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Enhancing antibacterial property: Tetraethyl orthosilicate influence on quaternised chitosan/PVA film

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ABSTRACT

As a food packaging material, a biofilm barricades the food from contaminants. A film formulated from biopolymers with antibacterial properties is useful and holds high potential as a food packaging alternative since biopolymers are degradable, compostable, and made from renewable resources. Chitosan has been reported to demonstrate antimicrobial properties which are efficient against gram-positive and gram-negative bacteria. However, in its pure form, chitosan has poor solubility in most solvents and is limited to acidic environments only. Thus, in this study, chitosan was quaternised using hexadecyl trimethyl ammonium bromide to enhance its hydrophilicity and its antibacterial effectiveness. A film was formulated with a blend of quaternised chitosan with polyvinyl alcohol and tetraethyl orthosilicate. The FTIR results revealed the presence of peaks around 1400 cm^{-1} representing the asymmetric bending of the trimethylammonium group and 950 cm^{-1} corresponding to the quaternary ammonium group which show the success of the quaternisation process and the crosslinking with tetraethyl orthosilicate (TEOS) results in improving the hydrophilicity of the film. By using the technique of diffusion on the disc examined against E. coli, it was observed that all formulated films regardless of the presence of TEOS have antibacterial properties. The fabricated films can be used as food packaging alternative materials.

1. INTRODUCTION

Due to the increase in public awareness of food safety, the fabrication and development of anti-bacterial thin film (TF) has drawn the attention of research. Biopolymers like polysaccharides, proteins, and fats

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have been used in food packaging development in recent years. This is because food packaging that is made from biomaterial is fully compatible, compostable, suitable for recycling, and originated from renewable sources (Khodaman et al., 2022). As a food packaging, an antibacterial thin film composite can slow down food deterioration, enhance food quality, and prolong its shelf life (Khodaman et al., 2022; Liang et al., 2023). Commonly food preservation would use preservative agents. However, some of the preservative agents are carcinogenic (Liang et al., 2023) Therefore, fabricating an antibacterial thin film composite would be useful (Bi et al., 2021; Liang et al., 2023).

Chitosan is one of the biomaterials that can form a thin film for film packaging material. Chitosan is obtained from deacetylation of chitin. Chitosan is known to have antimicrobial activity, polyelectrolyte behaviour, high viscosity, fat, dyes, and mineral binding properties, film-forming capability, biodegradability, antioxidant property, hypolipidemic activity and accelerating wound healing (Alfuraydi et al., 2022; Caroni et al., 2021; Hefni et al., 2022). Because of the electrostatic interactions between the polycationic structure of chitosan and the anionic cell walls of bacteria, the protonated amino groups of chitosan have an antibiotic effect (Ali et al, 2019). Additionally, because the quaternising agent reduces the chitosan's hydrogen bonds, quaternised chitosan exhibits improved antibacterial characteristics (Aziz et al., 2023) (Khalaji et al., 2021). This makes chitosan receive increasing attention in many fields of research (Alfuraydi et al., 2022; Ke et al., 2021). However, chitosan has poor solubility in most solvents at neutral and basic conditions, which significantly limits its application (Omer et al., 2021). These limitations can be eliminated through modifications of chitosan physiochemical, including, grafting, crosslinking, quaternisation, and polymer blending (Omer et al., 2021; Wardhono et al., 2022). A structural modification through the quaternisation process increases the solubility and capacity of chitosan to bind both positively and negatively charged species by transforming the primary amine groups on C2 into quaternary salts with a stable positive charge (Andreica et al, 2020). In terms of the solubility enhancement of the quaternised chitosan over a broader range of pH levels, the quaternisation converts the main amino group in chitosan to a soluble quaternary ammonium ion (Aziz et al., 2023)(Omer et al., 2021).

Polymer blending of chitosan with other polar materials for example polyvinyl alcohol (PVA) on the other hand is expected to improve the mechanical properties of the resultant polymer (Li et al., 2019). PVA is a synthetic vinyl polymer that has many favourable properties such as being water-soluble, chemically stable, inherently non-toxic, hydrophilic, elastic, and able to form gel and film (Alfuraydi et al., 2022). As a film, PVA has a high strength and is resistant to acids and alkali. The subsequent crosslinking process of the polymer blend is carried out to produce a film with good thermal and mechanical stabilities (Kassim Shaari et al., 2019). In this study, tetraethylorthosilicate was used as the crosslinker.

