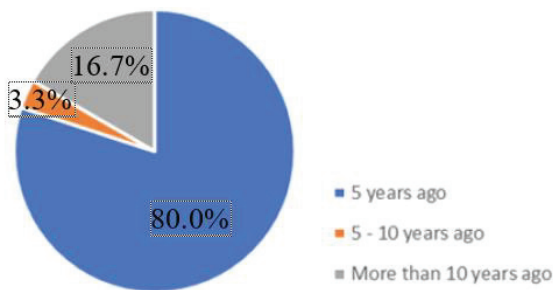


Fig. 1. Farmers' response (%) to the appearance of weedy rice in Thap Muoi district.

Regarding the morphological traits of weedy rice, farmers observed a higher prevalence of weedy rice in transplanted and broadcast-sown rice fields compared to direct-seeded ones. They noted that weedy rice seeds are easily shed and recognised it as a wild grass species in rice fields. Some weedy rice varieties lack awns and display high germination rates upon soil contact. Farmers could easily identify weedy rice seeds due to their characteristic colour, which persists even after milling. However, when queried about specific characteristics related to seed dormancy and persistence, such as the shorter stature of weedy rice compared to cultivated rice and the longevity of weedy rice seeds in the soil (over 1 year), 33,3 to 51,7% of farmers were not aware of these traits (Table 1). This lack of knowledge might lead farmers to misidentify weedy rice outside rice fields.

Table 1. Survey results of some characteristics of weedy rice in Thap Muoi district, Dong Thap province.

No.	Attributes of weedy rice	Number of responses and percentage (%) of surveys			
		Known	Percentage	Unknown	Percentage
1	Weedy rice is commonly found in fallow rice fields rather than in planted or cultivated fields	57	95,0%	3	5,0%
2	Weedy rice seeds exhibit a state of dormancy	29	48,3%	31	51,7%
3	Weedy rice seeds are prone to shattering	60	100,0%	0	0,0%
4	Weedy rice plants have shorter height compared to cultivated rice	39	65,0%	21	35,0%
5	Weedy rice is a type of weed found in rice fields	58	96,7%	2	3,3%
6	Some types of weedy rice do not have awns (beard-like structures on seeds)	60	100,0%	0	0,0%
7	Weedy rice seeds readily germinate and sprout when they fall to the ground	59	98,3%	1	1,7%
8	Weedy rice seeds have distinct coloration	60	100,0%	0	0,0%
9	Even seeds after milling, weedy rice retain their colour	58	96,7%	2	3,3%
10	Weedy rice can be consumed as food	55	91,7%	5	8,3%
11	Weedy rice can be cultivated as normal rice	40	66,7%	20	33,3%
12	The lifespan of weedy rice in the soil can exceed one year	33	55,0%	27	45,0%

The survey findings demonstrated varying degrees of weedy rice infestation, ranging from mild (2-5 plants/m²) to severe (11-20 plants/m²), with 95% of the fields being affected. Notably, 40% of the fields exhibited an infestation level of 11-20 plants/m² (Fig. 2). Only 3,3% of farmers reported the presence of a single weedy rice species in their rice fields, while 58 farmers acknowledged the coexistence of two or more weedy rice species in their fields (Fig. 3).

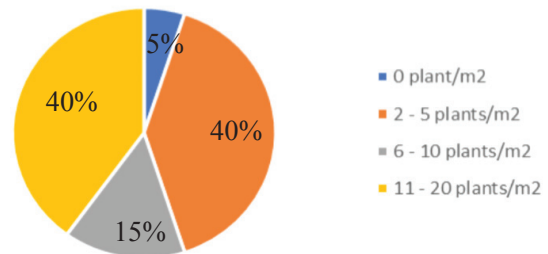


Fig. 2. Density of weedy rice infection (%) in Thap Muoi district.

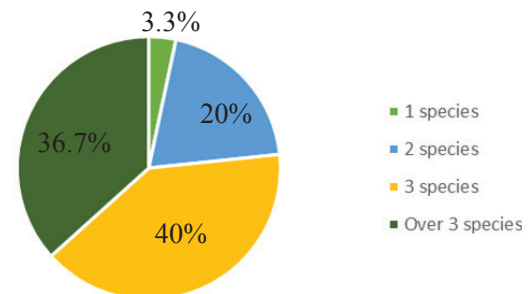


Fig. 3. Farmers' observation (%) of the number of weedy rice species appearing in rice fields in Thap Muoi district.

The survey showed that 55 households used certified rice varieties for cultivation, accounting for 91,7%, while 5 households reused planted rice to make seeds for the next crop (8,3%). This also increases the possibility of spreading weedy rice from the previous crop to the next crop. There are 4 main rice varieties grown in Thap Muoi including OM5451, OM18, OM9582, and IR4625. Specifically, OM18 (48,3%) rice variety is most commonly grown in Thap Muoi district (Fig. 4).

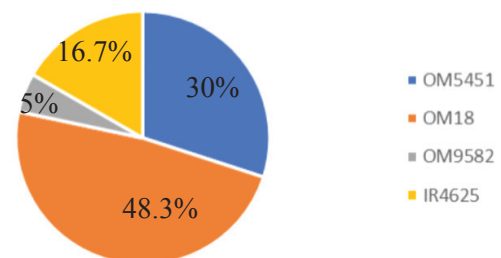


Fig. 4. Observation of rice varieties (%) grown in Thap Muoi district.

Regarding agricultural practices, 100% of farmers practiced triple cropping of rice per year and synchronised

their rice planting schedule based on local water availability. The interval between harvest and subsequent planting ranged from 7 to 10 days. With regard to crop residue management and weedy rice at the beginning of the planting season, only 24% of farmers burned straw before ploughing the field. The majority of farmers incorporated straw into the soil immediately after harvest and then flooded the fields for 5 to 7 days. This practice might bury weedy rice seeds from the previous crop, resulting in an increased weed seed bank in the soil. After 1 to 2 days, they allow the land to emerge, during which buried weeds start to germinate. Land shaft and grooving follow, with proper channels facilitating water management and herbicide application. Sowing is done after these preparations, enabling farmers to manage weed growth from the start of the crop cycle. However, weedy rice seeds can last in the soil and emerge in the next rice season, affecting the rice yield. Notably, 100% of farmers did not clean their combined harvesters before rice harvest, potentially contributing to the spread of weedy rice from one field to another.

In terms of weed management, the primary herbicides used by the majority of farmers (91,7%) were pretilachlor and butachlor, applied at 5 different timings. Most farmers (95,0%) applied the herbicides between 0 to 4 days after planting (DAP), using a single application. Only 8,3% of farmers applied pretilachlor twice, one day before planting and two days after planting (Table 2). Considering the close relation of weedy rice and cultivated rice, both belonging to the same species [18], early pre-emergence herbicide application on rice to manage weedy rice is crucial for effective weed control.

Table 2. Chemical methods used by farmers to manage weedy rice in Thap Muoi district, Dong Thap province.

No	Herbicide active ingredient	Treatment	No. of farmers applying	Farmers applying (%)
1	Pretilachlor 300 g/l Fencloirim 100 g/l	Spray 1-1.2 l/ha after 2 DAP, irrigate field 5 DAP.	23	38,3
2	Pretilachlor 300 g/l Fencloirim 100 g/l	Spray 1-1.2 l/ha 1DBP; repeat 2 DAP and irrigate field 5 DAP.	5	8,3
3	Pretilachlor 360 g/l Fencloirim 150 g/l	Spray 1-1.2 l/ha 0-4 DAP. Irrigate field after 3 to 5 days	8	13,3
4	Butachlor 620 g/l	Spray 1,2 l/ha 1-4 DAP, then irrigate field 5-7 DAP	20	33,3
5	Butachlor 600 g/l	Spray 1 l/ha 0 to 3 DAP. Then add irrigate field 5-7 DAP.	4	6,7

The survey revealed an average yield of 7,06 t/ha from the 60 households during the Summer-Autumn crop of 2022, which was 0,24 t lower than the average yield in the previous Summer-Autumn crop of 2021 in Thap Muoi district (7.30 t/ha) (Department of Agriculture and Rural Development of Dong Thap province, 2021). These findings suggest that the decrease in yield during the Summer-Autumn crop of 2022

could be influenced by various factors, including weather conditions, pests, and weeds, including weedy rice.

General Morphological Characteristics of Weedy Rice Strains:

- Growth duration (GD): All 7 weedy rice strains exhibited a significantly shorter GD compared to the cultivated rice OM18 (103 days). The GD ranged from 84 to 94 days, with an average of approximately 89 days after sowing (DAS). This particular trait observed in weedy rice strains within Thap Muoi district is consistent with the findings reported in the study conducted by [19].

- Plant height: The plant height of the weedy rice strains demonstrated considerable variability, with strain WR5 attaining the tallest height at 150,0 cm and strain WR6 being the shortest at 104.4 cm. The average plant height was 128,4 cm, which exceeded the average height of the OM18 rice variety (108 cm). Most weedy rice strains exhibited taller average plant heights than the cultivated OM18 rice, except for strain WR6, which displayed a lower average height. A study by A.D. Morat, et al. (2018) [20] also corroborated

Table 3. Comparison of agronomic characteristics between weedy rice strains and commonly cultivated rice variety OM18.

Description of basic characteristic	WR1	WR2	WR3	WR4	WR5	WR6	WR7	OM18
1. GD (days)	94	89	84	89	91	87	89	103
2. Plant height (cm)	126.8±3.2	148.7±3.0	141.0±2.8	114.1±2.9	150±2.6	104.4±2.9	114.1±2.9	108.4±2.6
3. Stem angle	Medium	Erect	Erect	Erect	Medium	Medium	Erect	Erect
4. Tillers per plant	16±2	20±3	18±1	15±3	13±2	23±3	21±3	19±2
5. Panicles per plant	13±1	9±1	12±1	10±2	12±2	18±1	16±2	13±2
6. Flag leaf length (cm)	44.3±2.32	42.1±2.85	34.0±1.42	35.0±1.49	33.9±1.58	41.0±1.92	35.0±1.49	32.3±3.5
7. Chlorophyll content (SPAD)	38.0±1.07	33.7±6.93	36.5±2.24	36.5±2.67	38.1±1.82	38.8±2.15	40.0±2.12	39.2±2.43
8. Panicle type	Compact	Compact	Compact	Compact	Compact	Medium	Compact	Compact
9. Panicle length (cm)	27.2±1.2	21.8±0.6	26.5±1.9	21.0±1	31.3±2.0	22.9±0.7	21.0±1	22.8±0.4
10. Seed shattering characteristics	Difficult	Quite easy	Easy	Easy	Easy	Easy	Easy	Difficult
11. Hull colour	Bright yellow	Bright yellow	Black	Bright yellow	Brown	Black	Straw yellow	Straw yellow
12. Grain colour	Red	Red	Red	Red	Red	Red	Red	White
13. Grain length (mm)	9.20	8.16	7.72	10.20	8.40	7.84	8.54	9.80
14. Grain width (mm)	2.10	2.49	2.76	2.36	2.16	2.20	2.56	2.04
15. Awn length (mm)	0	0	0	2-12	9-27	12-38	41-63	0
16. 1000-grain weight	25,11	16,94	14,88	28,39	15,56	15,96	18,64	23,70

that weedy rice strains generally possess greater plant height compared to cultivated rice. This trait suggests that weedy rice exhibits potential adaptability and evolution under changing climate conditions and rice cultivation practices in the Mekong delta region.

- **Tillering ability:** The weedy rice strains demonstrated similar tillering capabilities ranging from 13 to 23 tillers/plant, with an average of 18 tillers/plant, though slightly lower than the average tillering of the OM18 rice, which had 19 tillers/plant. The average number of panicles/plant for the weedy rice strains was 12.9, closely resembling the average panicle count of the OM18 rice (13 panicles/plant) (Table 3). With comparable tillering and panicle numbers to cultivated rice, weedy rice exhibits the ability to competitively acquire nutrients, water, and light within the agricultural field.

- **Leaf shape:** Certain weedy rice strains displayed elongated, narrow, and thin leaves. This particular leaf morphology was evident during the vegetative growth stage. The average length of the flag leaf for weedy rice was 37.9 cm, with the longest leaf measuring 44.3 cm and the shortest 33.9 cm, all exceeding the flag leaf length of OM18 (32.3 cm).

- **Leaf colour:** During the vegetative growth stage, some weedy rice strains exhibited lighter leaf coloration compared to cultivated rice, facilitating early differentiation by farmers. The average chlorophyll content index (SPAD) for the weedy rice strains was 37.4, lower than that of the cultivated OM18 rice (39.2) (n=10)

- **Seed shape and colour:** The OM18 rice variety produced elongated and slender seeds with a length-to-width ratio exceeding 4. The seed colour was straw yellow, while the brown rice exhibited a white hue, with a 1000-grain weight ranging from 25-26 g. Conversely, the weedy rice seeds displayed diverse shapes, including round, medium-long, and long. The seed coloration also exhibited variation, with certain seeds appearing black, yellow, and occasionally presenting multiple colours within the same panicle. Red rice was prevalent among the rice samples. The 1000-grain weight of the weedy rice strains ranged from 14.88 to 28.39 g, reflecting variations in maturation rates and shattering tendencies.

- **Awn and awn colour on weedy rice seeds:** The weedy rice strains exhibited considerable diversity in awn shape and length. Some strains possessed awns with lengths ranging from 0 to 63 mm, while others lacked awns, akin to the OM18 rice seeds (Table 3, Fig. 5).

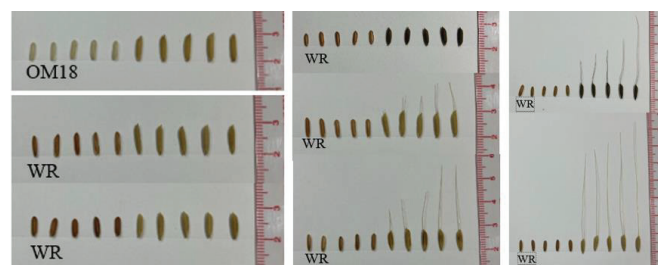


Fig 5. Weedy rice seeds.

- **Seed shattering characteristic:** Most weedy rice strains experienced seed shattering prior to physiological maturity (80% of seed weight). Easy seed shattering is the principal cause of yield loss in rice fields as weedy rice strains vie for nutrients, water, and light akin to cultivated rice. Survey results indicated that numerous weedy rice strains-initiated shattering approximately 15 days after the milk stage, with shattering intensity increasing towards the ripening stage. The average seed shattering rate was 50%, with certain strains exhibiting shattering rates as high as 90%. The well-filled seeds situated at the apex of the panicles were particularly susceptible to shattering, while only a few small, suspended seeds remained near the rachis at the base of the panicle.

4. Discussion

Based on the survey conducted with 60 farmers in Thap Muoi district, Dong Thap province, Vietnam, it was observed that although the farmers had limited education levels, they demonstrated awareness of weedy rice. Weedy rice has been present in the region for several decades, but its occurrence has notably increased in recent years, particularly in fallow fields. However, more than 50% of the farmers lacked knowledge about specific characteristics of weedy rice, such as seed dormancy, the potential for simultaneous harvesting with cultivated rice, its persistence in the soil for over one year, and the typical shorter stature compared to cultivated rice.

The escalating presence of weedy rice over the past five years can be attributed to farmers' incomplete understanding of all aspects of weedy rice, with recognition limited to easily identifiable traits. This, combined with a higher frequency of rice cultivation within the year, has led to changes in farming practices, including failure to clean harvesters before re-seeding and leaving seeds for subsequent crops. Additionally, ploughing rice straw into the soil instead of burning it has contributed to the proliferation of weedy rice in rice fields [21]. The limited interval for land preparation before sowing (5 to 7 days) does not provide sufficient time

to completely eliminate weedy rice seeds. Moreover, post-sowing herbicide application has proven ineffective due to the shared species between weedy rice and cultivated rice, resulting in normal growth of weedy rice. The average yield of the Summer-Autumn crop in 2022 has decreased compared to the previous year, and the influence of weedy rice on the yield cannot be disregarded.

Upon observing and analysing the characteristics of 7 representative weedy rice strains and one common cultivated variety (OM18), a diverse range of morphological and biological traits were identified among the weedy rice strains collected in Thap Muoi district. Distinct characteristics, such as plant height, leaf colour and shape, stem structure, presence of awns, and hull colour, were found useful in differentiating weedy rice strains from cultivated rice varieties at various growth stages in the field.

Overall, the study highlights the importance of raising farmers' awareness and understanding of the complete range of weedy rice characteristics to implement effective management strategies. Additionally, optimising land preparation practices and employing proper post-sowing weed control measures are critical to mitigate the spread of weedy rice and minimise its impact on rice yields.

