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Swelling and Thermal Characteristics of Genipin Crosslinked Chitosan and Poly(vinyl pyrrolidone) Hydrogels

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Summary

Chitosan / poly(vinyl pyrrolidone) (PVP) were used to prepare semi-interpenetrating polymeric networks. The hydrogels were crosslinked using genipin, a non-toxic cross-linking agent extracted from the fruits of *Gardenia jasminoides Ellis*. Swelling properties of these hydrogels were studied in media of different pH's and temperatures. States of water in the swollen hydrogels at 25°C and pH 7 were determined using Differential Scanning Calorimetry (DSC). The swelling behaviour of the hydrogels was found to be dependent on the temperature and the pH of the swelling medium. The total water content in the hydrogels was found to increase with increasing PVP content.

Keywords: chitosan, poly(vinyl pyrrolidone), hydrogels, hydrophilic polymers, swelling behaviour

Introduction

Hydrogels are crosslinked hydrophilic macromolecular networks which can absorb large amounts of water or biological fluids within their structure without disintegrating. The swelling characteristics of the hydrogels are dominated by several factors such as the nature of the polymer, the hydrogel microstructure, temperature, pH, solvent composition, ionic concentration, electric field and magnetic field.

The key properties that make hydrogels valuable in their applications are; the degree of swelling, sorption kinetics, solute permeability, and their in vivo performance characteristics. The degree of crosslinking is an important property which directly influences these properties.

A swollen hydrogel is considered to contain three types of water. 'Free water' which freezes at normal freezing point, 'intermediate freeing water' which freezes below the usual freezing point and 'unfrozen bound water' which does not freeze [1]. Many techniques such as NMR [2], DSC [3], IR [4] and Positron annihilation life time spectroscopy (PALS) [5,6] etc., have been employed to study the states of water in the

hydrogels. PALS technique has revealed that the molecular cavity in the gels is also a contributing factor responsible for water absorption in the hydrogels through diffusion.

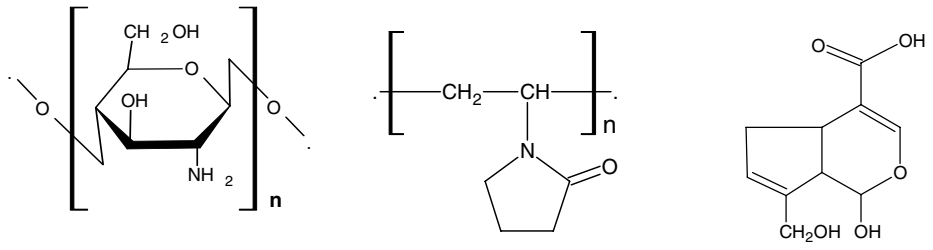
Hydrogels of natural polymers have attracted much interest because they are biocompatible. A wide range of natural polymers have been used to prepare hydrogels which are being tried as candidates for wound healing [7], carriers for the release of drugs [8,9], agriculture and food processing industry.

Chitosan, Fig 1a, the deacetylated form of chitin, is currently receiving a lot of interest in medical and pharmaceutical application because it is non-toxic, biocompatible and biodegradable [10]. A number of hydrogels containing chitosan has been reported [11,12] and have been tested for various applications. Since chitosan is soluble in acidic medium, chitosan gels are often crosslinked to give some stability in its structure.

Glutaraldehyde, formaldehyde, epoxy compounds, dialdehyde, etc. are the commonly used crosslinking agents.However, these compounds are chemically synthesized and are not free from the problems of physiological toxicology [13] and is of concern where the hydrogel is used for biomedical application.

