Properties of polyvinyl pyrrolidone/β-chitosan hydrogel membranes and their biocompatibility evaluation by haemorheological method

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The semi-IPN hydrogel membranes of polyvinyl pyrrolidone (PVP) and β-chitosan were synthesized by crosslinking β-chitosan with glutaraldehyde. Hydrogel membranes were characterized by spectroscopic, swelling, thermal and mechanical properties. The *in vitro* biocompatibility of hydrogel membranes was studied by haemorheological method. These hydrogels have water contents in the range of 60–70% with a high fraction contributed by free water (> 45%). The gel composition, amount of cross-linking agent and swelling temperature plays an important role in swelling kinetics of these semi-IPN membranes. Melting temperature (T_m) of membranes increased with a decrease in endothermic peak with increasing β-chitosan content. The tensile strength of membranes in the dry state was found to be high (29–43 MPa) and it increased with increasing β-chitosan content. The *in vitro* haemorheological studies indicated the biocompatible nature of membranes with no significant changes in whole blood and plasma viscosity and red blood cell rigidity.

1. Introduction

Hydrogels are polymeric networks which swell in aqueous media at physiological temperature, pH, and ionic strength. Many hydrogels have potential biomedical applications since they have been proved to be biocompatible, promote cell growth and tissue regeneration [1, 2]. The utility of biopolymers for slow drug delivery systems, tissue substitutes and cell attachment matrices has been investigated because of their compatibility with body environment [3, 4]. β-chitosan is a deacetylated product of β-chitin, which is an abundantly found polysaccharide in nature. β-chitosan and polyether semi-IPN networks were synthesized and studied for their swelling properties [5, 6]. The use of β-chitosan or β-chitosan-poly (ethylene oxide) hydrogels in slow drug delivery systems and in the entrapment of enzymes has been investigated [7–9]. The semi-IPN networks of macromer prepared from poly(ethylene glycol) and β-chitin or β-chitosan have been found to posses good mechanical, thermal and swelling properties [10, 11]. Water soluble β-chitosan membranes cross-linked with glutaraldehyde have also been investigated [12]. β-chitosan has been used as wound dressing since it allows normal cellular components of skin to regenerate and prevent scar tissue formation [13] and also as a scaffold in orthopaedics [14]. β-chitosan has hemostatic activity but its hemocompatibility can be improved by chemical modifications leading to sulfate esters or by complexation/ interpenetration of anionic polysaccharides [14, 15]. It also possesses immunomodulating properties for the activation of non-specific host resistance against various infections by stimulating the production of monokines [16]. PVP, a synthetic polymer has good biocompatibility and is used as a blood plasma expander [17] and as a vitreous humor substitute [18]. The UV cured films of n-vinyl pyrrolidone (monomer of PVP) are proposed as a potential bioadhesive wound dressing matrix when blended with other polymeric materials [19]. The evaluation of haemorheological parameters to screen the materials for their biocompatibility has been reported [20, 21].

The objectives of the present report were to characterize PVP/β-chitosan semi-IPN membranes and to evaluate the biocompatibility of the hydrogels by haemorheological parameters.

2. Materials and methods

2.1. Materials

β-chitosan and polyvinyl pyrrolidone (PVP) (Mw = 40 000) were purchased from ICN Biomedicals Inc. (Ohio, USA) and from SRL Laboratories (Mumbai, India).

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2.2. Membrane casting

β-chitosan solution (2% w/v) was prepared by dissolving β-chitosan in an aqueous solution of acetic acid (2% v/v). PVP solution (4% w/v) was prepared in double distilled water and various compositions (1 : 1, 2 : 1 w/w) of β-chitosan and PVP were prepared as denoted in Table I. An aqueous solution of glutaraldehyde (0.5% of final volume) was then added with continuous stirring. Membranes were cast in a casting apparatus and kept for drying at room temperature ($30 \pm 2°C$) for 48 h. After drying they were soaked in 0.1 N NaOH solution for 5 min, washed with double distilled water, followed by absolute ethanol and dried at 45 °C for 24 h. They were stored in vacuum until used.

2.3. Characterization

Spectroscopic structural elucidation of β-chitosan, PVP and semi-IPN membranes was performed by Fourier transform infrared spectroscopy (FTIR, Nicolet model Impact 400).

To measure the equilibrium water content (EWC), preweighed dry samples were dipped in double distilled deionized water. At various time intervals swollen samples were removed and weighed on a sensitive balance (Sartorius Analytic, A200S) after excess water from the surface was blotted by tissue paper. This procedure was repeated until there was no increase in weight of the samples even after immersion in water. The following equation was used to determine the equilibrium water content.

$$\text{EWC } (\%) = W_t - W_o/W_t \times 100 \qquad (1)$$

where W_t and W_o are the sample weight at time t and in the dry state, respectively. Effect on swelling kinetics of temperature, amount of cross-linker and composition of membranes was determined using the same procedure.

