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Characterization of Chitosan-poly (Ethylene Oxide) Blends as Haemodialysis Membrane

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Abstract: Blend membranes of chitosan and poly (ethylene oxide) with different molecular weights of 100,000 and 600,000 were prepared by the solution cast technique. The Chitosan-PEO blend membranes were produced to study their water adsorptions capacity and characteristics of the haemodialysis membrane application. An increase in the water adsorption capacity of chitosan-PEO blend membranes compared to the pure chitosan was due to the porous structure as evident from the scanning electron micrograph. Addition of PEO with higher molecular weight had reduced the percentage of water adsorption of the chitosan-PEO blend membranes. XRD results revealed that chitosan-PEO blend membrane with higher water adsorption ability shows lesser degree of amorphosity. Intermolecular interactions between chitosan and higher molecular PEO chains in the blend contributed to important alteration in chitosan structure as observed in the infrared spectroscopy which lessens the permeability of the membrane.

Key words: Chitosan, poly-ethylene oxide, biocompatibility, haemodialysis membrane

INTRODUCTION

Chitosan is a natural mucopolysaccharide of marine origin having structural characteristics similar to glycosaminoglycans that is present in the exoskeleton of crustacean[1], arthropod and fungi[2]. It consists of a linear (1→ 4)-linked 2-amino-2-deoxy-β-D glucan and can be chemically prepared from naturally occurring chitin i.e. its N-acetyl product by treatment with alkali at elevated temperature[3]. The structure of chitosan is shown below in Fig. 1.

Chitosan has been used for a wide variety of biomedical applications, such as in the drug delivery system[4], charcoal encapsulated chitosan beads for toxin removal[5], dental and orthopedic materials[6,7]. This is primarily due to its biodegradable, nontoxic and biocompatible features[1]. Other applications in biomedical field includes fibers for fat blocker, digestible sutures, liposome stabilization, anti bacterial, anti viral and anti tumor agents, haemostatic and hypocholestertemic and hypolipidemic agent[3].

Poly (ethylene oxide) has been characterized as having a moderate tensile modulus, high elongation and ability to orient when stressed. Its solubility in various solvents is a function of its molecular weight and temperature. In general higher molecular weight PEO is harder to dissolve. Industrial applications of PEO include in fire fighting and also as adhesives[8].

Chitosan as a membrane alone has high mechanical strength, permeability to urea, amino acids and creatinine and able to reject high molecular weight compounds. It is impermeable to serum proteins which suggest the prevention of toxic metals into blood stream[2,9]. However, Mallete *et al.*[10,11] reported that chitosan solution formed a coagulum when in contact with blood and its haemostatic property involved agglutination of red blood cells.

De Chrico *et al.*[2] started the pioneering work to improve the problems by blending chitosan with polyhexamethyleneadipamide in 99% formic acid solution. Henceforward, other chitosan blends had been developed such as chitosan-polyvinyl alcohol blend membranes[12], chitosan-albumin blend membranes[13] and chitosan-poly (ethylene oxide) blend membranes[14].

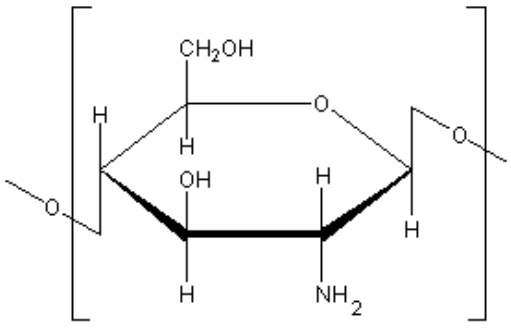

Fig. 1: Structure of Chitosan

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The permeability and blood compatibility of chitosan-poly (ethylene oxide) blend membranes using different molecular weights of PEO have been studied and the results show that the permeability coefficients of urea and other solutes through the membranes are much higher compared to the pure chitosan membrane and Cuprophan i.e. commercially available haemodialysis membrane. The platelet adhesion and activation were also significantly reduced on chitosan-PEO membranes[14].