5. Conclusions and recommendations

5.1. Conclusions

a) The education level of farmers in Thap Muoi district, Dong Thap province, Vietnam, is still low, but most of them are familiar with weedy rice.

b) Weedy rice has been present in the area for several decades, though its occurrence has significantly increased in the past 5 years, especially in fallow fields.

c) More than 50% of farmers are unaware of some characteristics of weedy rice, leading to ineffective management and widespread distribution of weedy rice in rice fields.

d) Changes in farming practices, such as not cleaning harvesters before reseeding, reserving seeds for the next crop, and ploughing rice straw into the soil after harvest, also contribute to the spread of weedy rice in rice fields.

e) The duration of land preparation before sowing is only 5 to 7 days, which is not enough to completely eliminate weedy rice seeds. The use of herbicides for post-sowing management is not highly effective, as weedy rice and cultivated rice are of the same species, leading to normal growth of weedy rice.

f) The average yield in the Summer-Autumn crop of 2022 decreased compared to the same period in the previous year, and the influence of weedy rice cannot be ruled out as a possible cause.

g) Observations and analysis of the characteristics of 7 typical weedy rice strains and one popular cultivated rice strain, OM18, revealed a diversity of morphological and biological traits among weedy rice strains in the region.

h) Characteristics such as plant height, leaf colour and shape, stem shape, presence of awns, and hull colour can be used to differentiate between different weedy rice strains and the cultivated rice strain at various growth stages in the field.

i) Most weedy rice strains have taller plant height and shorter GD compared to the cultivated rice strain, OM18.

j) The weedy rice strains show a high propensity for seed shattering and early seed shattering, but there were differences in the difficulty of seed shattering among the different strains.

k) The diversity, as well as similarities in morphological and biological traits among weedy rice strains compared to cultivated rice strains, indicate a highly diverse evolution and rapid adaptation of weedy rice to the current climate change and rice farming conditions. This may lead to increased spread and distribution of weedy rice, posing challenges in weedy rice control and potentially increasing threats to rice production and exports in the Mekong delta region and the entire country.

5.2. Recommendations

a) Enhance farmers' education and provide them with more information about the characteristics of weedy rice to improve their understanding and effective management of weedy rice in farming practices.

b) Provide guidance and training to farmers on proper cleaning of harvesters before harvesting and proper handling of rice straw after harvest to reduce the risk of weedy rice spread in rice fields.

c) Conduct research and apply more effective weed management methods that are suitable for the biological characteristics of weedy rice in the region, in order to minimise the impact of weedy rice on rice crop yields.

d) Strengthen monitoring and tracking of weedy rice development in rice fields to implement timely preventive and management measures for weedy rice.

e) Investigate and analyse the factors contributing to reduced rice crop yield in the Summer-Autumn crop of 2022 to determine the extent of weedy rice's impact and propose specific solutions to improve yields in the future.

CRedit author statement

Cong Vinh Kieu: Data curation, Writing original draft preparation; Ho Le Thi: Conceptualisation, Methodology, Investigation, Writing - Reviewing and Editing.

COMPETING INTERESTS

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

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Application of deep learning in chest X-ray abnormality detection

Nhan Ngo^{1,2}, Toi Vo^{1,2}, Lua Ngo^{1,2*}

¹*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*

²*Vietnam National University - Ho Chi Minh City, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam*

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Abstract:

Lung diseases are the most common diseases worldwide, especially in Vietnam. Certain thoracic lung diseases can even lead to dangerous conditions for patients. X-ray are a useful imaging modality for detecting the abnormalities in the chest area. Furthermore, artificial intelligence can improve the detection of abnormalities in X-ray images, reduce misdiagnosis, close the knowledge gap between doctors, and alleviate the pressure on doctors. Therefore, this study aims to apply deep learning techniques to detect abnormalities in chest X-ray images and use data science and statistical approaches to improve the performance of the deep learning model. The data was explored and processed to obtain high quality data with optimal characteristics. We then applied data augmentation and optimization to the RetinaNet model with ResNet101 in a Feature Pyramid Network (FPN) backbone to achieve the best performance. Our model achieved mean average precision of 0.55 at a threshold of 0.5 (mAP@0.5) in a validation set, which included five diseases: aortic enlargement, cardiomegaly, interstitial lung disease, infiltration, and nodule/mass.

Keywords: deep learning, RetinaNet model, thoracic lung diseases, X-ray.

Classification numbers: 3.2, 3.6

1. Introduction

The term “lung disease” refers to a variety of disorders that affect the lungs, such as asthma, chronic obstructive pulmonary disease (COPD), infections like influenza, pneumonia, tuberculosis, lung cancer, and other breathing problems. Some lung diseases can even result in respiratory failure [1]. According to the 2017 Behavioral Risk Factor Surveillance System (BRFSS) survey, approximately 22.5 million (9.1 percent) adults residing in the United States and 7.9 percent of children from twenty-seven states and the District of Columbia reported currently having asthma. About 16.3 million adults (6.6 percent) reported ever being diagnosed with COPD. Close to 33.2 million adults (13.4% reported being diagnosed with chronic lung disease [2].

In most of the cases, doctors rely on X-ray images to diagnose lung diseases. However, they face many daily challenges, with perhaps the greatest difficulty being the large number of chest radiographs to be reviewed. Additionally, the interpretation of X-rays image can lead to medical misdiagnosis, even among the best practicing doctors [3].

Among the available studies on detecting lung diseases in chest X-ray (CXR), one of the most interesting is that of M.T. Islam, et al. (2017) [4]. Their study used ensemble deep learning models to improve the classification of cardiomegaly compared to a single deep learning model. Although the study had an impressive accuracy of 93.0% and an area under the ROC curve

(AUC) score [5] of 0.97, it mainly used image classification methods to detect cardiomegaly disease. Therefore, the method in that study is difficult to apply for the detection of many other lung diseases. Another study by H. Huang, et al. (2021) [6] used the Yolo [7] model to detect fourteen lung diseases from the VinBigData dataset [3]. However, the image processing stage in the Huang study only removed images without disease and resized images to 512x512 pixels. The resulting mean average precision at a threshold of 0.5 was not high, with a baseline model of 0.21 and 0.34 after model selection and hyperparameters tuning.

This study applies a state-of-the-art deep learning model to detect lung diseases in five out of fourteen categories in the VinBigData dataset, including aortic enlargement [8], cardiomegaly [9], interstitial lung disease (ILD) [10], infiltration [11] and nodule/mass [12]. Transfer learning techniques were applied by employing a pre-trained RetinaNet [13] model with a ResNet101 [14] backbone, in combination with the smooth L1 loss function [15] for bounding box regression and focal loss (FL) [13] for image classification. Weighted box fusion (WBF) [16] was applied in the first stage to eliminate overlapping ground truth bounding boxes and in last stage to eliminate overlapping predicted bounding boxes. The FPN [13] and FL-based RetinaNet provide an end-to-end approach that achieves high accuracy. The evaluated score from multiple metrics of this solution is promising, accurate, and can be encapsulated via a website to aid doctors in thoracic lung disease detection.

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

2. Materials and methods

The original dataset, consisting of 18,000 poster-anterior (PA) chest X-ray (CXR) scans in DICOM format with total size is 191.82 GB, was supplied directly by VinBigData [3]. However, this study utilised the dataset indirectly provided by VinBigData, which was converted to JPEG images with a total size of 3.4 GB [17]. This pure Vietnamese dataset includes 15,000 CXR images for training and 3,000 images used to evaluate algorithm models. The data were collected from 108 Military Central Hospital and Hanoi Medical University Hospital, with each image read and diagnosed by professional clinicians.

Of the 18,000 X-ray images provided, 15,000 images were used for the training set, with each image having multiple annotations by doctors via a CSV file. The remaining 3,000 images were used for the test set without any annotations. All images in the training set were labelled for the presence of 14 diseases: aortic enlargement, atelectasis [18], calcification [19], cardiomegaly, consolidation [20], ILD, infiltration, lung opacity [21], nodule/mass, other lesion, pleural effusion [22], pleural thickening [23], pneumothorax [24], and pulmonary fibrosis [25]. The “No finding” class was used to indicate the absence of all the above findings.

The annotations for the training data consist of one row for each object, including the type of disease and offset value of the ground truth bounding box. The following columns are included with each row: *image_id*: image filename, *class_name*: the name of the diagnosed disease of the object (or No finding), *class_id*: the ID of the disease of detected object, *rad_id*: the ID of doctor who made the observation, and *x_min*, *y_min*, *x_max*, *y_max*: the offset values of the object's bounding box. If the *class_name* is “No finding,” then the values in the columns (*x_min*, *y_min*, *x_max*, *y_max*) are not a number (NaN) as shown in Table 1.

Table 1. Sample ground truth from the doctors.

image_id	class_name	class_id	rad_id	x_min	y_min	x_max	y_max
50a418190bc3fb1ef1633b9678929b3	No finding	14	R11				
21a10246a5ec7af151081d0cd6d65dc9	No finding	14	R17				
9a5094b2563a1ef3ff50dc5c7ff71345	Cardiomegaly	3	R10	230.0	458.0	551.0	610.0
051132a778c61a86eb147c7c6f564dfe	Aortic enlargement	0	R10	421.0	247.0	537.0	339.0
063319de25ce7ed9b1c6b8881290140	No finding	14	R10				

2.1. Data analysis

The original CSV file had 67914 rows with 15,000 images, with each row containing the annotation of each image's ID by an individual doctor. As a result, each image was diagnosed by many doctors. The positive class names are the 14 diseases, which account for 36,096 (53%) annotations and 4,394 (29%) images. The rest of the training data belongs to the negative class, which is “No finding” - as shown in Fig. 1.

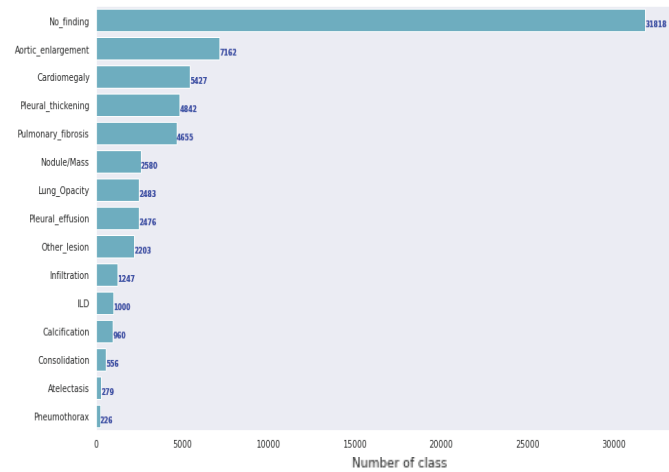


Fig. 1. The number of classes.

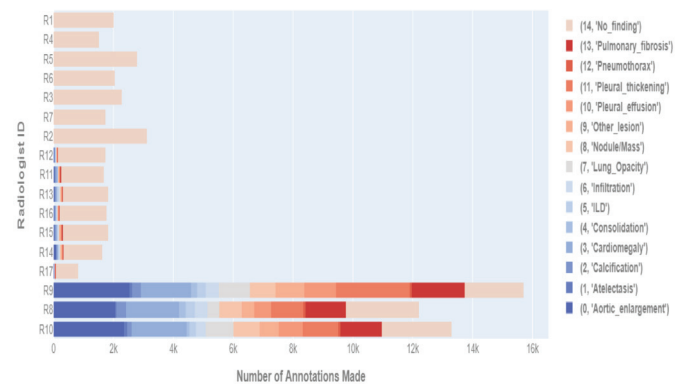


Fig. 2. The number of annotations of each doctor.

Figure 2 illustrates the relationship between the number of classes and the doctors' contribution by plotting their contributions. The dominance of the “No finding” class is due to the difference in the doctors' contributions. Fourteen out of the seventeen doctors have 80% of the “No finding class” in their contribution, and six of them have 100% of the “No finding” class. Meanwhile, certain doctors mainly annotated all images with positive classes (i.e., those that are not “No finding”).

In object detection, the negative class name is not used to train model, so all annotations of the negative class need to be dropped. Based on our analysis of the explored data and data description, we divided the doctors with positive classes into two groups. All annotations of doctor number 8 (R8), doctor number 9 (R9), and doctor number 10 (R10) contain almost all images with positive classes, 4,146 images, which account for 94.35% of the images. All annotations from doctor number 11 (R11) through doctor number 17 (R17) are included in 248 images with positive classes that are not the same images of the first group (of R8, R9, R10).

Based on the heatmap of the bounding boxes of the classes, the diagnostic consistency of three doctors and disease location, the first group is considered a standard dataset for training models. In addition, both groups of doctors have mismatched annotations. Pneumothorax is known as a collapsed lung, but as shown in Fig. 3, the annotations from R10 indicate the pneumothorax is detected in

the middle bone. R10 also labelled that location as “Other lesion,” which is why the pneumothorax and “Other lesion” have the same offset values of bounding boxes. This way of annotation has a problem. R10 may have intended to label that location as “Other lesion” but mistakenly labelled it as pneumothorax, or they may have forgotten to remove the pneumothorax annotation.

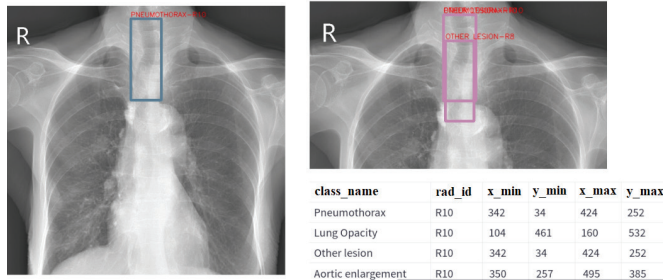


Fig. 3. Examples of wrong annotation from doctors.

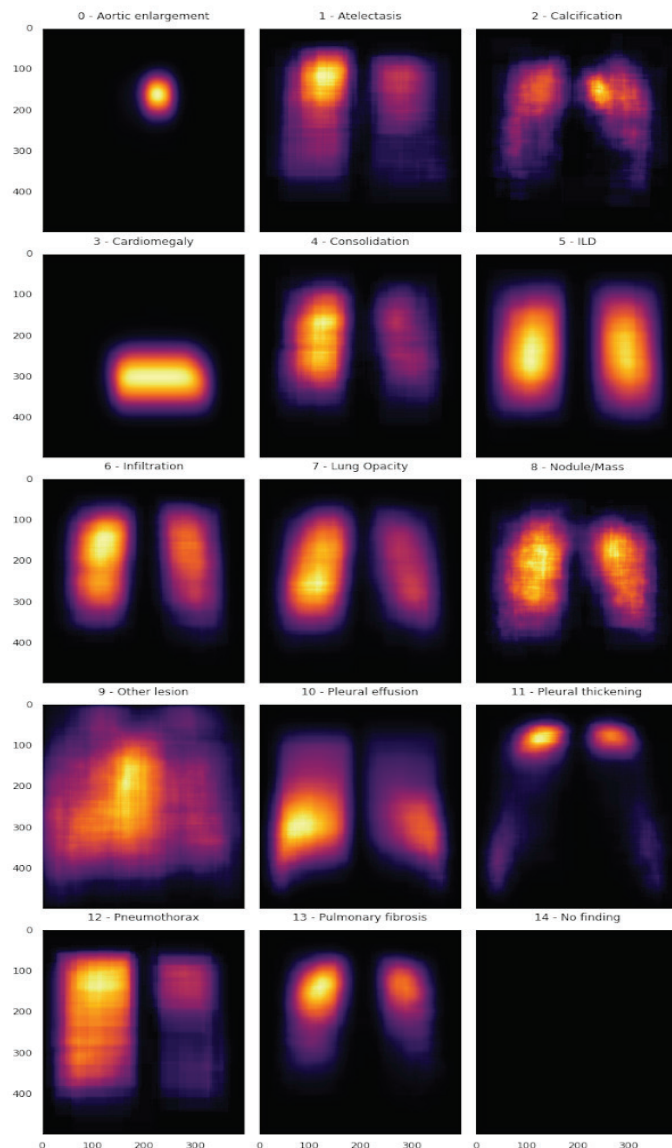


Fig. 4. Heat map for the 14 different diseases and “No finding”.

Figure 4 shows the heat map of all bounding boxes of 14 diseases in this dataset. Only two diseases, aortic enlargement and cardiomegaly, have centralized bounding boxes. The bounding boxes of the other diseases are too scattered. For instance, the ILD bounding boxes are located in two main regions (one on the left and one on the right side of the chest), but within these regions, the diseases can be labelled at different locations. The areas marked with ILD on the right side of Fig. 5, annotated by R11, are smaller than that annotated by R15. The reason for the difference in the bounding boxes for this ILD by different doctors is that some doctors may want to identify the damage clearly and draw the box right at the damaged tissue position, while other doctors draw the box bigger to encompass the entire location. The bounding boxes for pneumothorax, consolidation, and lung opacity are also located in two main regions, the left and right side of the chest, but are concentrated mainly on the right side. Another particular case that shows inconsistency in annotation is “Other lesion.” Although it mainly locates in the middle region, it can appear at many different regions, resulting in a high dispersion of the bounding box positions for “Other lesion.”

Figure 5 clearly shows the scattered property of the bounding box distributions. The ground truth bounding boxes of “Other lesion,” annotated by different doctors, are located in three different main regions, namely, lower right-hand area, right lung location, and the upper part of the neck. The “Other lesion” in the VinBigData dataset refers to a lesion of the thoracic lung that does not belong to the 13 other mentioned diseases (aortic enlargement, atelectasis, calcification, cardiomegaly, consolidation, ILD, infiltration, lung opacity, nodule/mass, pleural effusion, pleural thickening, pneumothorax, and pulmonary fibrosis). Another example that clearly shows inconsistency in annotation is the image on the right side of Fig. 5, which shows ILD disease. It can be observed that the sizes of bounding boxes annotated for ILD very greatly among different doctors.

