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Temperature and pH sensitive hydrogels composed of chitosan and poly(ethylene glycol)

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Summary

Chitosan based semi-interpenetrating polymer network (semi-IPN) hydrogels containing different amounts of poly(ethylene glycol) (PEG) were prepared. The crosslinking of the hydrogels was achieved by using a naturally occurring nontoxic cross-linking agent genipin. The swelling behaviour of these hydrogels was studied by immersing the films in deionized water at 25, 37 and 45°C and in media of different pHs at 37°C. Swelling was found to be dependent on temperature, pH of the medium and the amount of PEG in the gel. States of water in the hydrogels swollen in deionized water at 37°C were determined using Differential Scanning Calorimetry (DSC). The equilibrium water content and the amount of freezing water in the swollen hydrogels increased with the increase in PEG concentration in the gels.

Keywords

chitosan, poly(ethylene glycol), hydrogels, genipin, swelling behaviour, states of water

Introduction

Hydrogels are macromolecular networks, which can absorb large amounts of water while maintaining a distinct three-dimensional structure. Depending on the nature of the networks, hydrogels can be divided into three classes, namely entangled networks, covalently crosslinked networks and networks formed by secondary interactions [1]. Hydrogels are sensitive to environmental parameters such as pH, temperature, solvent composition, ionic concentration and electric fields. These properties make hydrogels an ideal class of materials for medical applications such as drug delivery [2] or scaffolds for cell or tissue culture [3,4]. For applications in the medical field, hydrogels prepared from natural polymers are preferred as these are known to be biodegradable and biocompatible.

Hydrogels of chitosan are currently receiving a lot of attention for medical and pharmaceutical applications, as chitosan is biocompatible, biodegradable, non-toxic and easily available. It is a deacetylated derivative of chitin, one of the most abundant polysaccharides in nature. It has been widely utilized, alone or in combination with

other polymers, for preparing hydrogels. It has been blended with hydrophilic polymers such as poly(vinyl alcohol) [5] and poly(vinylpyrrolidone) (PVP) [6,7] for improving hydrophilic character of the hydrogels.

Poly(ethylene glycol) (PEG) is also used in many biomedical applications due to of its outstanding physico-chemical and biological properties such as hydrophilicity, biocompatibility, and lack of toxicity [8]. Several studies have been published on hydrogels containing chitosan and PEG. Jiang and Han concluded from their viscometric studies that chitosan/PEG blends are compatible due to attractive intermolecular interactions between the polymer chains [9].

Chitosan and PEG based membranes were prepared using glucose mediating process [10]. The swelling measurements showed that the membrane swelled at pH 1.2 and shrank at pH 7.4. Recently a simple method for covalently crosslinking chitosan using PEG diacid as crosslinking agent has been proposed [8]. Networks were obtained by heating solutions of chitosan in aqueous PEG diacid solutions. In another approach PEG was grafted on chitosan and resulting material was used for preparing hydrogels [11].

In most of the studies on chitosan-based hydrogels, glutaraldehyde has been used as the crosslinking agent. However, the toxicity of this crosslinker has adverse effects on the biocompatibility of the obtained materials [12]. Thus, there is a need for a non-toxic and biocompatible crosslinking agents. We have been studying chitosan based hydrogels using a naturally crosslinking agent genipin which is known to be far less cytotoxic than glutaraldehyde [13]. Genipin effectively crosslinks polymers containing amino groups and the crosslinking mechanism has been well described by Butler *et al* [14]. We have reported the preparation and properties of genipin crosslinked hydgrogels of chitosan/PVP [7] and chitosan/PVA [15]. During the present study we prepared genipin crosslinked semi-interpenetrating networks of chitosan and PEG and studied the swelling behavior under different conditions.

Experimental

Materials

Chitosan with 85% degree of deacetylation was obtained from Fluka, U.K. Poly(ethylene glycol) with molecular weight 6000 and buffer tablets of pH 2, 4, 7, 9 and 10 were obtained from Aldrich. Genipin was purchased from Challenge Bioproducts Company. All chemicals were used as such without further purification.

