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Synthesis and properties of poly(ethylene glycol) macromer/β-chitosan hydrogels

Y. M. LEE*, S. S. KIM, S. H. KIM
Department of Industrial Chemistry, College of Engineering, Hanyang University, Seoul 133-791, South Korea

Semi-interpenetrating polymer network (semi-IPN) hydrogels composed of β-chitosan and poly(ethylene glycol) diacrylate macromer (PEGM) were synthesized and characterized for the application as potential biomedical materials. The mixture of PEGM and β-chitosan, dissolved in water including a small amount of acetic acid, was cast to prepare hydrogel films, followed by a subsequent crosslinking with 2,2-dimethoxy-2-phenylacetophenone as a non-toxic photoinitiator by ultraviolet irradiation. Photocrosslinked hydrogels exhibited relatively high equilibrium water content in the range 77–83% which is mainly attributed to the free water content rather than to the bound water, hydrogen bonded with components in semi-IPN hydrogels. The crystallinity, thermal properties and mechanical properties of semi-IPN hydrogels were studied. All the photocrosslinked hydrogels revealed a remarkable decrease in crystallinity. The glass transition temperatures, T_g, of crosslinked PEGM segment in semi-IPNs increased compared with poly(ethylene glycol) itself. However, with increasing β-chitosan content their T_g decreased owing to the higher degree of crosslinking. The tensile strengths of semi-IPNs in dry state were rather high, but those of hydrogels in wet state decreased drastically.

1. Introduction

Hydrogels are polymeric networks which retain a large amount of water within their crosslinked structure without dissolution and are found to be relatively tissue compatible, because their high water content and soft rubbery consistency can give them a certain resemblance to living tissue.

Sawhney *et al.* [1] synthesized bioerodible hydrogels based on photopolymerized poly(ethylene glycol) macromer (PEGM), which showed potential for use in macromolecular drug delivery. In the meantime, the use of natural polymers such as proteins and polysaccharides for the biomedical application has also attracted many investigations. This is because such systems are susceptible to enzymatic digestion inside the body. However, these naturally abundant materials also exhibit a limitation in their reactivity and processability. Many studies have attempted to overcome these shortcomings by chemical and physical alteration of natural polymers. Among these investigations, hydrogel types and interpenetrating polymer network (IPN) structures have been noted by several researchers [2–8] in recent years.

Recently, Yao and co-workers [9, 10] reported chitosan/polyether semi-IPN hydrogels crosslinked by glutaraldehyde and their swelling kinetics [9, 10]. Ramaraj and Radhakrishnan [11] studied the swelling and release of bromothymol blue using an IPN based on gelatin and polyacrylamide. More recently, we have reported the semi-IPN composed of β-chitin and PEGM [12–14].

Chitin has been known as a potentially useful biopolymer produced in a huge amount in nature. There have been many studies of chitin as a biocompatible material. Most chitin studies, however, are based on readily accessible α-chitin whose main chain arranges in an antiparallel fashion with strong intermolecular hydrogen bonding. According to Kurita *et al.* [15, 16], β-chitin has a much higher reactivity and versatility than ordinary α-chitin, because β-chitin has much weaker intermolecular hydrogen bonding ascribable to the parallel arrangement of the main chain and thus can be used effectively as a novel material in the biomedical field. Nevertheless, β-chitin still has a problem with solubility, because there are few strong acidic solvents to prepare solution such as formic acid which may irritate human tissues. However, β-chitosan, the deacetylated product of β-chitin, is dissolved in water by adding a small amount of acetic acid, so that the hydrogel can be prepared under milder conditions. Therefore, it might be expected that β-chitosan would be a strong candidate for hydrogel preparation in biomedical applications. Moreover, it has been shown that chitosan can be hydrolysed by lysozyme which is abundant in animal organs. Chitosan itself has some pharmaceutical activities such as antacid, antiulcer,

*Author to whom all correspondence should be addressed.

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hypocholesterolaemic activity, and suppression of growth of tumour cells. PEG is a water-soluble and non-toxic polymer which can be used to prepare acrylate-terminated macromer that could be crosslinked later [17].

There have been no reports to date on IPNs or semi-IPNs composed of β-chitosan and PEGM. Thus, the objective of the present study is to report the properties of semi-IPNs composed of β-chitosan and PEGM.

