Polyelectrolyte Complex (PEC) film based on chitosan as potential edible films and their antibacterial activity test

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Abstract. The synthesis of polyelectrolyte complexes (PEC) based on chitosan and determination of their antibacterial properties had been conducted. The microstructure of PECs obtained were described by SEM analysis, while inhibition activity of PECs against S. aureus and E. coli was determined by measuring inhibitory zone diameter. Characteristics of chitosan, alginate and κ-carrageenan as natural polymers which non-toxic, bio-degradable and safe to eat meet the edible film criteria. Chitosan as a polycationic interacts with alginate and κ-carrageenan as polyanionic under the appropriate conditions to form PEC film. Based on FTIR spectra, it was found that interaction of chitosan and alginate as well as chitosan and κ-carrageenan was an electrostatic interaction. Microstructure study using SEM found that PECs have irregular and fibrous surface structure. Based on their inhibitory activity against S. aureus and E. coli, PECs have the strongest antibacterial activity compared to their original polymer. Therefore, PECs film could be excellent edible film for food coating that protect product from bacterial contamination.

1. Introduction

The use of packaging in food products is now an important requirement [1]. Packaging protects the product or foodstuff from physical impact and microbial contamination thereby extending its shelf life [2]. Petroleum-based synthetic packaging has been widely used today. Unfortunately, its non-degradability caused serious pollution problems [3]. Based on environmental safety considerations, edible film-based packaging materials have been developed [4,5].

Recently, there has been an increase in the development of bio-composite materials based on PEC that have the potential as edible films [6]. Besides its mechanical characteristics, edible films need to have resistance to food damage due to the presence of microorganisms. Chitosan has been known to be used as biodegradable film and food preservative that is resistant to microbes. The antibacterial properties of chitosan are obtained from a polymer structure that has a positively charged amine group [7–9]. However, chitosan has the disadvantage of leaching under acidic conditions and high water absorption. This weakness can be overcome through the formation of a PEC film [10,11].
In this work, a PEC as potential edible film based on chitosan (as polycationic) was made using carrageenan and alginate as polyanionic. Chitosan-carrageenan and chitosan-alginate polymers formed PEC through electrostatic interactions, without the use of crosslinking agents which are likely to be toxic [12]. The PEC film has the advantage of a dense structure that can inhibit the decay process by blocking oxygen inhalation [6]. The simple and easy manufacturing technique is suitable for large scale applications. The chitosan-carrageenan and chitosan-alginate films that were synthesized without any other toxic materials met the edible film criteria. The antibacterial properties of the formed films were also examined.

2. Materials and methods

2.1. Materials

Chitosan with 95 % degree of deacetylation (DDA) and low molecular mass (72 mPa.s, Mol. wt. 300–400 kDa) from crab shell, sodium alginate (45.3 mPa.s, Mol. wt. 10–20 kDa) from brown algae, κ-carrageenan (6.4 mPa.s, Mol. wt. 12-40 kDa) from red seaweed, S. aureus (ATCC 25923) and E. coli (ATCC 25922) were obtained from Sigma-Aldrich (UK), while glacial acetic acid (98%), sodium hydroxide, and hydrochloride acid (37%) were received from Merck (UK). Solutions were prepared with deionized water.

2.2. Chitosan-carrageenan film preparation

Chitosan-carrageenan film was made according to previous work by Carneiro et al. [13] by mixing at 1:1 molar ratio. Chitosan hydrosol was prepared by dissolving approximately 1.53 g of chitosan (0.38 % w/v) in 400 mL of 0.16 M HCl, while κ-carrageenan hydrosol was made by dissolution of 4.40 g of κ-carrageenan (0.63 % w/v) in 700 mL of deionized under stirring condition at 500 rpm (25 °C and 24 h). The addition of chitosan hydrosol to the κ-carrageenan hydrosol was done by using drop-wise fashion (approximately rate of 14 ml/min) in a 2 L Pyrex media bottle (Fisher Scientific, UK) under stirring at 500 rpm. Then, the mixture was cooled using a water bath at 25 °C for 15 min, followed by pH measurements using pH-meter (TOA Electronic Ltd model IM-20E). Polymer mixture was poured and molded into glass plate for drying at room temperature for 72 h.