Preparation of hydrogels

1.5 (w/v) % solution of chitosan in 1 (w/v) % acetic acid was obtained by stirring the contents at room temperature. The resulting viscous pale yellow chitosan solution was filtered to remove any undissolved matter. 5 (w/v) % solution of PEG and 0.5 (w/v) % solution of genipin were prepared in deionized water and were added to chitosan solution to obtain different mass ratios as shown in Table 1. The solutions were continuously stirred for approximately 30 minutes and then poured into polystryrene Petri dishes and left for 12 hours to dry at room temperature. The hydrogels were obtained in the form of thin films. The films were further dried in vacuum oven for a week and stored in a desiccator.

Table 1: Compositions of the semi-IPN prepared

Gel designation	chitosan (g)	PEG (g)	genipin (g)	Mass % of PEG in the gel
C6P1	0.60	0.10	0.02	14.3
C4P1	0.60	0.15	0.02	20.0
C3P1	0.60	0.20	0.02	25.0

Swelling Measurements

The swelling behavior of the hydrogels was measured in deionised water at 25°C, 37°C and 45°C. Pre-weighed hydrogel films of surface area approximately 100 mm^2 were placed in beakers containing deionised water and beakers were immersed in a water bath maintained at 25°C, 37°C or 45°C. The films were withdrawn from the solutions at different time intervals and the wet weight was determined after gently wiping the surface water with a filter paper and immediately weighing the films. Measurements were conducted in triplicates. The swelling ratio (SR) was calculated from the following equation:

$$SR(\%) = \left(\frac{W_w - W_d}{W_d} \right) \text{ x } 100 \qquad (\mathbf{1})$$

where W_w and W_d are the wet and dry weights of the films.

The above procedure was continued till no weight increase was observed for at least five measurements. Constant weight indicated that the gel had reached equilibrium swelling state. Equilibrium water content (EWC) of the hydrogels was calculated using the following equation:

$$EWC(\%) = \left(\frac{W_{eq} - W_d}{W_{eq}} \right) \text{ x } 100 \qquad (\mathbf{2})$$

W_{eq} represents the weight of the swollen sample in the equilibrium state.

For studying the swelling behavior of the gels in media of different pHs at 37°C, pre-weighed hydrogel samples were immersed in buffer solutions of pH 2, 4, 7, 9, and 10. The films were withdrawn and the SR and EWC were determined after removing the surface water as stated above.

Differential Scanning Calorimetry

The states of water in the hydrogels were investigated using Differential Scanning Calorimetry (Perkin-Elmer DSC 6). 1-5 mg of the gels swollen in deionized water at 37°C were placed in DSC pans and were scanned from –50°C to 20°C at a heating

rate of 1°C/min under nitrogen atmosphere. The amount of total freezing water was calculated from the melting enthalpies of endothermic peaks near 0°C.

Results and Discussion

Time dependent swelling behaviors of the hydrogels in deionised water (pH 7) at 25°C, 37°C, and 45°C have been plotted in Figures 1 - 3. All the hydrogels swelled rapidly and reached equilibrium within one hour. The swelling behavior plotted in these figures is the average of three trials.

