Synthesize of self-electrostatic interaction chitosan-carrageenan membrane and its properties

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Abstract. The preparation of polyelectrolyte complex (PEC) based on bio-composite materials often requires crosslinking agents to achieve the desired stability and properties of the material formed. PEC with opposite charges has the advantage in self-crosslinking through electrostatic interactions. Chitosan is a positively charged polysaccharide with -NH\textsubscript{3}\textsuperscript{+} group while κappa-carrageenan is a negatively charged polysaccharide with -OSO\textsubscript{3}\textsuperscript{−} group. This work focused on synthesizing chitosan-carrageenan membrane and determining its characteristics. The membrane was prepared by interacting chitosan and carrageenan hydrosols at a pH of 5. The obtained chitosan-carrageenan membrane had better physical-mechanical properties, including tensile strength (load), elongation (strain), and elasticity (modulus young), adsorption, and resistance of water, than the constituent polymers. The FTIR spectra indicated the presence of self interaction in membrane between protonated amine groups of chitosan and sulfate groups of carrageenan. The difference in surface morphology among chitosan-carrageenan membrane and its constituent membranes was confirmed by SEM analysis.

1. Introduction
Research interest in developing bio-composite materials as membranes that have better properties such as more effective, economical, stable for their applications such as enzyme immobilization matrices and edible films has increased in recent years [1,2]. Polysaccharides have been widely used as bio-composite materials, but generally, a chemical crosslinker is needed to improve their properties so that they are suitable for their intended use. Glutaraldehyde, as a commonly used chemical crosslinker is toxic and mutagenic [3], making it harmful for both researchers and the environment. Self-crosslinked through electrostatic self-interaction to form polyelectrolyte complex, which eliminates the use of crosslinker. Unlike covalently cross-linked macromolecules, polyelectrolyte complex (PEC) do not require a significant verification process in their formation. PEC is a dynamic material because of its electrostatic bonds, which are reversible and the charged groups depend on the pH, molar ratios of its constituents, ionic strength, and temperature. This dynamic structure gives unique structure and properties of PEC as a membrane [4,5].
In this study, PEC was prepared using chitosan and kappa-carrageenan. Chitosan is a polysaccharide that is found in abundance in nature. Chitosan, poly [β-(-1,4)-2 amino-2 deoxy-D-glucopyranose] is a product of chitin deacetylation [6]. The dissolution of this polymer in acidic water causes the amino group of chitosan to produce a positively charged group [7]. While carrageenan consists of β-D-galactose and 3,6-anhydro-α-D-galactose acid residues that are bound via α-1,3 and β-1,4-glycosidic bonds [8]. The sulfate groups become negatively charged when carrageenan is dispersed in water. Kappa (κ) carrageenan that consist of one sulfate group per disaccharide is one of three main commercial types of carrageenan beside iota (ι) and lambda (λ) carrageenan that consist of two sulfate and three groups per disaccharide, respectively [9]. Chitosan is polycationic, and carrageenan, which is polyanionic under the right conditions, can interact with one another through amino groups from chitosan and sulfate groups from carrageenan to form PEC bio-composite materials. The self-interaction in PECs is the same as ionic crosslinking [10,11].

Production and characterization of chitosan-carrageenan PEC have been reported in several studies. Nonthrombogenic materials, solvent cast chitosan-carrageenan PEC gels into films, have produced for blood contacting applications [12]. Three-dimensional structures made from chitosan-carrageenan PEC scaffolds have been previously reported by Araujo et al. [13]. In this work, chitosan-carrageenan PEC membrane was synthesized in a simple method with self-electrostatic interaction between oppositely charged in polyelectrolyte [3,14], which can reduce the possible toxicity and other undesirable effects of the crosslinker. Here, chitosan hydrosol and carrageenan hydrosol was mixed in the appropriate condition in order to the NH$_3^+$ side group of chitosan and OSO$_3^-$ side groups of carrageenan can interact in each other and form PEC membrane. The interaction in the membrane was studied by characterizing PECs using FTIR and SEM, and its physical-mechanical properties were assessed.