Therefore, we intend to correlate the surface and chemical characteristics of chitosan-PEO blend membranes to their permeability coefficients, which is important in developing haemodialysis membrane. This objective is hoped to be achieved through the characterization of the chitosan-PEO blend membranes using Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD) and Fourier Transform Infra Red (FTIR) techniques.

MATERIAL AND METHODS

Preparation of chitosan and chitosan-poly (ethylene oxide) (PEO) blend membranes: Chitosan and chitosan-PEO blend membranes were prepared by solution cast method. Chitosan (Fluka) and PEO of different molecular weight of 100,000 and 600,000 (Aldrich) were dissolved in 0.1 M acetic acid (BDH) to prepare a 2.0% (w/v) solution for each. The solutions were continuously stirred with a magnetic stirrer for about 8 hours at room temperature. Then, the viscous chitosan solutions were mixed with PEO solutions.

Subsequently, the homogenous solutions were poured onto a polystyrene Petri dish and were allowed to spread into a thin film of approximately 50μm thickness and left slowly at room temperature. After the drying process was completed, the dried membranes peeled off easily and were soaked in 1.0 M sodium hydroxide (BDH) for several hours to neutralize excess acetic acid followed by extensive washing. The membranes were dried until use.

Equilibrium hydration: Since the chitosan-PEO blend membranes were expected to be used as the dialysis membrane, the equilibrium hydration i.e. the water sorption capacities of a material were an important characteristic for the absorption of body fluid and for a transfer of cell nutrients and metabolites through the materials.

The water sorption capacities of chitosan-PEO blend membranes were determined by swelling the membranes in pH 7.4 of phosphate-buffered saline (PBS) solution (Sigma) containing 0.02% sodium azide (Ajax) as a preservative at 4^o for 24 hours. A known weight of a dried chitosan-PEO blend membrane was immersed in the media. The percentage of water adsorption from chitosan-PEO membrane was calculated:

$$E_{sw} = [(W_{wet}-W_{dry})/W_{dry}]\times 100\%$$

where E_{sw} is the percentage water adsorption of chitosan-PEO membranes at equilibrium. W_{wet} denotes the membranes weight at equilibrium water adsorption and W_{dry} is the initial weight of the membranes.

Scanning Electron Microscope (SEM): Morphological properties of the membranes such as surface porosity, roughness and texture were studied. In this study, dried chitosan-PEO blend membrane was mounted on a standard SEM sample holder and fixed to the base. The same procedure was also applied to the pure chitosan membrane, the PEO 100K membrane and the PEO 600K. The sample was then sputtered coated with gold-palladium coating using an SEM coating system before viewing under the Philips SEM 515 system.

X-ray diffraction (XRD): Identification and quantitative determination of the various crystalline compounds known as phases were studied using XRD. Thus, the existing phases of developed pure and blend membranes were studied. X-ray diffraction measurements on the membranes were performed with the Philips Expert Pro Diffractometer using CuKα radiation. Irradiation conditions were 30^o kV and 40mA for the scanning of diffraction angle 2θ between 10^o and 70^o.

Fourier transform infrared (FTIR): Characterization of the chemical structure of a polymer is done by using Fourier Transform Infra Red (FTIR) technique. The specific chemistries and orientation of the structure will be known from the IR spectrum. The membranes infra red spectra were measured using of the Perkin Elmer FTIR model 2000 spectrophotometer. The transmission spectrum was recorded in the wave number range from 4000 cm^{-1} to 600 cm^{-1}.

RESULTS AND DISCUSSION

Equilibrium hydration: The results obtained from the experiments indicate the exudates drainage ability of various chitosan-PEO blend membranes. From the calculation, the average water sorption of chitosan-PEO 600K blend membrane is 55.60% compared to the water sorption capability of chitosan-PEO 100K blend membrane with 58.78%. Both blends showed better water sorption characteristics than pure chitosan membrane which is 50.67% (Table 1).