2.3. Chitosan-alginate film preparation

The preparation of chitosan-alginate film was conducted by procedure described as previous work [10]. Briefly, hydrosol of chitosan at 1% (w/v) were prepared in 20 mL glacial acetic acid under stirring at 400 rpm using a magnetic stirrer (IKA Combimag REO Drehzahl Magnetic Stirrer Electronic, Germany), while alginate was dissolved in deionized water until hydrosol at concentration of 4% (w/v) was formed. Then, both alginate and chitosan hydrosols were left out overnight to remove air bubbles. The chitosan hydrosol (100 mL) was added to the alginate solution and added 8 mL of 32% HCl (100 mL) at 25°C, homogenized using a homogenizer (IKAT18Ultra Turrax, Germany) for 90 seconds. Following, NaOH 10% (w/v) was added till the pH of the suspension was elevated to 5.28 and homogenized again for 90 seconds. Mixture of polymer was poured and molded into glass plate for drying at room temperature for 72 h.

2.4. Antibacterial activity test

Antibacterial activity of PEC films against both E. coli and S. aureus was evaluated by using disc diffusion method as implemented by Kaya et al. [14]. As a reference standard 0.5 McFarland was used to adjust bacterial susceptibility. Suspension of bacterial culture was wiped onto a plate inside agar of Müeller-Hinton. On agar medium that has been inoculated with bacteria, paper discs were placed, and then loaded with 20 mL hydrosol film. It was incubated for 24 hours at 37°C. As positive control, it was used 10 mg gentamicin per disc and deionized water as a negative control. Inhibition zone diameter on a clear area of the paper disc was measured. All experiments were done in triplicate.
3. Results and discussions

3.1. Formation of PEC films

The formation of PEC films was confirmed by FTIR spectra. It was shown in Figure 1, along with their constituents polymers. Typical absorption peak of chitosan showed at 1643.0 and 1560.6 cm\(^{-1}\) while peak of \(\kappa\)-carrageenan was at 1231.1; 924.49; 846.48 cm\(^{-1}\). Shifting of sulphate group absorption in \(\kappa\)-carrageenan from 1231.1 cm\(^{-1}\) to 1208.2 cm\(^{-1}\) was observed in chitosan- \(\kappa\)-carrageenan film. Shifts also occurred at 1647.6 cm\(^{-1}\) (\(\kappa\)-carrageenan) and 1643.0 cm\(^{-1}\) (chitosan) absorption which were then combined into absorption at 1652.2 cm\(^{-1}\) in PEC with a stronger intensity. The formation of the chitosan- \(\kappa\)-carrageenan PEC was indicated by absorption at 1556.1 and 1208.2 cm\(^{-1}\). The loss of the absorption band at 1148.7 cm\(^{-1}\) as amine group characteristics indicated that the chitosan amine group has been protonated and interacted with the \(\kappa\)-carrageenan sulphate group. It was confirmed by the appearance of a peak in the area of 1556.1 cm\(^{-1}\) indicating the presence of the NH\(^3+\) ion. It was strengthened with absorption at 1208.2 cm\(^{-1}\) that indicated electrostatic interactions of chitosan- \(\kappa\)-carrageenan in molar ratio of 1:1. A pH 5 was chosen as the condition for chitosan- \(\kappa\)-carrageenan film synthesize. The amine groups of chitosan were protonated and interacted with the sulphate groups of \(\kappa\)-carrageenan under these condition. Chitosan is a weak base polymer with an intrinsic acid dissociation constant (pKa) of 6.5 [15], while \(\kappa\)-carrageenan consists of ionisable sulphate groups at pH above pKa of 2 [16]. It was found that optimal condition of chitosan-\(\kappa\)-carrageenan films formation at pH 5 with yield of 85% via ionic interaction.