In this work, a combination of PVA/QCs was used to make a film, which was then crosslinked using tetraethylorthosilicate (TEOS). The loadings of TEOS were varied to investigate the effect of the crosslinking process on the properties of the film. Using Fourier Transform Infrared Spectroscopy (FTIR), the film was characterized, and its antibacterial properties were assessed against *Escherichia coli*.

2. METHODOLOGY

2.1 Materials

Pellets of polyvinyl alcohol (PVA) ranging in hydrolysis degree from 87% to 89% (MW: 85000 – 124000) were purchased from Sigma Aldrich. Aman Semesta Enterprise, Shah Alam, Malaysia, provided the chitosan, which had a deacetylation degree of 84.412%. Dimethyl sulfoxide (DMSO) was supplied by R&M Chemicals, Subang, Malaysia. BT Science Sdn. Bhd. provided glacial acetic acid, hexadecyl trimethyl ammonium bromide (TMAB), potassium hydroxide pellet (KOH), tetraethyl orthosilicate (TEOS) with 99% purity, and 37% purity hydrochloric acid (HCL).

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2.2 Synthesis of quaternised chitosan (QCs)

2 g of glacial acetic acid was diluted with 98 g of distilled water. Then 0.5 g of chitosan was dissolved in 99.5 g of the solution. The mixture was stirred at 400 rpm and heated constantly at 90 ℃ for 4 hours. It was then left to cool to 65 ℃ before the addition of 15 g of TMAB. Then, 15 g of 1 M KOH solution was introduced to the solution. Using the same speed, the mixture was left under steady mixing for 4 hours at 65 ℃. Next, the resultant solution was cooled to room temperature before rinsing it with 500 mL of ethanol until the white quaternised chitosan started to precipitate. The mixture was then filtered using filter paper to separate the precipitate (Aziz et al., 2023).

2.3 Formulation of QCs/PVA/TEOS thin film

90 mL of DMSO was used to dissolve 10 g of PVA pellet. For six hours, the solution was allowed to homogenise while being stirred continuously at 400 rpm and 90 °C. The precipitate QCs and PVA solution were mixed at a weight ratio of 1:10, respectively. This was done by introducing 5 g of QCs white precipitate into a 50 g PVA solution. Then 0.227 g of TEOS as a crosslinker which represents 0.5 wt.% from the total polymer blend was added to the mixture followed by 1 ml of the catalyst HCL. The solution was continuously stirred and heated for 6 hours at 400 rpm and 60 ℃, respectively. The procedure was repeated using TEOS concentrations of 0 wt.%, 0.7 wt.% and 1 wt.% (Aziz et al., 2023). The detailed formulation is shown in Table 1.

Table 1. TEOS loading of the thin film

Membrane Code	Quaternised Chitosan (g)	PVA (g)	TEOS (g)
TF 1	5	50	0.00
TF 2	5	50	0.28
TF 3	5	50	0.39
TF 4	5	50	0.55

Source: Author's own data

2.4 Fabrication of a film

Using a film applicator, each prepared solution as indicated in Table 1 was spread onto a glass plate and formed into a 200 µm-thick film. The thin film was then dried in an oven set to 40 ℃ for the entire night (Aziz et al., 2023).

2.5 FTIR and antibacterial properties

The Perkin-Elmer Spectrum 2000 Fourier Transform Infrared Spectroscopy (FTIR) was utilised to investigate the variations in the functional group inside the polymer matrix of the film within the wavelength range of 515 cm^{-1} to 4000 cm^{-1}.

The antibacterial property of the QCs/PVA/TEOS thin film was tested against *E. coli*, a common gram-negative bacterium (Omer et al., 2021; Wang et al., 2021). The method used in the evaluation was the disc diffusion method. A transparent plastic film was used as a negative control. Luria-Bertani medium consisted of 1% peptone, 0.5% yeast extract, and 1 % NaCl was used to inoculate the *E. coli*. The petri dish containing the agar was divided into 4 quadrants to fit for 4 formulations as depicted in Table 1. Each film was placed inside the quadrant on the agar. After 24 hours of incubation period and at a temperature 37 °C, the zone of bacteria inhibition was examined.