Recently a new crosslinking agent, genipin Fig. 1c, has been successfully used [14]. It is obtained from its parent compound, geniposide, via enzymatic hydrolysis with β-glucosidase [15]. Geniposide is extracted from fruits of *Gardenia jasminoides Ellis* and *Genipa americana* [16] and used in traditional Chinese medicine. Extracts from the fruit has been used to form blue pigments used in food dye known as gardenia blue, when genipin reacts with primary amine groups in the presence of oxygen. It is an effective crosslinking agent for polymers containing amino groups [15] and has been found to be much less cytotoxic than glutaraldehyde [17].

PVP, Fig. 1b, is a highly amorphous water soluble polymer with a glass transition temperature of around 160°C. It is prepared through free radical polymerization of vinylpyrrolidone monomer. It has been considered as being a "miracle" polymer because of its many amazing applications. It is used in the preparation of synthetic blood plasma, construction of hydrogels through crosslinking and creation of thromboresistant hydrophilic gels [18]. UV-cured films have been proposed as potential bioadhesive wound dressing matrix [19]. PVP's viscous and lubricity properties have seen it being used as vitreous humor substitute [20].



a b c

Figure 1. Chemical structure of : a. Chitosan , b. Poly (vinylpyrolidone) , c. Genipin

Blends of chitosan and PVP have been reported to be miscible in the solid state by exhibiting a single composition dependent glass transition temperature [21,22] and hydrogen bonding was responsible for the miscibility [22]. However, viscometric study revealed the blend to be immiscible in the solution due to less interaction between the polymers but more affinity for the solvent [22]. Mechanical properties of these blends crosslinked by UV irradiation [23] were shown to be greatly affected when compared to the individual polymers and dependent on the time of irradiation and composition of sample. The swelling behaviour of the blends crosslinked by γ-irradiation [24] showed the gels to swell reversibly and was sensitive to pH, temperature, the irradiation dose and the amount of PVP in the gel. Another study [25] on this blend system in which ethyleneglycol diglycidyl ether (EGDE) was used as the crosslinking agent also found the swelling was sensitive to pH. No studies have been reported in the literature on Chitosan / PVP system in which genipin has been used as the crosslinking agent.

In the present study, we aimed to prepare genipin crosslinked hydrogels of chitosan and PVP of different weight ratios and investigate their swelling behaviour at various pH's and temperatures. The different states of water present in the swollen hydrogels were also determined using DSC.

Experimental

Materials

Chitosan with 85% degree of deacetylation (Mw: 600 000) was obtained from Fluka, U.K. PVP (Mw: 8.5 x 10^4–1.46 x 10^5) was purchased from Aldrich and genipin from Challenge Bioproducts Company, Taiwan (R.O.C). All chemicals were used without further purification.

Preparation of hydrogels

Chitosan was dissolved in 1% aqueous acetic acid solution at room temperature with continuous mechanical stirring for 24 hrs to obtain a 1.5% (w/v) solution. The viscous solution was filtered through a filter of mesh size 0.5mm to remove any undissolved matter. PVP was dissolved in deionised water at 85°C with continuous mechanical stirring for 1 hr to obtain a 5% (w/v) solution which was clear and homogeneous thus was not filtered.

Varying amounts of 5% PVP solution was added to the chitosan solution to obtain mixtures having chitosan : PVP weight ratios of 1:3, 1:1 and 3:1. Genipin solution of concentration 0.5% (w/v), was slowly added to the mixtures under constant stirring. The detailed composition and designations of the pregels are listed in Table 1.

Table 1. Compositions of genipin crosslinked chitosan:PVP hydrogels

Designation	Vol. of chitosan (cm^3)	Vol. of PVP (cm^3)	Vol. of genipin (cm^3)	Molar ratio Chs : PVP : Genipin
CLChs	40	0	4	1 : 0 : 0.025
ChsPVP1	40	4	4	1 **:** 0.43 **:** 0.025
ChsPVP2	40	12	4	1 **:** 1.28 **:** 0.025
ChsPVP3	40	36	4	1 **:** 3.84 **:** 0.025

The pregel solutions were poured into polystyrene petri dishes and allowed to undergo gelation at room temperature for 12 hrs. The hydrogels were then further dried at 55°C and obtained as thin films of thickness approximately 0.1mm. Films were vacuum dried at 50°C until further treatment.