The absorption at 3459 cm\(^{-1}\) indicated the presence of the -OH group of alginates and -NH\(^3+\) chitosan, 2924 and 2870 cm\(^{-1}\) are the absorption of CH (sp\(^3\)), 1578 cm\(^{-1}\) was the vibration of the -COO\(^-\) group. The loss of amine group absorption at 1157 cm\(^{-1}\) indicated that the chitosan amine group has been protonated and interacted with the carboxylate group of alginate [17]. This was strengthened by the appearance of peaks in the area of 1550 cm\(^{-1}\) which showed the presence of protonated amine (–NH\(^3+\) ion). Peak intensity at wave number of 1398 cm\(^{-1}\) also showed electrostatic interactions on alginate-chitosan film, with alginate and chitosan mass ratio of 1:1. The presence of the absorption band mentioned above showed the existence of ionic bonds of protonated amine and the carboxylate groups. Chitosan consists of amine groups which have intrinsic pKa value of 6.5, while alginates consist of carboxyl groups that undergo ionization at pH of the solution above its pKa of 4.7 [18]. Therefore, at pH 5.28 chitosan presence at protonated form while alginate is in carboxylate form which is negatively charged. When both hydrosols were mixed in a 1:1 ratio, electrostatic interaction of protonated amine and carboxylate has occurred. Mechanism of PEC formation was described in Figure 2.

The surface morphology of chitosan, carrageenan, alginate, chitosan- \(\kappa\)-carrageenan and chitosan- alginate films was studied through Scanning Electron Microscopy (SEM) as shown in Figure 3. Here, the morphology of chitosan- \(\kappa\)-carrageenan films is less homogeneous than chitosan or \(\kappa\)-carrageenan alone. The chitosan film appeared microscopically smooth and uniform, while the \(\kappa\)-carrageenan and alginate films showed the clearest and smoothest surface. While, that surface structure of both PEC films was irregular and fibrous. The irregularity in the structure of the chitosan- \(\kappa\)-carrageenan and chitosan-alginate films indicated that complex aggregates were formed due to ionic interactions.

One of important parameters in edible film as a food coating is its physico-mechanical properties including tensile strength, elongation break, and modulus young. These characteristic affect oxygen diffusion elerates oxidative damage and food protection from physical impact during the transport and storage process. The physico-mechanical properties of PEC as edible film has been studied in previous work [6,11]. It has been found that PEC has better physico-mechanical properties than its constituent polymers due to its tighter microstructure.
**Figure 1.** The FTIR spectra of (a) chitosan, (b) κ-carrageenan, (c) alginate, (d) chitosan-κ-carrageenan PEC and (e) chitosan-alginate PEC film

**Figure 2.** Mechanism of PEC formation [10,11].

**Figure 3.** SEM images of surface morphology of (a) chitosan, (b) κ-carrageenan, (c) alginate, (d) chitosan-carrageenan, (e) chitosan-alginate film with 3000x magnification.
3.2. Antibacterial activity test

Inhibitory activity of PECs and their constituent polymer against *S. aureus* and *E. coli* is shown in Figure 4. As shown in Figure 4, κ-carrageenan showed antibacterial activity against *E. coli* and *S. aureus* compared to the negative control of deionized water. The inhibitory effect on *E. coli* was higher than against *S. aureus*. The inhibitory effect by chitosan was found to be higher than that of κ-carrageenan in both types of bacteria. PECs have the strongest antibacterial activity compared to their original polymer. The antibacterial activity of the PECs can be due to the \(-\text{NH}_3^+\) group which contributes a positive charge to PECs. Cationic charge can interact electrostatically with the major components of positive and negative bacteria, namely anionic phospholipid dipalmytol phosphatidylglycerol [19]. The interaction of protonated amine and sulfate in chitosan-carrageenan or carboxylate in chitosan-alginate is electrostatic that are not as strong as covalent bonds, allowed protonated amino groups to interact with anionic phospholipid of bacteria [6]. This interaction increases the permeability of the cell wall membranes of both bacteria, encourages release of nucleic acid, glucose, lactate dehydrogenase from cell as well as interfere transport of nutrient to cells causing bacterial death [20].

![Figure 4. Antibacterial activity of films.](image)

4. Conclusion

PECs based on chitosan, alginate and κ-carrageenan were prepared. PECs were formed by electrostatic interaction of chitosan-alginate as well as chitosan-κ-carrageenan. Surface morphology and structure of formed PECs was more irregular and fibrous structure than their native polymer. Antibacterial activity of PECs were the strongest compare to their constituents polymers. Hence, this biomaterial can be applied as active and safe coating for food product.

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