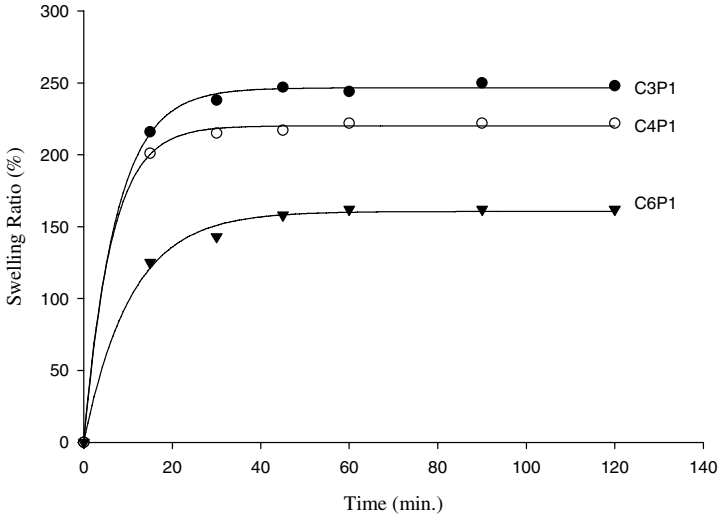

Figure 1: Swelling behaviour of chitosan/PEG hydrogels at pH 7 and 25°C

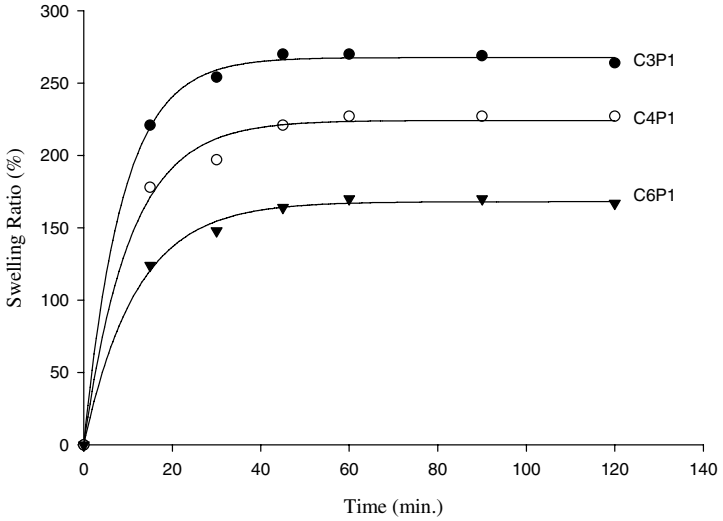

Figure 2: Swelling behaviour of chitosan/PEG hydrogels at pH 7 and 37°C

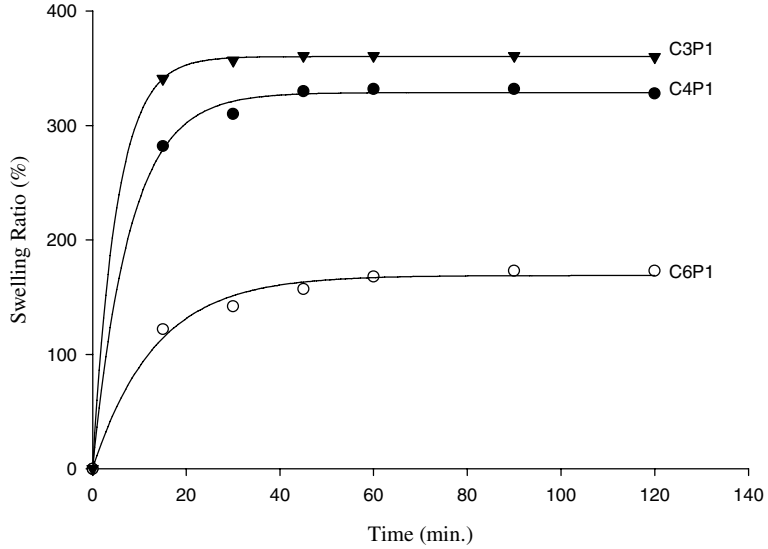

Figure 3: Swelling behaviour of chitosan/PEG hydrogels at pH 7 and 45°C

Figure 4 shows the equilibrium swelling behavior of the hydrogels in deionized water (pH 7) at different temperatures. The swelling ratios of the gels increased as the temperature increased indicating that at higher temperatures the secondary interactions between the polymer chains broke thus allowing more water to move into the matrix of the gel.