Swelling Measurements

The swelling behaviour of the hydrogel films was measured by swelling the films in buffer solutions of different pH (2.2, 4.2, 7.1, 8.9 and 10.0) ± 0.1 at 25°C ± 1°C . The swelling kinetics of the hydrogels in deionised water at 25, 35 and 45°C were also investigated. Each film sample (surface area approximately $1cm^2$) was weighed and placed in a preweighed stainless steel wire mesh of size 1mm. The mesh containing the film sample was then submerged into a 250 cm^3 beaker containing the swelling medium and covered with parafilm to avoid evaporation. At preset time intervals the films were withdrawn and their wet weights were determined after blotting with a filter paper followed by blowing with a stream of air to remove the surface water and immediately weighing the films. Swelling measurements were continued until a constant weight was observed. Average of three trials was taken and Q test was done on all measurements. The swelling ratio was calculated using equation 1.

$$E_{sr}\,(\%) = [(W_s - W_d) / W_d] \times 100 \quad (1)$$

where E_{sr} is the percentage water absorption of the films, W_d and W_s are the weights of the samples in the dry and swollen states respectively.

The equilibrium water content (EWC) was calculated using equation 2.

$$EWC\,(\%) = [(W_e - W_d) / W_e] \times 100 \quad (2)$$

where W_e represents the weight of the swollen state at equilibrium.

DSC Measurements

Perkin Elmer Pyris 6 DSC was used to investigate the different states of water in the hydrogels. Samples of mass approximately 1-5mg and diameter 5mm were weighed before and after swelling in deionised water for 12 hrs at 25°C and pH 7. Scanning was done from -50°C to 20°C with a heating rate of 5°C/min under nitrogen flow. Average of five measurements was taken and the Q test was performed on all measurements.

Results and Discussion

Hydrogels of chitosan/PVP crosslinked by genipin were pale pink in colour and changed to greenish blue upon drying. The time dependent swelling behaviors of the blend hydrogels in deionised water (pH = 7) at 25, 35 and 45°C are shown in Figs. 2-4. All hydrogels revealed a rapid increase in water content and reached equilibrium state in approximately 30 mins.

ChsPVP3 showed the highest swelling ratio at all temperatures while ChsPVP1 showed the lowest. The degree of swelling was observed to increase with increasing PVP content in the hydrogel.