2. Experimental procedures

2.1. Materials

Poly(ethylene glycol) (PEG) ($M_n = 6000$) was purchased from Showa Chemical Inc. and was dried by azeotropic distillation with benzene (Junsei Chemical Co. Ltd). Acryloyl chloride and the photoinitiator, 2,2-dimethoxy-2-phenylacetophenone, were obtained from Aldrich and were used without further purification. *n*-Hexane and acetic acid were purchased from Duksan Pharmaceutical Co. Ltd and Janssen Chimica, respectively. All other chemicals were reagent grade and were used as received.

2.2. Preparation of β-chitosan

β-chitin extracted from squid pen by the modified Hackmann method was treated for 2 h in 40% NaOH solution in a reactor at 110 °C under a nitrogen atmosphere [18]. The β-chitosan product obtained by the alkali treatment was washed in water to neutrality, the deacetylation being about 80% or less by the first treatment according to a previous study [19]. β-chitosan after being washed in water was treated again in an alkaline solution for further deacetylation. The alkali treatment and washing processes were repeated three times to obtain β-chitosan products which show deacetylation degree of over 90%. The degrees of deacetylation estimated from elemental analysis and infrared spectroscopy were 92 and 91%, respectively [20]. The viscometric number-averaged molecular weight of β-chitosan using the Mark–Houwink equation was in the range of 5.5×10^5 [21].

2.3. Synthesis of semi-interpenetrating polymer networks

2 wt % β-chitosan solution was prepared by dissolving β-chitosan in 2 wt% acetic acid aqueous solution and filtering with a 2G1 glass filter (Schott). By adding 10 wt% PEGM aqueous solution synthesized in our previous studies, various compositions (PEGM/β-chitosan, 1/1, 1/2 and 1/3 w/w) of PEGM/β-chitosan were prepared [12, 13]. Samples were denoted as in Table I. The PEGM–β-chitosan mixed solutions were bubbled for 30 min with nitrogen and 2,2-dimethoxy-2-phenylacetophenone (0.3 g) dissolved in 1 ml of *N*-vinylpyrrolidone was added. The solutions were poured up to a depth of 5 mm into Petri dishes stored in a box and exposed to a 450 W ultraviolet (UV) lamp (Ace Glass Co.) placed above the mould at a height of 20 cm for 2 h with nitrogen gas blowing until gelation occurred. The whole synthetic scheme is presented in Fig. 1.

2.4. Characterization

Fourier transform infrared (FTIR) spectroscopy (Nicolet model Magna IR 550) was used to confirm the structure of PEG, PEGM, β-chitosan and semi-IPNs. To measure the equilibrium water content (EWC), pre-weighed dry samples were immersed in distilled water. After the excess surface water had been removed with filter paper, the weights of swollen samples were measured. The procedure was repeated until there was no further weight increase on immersing the samples. The swelling procedure was so rapid that swelling kinetics were not detected. EWC was determined according to the following equation:

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

where W_s and W_d represent the weights of swollen and dry samples, respectively.

The crystallinity and melting endotherm of dry hydrogel were investigated by differential scanning calorimetry (DSC) (DuPont Instruments 910 DSC). All samples equilibrated at 20 °C were sealed in aluminium pans and rescanned up to 70 °C with a heating rate of 10 °C min^{-1} under nitrogen flow. The melting characters of free water were estimated by DSC in the temperature range from − 15 to 15 °C with a heating rate of 5 °C min^{-1}.

Dielectric analysis (DuPont Instruments DEA 2970) was employed to measure the glass transition temperatures of semi-IPNs. The measurements of the dielectric constant, ε′, and dielectric loss factor, ε″, were carried out at temperatures ranging from − 30

TABLE I Sample preparation and designation

Sample designation[a]	PEG macromer (wt%)	β-chitosan (wt%)
PEGM	100	0
PCN-1	50	50
PCN-2	33	67
PCN-3	25	75

[a] The PEG used has a molecular weight of 6000.

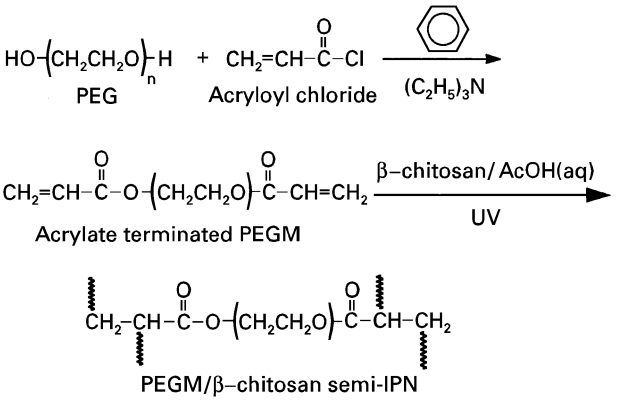

Figure 1 Synthetic scheme of PEGM/β-chitosan semi-IPN.

to 200 °C with frequencies of 100 Hz, 500 Hz and 1 KHz, the heating rate being 2 °C min^{-1}. Experiments were carried out on cast films of 3 cm diameter.