2. Materials and methods
Chitosan (72 mPa.s, 95% degree of deacetylation) from crab shell and κ-carrageenan (6.4 mPa.s) from red seaweed were purchased from Sigma-Aldrich, UK. While hydrochloride acid (37%), glacial acetic acid (98%), and sodium hydroxide were analytical grade and obtained from Merck (UK). Solutions were prepared with deionized water.

2.1. Synthesize of chitosan, carrageenan, and chitosan-carrageenan PEC membranes. Chitosan and carrageenan hydrosols were prepared according to previous methods [13]. Previously, increasing the pH of chitosan hydrosol was done by adding approximately 10 mL of 5 M NaOH. For chitosan membranes preparation, the chitosan hydrosol (9 mL) was dropped carefully using a 5 mL transfer pipette into a round silicone mold (id of 54 mm). Meanwhile, the carrageenan membrane was made by heating the carrageenan hydrosol in a water bath to 60 °C. In hot conditions, the hydrosol was poured into a round Teflon® mold (id 50 mm) which has been previously heated to 60 °C. It needs to be done to obtain a uniform membrane and minimize the initial gelification of carrageenan. In addition, chitosan and carrageenan hydrosols must be piped carefully to prevent the formation of air bubbles in the membrane. While, chitosan–carrageenan PEC was prepared based on the method reported by Carneiro et al. [12]. Unit molar ratio (UMR) of chitosan and carrageenan was 1:1 which was prepared by mixing of chitosan hydrosol (0.38%, 1.53 g of chitosan in 400 mL of 0.16 M HCl) and carrageenan hydrosol (0.63%, 4.40 g of κ-carrageenan in 700 mL of deionized water). Chitosan hydrosol at 25 °C was added dropwise at a rate of 14 mL/minute to carrageenan hydrosol in a 2 L Pyrex medium bottle (Fisher Scientific, UK) under magnetic stirring at 500 rpm. After all the chitosan was added, the mixture was cooled in a water bath at 25 °C for 15 minutes, then the pH was measured (TOA Electronics Ltd model IM-20E). Chitosan-carrageenan PEC membrane was fabricated by the same preparation of chitosan membrane. The hydrosols of chitosan, carrageenan and chitosan-carrageenan were then dried for two weeks. The remain reactan was removed by immersing the PEC membrane in 1 M NaOH and 1 M KCl solutions for 24 h and subsequently washed twice with
deionized water for 1 hour and 1 min respectively under shaking condition. All membranes were dried at room temperature for 24 hours.

2.2. Physical-mechanical properties methods.
Mechanical strength measurements, including the tensile strength, percentage of elongation at break, and elasticity (modulus young) were measured using a universal testing machine. Firstly, the membrane samples were prepared with size of 100 × 20 mm², then put into the autograph machine. The stress applied is measured in MPa while the test speed was set at 10 mm/min, and the measurements for the tensile strength, elongation at break and elasticity of each membrane were determined.

Water absorption (swelling test) and resistance test were determined using the gravimetric method. Before testing, the dried membranes were placed in a desiccator for one week. Immersing of the dried membranes was done in 10 mL of distilled water for 1 h for water absorption test and 6 h for resistance test. Next, the membranes were dried and weighed before and after immersing.

2.3. Characterization methods.
The FTIR spectra of membranes were characterized using Fourier transform infrared spectrometer (FT-IR 1600 Perkin Elmer Co Japan) in a wavenumber range of 4000-400 cm⁻¹ wavenumbers. The KBr pellet method was used with a compression pressure of 2500 Ib/m².

The surface morphology of membranes was examined by using a scanning electron microscope (Hitachi TM-3000) at 15 kV accelerating voltage and 500x magnification images. Before the samples were analyzed, samples need to be attached to a stub by using double-sided carbon tape, then coated with platinum using sputter coater ion (Hitachi E-1045) for 10 seconds.