Differential scanning calorimetry (DSC) was performed on a calorimeter (Dupont Instruments, 2100 DSC) to determine the melting endotherms and free water melting endotherms of dry and wet samples, respectively. All samples were equilibrated at 30 °C, in sealed aluminum pans and then scanned up to 180 °C with a heating rate of 10 °C min^{-1} under nitrogen flow. The melting characters of free water were determined in the temperature range from -40 °C to 30 °C with a heating rate of 5 °C min^{-1}.

Mechanical properties of hydrogels were tested using a Universal testing machine (Instron 4204). All tests were carried out following ASTM standards. The tensile strength and % strain at maximum load of membranes were measured in the dry state with extension speed of 5 mm min^{-1}. Reported values are the mean of three experiments and deviation from mean is $\pm$ 5%.

2.4. Biocompatibility evaluation by haemorheological parameters

Screening for biocompatibility was performed by haemorheological tests [21]. Blood from healthy human volunteers was collected in EDTA coated tubes and incubated with membranes (1 cm^2) at 37 °C for 45 min. A part of the treated blood was used to separate plasma and red blood cells (RBC). RBCs (450 µl) were suspended in Eagle's buffer (900 µl). Viscosity for whole blood, plasma and RBC suspension was detected by bob-and-cup arrangement viscometer (Contraves low shear 30 viscometer) at shear rate of 51.2. Blood samples that were incubated without membranes were used as control.

2.5. Statistical analysis

Jandel Scientific ''Sigma Stat'' statistical software (version 2.0) was used to perform statistical analysis. Data was obtained from three different experiments and represented as mean $\pm$ SEM (scanning electron microscopy). Mann-Whitney rank sum test was employed to determine statistical significance between samples.

3. Results and discussion

3.1. Fourier transform infrared analysis

FTIR spectra of β-chitosan, PVP and semi-IPN membranes HM1 and HM2 are shown in Fig. 1. The peak at 1596 cm^{-1} in spectra of β-chitosan is due to characteristic N-H bending vibration [22]. Spectra of PVP show a peak at 1288 cm^{-1}, a characteristic peak for C—N stretching. Disappearance of the peak at 1597 cm^{-1} and appearance of a new peak at 1648 cm^{-1} and 1643 cm^{-1} in HM1 and HM2 is attributed to the formation of C═N (imine linkage) due to cross-linking reaction between aldehyde group of glutaraldehyde and amino group of β-chitosan [23]. Appearance of a new peak ensures the proper cross-linking by glutaraldehyde to form semi-IPN network structure. HM1 and HM2 show a common peak at 1288 cm^{-1} which is attributed to PVP (C—N stretching vibration).

3.2. Swelling characteristics

The equilibrium water content and effect of composition on membranes HM1 and HM2 is shown in Fig. 2. EWC was 70% and 63% for HM1 and HM2 respectively, while total water content of β-chitosan was found to be around

TABLE I Sample composition, water contents, thermal properties and mechanical properties of semi-IPN hydrogels

Sample designation	β-Chitosan W_t %	PVP W_t %	Total water W_t %	Free water $W_t - W_b$ (%)	Bound water W_b (%)	T_m °C	H_f Jg^{-1}	Tensile strength MPa	% Strain at max. load
HM1	50	50	70	33	37	110	410	29.67	5.74
HM2	67	33	63	52	11	118	351	43.13	4.84

HM* has the same composition but twice the amount of cross-linker than HM1.

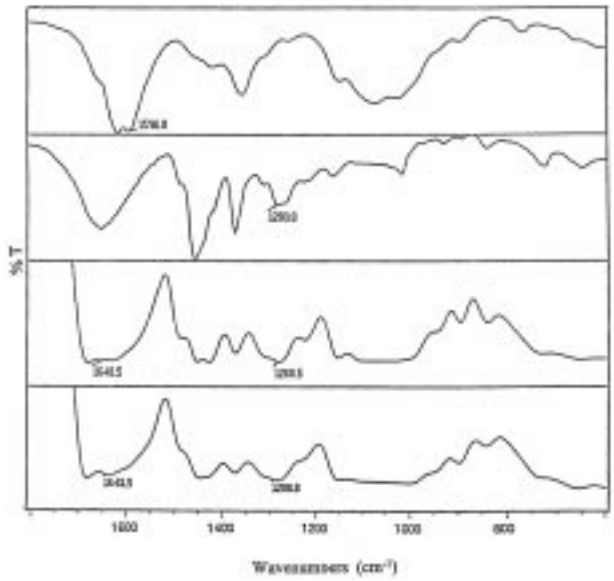

Figure 1 FTIR spectra of (a) β-chitosan, (b) PVP, (c) HM1 and (d) HM2.