A universal testing machine (Hounsfield 10 KM) measured the tensile strength and elongation at breaking in dry and wet states with an extension rate of 2 mm min^{-1}. For the wet-state measurement, the films were immersed into water until EWC was achieved. Reported values were the mean of the five measurements and the deviation from the mean is within $\pm$ 5%.

3. Results and discussion

3.1. Fourier transform infrared analysis

Fig. 2 shows FTIR spectra of semi-IPN, β-chitosan, PEGM and PEG. A new peak appears at 1730 cm^{-1} in PEGM which can be attributed to the formation of a carbonyl bond due to the reaction between acryloyl chloride and hydroxyl groups in PEG. Hence, the –OH stretching vibration peak at 3400 cm^{-1} decreased remarkably in the PEGM. Moreover, the peak at 1413 cm^{-1} indicates the stretching vibration of conjugated double bonds between C=C and C=O from end groups in PEGM. Accompanying the crosslinking procedure of terminal acryl groups in PEGM by UV irradiation, the peak at 1413 cm^{-1} might disappear in the semi-IPN. The peaks, however, are not identified well because those of β-chitosan in semi-IPN overlap those of PEGM. The spectrum of pure β-chitosan was subtracted from that of the semi-IPN to give the spectrum of PEGM in the semi-IPN. From the resulting subtraction spectrum, it was noticed that the 1413 cm^{-1} band was smoothly cancelled out, which demonstrated that the remaining PEGM was crosslinked relatively well.

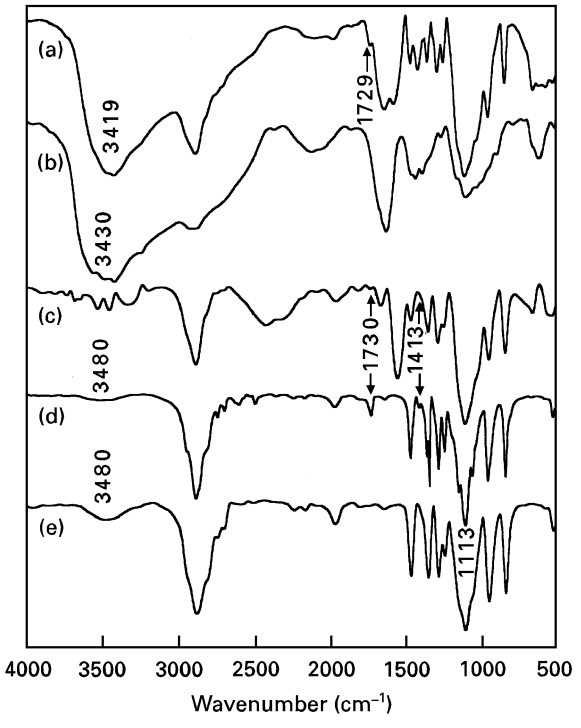

Figure 2 FTIR spectra: (a) semi-IPN; (b) β-chitosan; (c) subtraction; (d) PEGM; (e) PEG.

3.2. Swelling characteristics

All hydrogels swelled so rapidly on immersing the samples in water that the swelling kinetics could not be detected. In our previous studies, the EWC of β-chitin was around 48% and that of semi-IPN incorporated with PEGM was in the range 60–80% depending on the amount of β-chitin component [21]. As listed in Table II, in the case of semi-IPN composed of β-chitosan and PEGM, the EWC was much higher than that of semi-IPN incorporating β-chitin. For PCN-1, the degree of crosslinking may be the highest among the samples, resulting in the lowest EWC. This behaviour is in general agreement with previous results for IPN hydrogels, indicating that high crosslinking density leads to a low water content [9, 11].