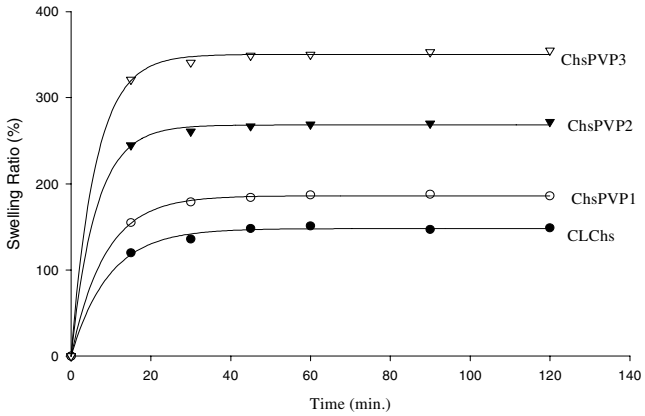


Figure 2. Swelling Ratio of CLChs and ChsPVP hydrogels at 25°C and pH 7

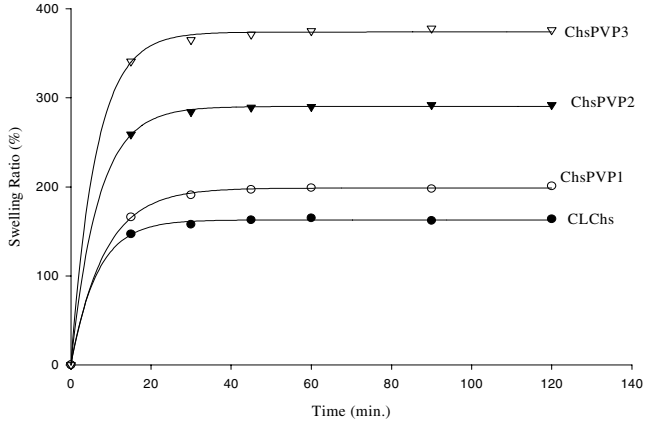


Figure 3. Swelling Ratio of CLChs and ChsPVP hydrogels at 35°C and pH 7

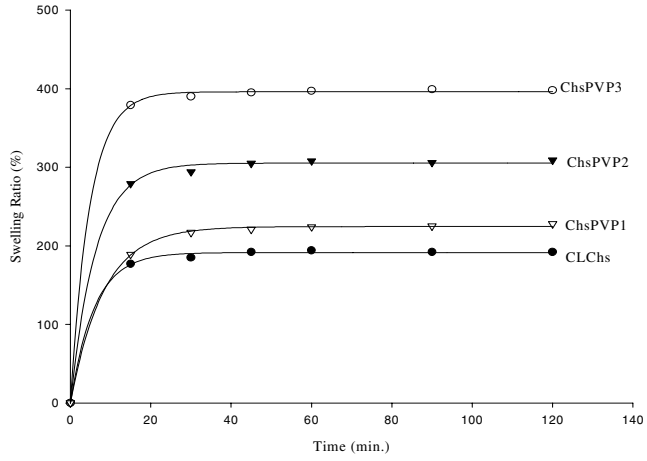


Figure 4. Swelling Ratio of CLChs and ChsPVP hydrogels at 45°C and pH 7

Swelling in hydrogels occurs when water molecules get absorbed by the gel. Initially swelling is due to water molecules forming hydrogen bonds with hydrophilic groups present in the polymer chains. More water then orientates around the bound water to form cage like structures or clusters. Finally, excess water enters freely into the gel network resulting in more swelling [26].

Swelling study carried out by Dergunov et. al. [24] on PVP/chitosan hydrogels crosslinked by γ irradiation, the degree of swelling was found to decrease with increasing PVP content. This is because of the way in which the crosslinking of the hydrogels was achieved. γ irradiation crosslinks PVP chains only and degrades chitosan to some extent. As the concentration of PVP increased, the degree of crosslinking also increased making the gel microstructure rigid resulting in decreased swelling.

Genipin is known to crosslink the amino groups of the chitosan chains only [15] and PVP is physically entangled in the chitosan network. High affinity of both chitosan and PVP for the solvent [22] results in large uptake of water into the gels. PVP rich gels show higher swelling as PVP is more hydrophilic than chitosan and the presence of the ring in PVP may create void volumes within the gel structure due to the irregular arrangement of the chains in the gel. Thus during swelling water molecules are able to diffuse in to fill up the void volumes without forming hydrogen bonds. This fact is also supported from DSC results.

The temperature dependent swelling behaviors of the hydrogel at pH 7 at 25, 35 and 45°C are summarized in Fig. 5. All hydrogels exhibited a temperature responsive behaviour. Swelling increased with increase in temperature. It is suggested that as the temperature is increased the intra and inter molecular hydrogen bonds in chitosan dissociates, thus allowing more water within the hydrogel network.

To study the effect of pH on the swelling behaviour, the hydrogels were allowed to swell to equilibrium at 25°C in an aqueous medium of pH 2.2, 4.2, 7.1, 8.9 and 10.0 ± 0.1. The degree of swelling is shown in Fig. 6.