Table 1: Relationship between types of membrane and water sorption capability

Types of Membrane	Water Sorption (%)
Pure Chitosan	50.67
Chitosan-PEO 600K Blend Membrane	55.60
Chitosan-PEO 100K Blend Membrane	58.78

Therefore, it was demonstrated that the water sorption ability of the chitosan-PEO 100K blend membranes were higher. Thus, the water sorption capability of the chitosan-PEO blend membranes is strongly dependent on the molecular weight of the PEO introduced.

Scanning electron microscope: The morphology of pure chitosan, pure poly (ethylene oxide) 600K and pure poly (ethylene oxide) 100K are featured in Fig. 2.

The scanning electron micrograph for the pure chitosan membrane revealed that the membrane is non-porous and the texture is plain without pores. From the micrograph under 10,000 times magnification, the surface of PEO 600K shows a wavy structure and a randomly distributed microstructure space that looked like a crack (Fig. 3). This structure suggested that PEO 600K particles might have failed to crystallize.

PEO 100K has a "branch" like structure, which has large spaces between the particles unlike its PEO 600K counterpart. This happened due to the longer evaporation time taken by PEO 100K which enabled it to crystallize (Fig. 4).

The surface structure of chitosan-PEO 600K blend membrane is rough and uneven. There were no pores or semi-pores available on the surface (Fig. 5).

For the chitosan-PEO 100K blend membrane, small pores were dominating the morphological structure of the membrane (Fig. 6).

From SEM micrographs, it is proven that the surface characteristic of the membrane plays an important role to allow water uptake. Blending PEO 100K with pure chitosan had improved the porosity of chitosan. Meanwhile, Chitosan-PEO 600K blend membrane has a rough and uneven structure which disallows more water molecules to be adsorbed. SEM micrographs also revealed that the surface structure of chitosan-PEO 600K blend has much similarity with PEO 600K which exhibited amorphous feature.

X-ray diffraction (XRD): The X-ray diffractograms for pure chitosan, pure PEO membranes and chitosan-PEO blend membranes are shown in Fig. 7 and 8. The peak existed at $2\theta = 20^{o}$ is a characteristic peak for chitosan. Sharp and intense diffraction lines are obvious for both PEO 100K and PEO 600K at $2\theta = 23^{o}$ and 19^{o}.

A sharp line at $2\theta = 20^{o}$ is available in both chitosan-PEO 600K blend membrane and chitosan-PEO 100K blend membrane diffractograms. A new peak is formed at $2\theta = 15^{o}$ for both blends which indicates the influence of PEO. We should notice that PEO 100K has a very sharp, steep and intense diffraction peaks at 19^{o} and 23^{o} when compared to PEO 600K. Thus, under the PEO 100K peaks influence, the intensity of chitosan-PEO 100K peak at $2\theta = 20^{o}$ and 15^{o} is much higher.

However, the amount of intensity shown in chitosan-PEO 100K blend is not proportionate with the peak in PEO 100K. We could predict that there were limited molecular interactions happen between chitosan and PEO 100K compared to the other blend. By comparison, chitosan-PEO 600K blend is more amorphous. A weak intensity peak was formed at $2\theta = 15^{o}$ for the blend and this reaffirmed our conclusion from the SEM micrographs.