Table II also shows the free-water and bound-water contents estimated from the DSC free-water melting endotherms of semi-IPN hydrogels. The fraction of free water in the total water was approximately calculated from the ratio of the endothermic peaks, which were detected between 0 and 10 °C for water-swollen hydrogel, and the melting endothermic heat of fusion (79.7 cal g^{-1}) for pure water using the following equation.

$$W_b = W_t - (W_f + W_{fb}) = W_t - Q_{endo}/Q_f \quad (2)$$

where Q_{endo} is the heat of fusion for ice (equal to 79.7 cal g^{-1}), and Q_f is the heat of fusion for the sample.

As can be seen in Table II, the free water contents of samples are more than 50% of the total water and increased with increasing β-chitosan content in the hydrogel.

3.3. Thermal properties

DSC thermograms (not shown here) exhibit the melting endotherms of PEGM and crosslinked PEGM segments in the semi-IPN. PEGM shows a sharp melting endothermic peak at around 63 °C, while weak and broad melting endothermic peaks of PEGM segments in the semi-IPN, caused by crosslinking reaction and semi-IPN formation, appeared between 50 and 54 °C. In the meantime, the melting temperature, T_m, of β-chitosan was not detected owing to the rigid backbone chain of β-chitosan. In fact, it degraded before melting, which is a typical phenomenon for many polysaccharides.

Table III summarizes the thermal properties and crystallinities of PEGM and semi-IPN hydrogels. It is clear that, as the samples are further crosslinked, the crystallinity (percentage) decreases in the semi-IPN

TABLE II Water contents of semi-IPN hydrogels

Sample	Total water W_t (%)	Bound water W_b (%)	Free water $W_f + W_{fb}$ (%)
PCN-1	77	30	47
PCN-2	81	40	41
PCN-3	83	41	42

TABLE III Thermal properties, crystallinities and mechanical properties of PEGM and semi-IPN hydrogels

Sample	T_m (°C)	ΔH_f (J g^{-1})	Crystallinity[a] (%)	Tensile strength (MPa)	
				Dry	Wet
PEGM	63	122	56	—	—
PCN-1	54	45	20	4.8	0.06
PCN-2	50	24	11	5.7	0.04
PCN-3	51	10	5	6.5	0.03

[a] Crystallinity (%) $= (\Delta H_f/\Delta H_f^0) \times 100$, where ΔH_f^0 is 219.24 J g^{-1} for 100% crystalline PEG.

system. The areas of the melting endotherms in semi-IPNs were markedly reduced after crosslinking. In other words, as photocrosslinking takes place in the mixture, the degree of crystallinity and reorientation of the PEG macromer in semi-IPN hydrogels decrease compared with the PEG macromer itself. Consequently, the T_m of PEG macromer in the semi-IPNs shifted to a lower temperature and the melting peaks became smaller than those of the PEG macromer itself. In general, the glass transition temperature, T_g, of crosslinked polymer is difficult to detect using the ordinary DSC technique. However, the T_g of β-chitin was reported to be around 170 °C by dynamic mechanical thermal analysis [22]. In this study, the more sensitive dielectric analysis (DEA) was employed to determine the T_g of each component in the semi-IPNs.

Fig. 3 exhibits the log(tan δ) to loss factor (log ε″) of the PCN series depending on temperature at 100 Hz, 500 Hz and 1 kHz. Three relaxation peaks appeared at around 10, 60 and 130 °C in the semi-IPN. Our recent study on the thermal analysis of β-chitosan by DEA showed that the T_g of β-chitosan was observed at around 135 °C. Accordingly, the temperature of the maximum tan δ, around 130 °C, is considered to be the T_g of β-chitosan. Another maximum tan δ temperature at 60 °C was thought to be the T_m of PEG macromer segments in semi-IPN as was seen from DSC analysis. The lower maximum tan δ temperature was taken to be the T_g of PEG macromer segments in semi-IPN hydrogels. In fact, the T_g of PEG macromer itself was known to be − 53 °C as was reported in our previous study [12]. DEA showed that the T_g in semi-IPNs was much higher than that of PEG macromer and nearly shifted up to room temperature as a consequence of the fact that the decrease in the steric effect hinders the micro-Brownian motion of chain segment with increasing frequencies. The maximum tan δ temperature of PEG macromer in PCN-1 is higher than that in PCN-3. As the β-chitosan content increases in the semi-IPNs, the number of crosslinkable acrylate end groups contributing to the lower T_g decreases.