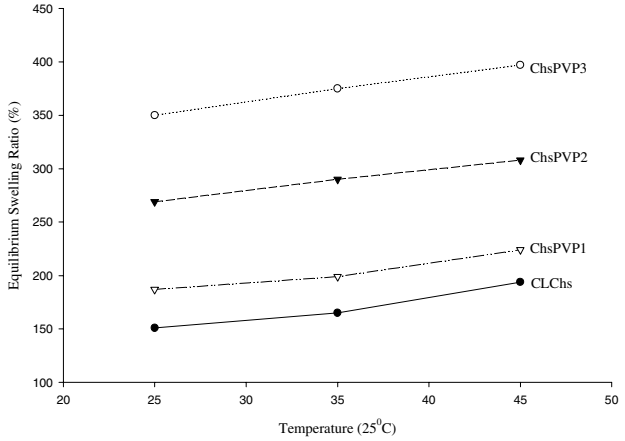


Figure 5. Equilibrium swelling ratio of CLChs and ChsPVP hydrogels at various temperatures at pH 7

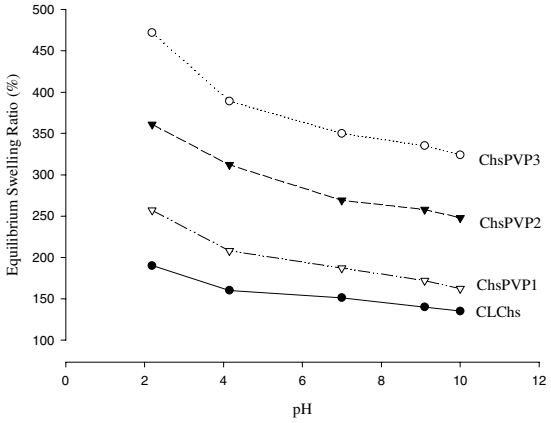


Figure 6. Equilibrium swelling ratio of CLChs and ChsPVP hydrogels at various pHs at 25°C

It was observed that at low pH there was pronounced effect on swelling compared to the neutral and alkaline media. ChsPVP3 showed the highest swelling followed by ChsPVP2 than ChsPVP1. The high swelling behaviour observed at low pH is due to the protonation of the amino groups of chitosan and the nitrogen of PVP. The protonation leads to polymer chain repulsion and dissociation of secondary interactions allowing more water into the gel network. As the pH increased, deprotonation took place, repulsion in the polymer chains receded, allowing shrinking to take place up to pH 7. In neutral and basic media, swelling was mainly driven by solvent diffusion and the swellability of the hydrogels remained relatively unchanged.

Comparing the pH and temperature dependencies of swelling of the hydrogels it was observed temperature sensitivity is very low at pH 7 but pH sensitivity is more extensive.

States of water in the hydrogels were determined using DSC. Three types of water are known to be present in the swollen hydrogels and are referred to as nonfreezing bound water, intermediate freezing water and free freezing water [27]. Nonfreezing bound water refers to immobilized water molecules which are bonded to the hydrophilic sites of the polymer chains and shows no melting peak. Intermediate freezing water molecules preferentially orientates around the bound water and have a melting endotherm below 0°C. Free freezing water molecules show greater degree of mobility and show a melting endotherm around 0°C.

The DSC heating scans of the hydrogels are shown in Fig 7. As expected, separate peaks for intermediate and free water were not observed. A broad melting endotherm around 0°C is attributed to the free freezing and intermediate freezing water. However, ChsPVP2 shows an exothermic peak around –8°C. We are not clear about the origin of this peak but believe it to be due to the relaxation of the polymer chains which occurs when the gel is heated. We also do not understand why this peak is not observed in ChsPVP1 and ChsPVP3.

The enthalpy values associated with the melting peaks (ΔH_{endo}) for various hydrogels are given in column 5 of Table 2.