3. Results and discussion

3.1. Synthesize of chitosan-carrageenan PEC membrane
PEC is a complex that forms when two oppositely charged polyelectrolyte solutions react in a solution system. The driving force for the formation of PEC is an electrostatic mechanism that includes charge neutralization and possible interactions (such as hydrogen bonding, Coulomb forces, van der Waals forces, and transfer forces) through multivalent counterions resulting in an attraction between the polyelectrolyte segments [15]. The formation of inter-polymer complexes by this electrostatic interaction occurs spontaneously [4], which can be easily distorted by the stirring effect. Initially, random bonds between polyelectrolytes occur, which are called primary complexes. Then, correction of the intracomplex and rearrangement of the bonds takes place to form a secondary complex. The final stage in the formation of PEC is an inter-complex aggregation. Hydrophobic interactions between secondary complexes cause their aggregation, with the aggregates of these complexes possibly in the form of large, entangled fibrils or tissues [16].

The molar ratio of chitosan and carrageenan at 1:1 was chosen, and it was optimum to form a good and stable PEC membrane. The strongest interaction between protonated amine of chitosan and sulfate group of carrageenan may obtain in this ratio, compared to other ratios. During the formation of the PEC membrane, pH 5 was chosen in the manufacture of the optimal chitosan-carrageenan membrane. At this pH, the -NH₂ chitosan groups are protonated, while the carrageenan groups are in the form of sulfate ions. Chitosan has an intrinsic acid dissociation constant (pKa) 6.5 [7], whereas pKa of carrageenan is 2 [9]. At extreme environmental pH, chitosan and carrageenan are degraded. The primary degradation mechanism of chitosan is caused by acid hydrolysis, while it is stable against the alkaline condition. Likewise, carrageenan hydrosol preparations at pH <4 should be avoided. Hence, in synthesize PEC membranes, the pH of the hydrosol chitosan must be adjusted to 4 before being added to carrageenan hydrosol and pH of 5 is the optimum pH for the formation of PEC.

3.2. Physical-mechanical properties methods.
The value of tensile strength and elongation of the membrane in Table 1 shows that the chitosan-carrageenan PEC membrane is quite elastic because it is not easily broken. The interaction of chitosan-carrageenan PEC membrane produced a combination of mechanical strength of chitosan and carrageenan polymers so that it had better mechanical strength. Electrostatic interaction increases tensile strength and elongation of PEC due to the membrane plasticization effect. The membrane mechanical strength test is carried out for the membrane in a dry and wet state (after diffusion). The mechanical strength of the membrane in wet conditions is important to know in relation to biomaterial applications. The reduced tensile strength of the membrane in wet conditions is possible due to the formation of interactions between water and membrane. The more hydrophilic groups per unit surface area of the membrane, the stronger the water interaction with the membrane. It provides less mechanical strength of membrane [17].

In Table 1, it was found that the chitosan-carrageenan PEC membrane has a greater modulus young value than chitosan and carrageenan only. The mechanical properties of mixed membranes are getting better properties and it is because the tight structure causes the distance between molecules in the membrane to get closer. The greater the modulus of young, the membrane has better ability to prevent damage caused by external forces, resulting in strong chitosan-carrageenan PEC membrane. This shows that the chitosan-carrageenan PEC membrane formed has a unique structure and properties so that it can be used in applications as a better supporting material (matrices).

Water absorption in membranes is generally similar to membrane swelling. Water absorption provides an illustration of the weight of water that can be absorbed on the membrane while swelling provides an overview of the development of membrane volume due to trapped water in the membrane. Absorption and membrane resistance values in Table 1 show that chitosan-carrageenan PEC membranes have greater water resistance and lower water absorption than carrageenan membranes. Self-electrostatic interaction that induced PEC formation may increase membrane hydrophobicity. Hence PEC has a higher resistance than its native membranes to insoluble in water. It was similar to the previous published [18] that higher hydrophobicity of membrane reducing affinity between water molecule and membrane, hence further decreasing the water uptake and penetration rate. Lower water uptake was also showed in PEC of chitosan/sodium alginate due to interaction between the opposite groups in PEC [19]. The development of chitosan-carrageenan membranes is probably due to the presence of OSO\textsuperscript{3}\textsuperscript{-} hydrophilic ions.