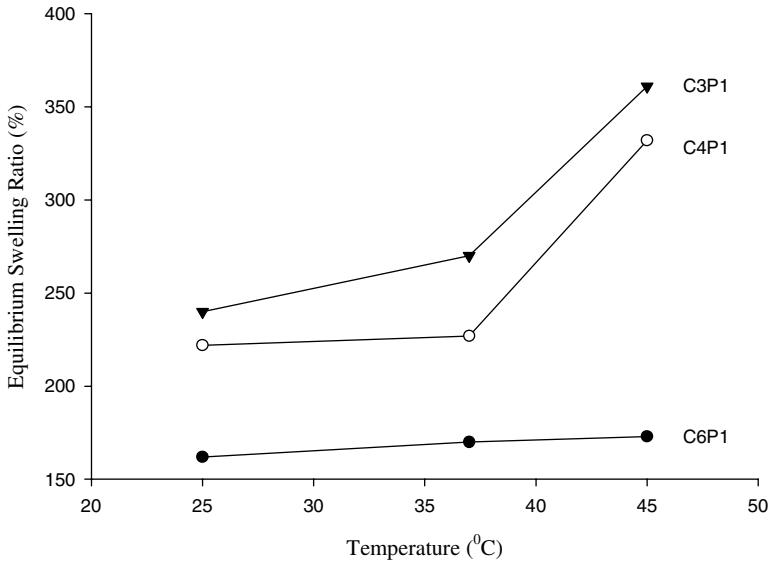

Figure 4: Swelling ratios of chitosn/PEG hydrogels at different temperatures at pH 7

The swelling response of the hydrogels exposed to media of pH 2, 4, 7, 9 and 10 is shown in Figure 5. It can be seen that the hydrogels swelled the most in acidic medium compared to the neutral or basic. In all the hydrogels, maximum swelling was observed at pH 2 and minimum at pH 10. The amount of PEG in the gel influenced the swelling and the hydrogel containing highest percentage of PEG (C3P1) swelled the most followed by C4P1 and C6P1.

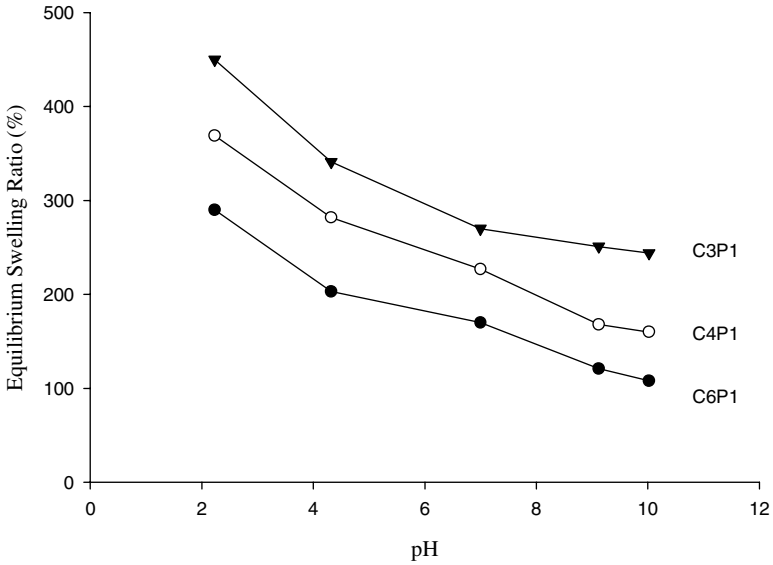

Figure 5: Swelling ratios of chitosn/PEG hydrogels at different pH's at 37°C

The effect of pH on the swelling of the chitosan hydrogels is explained on the basis of protonation of the amino groups of chitosan. In the acidic medium, the protonation of the amino groups leads to repulsion in the polymer chains, thus allowing more water in the hydrogel network. At higher pH, deprotonation of the animo groups takes place and repulsion in polymer chains is reduced. This results in the shrinking of the gels and therefore, the amount of water in the gel decreases.

Differential Scanning Calorimetry (DSC) can provide information about the states of water in the swollen hydrogels. Generally, the state of water in the polymer hydrogel can be divided into free water, freezing bound water and non-freezing bound water [16, 17]. Free water is the freezing water, which does not take part in hydrogen bonding with polymer chains and show a similar transition temperature and enthalpy of fusion in the DSC curves, as pure water does. Freezing bound water (intermediate water) is the water which interacts weakly with polymer molecules and has a melting endotherm below 0°C. Bound water is the non-freezing water as its molecules form hydrogen bounds with polymer chains. These water molecules are immobilized and show no freezing peak even up to –100°C.