Fig. 2: SEM image of the pure chitosan membrane under 10,000 time's magnification

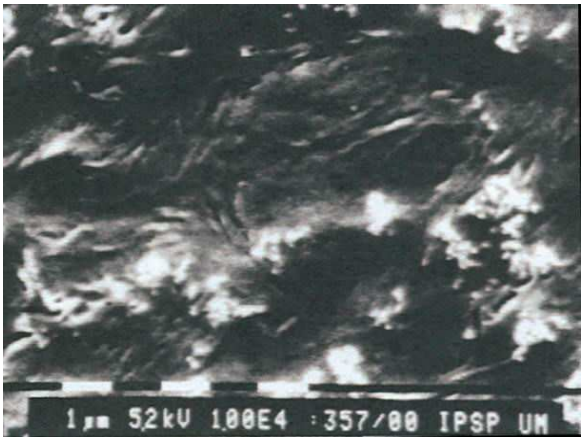

Fig. 3: SEM image of the pure PEO 600K membrane

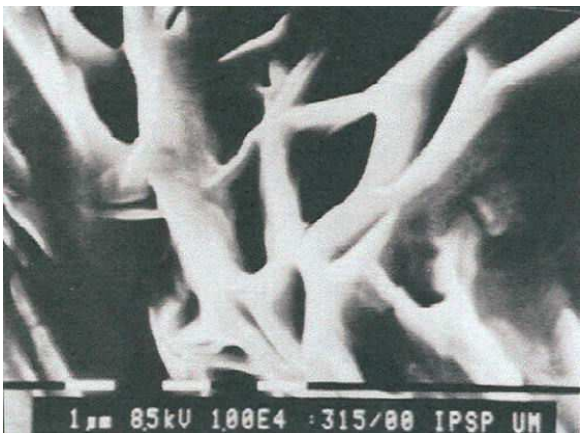

Fig. 4: SEM image of the pure PEO 100K membrane

Previous studies had revealed that by increasing the molecular weight of PEO, the water uptake percentage by the membrane would be improved. This improvement was due to the decrement in the crystallinity property of the chitosan and the intermolecular interactions of PEO with chitosan. However, the percentage started to decrease after PEO with very high molecular weight (for instance 600K) is blended with chitosan although crystallinity was further suppressed[14]. Therefore, we proposed that excess of membrane amorphosity also contributed to the deterioration of the membrane porosity thus prohibit the permeability of various solutes as proven in the XRD results.

Fourier transform infra red (FTIR): The IR spectra for pure chitosan, PEO 600K, PEO 100K, chitosan-PEO 600K blends and chitosan-PEO 100K blends are compared in Fig. 9 and 10. It is obvious from the spectra that molecular interactions did occur in both blends. However, it is clearly seen from the spectra that by adding PEO 600K, the chemical structure of chitosan had been changed significantly. Certain characteristic bands of chitosan had been eliminated or had been reduced their intensity severely.

The superposition of the both chitosan and PEO 600K spectra could be the cause for such phenomenon to happen. Furthermore, the introduction of higher molecular weight PEO had resulted in disturbing the chemical structure of the blend which might be responsible in introducing porosity of the membrane surface.

Meanwhile, the addition of PEO 100K had only enhanced the intensity of peaks in the blend. Certain features of PEO 100K had also been introduced. For instance, the formation of a peak at 1152 cm^{-1} which is due to the antisymmetric bridge C-O-C stretching vibrations[15] in PEO. This peak is absent in the pure chitosan spectrum. Thus, we suggest that the enhancement of certain features of chitosan might assist in improving the morphological and also material phase structure of the blend. As a comparison, this reaffirmed our assumption that molecular interaction in Chitosan-PEO 100K blend is lesser.

The first peak for chitosan at 898 cm^{-1} is assigned to the saccharide structure[16]. For the chitosan-PEO 100K blend IR spectra, the peak position is the same. However, the peak had moved to 843 cm^{-1} which in accordance with the peak existed at the same location in PEO 600K. This suggested that the saccharide structure have been disturbed[17] and the formation of the peak propose that the CH_2 are in rocking modes in gauche conformation[15].

For chitosan-PEO 600K blend, the band attributed to the vibration of $–NH_2$ group is missing at 1590 cm^{-1}[18]. Thus, the interaction between PEO 600K and chitosan is responsible to eliminate the NH_2 deformation band from the new formed blend.