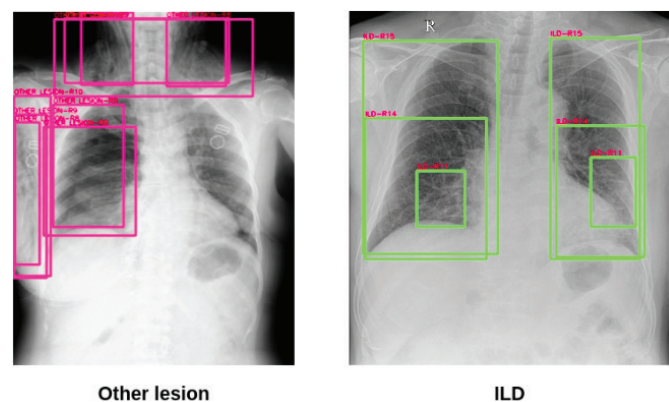


Fig. 5. Examples of inconsistency in doctors' diagnoses.

2.2. Data processing

Because of all of the differences in the size of the bounding boxes mentioned above, in this study, we did not eliminate the ground truth bounding boxes but modified them based on the

Intersection over Union (IoU) of all bounding boxes in the same class. There are several techniques depending on IoU, such as Non-Maximum Suppression (NMS) [26], Soft Non-Maximum Suppression (Soft-NMS) [27], and WBF [16] (Table 2). This study applied all three techniques to compare their results.

Table 2. Performance of NMS, Soft-NMS and WBF IoU techniques.

	Number of boxes
Original boxes	36,096
NMS@0.5	23,940 (↓ 33.7%)
Soft-NMS@0.5	32,273 (↓ 10.6%)
WBF@0.5	23,955 (↓ 33.64%)

Figure 6 explains the difference between NMS and WBF. WBF is based on all the ground truth bounding boxes of three doctors (R8, R9, R10) and generates the new bounding boxes. In another circumstance, the NMS chooses the bounding boxes with highest confidence score. However, in this case, all bounding boxes are the ground truths, and therefore all of the confidence scores of those boxes are equal to 1. Consequently, NMS chooses randomly. In Fig. 6, NMS randomly chose the ground truth bounding box of doctor R2. That is why the performance of WBF is better than that of NMS because it generates new bounding boxes based on all information on the ground truth bounding boxes of the doctors. Fig. 7 shows the different performances of NMS and WBF with an IoU threshold equal to 0.2. NMS eliminates the large “Other lesion” ground truth in the middle, while WBF generates new ground truth based on all ground truth bounding boxes, resulting in new large “Other lesion” ground truth bounding boxes on the left.

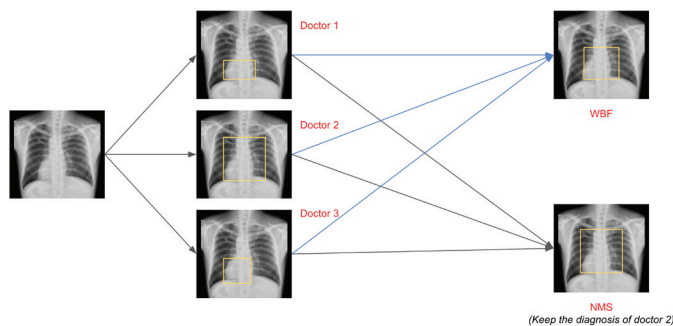


Fig. 6. Comparison of the NMS and WBF techniques.

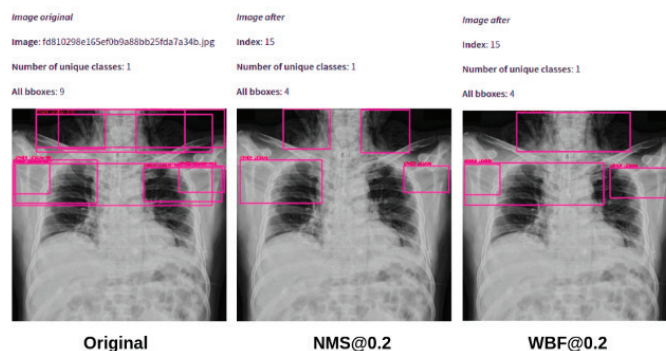


Fig. 7. Performance of NMS and WBF at an IoU threshold of 0.2.

Based on the data analysis section, the original dataset was split into two groups. The first group contains all annotations of R8, R9, and R10, which account for almost all of the images (94.35%) and is called the standard dataset. The second group contains all annotations from R11 through R17, which contains other parts of images and has many errors in the bounding boxes due to inconsistent diagnoses by doctors. After processing this group, it is called the Additional Dataset.

Figure 8 shows that the performance of WBF varies with different IoU thresholds. When the IoU is 0.3, WBF reduces several ground truth bounding boxes, while at an IoU of 0.2, the new ground truth bounding box on the top right contains the wrong location of the calcification disease. That is why we must calculate the IoU of each disease with different diagnoses of doctors and then obtain the average value of those IoUs. This study used the standard dataset to calculate the average value of all diseases except “Other lesion” and pleural thickening because those two diseases have dispersion in the heat map (Fig. 4). Our result of the IoU threshold is 0.4, as shown in Table 3.

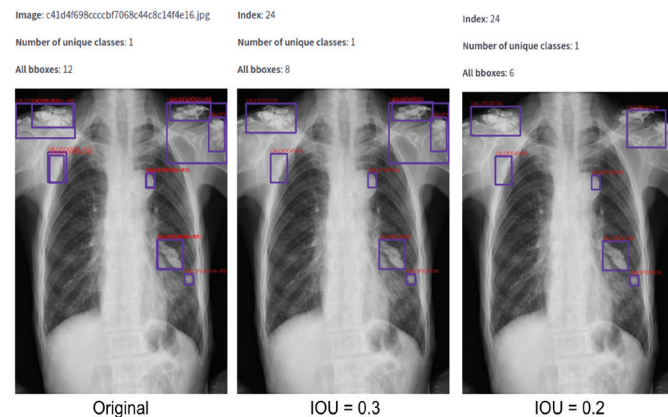


Fig. 8. WBF with different IoU values.

Table 3. Various IoU thresholds of WBF.

Class name	IOU in ground truth	IOU threshold
Aortic enlargement	0.688	0.3
Atelectasis	0.48	0.5
Calcification	0.55	0.5
Cardiomegaly	0.73	0.3
Consolidation	0.6	0.4
ILD	0.59	0.4
Infiltration	0.57	0.4
Lung Opacity	0.52	0.5
Nodule/Mass	0.64	0.4
Other lesion	0.5	0.5
Pleural effusion	0.5	0.5
Pleural effusion	0.47	0.5
Pneumothorax	0.68	0.5
Pulmonary fibrosis	0.5	0.5
Mean with except “Other lesion” and pleural thickening		0.4

To process all annotations from R11 to R17, min-max normalisation is first applied for the offset value of the ground truth bounding boxes. Then, the area of the ground truth bounding boxes is calculated after each disease normalization. The values are then averaged for each disease saved in the standard dataset. Table 4 compares the diagnoses of each doctor in the Additional Dataset. In Table 5, the standard dataset with doctor R11 is compared to the Additional Dataset to obtain suitable ground truth bounding boxes for R11.

Table 4. Mean bounding boxes area norm in the standard dataset.

Rad_id	Class name	Num bounding boxes	Mean bounding boxes area norm
R8_R9_R10	Aortic enlargement	6956	0.0145
	Atelectasis	230	0.04917
	Calcification	758	0.00764
	Cardiomegaly	5268	0.05997
	Consolidation	520	0.03731
	ILD	812	0.07503
	Infiltration	1189	0.03886
	Lung opacity	2382	0.02751
	Nodule/mass	2468	0.00427
	Other lesion	2035	0.0248
	Pleural effusion	2395	0.02423
	Pleural effusion	4741	0.00698
	Pneumothorax	219	0.09259
	Pulmonary fibrosis	4489	0.01511
Total annotations: 34462/36096			
Total images: 4146/4394			

Table 5. Standard dataset and R11 annotations.

Rad_id	Class name	Residual bounding boxes area norm (R11-R8_R9_R10)	Ratio bounding boxes area norm (R11/R8_R9_R10)
R8_R9_R10 and R11	Aortic enlargement	0.03701	3.55
	Atelectasis	0.0093	1.19
	Calcification	0.00049	1.06
	Cardiomegaly	0.04316	1.72
	Consolidation	0.01147	1.31
	ILD	-0.04563	0.39
	Infiltration	-0.03886	0
	Lung opacity	-0.01554	0.43511
	Nodule/mass	0.00597	2.39813
	Other lesion	0.01215	1.48992
	Pleural effusion	0.00826	1.34
	Pleural effusion	0.00946	2.36
	Pneumothorax	-0.08059	0.13
	Pulmonary fibrosis	0.0033	1.2184
Total annotations: 34462/36096			
Total images: 4146/4394			

After comparing all ground truth bounding boxes of doctors R11-R17 to obtain the novel Additional Dataset, it was added into the standard dataset to generate the Final data. Fig. 9 summarises the above data processing steps.

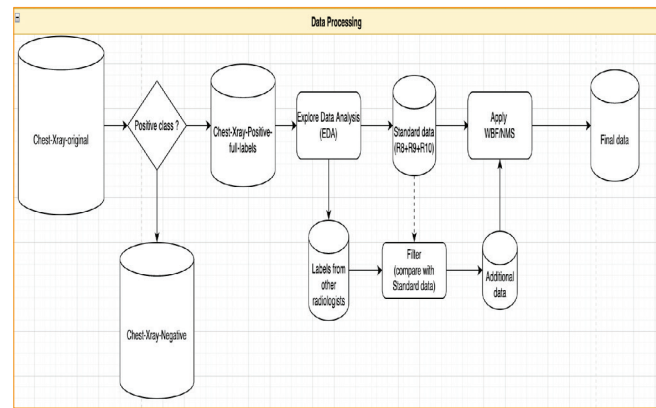


Fig. 9. Data processing steps.

After generating the Final data, several image augmentation techniques were applied such as padding, horizontal flip, and image rotation. Then, the data was randomly split with 80% used for the training dataset and 20% for validation dataset, as seen in Fig. 10.

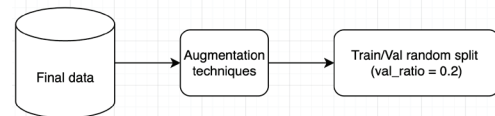


Fig. 10. Augmentation and Train-Val split.

Figure 11 summarises the process from analysing the data to splitting the training data and deploying it into production.

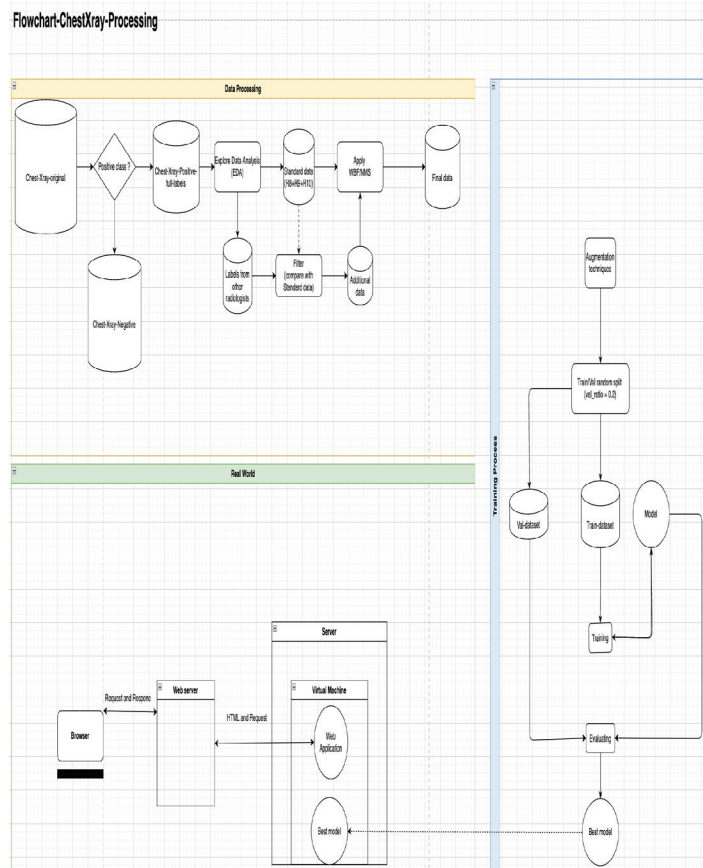


Fig. 11. Flowchart of the study.

2.3. Bounding box suppression techniques

In object detection models, having many overlapped bounding boxes can lead high recall values. Thus, the NMS technique is applied to eliminate redundant overlapping bounding boxes. NMS greedily selects high scoring detections and deletes nearby, less confident neighbours since they are likely to cover the same object. This algorithm is simple, fast, and surprisingly competitive compared to proposed alternatives [26]. The NMS algorithm is described in Algorithm 1.

Algorithm 1: Non-maximum suppression

input: $B = \{b_1, b_2, \dots, b_N\}$, $S = \{s_1, s_2, \dots, s_N\}$, N_t
 B : the list of bounding boxes of single class in individual image
 S : the list of confidence scores corresponding to bounding boxes
 N_t : the NMS threshold

begin
 $D \leftarrow \{\}$
while $B \neq \text{empty}$ **do**
 $m \leftarrow \text{argmax } S$
 $M \leftarrow b_m$
 $D \leftarrow D \cup M$
 $B \leftarrow B - M$
for b_i in B **do**
if $\text{iou}(M, b_i) \geq N_t$ **then**
 $B \leftarrow B - b_i$
 $S \leftarrow S - s_i$
end
end
end
return D, S
end

Soft-NMS is a variant of NMS. Instead of eliminating the redundant overlapping bounding boxes, Soft-NMS penalizes the redundant bounding boxes by reducing their confidence scores. The Soft-NMS algorithm is shown in Algorithm 2.

Algorithm 2: Soft non-maximum suppression

input: $B = \{b_1, b_2, \dots, b_N\}$, $S = \{s_1, s_2, \dots, s_N\}$, N_t
 B : the list of bounding boxes of single class in individual image
 S : the list of confidence scores corresponding to bounding boxes
 N_t : the NMS threshold

begin
 $D \leftarrow \{\}$
while $B \neq \text{empty}$ **do**
 $m \leftarrow \text{argmax } S$
 $M \leftarrow b_m$
 $D \leftarrow D \cup M$
 $B \leftarrow B - M$
for b_i in B **do**
 $s_i = \begin{cases} s_i & \text{if } \text{iou}(M, b_i) \leq N_t \\ s_i(1 - \text{iou}(M, b_i)) & \text{if } \text{iou}(M, b_i) \geq N_t \end{cases}$
end
end
return D, S
end

The WBF method is used to combine predictions of object detection models. Unlike the NMS and soft-NMS methods, which simply remove part of the predictions, the proposed WBF method uses the confidence scores of all the proposed bounding boxes to construct average boxes [16]. Algorithm 3 describes the WBF process.

Algorithm 3: Weighted boxes fusion

input: $B = \{b_1, b_2, \dots, b_N\}$, $S = \{s_1, s_2, \dots, s_N\}$, N_t
 B : the list of bounding boxes of single class in individual image
 S : the list of confidence scores corresponding to bounding boxes
 N_t : the NMS threshold

begin
 $WB \leftarrow \{\}$
 $WS \leftarrow \{\}$
 $FB \leftarrow \{\}$
 $FS \leftarrow \{\}$
while $B \neq \text{empty}$ **do**
 $m \leftarrow \text{argmax } S$
 $M \leftarrow b_m$
 $WB \leftarrow b_m$
 $WS \leftarrow s_m$
 $B \leftarrow B - M$
for b_i in B **do**
if $\text{iou}(M, b_i) \geq N_t$ **then**
 $WB \leftarrow b_i$
 $WS \leftarrow s_i$
else
 $FB \leftarrow b_i$
 $FS \leftarrow s_i$
end
end
 $FB \leftarrow f_b(WB)$
 $FS \leftarrow f_s(WS)$
end
end

In this algorithm, $WB = \{b_1, b_2, \dots, b_K\}$ is the list of overlapping bounding boxes with an IoU greater than the threshold (N_t). $WS = \{s_1, s_2, \dots, s_K\}$ is the list of confidence scores corresponding to WB with each $b_i = \{x_i^{\min}, x_i^{\max}, y_i^{\min}, y_i^{\max}\}$, and s_i is confidence score corresponding to b_i . The weighted boxes function f_b and confidence score rescale function f_s are given in detail below:

Function f_b :

$$x^{\min} = \frac{\sum_{i=1}^K s_i \times x_i^{\min}}{\sum_{i=1}^K s_i} \quad x^{\max} = \frac{\sum_{i=1}^K s_i \times x_i^{\max}}{\sum_{i=1}^K s_i}$$

$$y^{\min} = \frac{\sum_{i=1}^K s_i \times y_i^{\min}}{\sum_{i=1}^K s_i} \quad y^{\max} = \frac{\sum_{i=1}^K s_i \times y_i^{\max}}{\sum_{i=1}^K s_i}$$

Function f_s :

$$S = \frac{\sum_{i=1}^K s_i}{N}$$

K is the number of overlapped bounding boxes.

2.4. Methods

2.4.1. Transfer learning with RetinaNet

Within deep learning, transfer learning is a technique whereby a neural network model is first trained on a problem similar to the problem that is being solved. One or more layers from the trained model are then used in a new model trained on the problem of interest [28].

Based on the advantages of transfer learning and the goal of this study, a popular model called RetinaNet was employed, which was presented by T.Y. Lin, et al. (2017)

[13] from Facebook AI study. RetinaNet is a one-stage object detection model that utilizes a FL function to address class imbalance during training. FL applies a modulating term to the CE loss in to focus learning on hard negative examples [13]. RetinaNet's network architecture uses an FPN in the backbone, with the bottom-up being the ResNet architecture [14].