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3. RESULT AND DISCUSSION

3.1 Functional group

Fig. 1 shows the FTIR spectrum for each formulated membrane. Based on the figure, the presence of peak C-H at 1400 cm^{-1} represents the asymmetric bending of the trimethylammonium group from the quaternised chitosan (Akhmetova et al., 2022, Nhung et al., 2020, Luan et al., 2018, Cheah et al., 2018, Kalinov et al., 2015, Xue et al., 2010) and the C-N stretching vibration represents the quaternary ammonium group at around 950 cm^{-1} (Aziz et al., 2023, Cheah et al., 2018, Xue et al., 2010). These spectra show that the quaternary ammonium salt was successfully introduced to the films. The crosslinking structure of the film as a result of TEOS incorporation was observed at the peak around 1080 cm^{-1} (Aziz et al., 2023, Sagar et al., 2022, Khan et al., 2021), which was attributed to the Si-O-C bond stretching, which is expected to increase the thermal and integral stability of the films. An increasing trend was observed in the percent transmittance of the film from TF2 until TF4, which was attributed to the increase of TEOS loading in the formulation. A peak around 830 cm^{-1} was observed in the FTIR spectra, which represents the C-C bond vibration of PVA (Cheah et al., 2018). The hydrophilicity characteristic of the film is observed through the broad peaks around 3300 cm^{-1} (Nhung et al., 2020), where it was observed that crosslinking of films TF2 until TF4 did not reduce the intensity of the peak since silica has hydrophilic character (Akhmetova et al., 2022). The peaks at 2928 cm^{-1}, 2927 cm^{-1}, 2923 cm^{-1}, and 2922 cm^{-1} are due to the C-H bond vibration of the CH_3 and CH_2 group (Nhung et al., 2020). Peaks at 1717 cm^{-1}, 1714 cm^{-1}, and 1646 cm^{-1} could be attributed to the C-O and C=O (Cheah et al., 2018). The quaternisation process has resulted in a homogeneous solution of the quaternised chitosan which further proves the successful process of quaternisation.