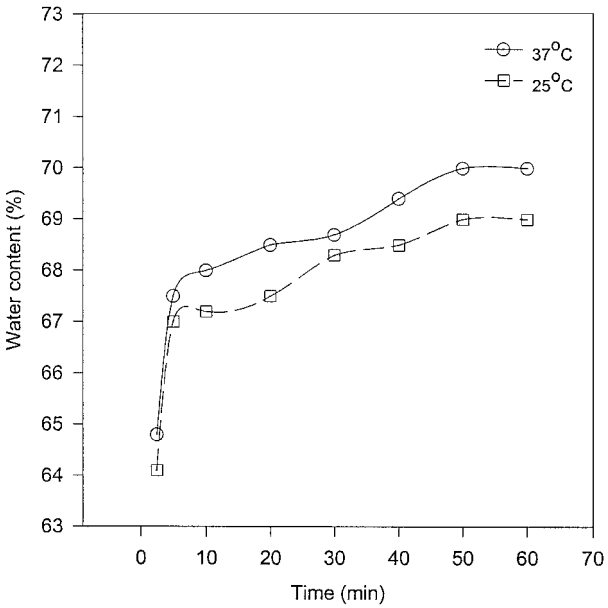

Figure 3 Effect of temperature on the swelling kinetics of semi-IPN (HM1) at 37 °C.

46%. Thus with increasing percentage of β-chitosan water content was decreasing, this can be due to the availability of more cross-linkable groups with increasing content of β-chitosan and thus forming a more cross-linked network. Fig. 3 demonstrates the effect of temperature on swelling of HM1 which was slightly more at 37 °C than at 25 °C. This is attributed to faster transport of water molecules and relaxation of semi-IPN network at higher temperature. The effect of amount of cross-linker on the swelling kinetics of HM1 is shown in Fig. 4. It was observed that from the beginning the swelling was less for membranes with a greater amount of cross-linker and this pattern continued till complete swelling was achieved. This could be due to an increase in cross-linking density which will have the effect of increasing the resistive force to chain elongation.

The bound water and free water content of membranes (Table I) is determined from DSC free water melting endotherms. Ratio of endothermic peak between 0 to 10 °C for water swollen samples and the endothermic heat of fusion (334 Jg^{-1}) for pure water determines the fraction of free water in total water.

The following equation was used to calculate the bound water content of semi-IPN membranes.

$$W_b = W_t - (W_t - W_{fb}) = W_t - Q_e/Q_f \qquad (2)$$

where Q_e is the heat of fusion for pure water and Q_f is the heat of fusion for the sample. It is seen from the table that the free water content of the membranes increased as β-chitosan content increased in the hydrogels.

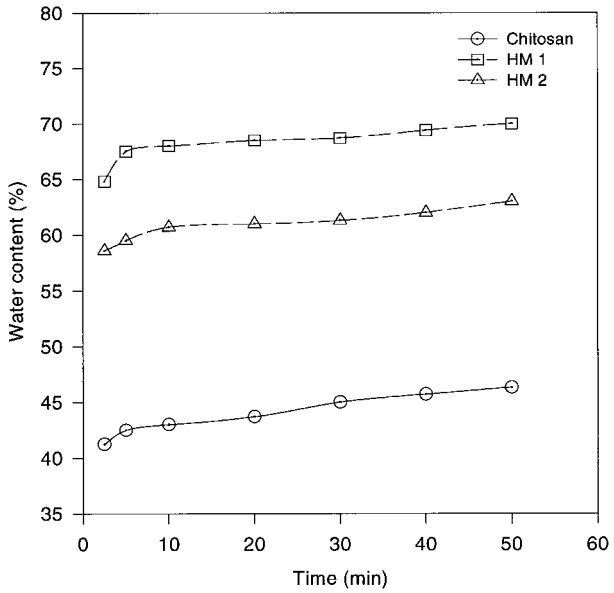

Figure 2 EWC and effect of composition on swelling kinetics of semi-IPN hydrogels.