2.4.2. Loss functions

Loss functions are one most important aspects of a deep learning model. They compute the distance (or error) between the actual values and predictions of the models to optimize parameter values in the models. In this study, two loss functions are applied, namely, FL for classification task and Smooth L1 Loss [15] for regression tasks.

FL is designed to address one-stage object detection scenarios in which an extreme imbalance between foreground and background classes during training exists [13]. The formula for FL is:

$$FL(p_t) = -\alpha_t(1 - p_t)^\gamma \times \log(p_t) \quad (1)$$

where p is the model's estimated probability for the class with a label of $y=1$ in binary classification: $p_t = \begin{cases} p & \text{if } y = 1 \\ 1 - p & \text{otherwise} \end{cases}$, α_t is the balance variant of FL to address the imbalance problem, and γ is the tuneable focusing parameter. When $\gamma=0$, FL is equivalent to cross-entropy (CE) [29] and as γ increases, the effect of the modulating factor is likewise increased [13].

Smooth L1 loss for object detection was originally proposed in Fast R-CNN [15]. Smooth L1 loss is used to make bounding box regression more robust by replacing the excessively strict L2 loss. The formula of Smooth L1 Loss is:

$$L_{loc}(t^u, v) = \sum_{i \in \{x, y, w, h\}} smooth_{L1}(t_i^u - v_i) \quad (2)$$

where u is ground-truth of class u ; v is ground-truth offset values of the bounding box, and

$$smooth_{L1}(t_i^u - v_i) = \begin{cases} 0.5(t_i^u - v_i)^2 & \text{if } |t_i^u - v_i| < 1 \\ |t_i^u - v_i| - 0.5 & \text{otherwise} \end{cases} \quad (3)$$

2.4.3. Metrics evaluation

In object detection, some applicable evaluation metrics are Intersection over Union (IoU), common statistic metrics such as True Positive (TP), True Negative (TN), False Positive (FP), False Negative (FN), Precision, Recall, confidence score, and mean Average Precision (mAP). Min-max normalisation [30] is used to standardise data in the data processing.

IoU is a metric evaluation that describes the extent of overlap of two bounding boxes, and a simple implementation of IoU is described in Fig. 12.

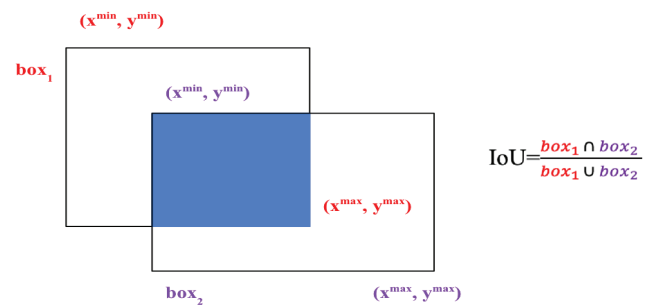


Fig. 12. Intersection over union formula.

TP is the total number of correct positive predictions of the model, TN is the total number of correct negative predictions of model, FP is the total number of incorrect positive predictions of model, and FN is the total number of incorrect negative predictions of model. However, in an object detection problem, TP is the number of predicted bounding boxes of the model with an IoU of the ground truth bounding box greater than the IoU threshold (in most cases the IoU threshold is 0.5). FP is the number of predicted bounding boxes of the model with an IoU lower than the IoU threshold, FN is number of models that cannot predict an object in the image, and TN is the number of models that correctly predict background in the image but in the object detection problem.

The precision in object detection is the ratio of correctly predicted bounding boxes of the model and all bounding boxes predicted by the model. Meanwhile, recall is the ratio of the correct bounding boxes predicted by the model and all ground truth bounding boxes.

$$precision = \frac{\text{correct bounding boxes prediction}}{\text{all bounding boxes prediction}} \quad (4)$$

$$recall = \frac{\text{correct bounding boxes prediction}}{\text{all ground truth bound boxes}} \quad (5)$$

The confidence score describes how the model predicts this bounding box for each class. A high confidence score means that the bounding boxes are accurately predicting the location and classification of the objects. The formula for confidence score is show below:

$$s = P(\text{object}) \times IoU(\text{object}, \text{groundtruth}) \quad (6)$$

Figure 13 illustrates how the confidence score is calculated in object detection.

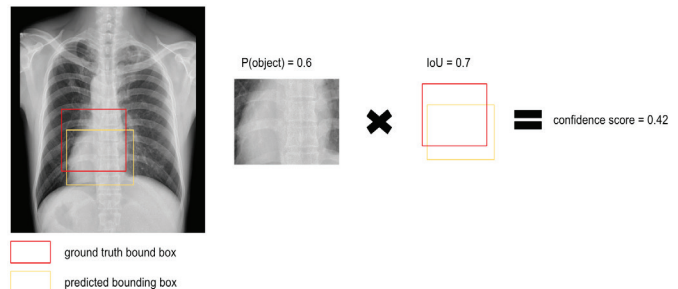


Fig. 13. Confidence score calculation method.

Average Precision (AP) is the average precision corresponding to the recall when changing the confidence score threshold. The confidence score threshold is the threshold used to control the prediction of model. For example, if the predicted bounding boxes have a confidence score lower than the confidence score threshold, then the predicted bounding boxes cannot be displayed as a prediction of the model, thus precision and recall are affected. Therefore, AP is the average precision when changing the confidence score from 1.0 to 0.

The mAP is the average AP of all the classes. The formula for mAP is

$$mAP = \frac{\sum_{i=1}^N AP_i}{N} \quad (7)$$

where N is total number of classes in the dataset, i is the confidence score from 1.0 to 0. The mAP value is affected by the IoU threshold because when changing the IoU threshold, it changes the value of TP and FP. Thus, $mAP@(\text{IoU threshold})$ is used to denote the mAP value corresponding to IoU threshold value. Common types of mAP are $mAP@0.5$ mean mAP with IoU threshold is 0.5 and $mAP@[0.5:0.95]$ means that the average of mAP with IoU threshold from 0.5 to 0.95 with increase step is 0.5.

The purpose of normalisation is to have every data point on the same scale, and min-max normalisation is one of the most common methods of data normalization. The min-max normalisation procedure scales the minimum value to 0 and the maximum value to 1. Every other value is transformed into a decimal value between 0 and 1. In this study, min-max normalisation were applied to the offset values of the ground truth bounding boxes instead of the X-ray images. The min-max normalization method for offset values of ground truth bounding boxes is given as follows:

$$x_i^{\min_{norm}} = \frac{x_i^{\min}}{image_width_i} \quad (8)$$

$$y_i^{\min_{norm}} = \frac{y_i^{\min}}{image_height_i} \quad (9)$$

$$x_i^{\max_{norm}} = \frac{x_i^{\max}}{image_width_i} \quad (10)$$

$$y_i^{\max_{norm}} = \frac{y_i^{\max}}{image_height_i} \quad (11)$$

where i is the i^{th} image, and the area of ground truth bounding box after normalization is:

$$area_i^{norm} = (x_i^{\max_{norm}} - x_i^{\min_{norm}}) \times (y_i^{\max_{norm}} - y_i^{\min_{norm}}) \quad (12)$$

3. Implementation of model

3.1. Model overview

As shown in Fig. 14, the training process of our system employed the RetinaNet model. Furthermore, based on experimental results, ResNet101 was chosen as the best backbone for RetinaNet in this study.

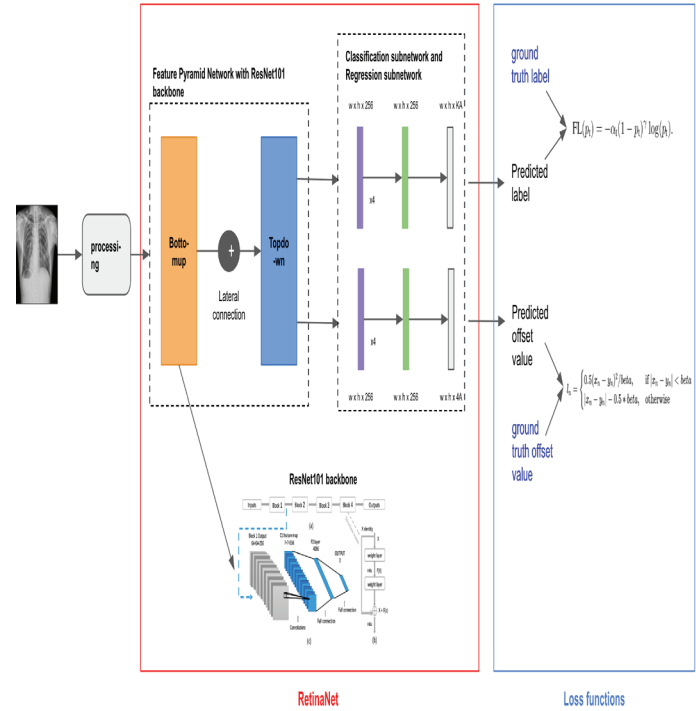


Fig. 14. Model architecture.

3.2. Implementation

3.2.1. FPN with ResNet101 backbone

Figure 15 illustrates the working mechanism of FPN with the ResNet101 backbone. As seen on the left-hand side of Fig. 15, the bottom-up pathway contains a five-stage feature map (C1-C5), which has different scales and channels. The right-hand side shows the top-down structure of FPN, which contains five components (P2-P6), with each component having a lateral connection with the corresponding stage of the feature map of the bottom-up pathway. Components P2-P6 have 256 channels, which are the output of the FPN. The purpose of FPN is to extract the most informative data from the image.

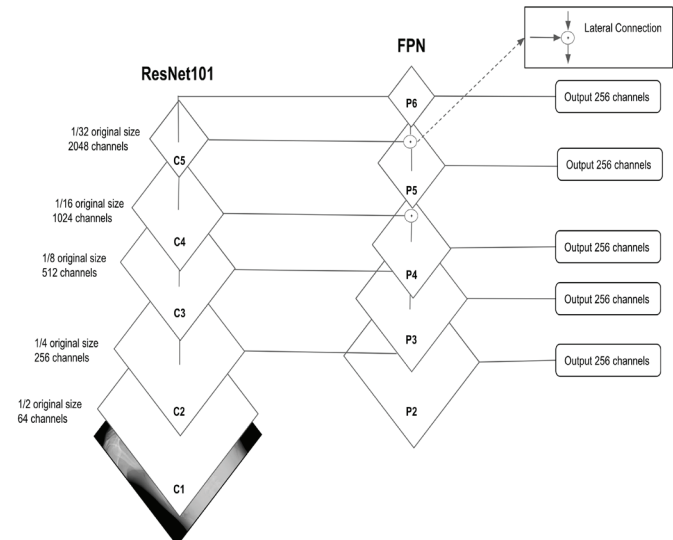


Fig. 15. The FPN with ResNet101 backbone.

Table 6. Performance of RetinaNet with ResNet50 backbone of the 14 diseases.

Class	mAP@[0.5:0.95]
Aortic enlargement	0.5457
Atelectasis	0.0456
Calcification	0.0023
Cardiomegaly	0.5170
Consolidation	0.0311
ILD	0.0508
Infiltration	0.0568
Lung Opacity	0.0398
Nodule/Mass	0.0454
Other lesion	0.0015
Pleural effusion	0.0926
Pleural thickening	0.0323
Pneumothorax	0.0012
Pulmonary fibrosis	0.0322
mAP@[0.5:0.95]: 0.1067	
mAP@0.5: 0.2174	

3.2.2. Classification and regression subnetworks

The outputs of RetinaNet are the predictive class labels and the offset value of bounding boxes corresponding to the predictive class labels. Therefore, RetinaNet has two subnetworks for the output: one of them treats the region of interest as image classification and the other treats bounding boxes regression.

4. Results and discussion

4.1. Experiment with all diseases

RetinaNet with ResNet50 and an FPN backbone (3x) gave the best results in the validation set without augmentation after 2500 epochs by applying Detectron2 from the Facebook AI study [31]. Table 6 shows the performance of the model with mAP@[0.5:0.95].

4.2. Experiment with 5 diseases

Based on the results from Table 6 and the problems in data processing, this study selected 5 of the 14 classes that were highly trainable: aortic enlargement, cardiomegaly, ILD, infiltration and nodule/mass. Because of the dataset imbalance,

Table 7. Performance of RetinaNet with different backbones and augmentation methods.

Backbone of FPN	Augmentation	mAP@[0.5:0.95]	mAP@0.5
ResNet50	None	0.2627	0.4547
ResNet50	Horizontal flip + Rotation	0.3052	0.535
ResNet101	Horizontal flip + Rotation	0.3193	0.5500

image augmentation methods like horizontal flip, rotation, and oversampling were deployed for the dataset. Table 7 shows the results of the model in the validation set. RetinaNet with ResNet101 and an FPN backbone gave the best result.

Figure 16 shows the loss functions of ResNet 101 with the FPN backbone during the training procedure. The loss function generally decreased; however, the decrease was not stable because the ground truths labelled by doctors were inconsistent, causing difficulty during the training process. The top right subfigure of Fig. 16 gives the learning rate, and this study applied the learning rate schedule technique [32] with a Cosine warm-up function.

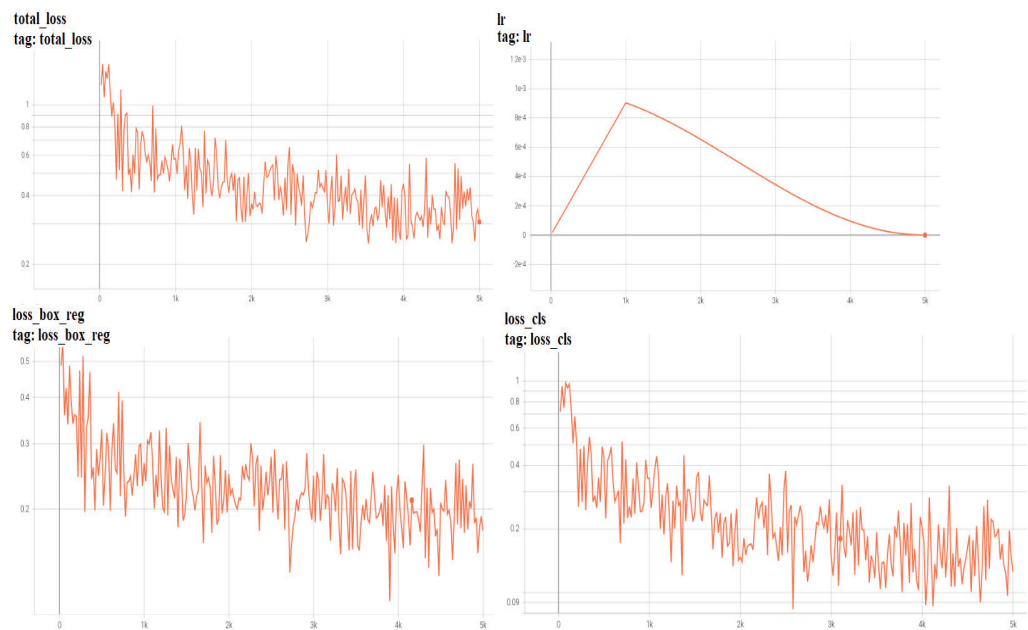


Fig. 16. Loss function and learning rate values during the training process.

4.3. Discussion

Table 8 compares the mAP of the 14 lung disease predictions from the proposed method and other publications.

Table 8. Comparison of the proposed method and other studies.

Study	Score
Our method	0.22 (mAP@0.5)
H. Huang, et al. (2021) [6]	0.21 (mAP@0.5)
Leader in Kaggle competition	0.31 (mAP@0.4)

In H. Huang, et al.'s study (2021) [6], the same dataset collected by VinBigData [3] was used, but with full quality DICOM-format images, while this study utilized lower quality (3x lower) JPEG-format images [17]. In the processing stage of H. Huang's study, the "No finding" class was removed, and images were rescaled to 512x512 pixels as input. The H. Huang, et al.'s study (2021) [6] used the Yolo-v5 [33] model as a baseline. Although the Yolo model is a popular model for object detection, it struggles with the various sizes of abnormalities in each image and new or unusual aspect ratios of images in this type of medical problem. Furthermore, Yolo struggles with imbalanced datasets and small labels inside large labels. The VinBigData Kaggle Competition (2022) [34] published a leaderboard with the winners of the competition, but unfortunately they did not propose any method or publication to explain CXR abnormality detection solution.

Although the proposed study is not among the top performers, the suggested method still has several advantages and proposes new ways to explore and process datasets to generate new datasets with more consistent and homogeneous properties. Firstly, the proposed method analyses the dataset through data science and statistical approaches. Secondly, it generates new ground truths by applying WBF and comparing the labels of doctor to reduce inconsistency in the dataset. Finally, it applies several optimization techniques such as learning rate schedule and different loss functions to boost the model performance.

5. Conclusions

This study has proposed a new method to detect abnormalities in CXR images using the RetinaNet model and applying WBF and statistical analysis to process data for improving the performance of the deep learning model. The performance of the proposed study achieved 0.22 mAP@0.5 for 14 diseases and 0.55 for 5 potential diseases (aortic enlargement, cardiomegaly, ILD, infiltration and nodule/mass). Overall, the achieved results indicate that the proposed method has promising potential for the development of processing-based approaches for the automatic detection of abnormalities in CXR images.

CRedit author statement

Nhan Ngo: Conceptualisation, Methodology, Software, Resources, Writing, Reviewing, Editing; Toi Vo: Reviewing, Editing; Lua Ngo: Supervision, Reviewing, Editing.