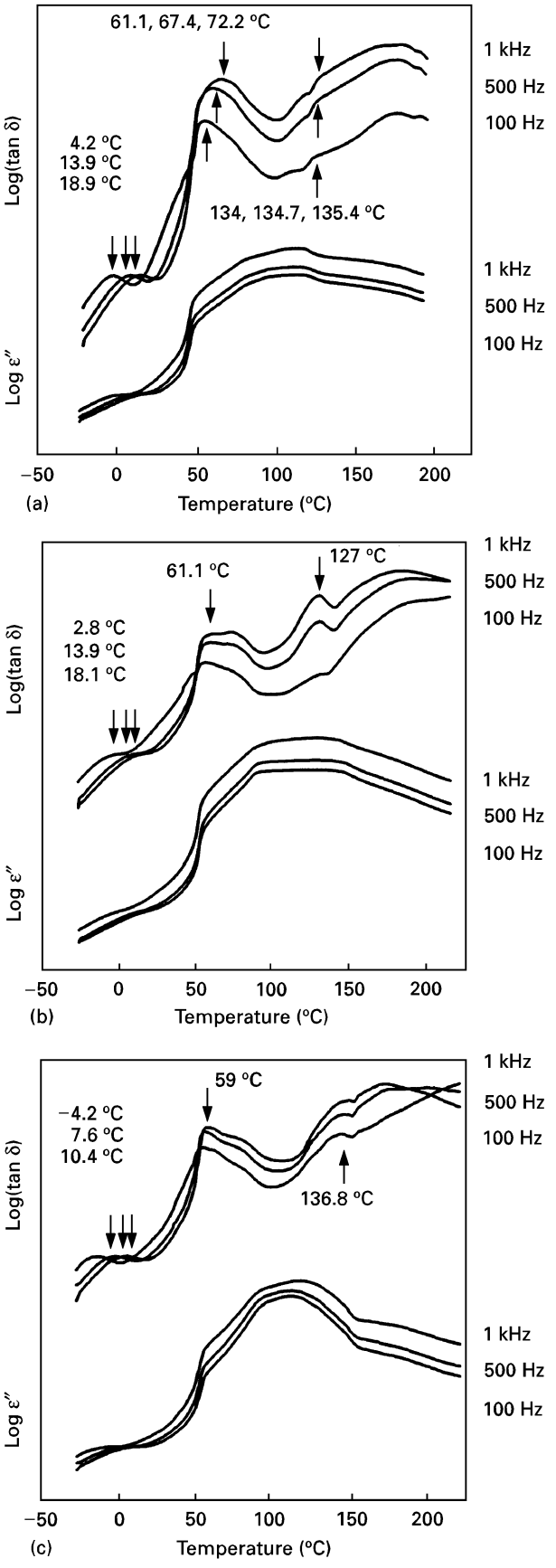

Figure 3 Dielectric analysis of semi-IPN hydrogels: (a) PCN-1; (b) PCN-2; (c) PCN-3.

3.4. Mechanical properties

The mechanical strength of semi-IPNs is also shown in Table III. The tensile strength of semi-IPNs in the dry state was proved to be relatively good. As the β-chitosan content increases, the tensile strength increases to 6.5 MPa. In the wet state, however, the tensile strength of semi-IPN hydrogels was too weak.

This result is contrary to what we expected. It may be caused by the high water content of β-chitosan (about 95%). Therefore, it is assumed that the mechanical strength in the wet state should be improved if both the β-chitosan and the PEG macromer are crosslinked fully to prepare IPN hydrogels by glutaldehyde and/or ultraviolet irradiation. The preparation and properties of full IPNs composed of β-chitosan and PEG macromer are under investigation and will be reported shortly.

4. Conclusions

To prepare a polymeric biomedical material, semi-IPN hydrogels composed of β-chitosan and PEG diacrylate macromer were synthesized by UV irradiation and their properties were studied. Hydrophilic PEG diacrylate macromer segments were crosslinked with β-chitosan. All hydrogels exhibited a high EWC in the range 77–83%. Upon crosslinking, the degree of crystallinity of PEG macromer chains in the semi-IPNs decreased markedly because of the reduced re-orientation of the PEG macromer and β-chitosan. Moreover, the glass transition temperature of PEG macromer segments in semi-IPNs increased nearly up to room temperature. The mechanical strength in the dry state became higher with increasing β-chitosan content. Although the mechanical strength of semi-IPNs in the wet state was much lower than expected, this seemed to be improved by preparing the full IPN system. We are continuing our preparation of full IPN systems, and this will be reported in the near future.

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