**Table 1. The physical-mechanical properties of the membrane**

| The physical-mechanical properties | Chitosan | Carrageenan | PEC membrane |
|-----------------------------------|----------|-------------|--------------|
| Thickness membrane /mm            | 0.28±0.21| 0.32±0.22   | 0.24±0.19    |
| Tensile strength (dry state) /MPa  | 26.45 ±  | 38.66±2.34  | 30.16±0.66   |
| Tensile strength (dry state) /MPa  | 0.92     |             |              |
| Tensile strength (wet state) /MPa | 4.81 ± 0.45 | 0.2±0.17 | 0.7±1.88     |
| Elongation (dry state) /%          | 3.60 ± 0.24 | 4.6± 0.24 | 6.07 ± 0.66  |
| Elongation (wet state) /%          | 4.30 ± 2.03 | 7.7± 2.03 | 8.44 ± 0.13  |
| Modulus young /(N/mm\textsuperscript{2}) | 16.12   | 1.75       | 13.93        |
| Water absorption /%                | 98       | 2090       | 150          |
| Water resistance /%                | 99       | 0          | 95           |

3.3. Characterization methods.

In this section, the formation of PEC was confirmed by the FTIR spectra that are shown in Figure 1. The absorption at 1643.0 cm\textsuperscript{-1} is characteristic absorption bands for chitosan that indicate the amide I of chitosan, while sulphate groups of carrageenan was showed at 1231.1; \textsuperscript{924.49} and 846.48 cm\textsuperscript{-1}. Sulphate groups in carrageenan (1231.1 cm\textsuperscript{-1}) was shifted to 1208.2 cm\textsuperscript{-1} after PEC was formed, indicated that this group was involved in complexing. A shift of absorption at 1647.6 cm\textsuperscript{-1} (carrageenan) and 1643.0 cm\textsuperscript{-1} (amide I peak of chitosan) to 1652.2 cm\textsuperscript{-1} with stronger intensity was
occurred in PEC spectra. The absence of peak at 1148.7 cm\(^{-1}\) which characterizes the amine group, indicates that it is protonated and interacts with sulphate group of carrageenan. This is confirmed by the appearance of a peak in 1556.1 cm\(^{-1}\) which indicates the existence of NH\(_3^+\) ion. Regarding the mentioned absorption above, an ionic bond between the protonated amine group and the sulfate group existed for chitosan-carrageenan PEC formation. Similar results were also reported by Li et al. [20] that the appearance of protonated amine peaks, a shift in the amide I peak of chitosan, and reduction in sulfate peak intensity indicates an interaction between the two groups in the formation of the chitosan-carrageenan polyelectrolyte complex.

![Figure 1. The FTIR spectra of (a) chitosan, (b) carrageenan, (c) chitosan-carrageenan PEC membrane.](image)

The membrane surface morphology was characterized by SEM analysis (Figure 2). The membrane surface of chitosan and carrageenan is more homogeneous than the formed PEC membrane. The carrageenan membrane featured the clearest and smoothest surface, followed by chitosan and PEC membranes. It was shown that the chitosan-carrageenan PEC membrane had an irregular and fibrous surface, indicating that complex aggregates were formed due to ionic interactions.

![Figure 2. SEM images of surface morphology of (a) chitosan, (b) carrageenan, (c) chitosan-carrageenan PEC membrane.](image)

4. Conclusions
The chitosan-carrageenan membrane was prepared at pH 5 with a molar ratio of 1: 1. The membrane was formed as a result of the self-electrostatic interaction of protonated amines and sulphate ions in the chitosan-carrageenan membrane. SEM analysis shows that the chitosan-carrageenan membrane has a different surface morphology from the constituent membranes. It was found that the membrane
formed had better properties than the original polymer in terms of the tensile strength (load), elongation (strain), and elasticity (modulus young), adsorption, and resistance to water.

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