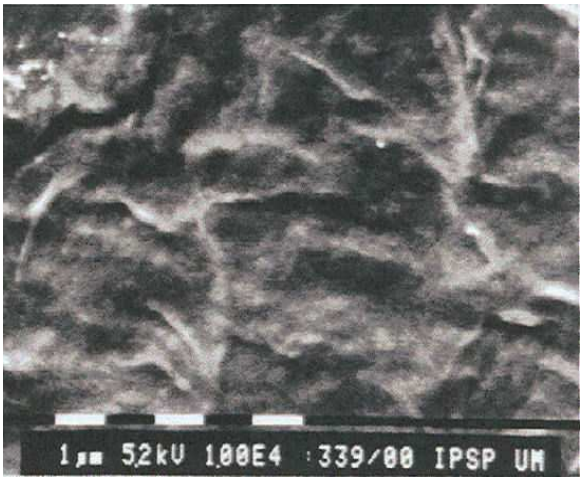

Fig. 5: SEM image of the chitosan-PEO 600K blend membrane

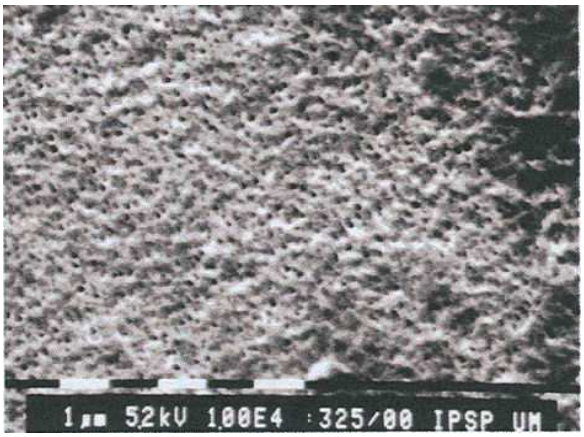

Fig. 6: SEM image of the chitosan-PEO 100K blend membrane

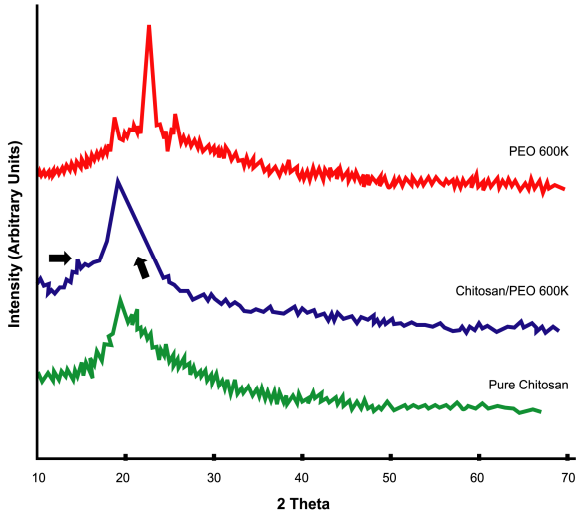

Fig. 7: X-ray diffractograms of pure PEO 600K, Chitosan-PEO 600K blend and pure Chitosan

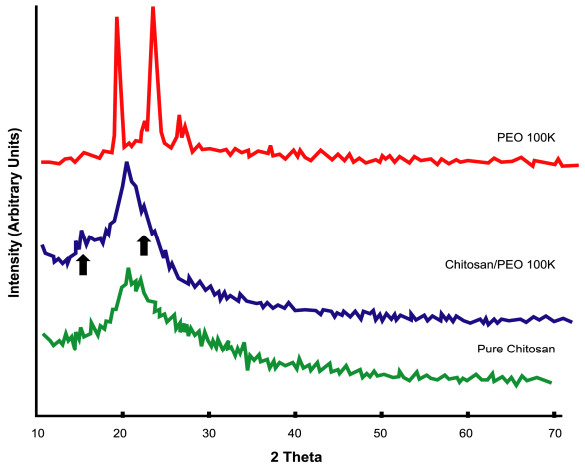

Fig. 8: X-ray diffractograms of pure PEO 100K, Chitosan-PEO 100K blend and pure Chitosan