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COMPETING INTERESTS

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

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Nutritional effects on biomass and metabolic products by *Aurantiochytrium* sp.

Pham Thi Mien^{1*}, Dao Viet Ha¹, Cornelis Verduyn²

¹Institute of Oceanography, Vietnam Academy of Science and Technology, 1 Cau Da Street, Vinh Nguyen Ward, Nha Trang City, Khanh Hoa Province, Vietnam

²Biotechnology Department, Mahidol University, 999 Phuttamonthon 4 Road, Salaya Ward, Nakhon Pathom Province, Bangkok, Thailand

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Abstract:

Docosahexaenoic acid, or Docosahexaenoic acid (DHA), plays a pivotal role in biological functions and is beneficial for both humans and animals. Traditionally, DHA is sourced from fish and fish oil. Due to the disadvantages associated with these sources, such as marine pollution and variable composition, there is a pressing need to explore alternative, reliable sources for DHA. *Aurantiochytrium* sp. has been identified as a promising candidate for DHA production. The primary aim of this study was to cultivate three strains in previously published media and, subsequently, to develop defined or semi-defined media for *Aurantiochytrium* sp.. These strains were grown in the selected media, and the dry weight of the cells was measured. Total fatty acids were extracted and analysed using gas chromatography. All three strains demonstrated satisfactory growth in media that incorporated glucose as a carbon source and monosodium glutamate as a nitrogen source. Strain B072 achieved the highest DHA concentration, with a peak of 5.0 g/l, and could accumulate lipids up to 57.57% (w/w). The DHA content in the biomass was 21.43% (w/w), with a biomass yield of 23.35 g/l. Vitamins and trace elements positively influenced the growth and DHA production of *Aurantiochytrium* sp.. Furthermore, strain B072 efficiently utilised ammonium for growth and fatty acid production.

Keywords: *Aurantiochytrium* sp., defined media, DHA production, metabolites, nutrient.

Classification numbers: 3.4, 3.5

1. Introduction

There is little doubt that omega-3 fatty acids hold significant importance in human nutrition. DHA is a primary structural component of the phospholipid membranes of tissues throughout the body, particularly prevalent in the retina, the grey matter of the brain, and the spermatozoa. Notably, in these areas, DHA (C22:6n-3) constitutes up to 36.4% of total fatty acid [1, 2]. As components of cell membranes, omega-3 fatty acids enhance membrane fluidity, which influences a variety of membrane functions, including eicosanoid signalling, ion channel modulation, and regulation of gene expression [3, 4]. Dietary omega-3 fatty acids reduce the risk of cardiovascular disease (CVD). Both epidemiological and interventional studies have shown the beneficial effects of omega-3 fatty acids on various CVD endpoints, encompassing all CVD (defined as all coronary artery disease [CAD], both fatal and nonfatal myocardial infarction, and stroke combined) [5, 6]. DHA is crucial for the growth and functional development of the brain in infants. This fatty acid is transferred across the placenta and accumulates in the brain and other organs during foetal development. Depletion of DHA from the

retina and brain results in diminished visual function and learning deficits, which may stem from DHA's essential role in membrane-dependent signalling pathways and neurotransmitter metabolism. DHA is also necessary for maintaining normal brain function in adults. A diet rich in DHA enhances learning ability, while DHA deficiencies correlate with learning deficits. The brain prefers DHA over other fatty acids. DHA turnover in the brain is rapid, more so than often realised. The visual acuity of healthy, full-term, formula-fed infants is enhanced when their formula contains DHA [7]. Over the past 50 years, numerous infants have consumed formula diets devoid of DHA and other omega-3 fatty acids. DHA deficiencies are linked with foetal alcohol syndrome, attention deficit hyperactivity disorder, cystic fibrosis, unipolar depression, and aggressive hostility. A decrease in the concentration of DHA in the brain are associated with cognitive degradation during ageing and with the onset of sporadic Alzheimer's disease [7, 8]. Are there detrimental effects for humans supplementing their diet with DHA? Numerous reports address this issue. Generally, DHA showed no toxicity when supplemented at low concentrations. For instance, oil from *Schizochytrium limacinum* strain FCC-3204, rich in DHA, was proposed

*Corresponding author: Email: mien.pham@gmail.com

as a novel food (NF). The applicant recommended its use in infant formulas (IF) and follow-on formulas (FOF) with DHA at levels of 20-50 mg/100 kcal. It was concluded that the NF is safe under the proposed conditions [9]. Docosahexaenoic acid-rich microalgae (DRM) from *Schizochytrium* sp. was given to groups of male and female Sprague-Dawley rats for at least 13 weeks. This study found that DRM supplementation showed no adverse effects in rats relevant to humans at dosages up to 4000 mg/kg/d [10]. The fatty acid profiles of microorganisms are swayed by environmental conditions. The medium's composition is one of the most important environmental criteria in determining final lipid composition. The concentration of carbon and nitrogen in the medium can affect DHA and lipid content, and a high carbon-to-nitrogen ratio often enhances the total lipid content and DHA yields [11].

Carbon and nitrogen sources, along with other cultivation conditions, were explored in batch fermentation to optimise DHA production from strains of the genera *Schizochytrium* and *Thraustochytriidae* [12]. Monosaccharides, glucose, and fructose proved beneficial for cell growth. Glucose emerged as the most effective carbon source for biomass, lipid, and DHA production. Though *Schizochytrium* sp. S31 could utilise disaccharides such as lactose, maltose, sucrose, and soluble starch for cell growth and lipid production, they were suboptimal for DHA production [13]. The highest DHA content (28% w/w) was noted with glycerol as a carbon source, whereas maltose and lactose yielded poor growth [14]. While glucose was generally preferred, some strains favoured fructose. For example, fructose emerged as the most suitable substrate for biomass, lipid, and DHA content in *Aurantiochytrium* sp. SW1 [15]. Both glucose and starch yielded high DHA levels, with 0.41 and 0.65 g/l, respectively, and total fatty acids of 40.8 and 49.0% (w/w) [16]. The effects of different carbon sources were studied in *Thraustochytrium* sp. ATCC 20892, with glucose producing optimal DHA yields even though biomass yields with glucose, starch, and linseed oil were consistent. However, maltose and sucrose as carbon sources resulted in inferior biomass and DHA yields, as observed by A. Singh, et al. (1996) [17]. This study seeks to investigate the influence of trace elements and vitamins, alongside defined MSG media, on the growth and DHA production in the valuable genus *Aurantiochytrium*.

2. Materials and methods

2.1. Materials

Preparation of inoculum: *S. mangrovei* Sk-02 (Sk-02), *S. mangrovei* strain B072, and *S. limacinum* B013 (B013) were employed in this study. These strains are most likely new species in the genus *Aurantiochytrium* [18, 19]. Pure

cultures were maintained on slants in solid GPY-medium containing 15 g/l agar and artificial sea salts (ASS, Sigma, USA) at 20°C, and were sub-cultured monthly. To prepare the inoculum, cells from a slant were streaked onto an agar plate and incubated at 25°C for two days. Using a Pasteur pipette, circular 1 cm diameter wells were made in the agar, and the plate was flushed with 10 ml of sterile ASS adjusted to 15%. After 2-3 h, zoospores accumulated in the wells, and this zoospore suspension served as the inoculum. 1 ml of zoospore suspension was transferred into 50 ml of sterile broth medium containing 1 g of Difco yeast extract (DYE), 1 g of peptone (Difco), 10 g of glucose, and 1 l of ASS at 15% in a 250 ml Erlenmeyer flask. The flasks were incubated at 25°C and agitated at 200 rpm for 24 h. These cultures were used as inoculums for subsequent cultivation. The broth in the inoculum flasks was harvested and centrifuged at 5,000 rpm for 10 min, then washed with 2-morpholino ethanesulfonic acid (MES). Afterwards, 5 ml of the cells were transferred into 100 ml of the subsequent medium.

Vitamins and trace elements were standardised from various literature sources [20]. A stock solution of a vitamin mixture was prepared, filtered using 0.2 µm filter paper (Minisart-Sartorius), and stored in a refrigerator. The components of the vitamin mixture per litre included: biotin 0.05 mg, para-aminobenzoic acid 0.20 mg, myo-inositol 25 mg, and 1 mg each of calcium pantothenate, nicotinic acid, thiamine HCl (B1), pyridoxine HCl (B6), riboflavin (B2), and cyanocobalamin (B12). The trace elements consisted of: ZnSO₄·7H₂O 4.5 mg, CoCl₂·6H₂O 0.3 mg, MnCl₂·4H₂O 1.0 mg, CuSO₄·5H₂O 0.3 mg, CaCl₂·2H₂O 4.5 mg, FeSO₄·7H₂O 3.0 mg, and Na₂MoO₄·2H₂O 0.4 mg per litre. The components of the media are detailed in Tables 1 and 2.

Table 1. Components of defined MSG media M19-19E.

Component (g/l)	M19	M19A	M19B	M19C	M19D	M19E
Glucose	60	60	60	60	60	60
MSG	10	10	10	10	10	10
ASS	15	15	15	15	-	-
K ₂ HPO ₄	0.3	0.3	0.3	0.6	0.3	0.3
NaCl	-	-	-	-	11.98	24
MgSO ₄ ·7H ₂ O	-	-	-	-	3.08	12
CaCl ₂	-	-	-	-	-	1
KCl	-	-	-	-	-	0.75
Vitamins	+	Reduce 5-fold	Reduce 20-fold	+	+	+
Trace elements	+	+	+	+	+	+

Table 2. Components of (NH₄)₂SO₄-MSG and MSG (5 g/l) media M20 and M27.

Component (g/l)	M20	M21	M22	M23	M24	M25	M26	M27
Glucose	30	30	30	60	30	30	30	30
DYE	2.5	-	5	-	2.5	-	-	-
(NH ₄) ₂ SO ₄	1.25	2.5	-	2.5	-	-	1.25	-
K ₂ HPO ₄	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
NaNO ₃	-	-	-	-	1.5	3	-	-
MSG	-	-	-	-	-	-	2.5	5
ASS	15	15	15	15	15	15	15	15
Vitamins	+	+	+	+	+	+	+	+
Trace elements	+	+	+	+	+	+	+	+

2.2. Dry weight determination

3 ml of broth culture was transferred into weighed centrifuge Eppendorf tubes, then centrifuged at 12,000 rpm for 10 min. The supernatant was subsequently separated. Harvested cells were rinsed thrice with distilled water, freeze-dried for 24 h, and weighed. Biomass values are presented as the means of three measurements along with their standard deviation [21]. Supernatants from the biomass were employed for glucose determination using a commercial enzymatic Glucose KIT (Human, Germany).

2.2.1. Fatty acids analysis

Freeze-dried cells (20 mg) were weighed and placed into dark vials. They were then subjected to acid-catalysed transesterification in 2 ml of 4% sulphuric acid in methanol. Heptadecanoic acid (C17-Sigma) acted as the internal standard, with butylated hydroxytoluene (BHT) added as an antioxidant (0.1 g/l). After vortex mixing, the vials were incubated in a water bath at 90°C for 1 h. Post-cooling to room temperature, the mixture was extracted twice with a combined 1 ml of hexane and 1 ml of distilled water. Anhydrous sodium sulphate (Na₂SO₄) grains were added to absorb any residual water. Extracted samples were introduced into the gas chromatograph (GC-17A-Osaka, Japan). Fatty acid methyl esters were differentiated using gas chromatography fitted with a flame ionisation detector (FID) and an Omegawax 250™ 30 m×0.25 mm×0.25 µm film thickness column. The injector was set at 250°C, accepting an injector volume of 1 µl. The column temperature was held at 200°C for first 10 min then increased up to 230°C at a rate of 10°C/min, where it remained for 14 min. Helium (He) served as the carrier gas. Fatty acid methyl esters were identified by chromatographic comparison with authentic standards (DHA-Sigma). The quantities of fatty acid methyl esters were derived from the peak areas in relation to the internal standard C17.

2.2.2. Calculations and definitions

Total fatty acid (TFA) content (% w/w) is calculated as: [Total fatty acid content (g) x 100]/Biomass (g). Percentages

of individual fatty acids were determined in both the biomass and lipid. Individual fatty acids in biomass (% w/w) is: [Each fatty acid (g/l) x 100]/Biomass (g/l). Individual fatty acids in lipid (% of TFA) is: [Each fatty acid (g) x 100]/Total fatty acid content (g). TFA (g/l) is: [TFA (%w/w) x Biomass (g/l)]/100. DHA (g/l) is: [DHA (% w/w) x Biomass (g/l)]/100.

Statistical analysis entailed a one-way analysis of variance (ANOVA). If the ANOVA yielded significant results (p≤0.05), the Student-Keuls-Newman tests were applied to discern pairwise treatment differences.

3. Results

3.1. Growth characteristics and fatty acids formation of three strains in MSG medium M19

Three strains were cultivated in medium M19 (Fig. 1). All strains demonstrated favourable growth in medium M19, where MSG functioned as the sole nitrogen source. The strain Sk-02 peaked in biomass when glucose was fully consumed. In contrast, strain B072 achieved its highest biomass at 22.4 g/l, and B013 reached 19.2 g/l when glucose was nearly depleted.

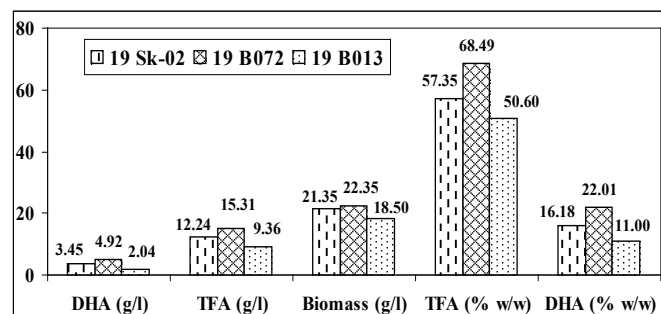


Fig. 1. DHA, DW, TFA of three strains in medium M19.

B072 was cultivated in media M19A, M19B, and M19C. In medium M19C, strain B072 attained its maximum biomass of 23.35 g/l. In other media, B072 consistently reached its peak biomass when glucose was entirely exhausted (Fig. 2).

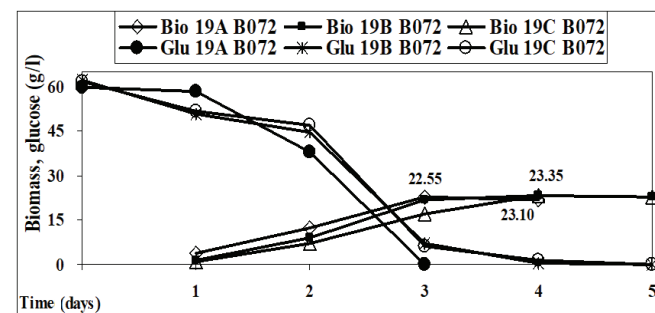


Fig. 2. Biomass and glucose levels for B072 modified MSG media M19A-C.

When cultivated in medium M19A (with vitamins reduced fivefold), B072 reached a maximum biomass and accumulated lipid up to 64.9% (w/w) with a DHA content of 17.74% (w/w). Consequently, DHA yield was 4.0 g/l, and the total fatty acids reached 14.6 g/l. In medium M19B (with vitamins reduced by a factor of 20), the biomass peaked at 23.1 g/l, accumulating lipid up to 47.8% (w/w) with a DHA content of 14.96% (w/w). Thus, DHA amounted to 3.46 g/l and total fatty acids to 11.04 g/l. When compared to medium M19, outcomes from M19A and M19B were inferior. These data clearly demonstrate the influence of vitamins on the growth and fatty acid formation of B072.

B072 was subsequently cultivated in medium M19C (Table 1). This medium differed from M19 by containing 0.6 g/l of K_2HPO_4 instead of 0.3 g/l. Results showed modest increases in both biomass (23.35 g/l) and DHA (5.0 g/l), with no significant difference in DHA level compared to medium M19.

In medium M19, where ASS functioned as the primary sodium source, B072 displayed robust growth (Fig. 3). To devise a cost-effective medium, ASS was replaced with different sea salt components, leading to the creation of media M19D and M19E.

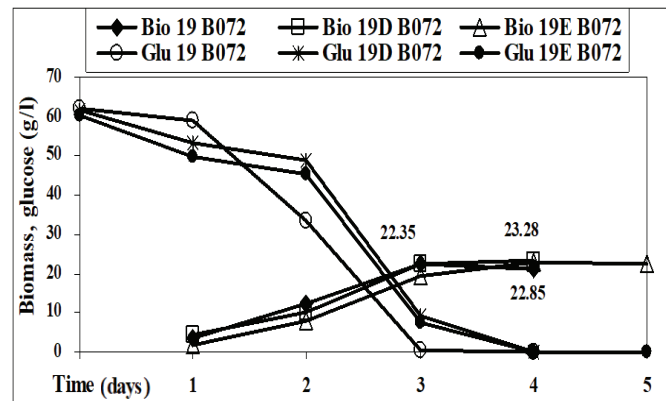


Fig. 3. Biomass and glucose levels for B072 in control medium M19 and media with individual salts replacing ASS (M19D and M19E).

B072 attained maximum biomass levels of 22.35 g/l in M19, 23.28 g/l in M19D, and 22.85 g/l in M19E. In M19, B072 achieved a DHA maximum of 4.9 g/l and a biomass of 22.35 g/l. In M19D, the figures were 4.4 g/l for DHA and 22.5 g/l for biomass, while in M19E, DHA and biomass values were 4.05 and 22.33 g/l, respectively. Results highlighted that while there was a significant ($p \leq 0.05$) reduction in DHA, biomass yield remained unaffected when specific salts replaced ASS. The study's primary focus remained on B072 due to its promising results in prior experiments.