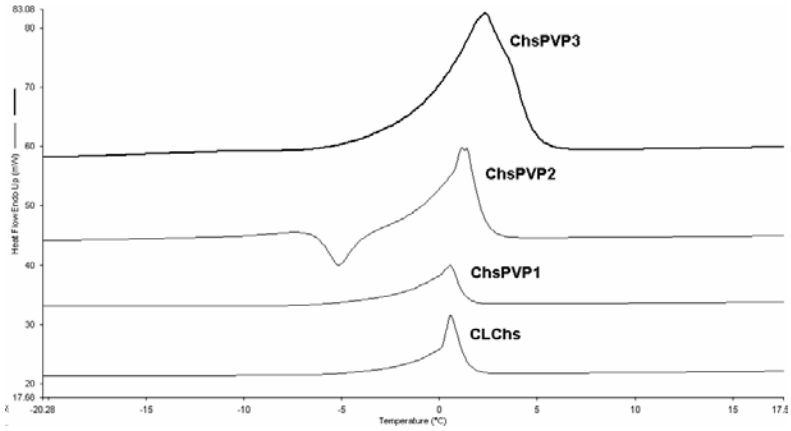


Figure 7. DSC heating scans of the hydrogels

The amount of bound water was calculated using equation 3.

$$W_b(\%) = W_t - (W_f + W_{if}) \quad (3)$$

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; and W_t is the equilibrium water content (EWC).

The total amount of freezing water can be estimated by taking the ratio of the enthalpy change associated with the endothermic peak (ΔH_{endo}) for intermediate and free water to the enthalpy of fusion (ΔH_{fus}) for pure water (333.3J/g).

$$(W_f + W_{if}) = (\Delta H_{endo}/\Delta H_{fus}) \times 100 \quad (4)$$

EWC, total freezing and bound water contents of various hydrogels are listed in Table 2.

The equilibrium water content and the total freezing water were found to increase with increasing content of PVP in the hydrogels. Non-freezing bound water was also found to increase with increasing PVP content but was not that pronounced.

Increase in free freezing water is due to the diffusion of water molecules into the gel network and also due to the water molecules orientating around the bound water to form cage like structures. On the other hand the slight increase in non-freezing bound water is due to the binding of water molecules to the hydrophilic groups of the polymer chains through hydrogen bonds and also through diffusion into the cavities in the gel network created by crosslinking [1].

Table 2. Water State of crosslinked chitosan hydrogels calculated by DSC analysis

Blends	EWC (%)	Bound water (%)	Free water (%)	ΔH (J/g)	Temperature at peak (°C)
ChsPVP3	76.29	19.04	57.25	192.14	2.5
ChsPVP2	68.63	18.95	49.68	-8.91	-7.5
				154.37	1
ChsPVP1	55.57	15.35	40.22	134.24	0.6
Chs	57.71	11.05	46.66	124.57	0.5

Conclusion

Hydrogels of chitosan and PVP were prepared and crosslinked with genipin. The swelling kinetics of the hydrogels exhibited a swelling response to stimuli such as temperature and pH. PVP rich hydrogels swelled the most because increasing amounts of PVP makes the gel structure less compact and more inhomogeneous. Maximum swelling was observed at low pH and high temperature due to the protonation of the amino group in chitosan the nitrogen of PVP and repulsion and disassociation of secondary interactions of the polymer chains respectively. The amount of free and intermediate freezing and nonfreezing bound water was investigated by DSC. The results showed that as the concentration of PVP in the hydrogels increased, the amount of freezing water in the swollen hydrogels also increased whereas the amount of nonfreezing bound water increased slightly. The increase in swelling and free freezing water in the hydrogels as the PVP content increases is due to the diffusion of water molecules into the gel network and also due to the water molecules orientating around the bound water to form cage like structures. The slight increase in non-freezing bound water is due to the binding of water molecules to the hydrophilic groups of the polymer chains through hydrogen bonds and also through diffusion into the cavities in the gel network created by crosslinking.

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