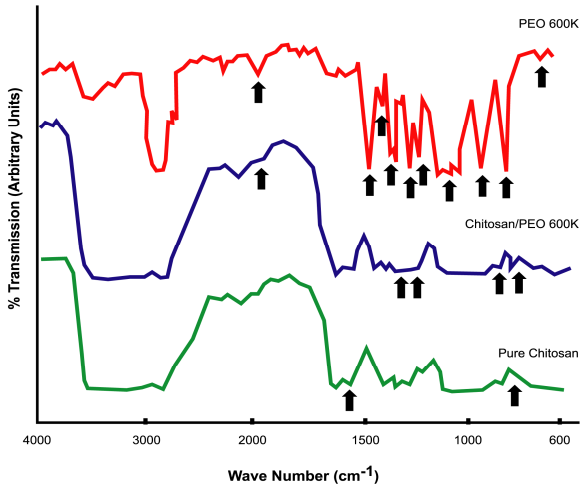

Fig. 9: IR spectra for PEO 600K, Chitosan-PEO 600K blend and pure Chitosan

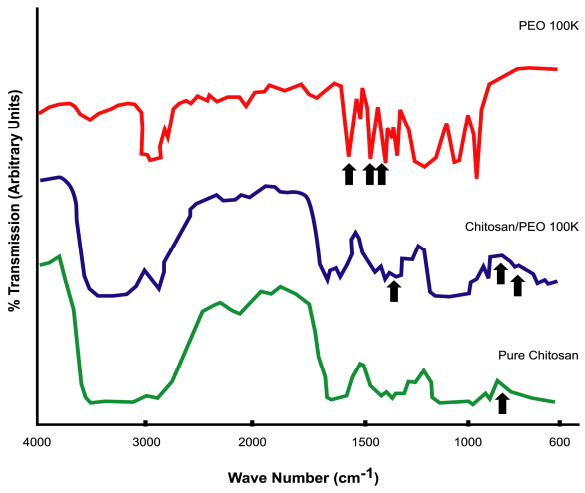

Fig. 10: IR spectra for PEO 100K, Chitosan-PEO 100K blend and pure Chitosan

This would be attributed to the increase of hydrogen bonding[18]. The chitosan-PEO 100K blend shows a more intense peak than the pure chitosan at the position but was shifted 4cm^{-1} from 1590 cm^{-1}. This indicates the complexation between PEO 100K and chitosan had happen. Furthermore, the effect of PEO 100K addition had contributed to the enhancement of both NH_2 band (1586 cm^{-1}) and also amide I (1654 cm^{-1}) peaks which is clearly distinctive in the blend spectra compared to the pure chitosan.

Another missing peak from the chitosan-PEO 600K blend spectra is the asymmetric CH_2 twisting peak which is located at ~1260 cm^{-1}[18]. Both pure chitosan and chitosan-PEO 100K blend exhibits the peak with similar intensity, although the band was shifted for 3cm^{-1} in the blend. An OH and CH deformation band is also absent in the chitosan-PEO 600K blend at 1320 cm^{-1} and a slight shift from 1323 cm^{-1} to 1324 cm^{-1} is observed in chitosan-PEO 100K blend[18].

CONCLUSION

Experimental results had shown by adding lower molecular weight PEO had improved the porosity of chitosan. Formerly, only the increment of crystallinity is blamed for limiting porosity [14], but from our study, the increase of amorphosity due to the introduction of higher molecular weight PEO could also reduce the water adsorption capability. Higher molecular weight PEO had not only increases the amorphosity of the material but also significantly changed the chitosan molecular structure which is exhibited in our FTIR results. The result from these molecular interactions in the blend had decreased the ability to adsorb water, thus, limiting permeability of the membrane.

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