3.2. Growth characteristics and fatty acids formation of B072 in modified MSG media

B072 was cultivated in media M19A, M19B, and M19C. The strain B072 achieved its peak biomass level at 23.35 g/l in medium M19C. Similarly, in other media, B072 consistently reached its peak biomass when glucose was fully consumed.

In medium M19A, where vitamins were reduced fivefold, B072 reached a maximum biomass of 22.6 g/l and accumulated lipids up to 64.89% (w/w). The DHA content in the lipid was 17.74% (w/w), resulting in a DHA yield of 4.0 g/l and a total fatty acid count of 14.6 g/l. When cultivated in medium M19B, where vitamins were reduced by a factor of 20, the biomass peaked at 23.1 g/l. The lipid accumulation was 47.8% (w/w), and the DHA content was 14.96% (w/w). As a result, DHA reached 3.46 g/l and total fatty acids totalled 11.04 g/l. Compared to medium M19, the results from mediums M19A and M19B were consistently lower. It is evident from these results that the vitamin content influences the growth and fatty acid formation in B072.

B072 was subsequently cultivated in medium M19C, which was a modification of medium M19, containing 0.6 g/l of K_2HPO_4 instead of the 0.3 g/l found in M19. The results, as illustrated in Table 3, indicated slight increases in biomass (23.35 g/l) and DHA (5.0 g/l). Nevertheless, the difference in DHA levels compared to medium M19 (the control medium) was not significant.

Table 3. Metabolites of strain B072 in M19-M19E.

Strain B072	M19 Day 3	M19A Day 3	M19B Day 4	M19C Day 4	M19D Day 3	M19E Day 5
Biomass (g/l)	22.35	22.55	23.10	23.35	22.50	22.33
TFA (g/l)	15.31	14.63	11.04	13.44	15.12	13.52
DHA (g/l)	4.92	4.00	3.46	5.00	4.40	4.05
TFA (%w/w)	68.49	64.89	47.80	57.57	67.19	60.55
DHA (%w/w)	22.01	17.74	14.96	21.43	19.56	18.14
FA compositions (% of TFA)						
C12	-	0.51	0.00	0.34	-	0.60
C14	7.81	10.56	8.20	7.23	8.37	11.34
C15	0.67	0.58	0.74	1.00	0.61	0.57
C16	51.78	53.67	51.70	45.21	54.90	50.11
C18	0.98	0.88	0.96	0.78	0.96	0.86
C20:3n3	0.70	0.63	0.66	0.94	0.61	0.79
C20:4n6	-	0.38	0.00	0.44	-	0.41
C20:5n3	-	0.00	0.00	0.44	-	0.47
DPA	5.91	5.45	6.45	6.40	5.45	4.90
DHA	32.14	27.34	31.29	37.22	29.11	29.97

3.3. Growth characteristics and fatty acids formation of B072 in DYE-ammonium sulphate $(\text{NH}_4)_2\text{SO}_4$ media M20-M23

Medium M20 comprised both DYE and $(\text{NH}_4)_2\text{SO}_4$. Medium M21 contained only $(\text{NH}_4)_2\text{SO}_4$, serving as the sole nitrogen source, while Medium M22 had only DYE. Medium M23 was identical to M21 but had 60 g/l glucose instead of 30 g/l (Fig. 4).

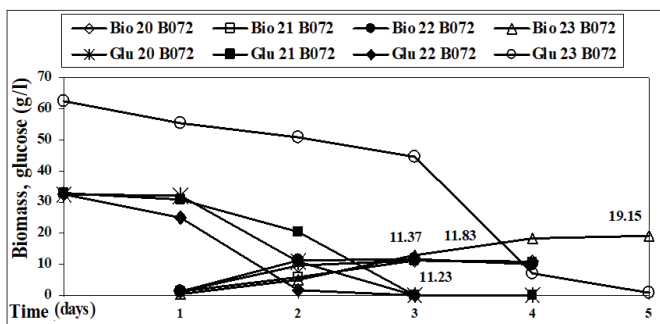


Fig. 4. Glucose and biomass levels of strain B072 in M20-M23 media.

In medium M21, B072 registered the lowest DHA level at a maximum of 0.88 g/l and the lowest lipid content in the biomass, reaching a peak of 42.33% (w/w). The DHA content in the biomass reached merely 8.46% (w/w). In medium M20 of DYE and inorganic nitrogen, B072 achieved a maximum of 1.28 g/l DHA and 11.27 g/l biomass, accumulating up to 53.38% (w/w) in total fatty acids. Medium M22, using DYE exclusively as the primary nitrogen source, yielded superior results compared to medium M21, which lacked any organic nitrogen sources. Strain B072 demonstrated substantial growth in medium M21 with 60 g/l glucose and ammonium sulphate as the exclusive nitrogen source, resulting in peak DHA and biomass levels of 2.73 and 19.15 g/l, respectively (Table 4).

Table 4. Biomass and metabolites of the strain B072 in M20-23.

Media	Strains	Biomass (g/l)	TFA (g/l)	DHA (g/l)	TFA % w/w	DHA % w/w	DHA % of TFA
M20	B072	11.27±0.56	6.07	1.28	53.38	11.27	23.91
M21	B072	11.23±0.61	4.74	0.88	42.33	8.46	19.98
M22	B072	11.83±0.59	6.14	1.65	65.30	16.13	28.00
M23	B072	19.15±1.05	11.10	2.73	60.16	14.78	24.99

3.4. Growth characteristics and fatty acids formation of B072 in DYE-sodium nitrate (NaNO_3) media M24 and M25

The constituents of mediums M22, M24, and M25 are presented in Table 5. While medium M22 exclusively utilised DYE as a nitrogen source, B072 reached peak biomasses of 10.93 and 11.93 g/l in media M24 and M27, respectively. By the third day of cultivation, glucose was entirely consumed.

B072 demonstrated growth in medium M25, where NaNO_3 acted as the sole nitrogen source. However, this strain yielded significantly lower biomass and DHA levels. The addition of DYE to medium M24 enabled this strain to grow more effectively, producing a higher DHA quantity than in medium M25, which contained only NaNO_3 as a nitrogen source. In medium M24, B072 achieved peak levels of 10.93 g/l biomass and 1.89 g/l DHA.

Table 5. Biomass and metabolites of three strains in M24-M27.

Media	Strains	Biomass (g/l)	TFA (g/l)	DHA (g/l)	TFA % w/w	DHA % w/w	DHA % of TFA
M24	B072	10.93±0.66	6.77	1.89	70.18	17.45	29.46
M25	B072	5.40±0.25	3.68	0.85	68.19	15.67	24.17
M26	B072	12.07±0.66	6.38	1.79	52.86	14.87	28.13
M27	B072	11.93±0.61	7.10	1.44	59.51	13.80	25.75

3.5. Growth characteristics and fatty acids formation of B072 in ammonium sulphate-MSG media M26 and M27

B072 was cultivated in medium M26, which combined the nitrogen sources of MSG and ammonium sulphate $(\text{NH}_4)_2\text{SO}_4$. B072 achieved its peak biomass level of 12.07 g/l when glucose was fully depleted, accumulating lipids up to 52.86% (w/w). The DHA content in the biomass was 14.78% (w/w). Therefore, this strain reached a maximum, translating to a DHA yield of 1.79 g/l DHA. In medium M27, which used MSG as the sole nitrogen source, B072 reached a biomass peak of 11.93 g/l and accumulated lipids up to 59.51% (w/w) with a DHA content of 13.80% (w/w), yielding 1.44 g/l DHA. These results conclusively demonstrate that B072 can effectively grow and produce DHA in media with inorganic nitrogen sources, such as ammonium.

4. Discussion

In previous experiment, all media were prepared either without yeast extract or with very minimal amounts to minimise the presence of amino acids, vitamins, and trace elements found in DYE. This approach was taken to evaluate the effects of vitamins and trace elements. The results demonstrated significantly low biomass and DHA levels for all three strains. These findings underscored the role of vitamins and trace elements on the growth and DHA production of Sk-02, B072, and B013, especially when comparing the biomass and DHA levels of these strains in pairs of media M12/M13 and M14/M15 [22]. I. Iida, et al. (1996) [23] revealed that vitamins and trace elements positively influenced the growth and DHA production of *T. aureum*. The significance of vitamins and trace elements was also corroborated for *S. mangrovei* Sk-02 [15].

ASS was substituted with sea salt components to reduce

costs and allow better control of individual salt levels. Using sea salt components could achieve similar results as ASS, particularly for strain B072. Another study found that DHA weight contents decreased with higher temperatures. The influence of salinity was ambiguous. The peak DHA content (28% w/w) was observed with glycerol; biomass and DHA levels reached their zenith at 25 g/l ASS. Replacing ASS with different sodium salts led to reduced biomass and DHA yields [24].

All three strains were cultivated in a defined MSG medium and its variants. These strains thrived relatively well, producing substantial amounts of DHA in the defined MSG medium M19 with MSG as the lone nitrogen source. The role of vitamins and trace elements became evident as B072 exhibited reduced biomass and DHA in vitamin-deficient media (M19A and M19B). The phosphate effect was assessed in medium M19C, which contained 0.6 instead of 0.3 g/l, as in medium M19. Both biomass and DHA saw minor increments. The replacement of ASS with a streamlined sea salt component formulation yielded equivalent results in biomass and DHA production (M19D, E vs M19). Moreover, medium M19D, containing a reduced amount of NaCl, may be viewed as a cost-effective option for large-scale fermentation.

Lastly, B072 cultivation was explored in media with combined nitrogen sources (ammonium sulphate-DYE, sodium nitrate-DYE, and MSG-DYE media). Table 8 results indicated that the ammonium sulphate-DYE medium resulted in superior DHA levels compared to the sole ammonium sulphate medium. A similar trend was observed in the nitrate-DYE medium. In juxtaposition with results from medium M22, which only contained DYE, it is inferred that B072 can utilise both ammonium and nitrate as nitrogen sources. Nonetheless, nitrate did not emerge as a suitable nitrogen source for B072. The findings in Table 5 illustrated that with nitrate as the primary nitrogen source, this strain assimilated glucose quite slowly, resulting in low biomass levels (M25). The nitrogen utilisation of *S. mangrovei* B072 paralleled that of *T. aureum* [25], *S. limacinum* SR21 [26], and *S. mangrovei* Sk-02 [12]. This study's outcomes signified that monosodium glutamate combined with ammonium sulphate was more efficacious as nitrogen sources for DHA production compared to monosodium glutamate on its own. This aligns with previous reports [27]. Notably, for *T. aureum*, optimal DHA levels were achieved by mixing monosodium glutamate with yeast extract [23]. In a different study, six media with diverse nitrogen sources were examined to pinpoint the most favourable medium for producing high quantities of DHA, biomass, and overall fatty acid content. The peak biomass (17.1 g/l) and DHA

(2 g/l) yields were achieved in a fed-batch system using sodium glutamate, $(\text{NH}_4)\text{SO}_4$, KH_2PO_4 , and MgSO_4 , after a 12-day incubation in the fed-batch medium of *T. roseum*. Here, the total lipid content stood at 48.8% (w/w) [17]. Similarly, a *Thraustochytrium* sp., closely related to *T. striatum* T91-6, managed to produce up to 28 g/l biomass, 81.7% (w/w) TFA, 31.4% (w/w) biomass DHA, and 4.6 g/l DHA under optimal cultivation condition with conditions; a shaker at 130 rpm and 25°C in a medium comprising 60 g/l glucose, 10 g/l yeast extract, and 2 g/l sea salt [28].

5. Conclusions

Aurantiochytrium sp. thrived and efficiently produced DHA in media sea salt components (sodium chloride, magnesium sulphate, sodium sulphate) and when replacing ASS media. Strain B072 was adept at utilising both $(\text{NH}_4)_2\text{SO}_4$ and NaNO_3 for growth and DHA production. Additionally, this strain harnessed ammonium for growth and fatty acid synthesis. Although strain B072 could grow using NaNO_3 as the sole nitrogen source, it resulted in meagre biomass and DHA production. Introducing yeast extract into the medium made strain B072 flourish and produce more DHA compared to a medium with only NaNO_3 as the nitrogen source, indicating the inadequacy of NaNO_3 as a primary nitrogen source for B072. Glutamate emerged as a potent nitrogen source for a defined medium, especially for B072. These findings suggest that defined media supplemented with vitamins and trace elements could be ideal candidates for *Aurantiochytrium* with an aim to enhance DHA production.

CRedit author statement

Pham Thi Mien: Methodology, Investigation, Writing original draft preparation; Dao Viet Ha: Data curation, Resources, Validation; Cornelis Verduyn: Conceptualisation, Resources, 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.

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Isolation of *Weizmannia coagulans* and probiotic characterisation of strains

Thuy Duong Pham, Thi Lan Anh Hoang, Thi Le Quyen Tran, Thanh Trung Trinh*

Institute of Microbiology and Biotechnology, Vietnam National University - Hanoi,
144 Xuan Thuy Street, Dich Vong Hau Ward, Cau Giay District, Hanoi, Vietnam

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Abstract:

Weizmannia coagulans is a potential bacterial candidate for probiotics because of their advantageous characteristics in endospore formation, antibacterial production, and other beneficial human health effects. In this study, we isolated *W. coagulans* strains from different sample types collected in Hanoi and examined them for probiotic characteristics. From 40 samples, 5 strains were isolated from cow faeces. All *W. coagulans* strains had non-haemolytic activity and were susceptible to seven tested antibiotics. Cell-free supernatants of *W. coagulans* strains cultured in MRS medium could inhibit some bacterial pathogens such as *Enterococcus faecalis*, *Listeria monocytogenes*, *Vibrio vulnificus*, and *Aeromonas dhakensis*. The strains showed antioxidant activity with scavenging DPPH rates ranging from 72 to 82%. Three strains were able to adhere to the HT-29 cell line with adhesion rates ranging from 46 to 62.59%. The spores and vegetative cells of the three strains also showed great stress tolerance (survival rates >90%) against simulated gastrointestinal fluid. In liquid media containing 0.1% pancreatin and 0.3% bile salt, spores of VTCC 12782 and VTCC 12779 could germinate at rates of 25 and 2%, respectively. With the most beneficial characteristics, *W. coagulans* VTCC 12782 is suggested as a potential candidate for the development of probiotic products in the future.

Keywords: beneficial properties, probiotic, Vietnam, *W. coagulans*.

Classification numbers: 3.4, 3.5

1. Introduction

Probiotics are “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” [1]. Recent studies on probiotic microorganisms mainly focus on some genera of *Lactobacillus*, *Bifidobacterium*, *Propionibacterium*, *Streptococcus*, and *Saccharomyces* [2]. These microorganisms are not able to survive in hostile conditions such as high temperatures and dry environments during the manufacturing processes [3]. Therefore, spore-forming bacteria with their higher stress tolerance and better survival during storage have attracted great attention in the probiotic production.

Weizmannia coagulans (formerly known as *Bacillus coagulans*) was firstly isolated in 1915 by B.W. Hammer (1915) [4] from spoiled canned milk. By 2020, they are classified in the genus *Weizmannia* [5]. The bacterium occupied a broad range of environments, including many types of food (milk, potato, pickle, corn, and tomato), animal faeces (broiler, calf, and piglet), compost, and silage [6-9].

Similar to other spore-forming bacteria, spores of *W. coagulans* can survive in extreme conditions like high temperatures and dry storage [10]. It also has great viability

in the gastrointestinal tract and good adhesion capacity to intestinal epithelial cells [9, 11]. In addition, the bacterium can produce some antibacterial substances, such as coagulin and lactosporin [12, 13], against some pathogenic bacteria like *Enterococcus* sp., *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus* [9, 14]. With these advantageous characteristics, *W. coagulans* is considered a potential probiotic candidate.

Several strains of *W. coagulans* have been identified as Generally Recognized as Safe (GRAS). The bacterium is also on the Qualified Presumption of Safety (QPS) list and authorized for human use [15]. Recently, an increasing number of studies have reported that probiotics derived from the original target host have evidently been revealed to be more efficacious than other sources (allochthonous) [16]. Therefore, exploiting native probiotic microorganisms should be considered. In Vietnam, the isolation of *W. coagulans* has only been reported from fermented cocoa beans [17] and Nam Dinh seawater [18]. Studies on *W. coagulans* isolated from other sources, and the characterization of bacterial probiotic properties, are limited. In this study, we aim to isolate, identify, and evaluate the probiotic properties of some indigenous *W. coagulans* strains.

*Corresponding author: Email: tttrung@vnu.edu.vn

2. Materials and methods

2.1. Microorganisms and cell line

Pathogenic bacteria including *Aeromonas dhakensis* VTCC 70106, *Enterococcus faecalis* VTCC 70177, *Escherichia coli* VTCC 12272, *Listeria monocytogenes* VTCC 70147, *Staphylococcus aureus* VTCC 12275, and *Vibrio vulnificus* VTCC 70092 were obtained from the Vietnam Type Culture Collection (VTCC), Institute of Microbiology and Biotechnology, Vietnam National University, Hanoi, Vietnam. These strains were stored at -70°C in nutrient broth (NB; Becton, Dickinson and Company, USA) supplemented with 20% glycerol (Fisher Scientific, UK) until use.