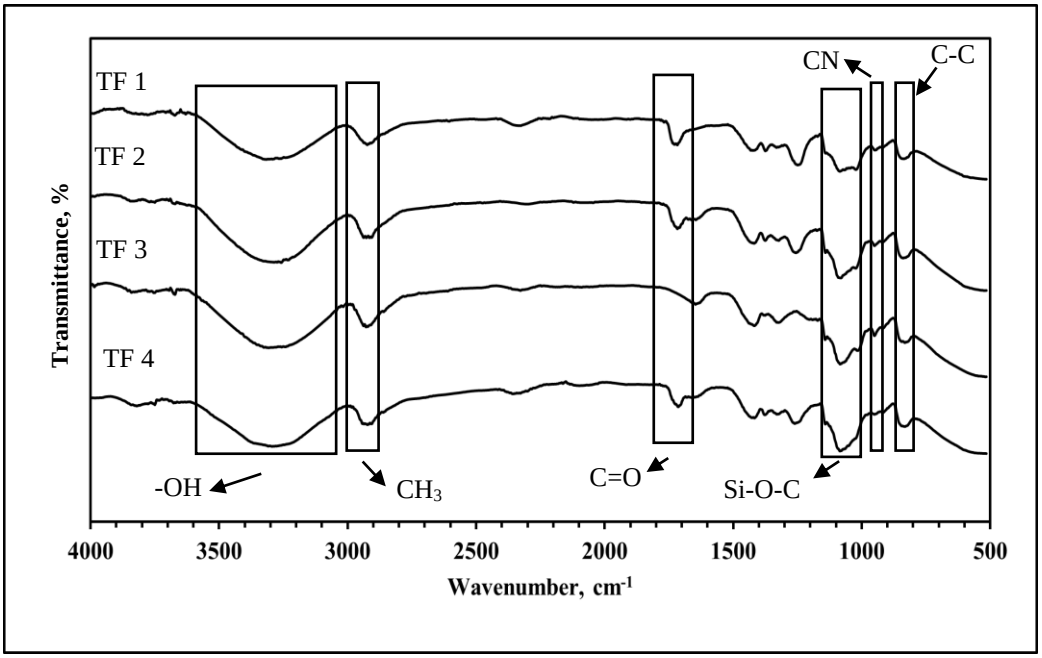

Fig. 1. FTIR spectra of films from different formulations

Source: Author's own data

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3.2 Antibacterial property

Fig. 2 shows the area of inhibition of all films. Based on the figure, all films exhibited the same area of inhibition, which was 6 mm. These results portrayed the antibacterial property of the film from quaternised chitosan regardless of the subsequent crosslinking process. This finding was similar to Wu et al. (2022), where a composite from QCs/PVA nanofiber membrane has an antibacterial activity against *E. coli*. Luo et al., 2022 performed a related investigation where the quaternised chitosan was blended with PVA at a ratio of 20/80 and 40/60. The inhibition zone was reported to be 9 mm and 11 mm, respectively, as compared to the 6 mm inhibition zone obtained in the current study. The low inhibitory zone in this study can be attributed to the lower QCs/PVA blending ratio of 5/50 and QCs concentration at 2% compared to 5% in that research. Matica et al., 2019 described that the antibacterial action of quaternised was caused by the disruption of the bacterial cell wall, interaction with the microbial DNA, and chelation of nutrients. As the quaternary ammonium salt group from quaternised chitosan gives a positive charge to the film, the film interacts electrostatically with the negatively charged cell wall of *E. coli*, which subsequently destabilises the cell wall and causes cell rupture (Matica et al., 2019). Based on Fig. 1, the incorporation of TEOS did not interfere with the antibacterial properties of the quaternised chitosan yet it provides a good crosslinking structure of the polymer film to ensure better integral stability.

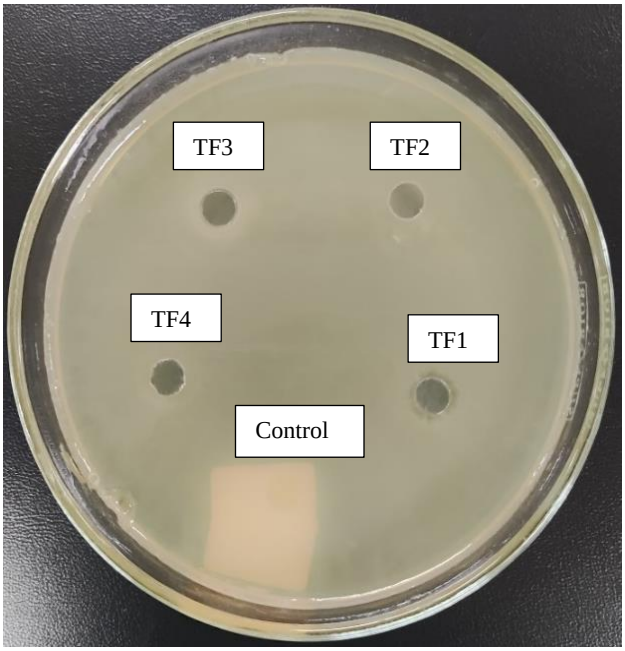

Fig. 2. Inhibition zone of films

Source: Author's own data

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4. CONCLUSION

The quaternisation process of the chitosan has been successfully conducted as it resulted in a homogeneous solution and the FTIR spectra depict the functional groups resulting from the process. All formulated films have equal antibacterial effectiveness toward *E. coli*. The cross-linking process using TEOS did not jeopardise the antibacterial property of the film despite additional advantages in terms of enhancing the crosslinking of the resultant film that will provide better integral stability. The fabricated films have a great potential to be used as food packaging alternative materials.

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CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

AUTHORS' CONTRIBUTIONS

Mohd Akasyah Sulaiman: Conceptualisation, methodology, formal analysis, investigation, and writing-original draft; **Norin Zamiah Kassim Shaari**: Project administration, conceptualization, validation; supervision, writing- review and editing, and validation.

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