Figure 6 shows the DSC heating scans of the hydrogels fully swollen in deionized water at 37°C.

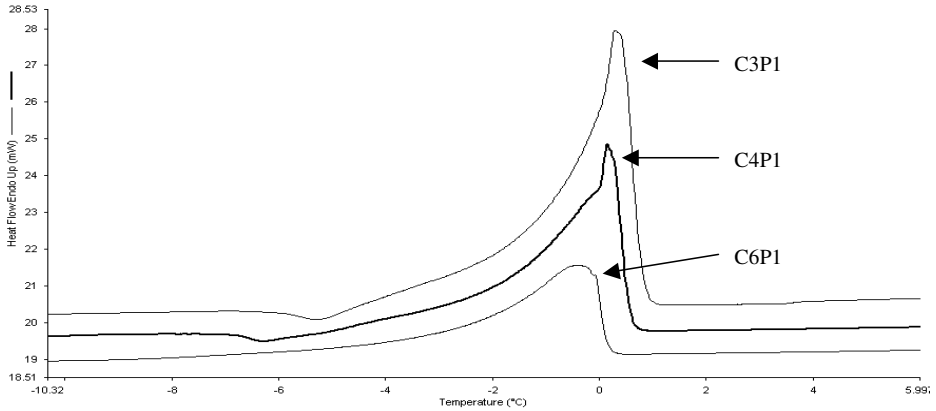

Figure 6: DSC heating scans of the hydrogels swollen in deionised water at 37°C

The enthalpy values associated with the melting peaks (ΔH_{endo}) for various hydrogels were used to calculate the free and bound water in the hydrogels. EWC represents the percentage of total water present in the hydrogel and is sum of the amount of the three types of water.

$$EWC = W_b + (W_f + W_{if}) \quad (3)$$

where W_b is the amount of nonfreezing bound water, W_f and W_{if} are the amounts of free water and intermediate freezing water respectively.

The freezing water (intermediate and free water) in the gel was determined from the ratio of the enthalpy change associated with the endothermic peaks near 0°C and the enthalpy of fusion of pure water (333.7J/K).

It is assumed that the enthalpy of fusion of freezing water in the hydrogel is the same as that of ice. Bound water was obtained by subtracting freezing water from the EWC and these values are given in table 2.

Table 2: Water states of the hydrogels calculated from DSC analysis

Gel	Mass % PEG in the gel	EWC (%)	Freezing water (%)	Bound Water (%)
C6P1	14.3	61.9	47.8	14.1
C4P1	20.0	69.4	58.0	11.4
C3P1	25.0	72.0	67.5	4.5

The results indicate that the EWC and the amount of freezing water in the gels increase as the percentage of PEG increases. The highest EWC and freezing water was observed in the hydrogel C3P1 containing the highest percentage of PEG. The hydrogel C6P1, containing the lowest percentage of PEG, showed lowest EWC and the amount of freezing water. This behavior suggests that PEG increases the hydrophilic character of the hydrogels. The hydrogel containing higher percentage of

chitosan seem to form a relatively compact structure. Since only chitosan is crosslinked by genipin, higher percentage of chitosan would mean availability of more crosslinking groups, thus resulting in a compact structure.

Conclusion

Chitosan hydrogels containing varying amounts of poly (ethelyne glycol) were prepared by crosslinking using a naturally occurring nontoxic cross-linking agent; genipin. Swelling properties of the hydrogels were studied at different temperatures as well as in media of different pHs. The swelling ratio increased as the temperature increased due to weakening of secondary interactions. At lower pH, more swelling of the hydrogels was observed compared to high pH because of protonation of amino acids in the acidic medium. The amounts of freezing water and bound water were investigated using Perkin-Elmer DSC 6. The hydrogel with the highest percentage of PEG showed the highest EWC and amount of free water. With the increase in the percentage of PEG in the gels, the amount of freezing water as well as EWC increased whereas the amount of bound water decreased.

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