A human colon adenocarcinoma cell line (HT-29) was kindly provided by Dr. P.T.T. Huong (Key Laboratory of Enzyme and Protein Technology, Vietnam National University, Hanoi).

2.2. Sampling and isolation of *W. coagulans*

In March 2021, 40 human and dairy cow samples were collected, including human breast milk (n=10), healthy vaginal secretion (n=10), and newborn faeces (n=10) at Hanoi Obstetrics and Gynaecology Hospital and raw cow milk (n=5) and cow faeces (n=5) at a dairy farm in Ba Vi, Hanoi. The milk samples were taken into sterile falcon tubes, the vaginal secretion samples were obtained by sterile swab sticks and the freshly voided faecal samples were collected in clean, dry, screw-top containers. All samples were transported immediately to the laboratory in a cool box and were processed the same day.

Human vaginal secretion and milk samples were spread directly onto nutrient agar (NA, Becton, Dickinson and Company, USA) plates. One gram of newborn or cow faeces was homogenized into 9 mL of sterile water. Then the diluted samples were heated at 80°C for 10 min before enrichment in the NB medium at 42°C for 24 h [6]. The enriched broths were diluted and plated out on the NA plates. The plates were incubated under aerobic and anaerobic conditions for 48-72 h. Then, suspected *W. coagulans* colonies with swollen sporangium were picked up and stored at -70°C as previously described. *W. coagulans* GBI-30, 6086 (BC30) obtained from a commercial probiotic Schiff was used as a reference strain in all experiments.

2.3. Identification of isolated strains

The identification of isolated strains was confirmed by 16S rRNA sequence analysis. Briefly, the PCR reaction was performed using universal primers of 8F

(5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). Successful PCR products were sequenced at First Base Laboratories (Malaysia). The 16S rRNA of *W. coagulans* strains were submitted to NCBI GenBank under accession numbers from OP592207 to OP592211. The 16S rRNA gene sequences were searched on the EzBioCloud database (<https://www.ezbiocloud.net>). Phylogenetic analysis was performed on MEGA X software using the maximum likelihood method in a Tamura 3-parameter model. Bootstrap analysis with 1000 re-samplings was assessed to confirm the consistency of the phylogram [5].

2.4. Safety test

Haemolysis activity was determined by streaking *W. coagulans* strains on the Columbia agar plates containing 5% (w/v) sheep blood (MELAB, Vietnam) [9]. After incubation at 37°C for 24 h, the strains formed a green zone, clear zone, and no clearance zone around the colony, which were classified as α -haemolysis, β -haemolysis, and γ -haemolysis, respectively. The antibiotic susceptibility of the bacterial strains was investigated by the Kirby-Bauer disk diffusion method [19]. The fresh bacterial cells (approximately 10⁸ CFU/ml) of *W. coagulans*, prepared from overnight cultures, were spread on Mueller-Hinton (MH; Becton, Dickinson and Company, USA) agar plates. Antibiotic discs (Oxoid, USA) containing chloramphenicol (30 μ g), erythromycin (15 μ g), gentamicin (10 μ g), neomycin (30 μ g), streptomycin (10 μ g), tetracycline (30 μ g), or vancomycin (30 μ g) were placed on pre-inoculated plates. Plates were then incubated at 37°C for 20 h and the zone of inhibition diameter was measured.

2.5. Antibacterial activity

Well diffusion and agar plug diffusion methods were used to test the antibiotic production of the *W. coagulans* strains. For the first one, *W. coagulans* strains were shaken in trypticase soy broth (TSB, Becton, Dickinson and Company, USA) and de Man, Rogosa, and Sharpe (MRS, Becton, Dickinson and Company, USA) broth at 160 rpm. After shaking at 42°C overnight, the cell-free supernatants (CFSs) were collected by centrifugation at 8,000 rpm for 10 min and added into wells (d=5 mm) of the MH agar plates pre-inoculated with one of six pathogenic strains, namely, *E. faecalis* VTCC 70177, *L. monocytogenes* VTCC 70147, *E. coli* VTCC 12272, *S. aureus* VTCC 12275, *V. vulnificus* VTCC 70092, and *A. dhakensis* VTCC 70106. The plates were incubated at 37°C for 24 h and the clear diameter zone surrounding each well was measured (including the well) [14]. For the later one, *W. coagulans* strains were spread

on the trypticase soy agar (TSA, Becton, Dickinson and Company, USA) and MRS agar plates instead of the broths and the antibacterial activity was tested as described above.

2.6. Enzyme production of *W. coagulans*

The activity of amylase, cellulase, and protease was tested by spotting *W. coagulans* strains on NA plates supplemented with 0.2% soluble starch (Sigma-Aldrich, USA), 0.2% carboxymethylcellulose (Sigma-Aldrich, USA), and 0.1% casein (Sigma-Aldrich, USA), respectively [20]. The plates were incubated at 42°C for 48 h and stained with Gram's iodine solution (for amylase and cellulase) or Amido black solution (for protease) for visualization of the clear hydrolytic zones. The production of β -galactosidase was tested by streaking the bacteria on TSA plates containing 60 μ l X-gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside; Thermo Fisher Scientific, Italy), which was diluted to a final concentration of 20 mg/ml in DMF (N, N- dimethylformamide; Wako, Japan) and 10 μ l of IPTG (isopropyl-thio- β -D galactopyranoside; Thermo Fisher Scientific, Lithuania) solution as inducer [21]. β -galactosidase-producing strains appeared in blue colonies while non-producing enzyme strains appeared in white colonies.

2.7. Antioxidant activity

Antioxidant activities were determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [22]. Briefly, a volume of 1.5 ml of each CFSs prepared from the TSB culture was added into tubes containing 1.5 ml of 0.2 mM DPPH (TCI chemical, Japan). A tube containing 1.5 ml of methanol and 1.5 ml of 0.2 mM DPPH was used as the blank. All tubes were incubated at 37°C for 30 min in the dark and the absorbance was measured at 517 nm using a UV-Vis spectrophotometer (DU® 730, Beckman Coulter, USA). The percentage of scavenged DPPH radical was calculated using the following formula:

$$\text{Scavenging effect (\%)} = \left(1 - \frac{A_{517}(\text{sample})}{A_{517}(\text{blank})}\right) \times 100$$

2.8. Adhesion capacity

HT-29 cell line was cultured at a density of approximately 2×10^5 cells per well in 24-well plates using Dulbecco's Modified Eagle Medium (DMEM; PAN-Biotech, Germany), which contained 1 g/l glucose, 10% (v/v) fetal bovine serum (Gibco, USA), and 1% (v/v) penicillin/streptomycin (Corning, USA) [11]. After overnight incubation at 37°C, the medium was removed, and the cell monolayer was washed once with phosphate buffered saline (PBS, pH 7.4). Subsequently, 900 μ l of DMEM with 1 g/l glucose and 100

μ l of vegetative cell suspension were added into the wells and incubated at 37°C for 2 h with 5% CO₂. The wells were washed twice with PBS (pH 7.4), added to 200 μ l of 1% (v/v) triton X-100 (Merk, Germany), and incubated at 37°C for 5 min. The suspension in each well was diluted and then plated out on TSA plates to determine the number of adherent bacteria.

2.9. Stress tolerance to simulated digestion conditions

2.9.1. Preparation of cell and spore suspensions

Cell and spore suspensions were prepared as described by L.H. Duc, et al. (2003) [23]. Briefly, *W. coagulans* strains were cultured in TSB medium overnight. The culture broths were centrifuged at 8,000 rpm at 25°C for 10 min to collect the cells. The cell pellets were washed twice and resuspended in sterile PBS (pH 7.4) to obtain an optical density (OD) of 1.0 at 600 nm using a spectrophotometer (Ultrospec 10, Biochrom, USA). To produce spores, *W. coagulans* strains were cultured in a suitable broth after an appropriate time. The spore pellets were collected by centrifuging at 8,000 rpm for 10 min at 25°C and resuspended in sterile PBS (pH 7.4). The spore suspensions were heated at 80°C for 10 min and adjusted to obtain spore densities at log 7 CFU/ml.

2.9.2. Acid tolerance of the *W. coagulans* spores

The spore suspensions of *W. coagulans* were treated in PBS (pH 2.5) at 37°C for 2 h [24]. Then, the bacteria were counted, and the acid tolerance was determined using the following formula:

$$\text{Survival rate (\%)} = \frac{\log \text{CFU after treatment}}{\log \text{CFU before treatment}} \times 100$$

2.9.3. Survival of the *W. coagulans* strains in the simulated gastrointestinal tract

The spore suspensions of *W. coagulans* were incubated in both the simulated gastric juice (0.3% w/v pepsin (Sigma-Aldrich, USA), pH 2.5) for 2 h and the simulated small intestine fluid (0.3% (w/v) bile salts (Biobasic, Canada), 0.1% (w/v) pancreatin (Sigma-Aldrich, USA), pH 8.0) for 6 h [25]. The vegetative cell suspensions were only treated with the simulated small intestine fluid. Then, spores and vegetative cells were counted, and the survival rates were calculated using the following formula:

$$\text{Survival rate (\%)} = \frac{\log \text{CFU at } T_n}{\log \text{CFU at } T_0} \times 100$$

where T_n is the time after incubation with simulated gastric juice or small intestine fluid; T_0 is the initial time before incubation.

2.9.4. Effect of bile concentrations on the growth of vegetative *W. coagulans* cells

The TSB medium prepared with different concentrations of 0.1, 0.2, and 0.3% (w/v) bile salt were inoculated with the overnight cultures under anaerobic conditions at 37°C for 24 h. The growth of bacteria was monitored by measuring the OD₆₀₀. The TSB medium without bile salt was used as the positive control [24].

2.9.5. Germination in the simulated small intestine

The germination ability was conducted using the method of C.M. Theriot, et al. (2016) [26] with some modifications. Briefly, the spore suspensions were added to TSB medium supplemented with 0.1% w/v pancreatin (pH 8.0) and bile salt at concentrations of 0, 0.1, 0.2, and 0.3% (w/v). All tubes were incubated at 37°C for 24 h and the total bacteria were counted. The remaining spores were counted after a heating step at 80°C for 10 min.

2.10. Statistical analysis

Data from all experiments were expressed as mean ± standard error of the mean of triplicates. Differences between the two mean values were calculated by the student's *t*-test. The difference was considered to be statistically significant at *p*<0.05. All statistical analyses were performed using GraphPad Prism version 8.4.3.

3. Results and discussion

3.1. Isolation and identification of *W. coagulans*

From 40 samples, 150 bacterial colonies were collected. Of those, 22 gram-positive bacilli were recorded with 18 spore-forming strains. Ten strains were suspected as *W. coagulans* based on a slightly swollen sporangium of the cells [6]. The 16S rRNA sequences of were analysed and five strains were identified as *W. coagulans* with the DNA similarity range from 99.43 to 99.71%. All *W. coagulans* strains were isolated from cow faeces under both aerobic and anaerobic conditions. On the phylogenetic tree, five strains formed a clade with *W. coagulans* ATCC 7050^T (Fig. 1).

The presence of *Bacillus* in breast milk samples has been found in a few healthy women by analysing the metagenome [27, 28], although this genus has not been reported in vaginal fluid samples. Microbial diversity in breast milk could be influenced by geographic, genetic, dietary factors, and the lactation period [27]. Newborns are colonized by maternal microbiota via birth-associated exposure and breastfeeding [29] and the composition of these pioneer communities exhibits individual differences in early life

depending on maternal microbiota. This could be explained by the absence of *W. coagulans* in newborn faeces in our study. However, a relatively small sample size in this study might limit the investigation. Involvement of larger human and livestock populations at different time points of life are further needed to confirm the ecology of *W. coagulans*.

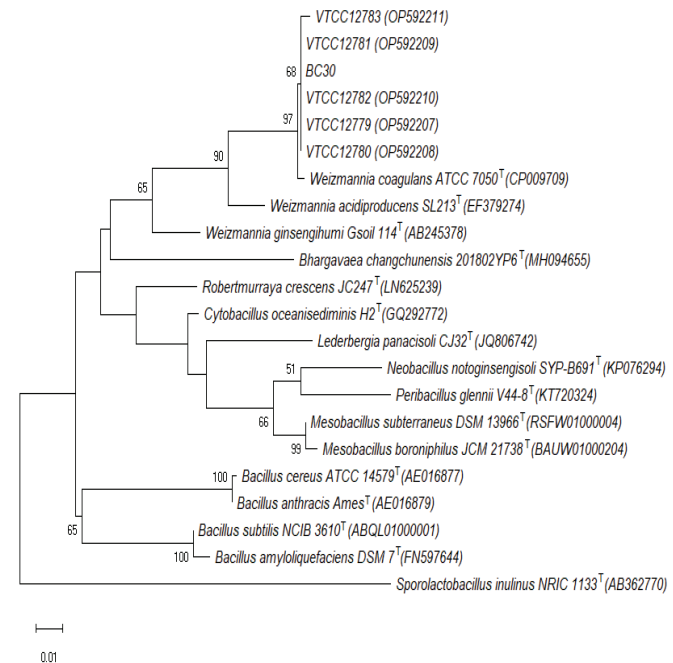


Fig. 1. Maximum likelihood phylogenetic tree based on the 16S rRNA gene sequences showing the relationships between our *W. coagulans* strains and some representatives of the *Bacillaceae* family. Accession numbers are shown in parentheses. The numbers at the nodes indicate bootstrap values as percentages of 1,000 replicates. *Sporolactobacillus inulinus* was used as the outgroup taxon to root the tree.

3.2. Safety of the *W. coagulans* strains

All five strains were γ-haemolysis, which is a safety characteristic of probiotic [30]. The five strains were sensitive to seven tested antibiotics except for erythromycin and chloramphenicol in which strains VTCC 12779 and VTCC 12783 were moderate susceptibilities (Table 1). In 2012, the QPS program added the requirement of antimicrobial resistance for probiotic GRAS certification because of the horizontal gene transfer from helpful bacteria to harmful bacteria in the host's intestines [30]. Haemolysis and antibiotic sensitivity are two important criteria in evaluating the safety of probiotic bacteria. However, these two criteria are not sufficient to conclude whether a bacterial strain is safe to use. Therefore, other criteria such as production of D(-)-lactate, deconjugation of bile salts, and virulence factors (gelatinase, DNase...) also need to be evaluated [1].

Table 1. Antibiotic resistance of the *W. coagulans* strains.

Antibiotic	Zone diameter (mm)					
	VTCC 12779	VTCC12780	VTCC 12781	VTCC 12782	VTCC 12783	BC30
Chloramphenicol (30 µg)	34.67±0.67 (S)	37.33±1.45 (S)	33.00±1.00 (S)	36.67±1.67 (S)	15.33±0.67 (M)	27.67±1.45 (S)
Erythromycin (15 µg)	15.33±0.67 (M)	32.00±0.00 (S)	34.67±1.76 (S)	31.33±0.67 (S)	18.67±0.67 (M)	33.33±1.76 (S)
Gentamicin (10 µg)	39.33±0.67 (S)	42.33±1.45 (S)	32.67±0.33 (S)	48.33±0.88 (S)	22.67±0.33 (S)	37.00±1.00 (S)
Neomycin (30 µg)	35.67±0.33 (S)	44.33±0.33 (S)	37.00±1.53 (S)	44.67±0.33 (S)	21.67±0.88 (S)	26.67±0.67 (S)
Streptomycin (10 µg)	31.33±0.33 (S)	34.33±1.20 (S)	33.33±0.67 (S)	35.00±1.53 (S)	24.33±1.20 (S)	30.00±1.15 (S)
Tetracycline (30 µg)	32.67±1.76 (S)	43.00±1.53 (S)	35.33±0.67 (S)	32.33±1.45 (S)	23.33±2.40 (S)	24.00±0.57 (S)
Vancomycin (30 µg)	30.67±0.67 (S)	32.67±1.20 (S)	32.67±0.33 (S)	33.67±0.67 (S)	36.00±0.00 (S)	32.00±0.00 (S)

Values are expressed as mean ± SEM (n=3). The inhibition zone diameters were measured, and the results were expressed as susceptibility (S), moderate susceptibility (M), and resistance (R).

3.3. Antibacterial activity

Using the MRS medium, *W. coagulans* strains showed antibacterial activity against the tested bacteria (Table 2). Strains VTCC 12779, VTCC 12781, and BC30 exhibited great antibacterial activity against *E. faecalis* and *L. monocytogenes* by the well diffusion method, while all *W. coagulans* strains were against *V. vulnificus* and *A. dhakensis* by the agar diffusion method. This characteristic of probiotic bacteria may suppress pathogenic bacterial growth and colonization in the gastrointestinal tract of hosts

Table 2. Antibacterial activity of the *W. coagulans* strains.

Bacteria	Media	Zone of inhibition (mm)					
		AD	EF	EC	LM	SA	VV
VTCC 12779	MRS broth	-	+	-	++	-	-
	MRS agar	+	-	-	-	-	+
VTCC 12780	MRS broth	-	-	-	-	-	-
	MRS agar	+	-	-	-	-	+
VTCC 12781	MRS broth	-	+	-	++	+	-
	MRS agar	+	-	-	-	-	+
VTCC 12782	MRS broth	-	-	-	-	-	-
	MRS agar	+	-	-	-	-	+
VTCC 12783	MRS broth	-	-	-	-	-	-
	MRS agar	+	-	-	-	-	+
BC30	MRS broth	-	+	-	+	-	-
	MRS agar	+	-	-	-	-	+

-: no inhibition zone; +: inhibition zone ≤10 mm; ++: inhibition zone >10 mm; AD: *A. dhakensis*; EF: *E. faecalis*; EC: *E. coli*; LM: *L. monocytogenes*; SA: *S. aureus*; VV: *V. vulnificus*.