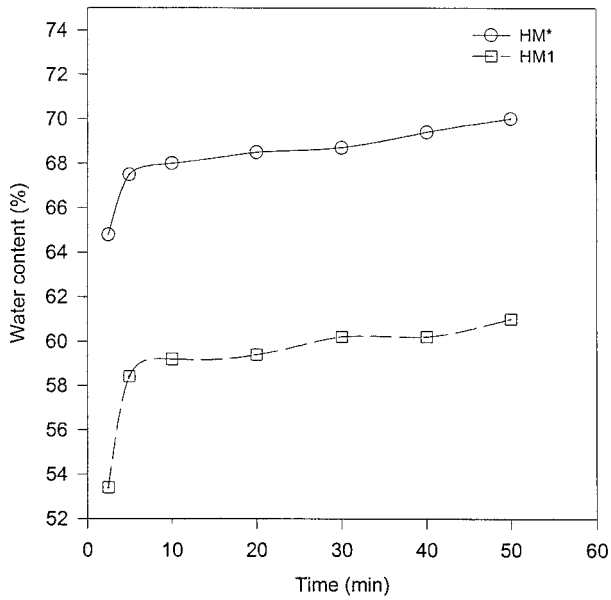

Figure 4 Effect of cross-linker on the swelling kinetics of semi-IPN (HM1) at 37 °C.

TABLE II Preliminary biocompatibility evaluation of semi-IPN by haemorheological parameters

Tests	Control (Cp)	Experimental (Cp)	p value
Whole blood viscosity			
HM1	5.10 ± 0.115	5.62 ± 0.051	0.10
HM2	5.10 ± 0.115	5.12 ± 0.309	1.00
Plasma viscosity			
HM1	1.32 ± 0.044	1.36 ± 0.051	1.00
HM2	1.31 ± 0.051	1.35 ± 0.268	1.00
RBC rigidity			
HM1	2.93 ± 0.232	3.22 ± 0.98	0.40
HM2	2.78 ± 0.14	3.21 ± 0.093	0.10

3.3. Thermal properties

Thermal properties of semi-IPN membranes are shown in Table I. The melting endotherms of PVP segments in the membranes appear at around 110 °C and 118 °C compared to 146 °C for PVP. Melting temperature for β-chitosan could not be detected due to the rigid backbone of β-chitosan. As cross-linking density increases the area of melting endotherms decreases with increase in melting temperature.

3.4. Mechanical properties

Table I shows the mechanical properties of hydrogel membranes. The tensile strength at breaking for semi-IPN membranes in dry state increased with an increase in content of β-chitosan. The unmodified chitosan membrane showed a tensile strength of 27.3 MPa, which is less than the tensile strength of both HM1 and HM2. This indicates that PVP has imparted additional strength to the hydrogel network due to its viscoelastic properties. Strain at maximum load shows a decrease from HM1 to HM2, which is due to the decrease in elasticity. Thus these membranes exhibit good mechanical properties.

3.5. Biocompatibility evaluation

The whole blood viscosity, plasma viscosity and RBC rigidity profile after incubating blood with semi-IPN membranes is shown in Table II. There was no significant change in the whole blood viscosity $(p = 0.1, 1.0)$ and plasma viscosity $(p = 1.0, 1.0)$ between control (incubation without membranes) and samples (incubated with membranes HM1 and HM2) respectively. This indicates that there could be no significant changes in the density of cellular component of blood and protein content of the plasma by adsorption of either of these on membranes. This also points out that there was no significant cell clumping in the blood, which otherwise would have changed the viscosity of blood incubated with membranes. The RBC rigidity had no significant differences $(p = 0.4, 0.1)$ between control and samples, indicating that hydrogel membranes had no effect on the plasma membrane rigidity. No haemolysis was observed after incubating hydrogel membranes with blood. All these haemorheological tests give a preliminary indication of good biocompatibility of β-chitosan/PVP semi-IPN membranes.

4. Conclusions

The hydrogel membranes from β-chitosan and PVP were synthesized by cross-linking β-chitosan with glutaraldehyde to prepare semi-IPN networks. Hydrogel membranes exhibited water content in the range of 60–70% with a high percentage of free water. The swelling kinetic behavior indicates that gel composition, amount of cross-linking agent and swelling temperature play an important role in swelling kinetics of the semi-IPN. Melting temperature (T_m) of membranes increased from 110–118 °C with decrease in endothermic peak area as cross-linkable groups increased. The mechanical strength of membranes in the dry state was found to be good and it improved with increasing content of β-chitosan. PVP seems to have contributed in improving mechanical strength of membranes due to its viscoelastic nature. Haemorheological studies indicated the membrane biocompatiblity. These semi-IPN membranes had good physical properties and could be promising candidates in biomedical applications.

Acknowledgments

The authors wish to acknowledge the School of Biomedical Engineering, Indian Institute of Technology, Bombay for funding this project. We are grateful to Prof. R. R. Puniyani and (Mrs) R. Gupte for their help in haemorheological studies. We also thank Dr Lele, Polymer Processing Division, National Chemical Laboratory for making the UTM facility in his laboratory available.

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Received 22 October 1998
and accepted 22 July 1999