[31]. A previous report on *W. coagulans* BDU3 also showed a wide spectrum of antimicrobial activity against pathogenic bacteria of *B. cereus*, *S. aureus*, *Enterococcus* sp., and *Micrococcus luteus* [14]. In the TSB medium, none of the *W. coagulans* strains showed antibacterial activity (data not shown). Variations in the culture medium often result in an alteration of antibiotic production, involving changes in both yields and the composition of the substances [32]. Therefore, the MRS medium might be a relevant culture medium for the production of antimicrobial substances by *W. coagulans*.

3.4. Enzyme production of *W. coagulans*

Amylase, cellulose, and protease activities ranged from 1.33 to 8.33 mm, 5.67 to 14.00 mm, and 4.00 to 12.00 mm, respectively (Table 3). VTCC 12783 showed the highest enzymatic activities. All strains were able to produce β-galactosidase. The results were consistent with previous reports. β-galactosidase was identified in extracellular protein extracts of *W. coagulans* MA-13 with a high expression level under culture containing 0.1% yeast extract and 0.1% lactose [33]. Moreover, *W. coagulans* MA-13 also produced soluble thermophilic cellulases in carboxymethyl-cellulose screening medium at 37°C and had an optimal operational activity at 50°C [34]. The production of extracellular, thermostable α-amylase by *W. coagulans* B49 was studied in solid state fermentation with a significant level of amylase produced in wheat bran [35]. The proteolytic activity of *W. coagulans* was also reported [36].

Table 3. Qualitative enzyme activity was measured by clear zone diameter.

Strain	Clear zone diameter ratio (mm)			β-galactosidase
	Amylase	Cellulase	Protease	
VTCC 12779	3.00±0.57	5.67±0.67	4.00±1.53	+
VTCC 12780	1.33±0.67	5.67±0.67	4.33±0.33	+
VTCC 12781	5.33±0.33	6.33±0.88	7.33±1.76	+
VTCC 12782	1.33±0.67	6.33±0.33	4.67±0.33	+
VTCC 12783	8.33±0.882	12.33±0.88	10.33±1.19	+
BC30	1.67±0.33	14.00±0.57	12.00±1.15	+
Mean ± SEM (n=3).				

3.5. Antioxidant activity

Oxidative stress can cause damage to lipids, proteins, and DNA when the levels of intracellular oxygen radicals are elevated. The scavenging DPPH rates of the *W. coagulans* strains ranged from 72 to 82% (Fig. 2). VTCC 12782 showed the strongest antioxidant activity

(82.97%), which was higher than BC30 (80.84%). The scavenging hydroxyl radical rates of our strains were higher than those of other species including *Pediococcus pentosaceus* SW01 (39.64%), *Lactobacillus pentosus* SW02 (34.31%), and *Lactobacillus plantarum* subsp. *plantarum* SW07 (28.63%) [22]. The antioxidant potential of *W. coagulans* was also reported through the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging ability [37].

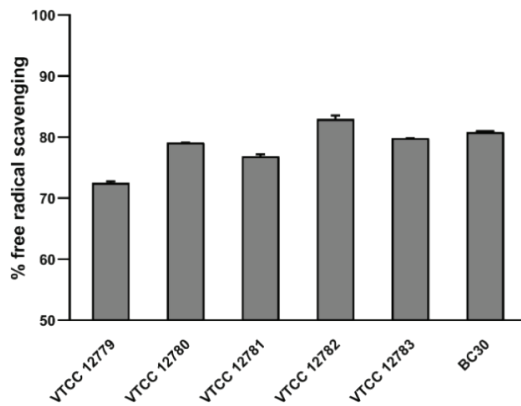


Fig. 2. Scavenging effect of the *W. coagulans* strains on DPPH free radical. Values are expressed as mean $\pm$ SEM (n=3).

3.6. Adhesion capacity

Three *W. coagulans* strains had remarkable adhesiveness toward intestine cells (Table 4). VTCC 12782 expressed the strongest adhesion (62.59%), followed by VTCC 12783 and VTCC 12779 with adhesion rates of 50.70 and 46%, respectively. This property ensures that probiotic bacteria colonize mucosal gut surfaces, interfere with pathogen binding, and interact with immune cells. *W. coagulans* isolated from calf faeces was able to adhere to INT407 cell monolayers [7]. *W. coagulans* CGMCC 9951 also exhibited strong adhesion to pig intestinal mucus with adhesion rates at 44.5, 48.9, 42.6 and 37.6% to the jejunum, ileum, transverse colon, and sigmoid colon, respectively [8].

Table 4. Adhesion of the *W. coagulans* strains to HT-29 cell line.

Adhesion period	VTCC 12779	VTCC 12780	VTCC 12781	VTCC 12782	VTCC 12783	BC30
Initial cell	6.99 $\pm$ 0.08	6.71 $\pm$ 0.13	7.13 $\pm$ 0.05	7.03 $\pm$ 0.08	7.16 $\pm$ 0.14	7.07 $\pm$ 0.13
Adhesion cell	3.20 $\pm$ 0.17	ND	ND	4.40 $\pm$ 0.22	3.63 $\pm$ 0.19	ND
Adhesion ability (%)	46	-	-	62.59	50.70	-

Values are expressed as mean $\pm$ SEM (n=3). The means are presented as log-transformed values of CFU/ml of the bacterial strains. ND: not detected.

3.7. Stress tolerance to simulated digestion conditions

Spores of *W. coagulans* enter the body through the oral route and safely travel to the stomach. Then, the spores germinate in the duodenum and grow in the upper part of the small intestine. *W. coagulans* cells subsequently go down to the large intestine and sporulation begins in the lower part of the colon [2]. Therefore, we challenged vegetative cells with small intestine fluid, while spores were treated with acid, simulated gastric juice, and small intestine fluid.

3.7.1. Survival of vegetative *W. coagulans* cells in the simulated small intestine fluid

Based on the adherence test, the three strains VTCC 12779, VTCC 12782, and VTCC 12783 were selected. Survival rates of the vegetative cells of VTCC 12779, VTCC 12782, and VTCC 12783 in the simulated small intestine fluid were 94.71, 81.93 and 95.21%, respectively (Table 5). In more detail, after 6 h of incubation, the spore densities decreased from log 7.18 CFU/ml to log 6.80 CFU/ml (VTCC 12779), from log 7.14 CFU/ml to log 5.85 CFU/ml (VTCC 12782), and from log 7.31 CFU/ml to log 6.96 CFU/ml (VTCC 12783). These survival rates were considerably higher than those previously reported for *L. acidophilus* M92 (survival rate of 57%) and *B. subtilis* (survival rate of 0.0002% after the first hour of exposure) [23, 25].

Table 5. Survival of the *W. coagulans* vegetative cells in the simulated small intestine fluid.

Incubation period	VTCC 12779	VTCC 12782	VTCC 12783	BC30
Initial cell, 0 h	7.18 $\pm$ 0.1	7.14 $\pm$ 0.10	7.31 $\pm$ 0.23	7.30 $\pm$ 0.13
Small intestine fluid, 6 h	6.80 $\pm$ 0.32	5.85 $\pm$ 0.62	6.96 $\pm$ 0.08	7.27 $\pm$ 0.13
Survival rate (%)	94.71	81.93	95.21	99.58

Values are expressed as mean $\pm$ SEM (n=3). The means are presented as log-transformed values of CFU/ml of the bacterial strains.

3.7.2. Effect of bile salts on the growth of the vegetative *W. coagulans* cells

Resistance to bile salts is an important property of probiotic bacteria to survive and grow in the intestinal tract [24]. Three strains exhibited growth at a bile salt concentration of 0.1% (Fig. 3), whereas no growth was observed at higher concentrations. The data were consistent with previous studies, which showed *W. coagulans* strains were sensitive or weakly tolerant to a bile salt concentration of 0.3% [24, 38]. Because of higher growth in bile salt concentration of 0.1%, VTCC 12779 and VTCC 12782 were selected for further experiments.

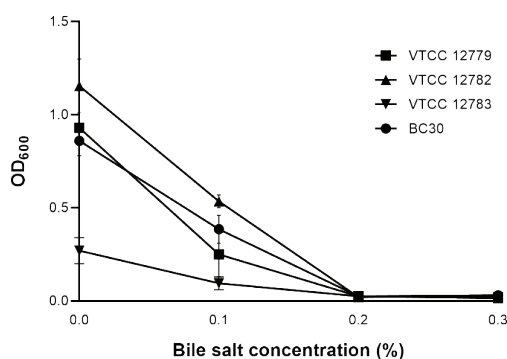


Fig. 3. Growth of the *W. coagulans* strains at different concentrations of bile salt. Values are expressed as mean $\pm$ SEM (n=3).

3.7.3. Acid tolerance of the *W. coagulans* spores

The survivability in gastric juice mainly depends on their tolerance to low pH. The tested *W. coagulans* spores showed survivability >90% at pH 2.5 (Table 6). After 2 h of incubation, the spore densities declined from log 7.69 CFU/ml to log 7.09 CFU/ml (VTCC 12779), from log 7.40 CFU/ml to log 7.14 CFU/ml (VTCC 12782), and from log 7.38 CFU/ml to log 6.81 CFU/ml (BC30). Spores of VTCC 12782 seemed to be more acid-tolerant than those of VTCC 12779 and BC30. Previous reports also showed high survival rates of spores of *W. coagulans* CGMCC (90.1%) and another *Bacillus* spp. (80%) [8, 24].

Table 6. Acid tolerance of the *W. coagulans* spores.

Incubation period	VTCC 12779	VTCC 12782	BC30
Initial spores, 0 h	7.69 $\pm$ 0.00	7.40 $\pm$ 0.04	7.38 $\pm$ 0.15
pH 2.5, 2 h	7.09 $\pm$ 0.12	7.14 $\pm$ 0.05	6.81 $\pm$ 0.23
Survival rate (%)	92.20	96.49	92.23

Values are expressed as mean $\pm$ SEM (n=3). The means are presented as log-transformed values of CFU/ml of the microbial strains.

3.7.4. Survival of the *W. coagulans* spores in the simulated gastrointestinal tract

Spores of VTCC 12779 were the most resistant to the simulated gastrointestinal tract, which decreased in viability from log 7.22 CFU/ml to log 7.17 CFU/ml (99.31% spore survival). The spore densities of VTCC 12782 decreased from log 7.59 CFU/ml to log 7.52 CFU/ml (99.08% spore survival) (Table 7). The survival rates of our *W. coagulans* strains were similar to that reported by T. Shinde, et al. (2019) [11] in which no significant decrease in *W. coagulans* spore count was detected after exposure to gastric conditions.

Table 7. Survival of the *W. coagulans* spores in the simulated gastric juice.

Incubation period	VTCC 12779	VTCC 12782	BC30
Initial spores, 0 h	7.22 $\pm$ 0.00	7.59 $\pm$ 0.04	7.37 $\pm$ 0.02
Simulated gastric juice, 2 h	7.17 $\pm$ 0.05	7.52 $\pm$ 0.18	7.22 $\pm$ 0.04
Survival rate (%)	99.31	99.08	97.96

Values are expressed as mean $\pm$ SEM (n=3). The means are presented as log-transformed values of CFU/ml of the microbial strains.

Spores of the tested strains also showed more than 99% survivability in small intestine fluid. After 6 h of incubation, the spore densities slightly increased from log 7.06 CFU/ml to log 7.07 CFU/ml (VTCC 12779) and from log 7.43 CFU/ml to log 7.77 CFU/ml (VTCC 12782) (Table 8). These results indicated that the spores of our strains had better survival ability in the simulated small intestine when compared to *B. subtilis* SC2362 (spore survival rate of 94%) [23]. For other species, *L. plantarum* and *L. brevis* strains exhibited survival rates in a range of 40 to 63% whereas *Pediococcus ethanolidurans* strains were sensitive to the simulated intestinal juice with no viability observed after 6 h of exposure [39].

Table 8. Survival of the *W. coagulans* spores in the simulated small intestine fluid.

Incubation period	VTCC 12779	VTCC 12782	BC30
Initial spores, 0 h	7.06 $\pm$ 0.16	7.43 $\pm$ 0.56	7.37 $\pm$ 0.02
Small intestine fluid, 6 h	7.07 $\pm$ 0.02	7.77 $\pm$ 0.02	7.32 $\pm$ 0.02
Survival rate (%)	100.14	104.58	99.32

Values are expressed as mean $\pm$ SEM (n=3). The means are presented as log-transformed values of CFU/ml of the microbial strains.

3.7.5. Germination of the *W. coagulans* spores in the simulated small intestine

The germination was tested based on the number of *W. coagulans* spores, which gradually decreased while the total number of bacteria increased (Fig. 4). Spores of VTCC 12782 reduced from 25 to 27% in all tubes containing different concentrations of bile salt while spores of VTCC 12779 reduced from 2 to 18%. Previous studies have reported the germination of *Bacillus* spp. spores, including *W. coagulans* in the gastrointestinal tract. According to L.H. Duc, et al. (2003) [23], the germination of *Bacillus* spores was expressed through the decrease of OD_{600nm} in AGK solution containing spores in the presence of bile salts. Using the *in vitro* model of the stomach and small intestine, the germinating percentage of *W. coagulans* BC30 spores was reported at 93%, which was higher than that of our strains. This might be due to the absence of germination triggers that were contained in the meal [40].

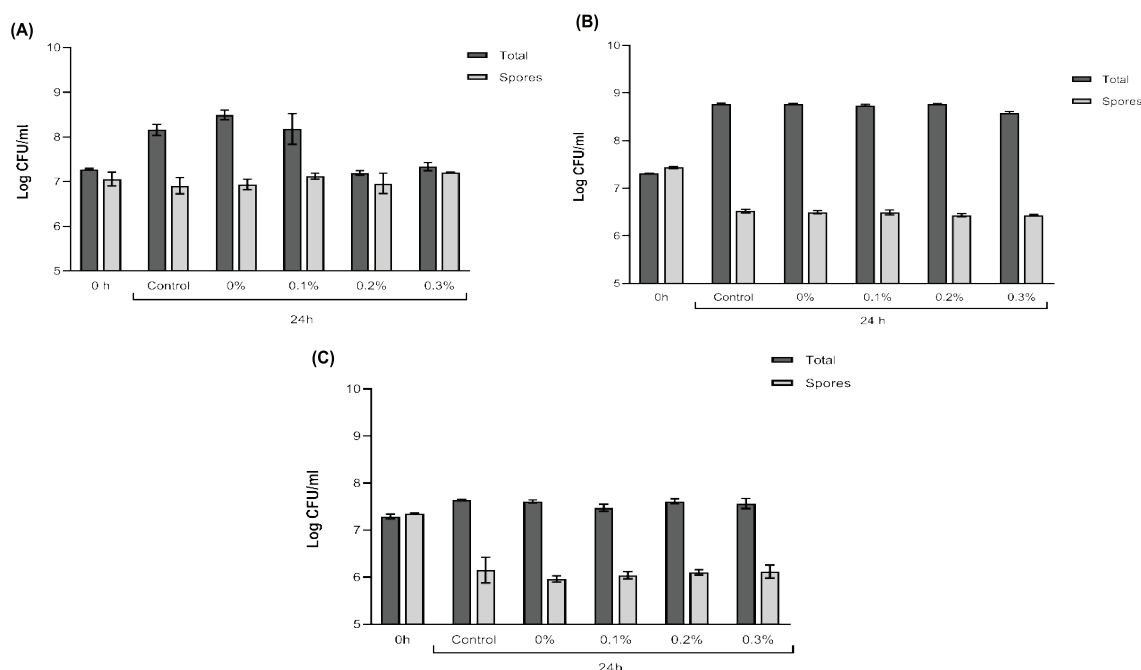


Fig. 4. Germination of the *W. coagulans* spores in the simulated small intestine. Spores of *W. coagulans* VTCC 12779 (A), VTCC 12782 (B), and BC30 (C) were treated in the simulated small intestinal condition and viabilities were assessed at after 24 h. Values are expressed as mean $\pm$ SEM (n=3).

4. Conclusions

In conclusion, from 40 human and cow samples, we isolated five *W. coagulans* strains from cow faeces. Not all strains showed haemolytic activity as well as resistance to tested antibiotics. Moreover, these strains were able to produce amylase, cellulase, protease, β -galactosidase, and antibacterial and antioxidant activity. The three strains VTCC 12779, VTCC 12782, and VTCC 12783 were able to adhere to HT-29 cells and survive in simulated intestinal conditions. VTCC 12779 and VTCC 12782 could grow in the presence of bile salt and had a high rate of spore survival in the gastrointestinal tract. The spores of VTCC 12782 exhibited the highest germination rate in the simulated small intestine. Therefore, strain VTCC 12782 is considered a potential candidate for probiotic production.

CRedit author statement

Thuy Duong Pham: Data collection, Experimental work, Writing; Thi Lan Anh Hoang: Study design, Reviewing and Editing; Thi Le Quyen Tran: Samples collection, Supporting data analysis; Thanh Trung Trinh: Supervision, Editing.

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COMPETING INTERESTS

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

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113 Tran Duy Hung Str., Cau Giay Dist., Hanoi, Vietnam

Tel: (84.24) 39436793; Fax: (84.24) 39436794

Email: vjste@most.gov.vn

Website: vietnamscience.vn or vietnamscience.vjst.vn

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