# Swelling properties of chitosan hydrogels

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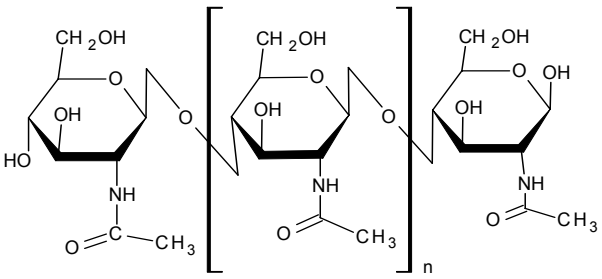
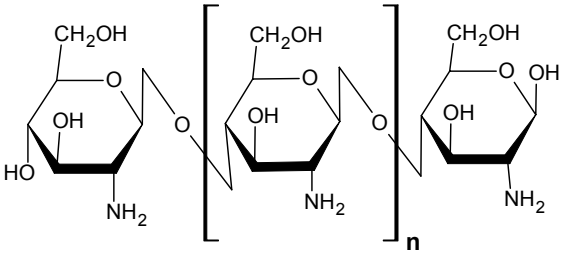
**ABSTRACT**
*Chitosan hydrogels were prepared by crosslinking chitosan with glutaraldehyde. The swelling behaviour of the cross-linked and uncross-linked hydrogels was measured by swelling the gels in media of different pH and at different temperatures. The swelling behavior was observed to be dependent on pH, temperature and the degree of crosslinking. The gel films were characterized by Fourier transform Infrared spectroscopy (FT-IR) and Differential Scanning Calorimetry (DSC). The glass transition temperature ($T_g$) and the amount of free water in the hydrogels decreased with increasing crosslinking in the hydrogels.*
***Keywords:*** *chitosan, hydrogel, swelling behaviour, thermal properties.*

# 1 INTRODUCTION

Hydrogels are crosslinked macromolecular network that swell in water or biological fluids. They have become a potential candidate for carriers of bioactive macromolecules, wound dressing and controlled release of drugs (Kawaguchi, 2000) in their swollen state. Their major disadvantage is their low mechanical strength. To reinforce their structure, the hydrogels are crosslinked. There are many compounds such as formaldehyde, epoxy compounds, dialdehyde and starch which are used as crosslinking agents. The most commonly used crosslinking agent is glutaraldehyde (Aly, 1998). A wide range of hydrophilic polymers has been examined for preparing hydrogels and chitosan is one of them.



**Chitin**



**Chitosan**

Chitosan is the deacetylated derivative of chitin which is a water insoluble polymer, (N-acetyl-d-glucosamine), found in nature, present in insect exoskeletons, outer shells of crabs, shrimps, lobsters etc. and fungal cell walls. The difference between chitin and chitosan chemical structure lies in the degree of deacetylation.

Chitosan is currently receiving enormous interest for medical and pharmaceutical applications due to its non-toxic, odorless, biocompatible in animal tissues and biodegradable properties (Borzacchielo *et al.* 2001).

Chitosan hydrogels, like other hydrogels, contain much water. Part of this water is tightly bound to the polymer (De Angelis *et al.* 2001) and rest is present as free water. Water in crosslinked and uncrosslinked chitosan gives rise to a three-dimensional network. Chitosan based hydrogels exhibit good biocompatibility, low degradation and processing ease. The ability of these hydrogels to swell and dehydrate depend on composition and environment which has been exploited to facilitate a range of applications such as drug release, its biodegradability and ability to form hydrogels. (Li Q *et al*. 1997).

Blending of chitosan with other polymers (Park and Nho, 2001; Shin *et al.* 2002; Zhu *et al*. 2002) and crosslinking are both convenient and effective methods of improving the physical and mechanical properties of chitosan for practical applications. Immunization studies carried out on rats using glutaraldehyde crosslinked chitosan spheres (Jameela *et al.* 1994) showed promising tolerance by the living tissues of the rat muscles.

In the present study chitosan was crosslinked with different amounts of glutaraldehyde to form hydrogels. The swelling behaviour of the gels in aqueous media at different temperatures and pHs have been examined and the amounts of free water and bound water has been determined. The glass transition temperature and molecular interaction has also been evaluated by DSC and FT-IR.

# 2 EXPERIMENT

## 2.1 POLYMERS AND REAGENTS

Chitosan with 85% degree of deacetylation (dd) was obtained from Fluka, U.K. and 25% glutaraldehyde was obtained from Unilab. Both were used without further purification.

## 2.2 PREPARATION OF CHITOSAN SOLUTIONS

Chitosan was dissolved in 1% aqueous acetic acid at room temperature and left overnight with continuous mechanical stirring to obtain a 1% (w/v) solution. The viscous, pale yellow chitosan solution was filtered through the stintered glass crucible to remove any undissolved matter.

## 2.3 PREPARATION OF GLUTARALDEHYDE CROSS – LINKED HYDROGELS

1% aqueous solution of glutaraldehyde in different mole ratios was added to the clear, pale yellow chitosan solution. The solution was stirred for 30 min at room temperature until it became increasingly viscous. The viscous solution was poured into a polystyrene petri dish and dried to room temperature overnight to form the hydrogel. The semi-dried hydrogels were further dried under vacuum at 55°C to completely remove the residual solvent. The hydrogel was obtained as a film whose thickness was about 0.1 mm.

Two crosslinked hydrogels were prepared with different chitosan **:** glutraraldehyde compositions. The detailed composition and designations are listed in Table 1.

**Table 1.** Compositions and designations of Chitosan/ glutaraldehyde hydrogels

| Designation | Vol. of chitosan [$cm^3$] | Vol. of crosslinker [$cm^3$] | Molar proportion [chitosan: crosslinker] |
|---|---|---|---|
| Uncrosslinked chitosan [NCLChs] | 40 | 0 | 1 **:** 0 |
| Crosslinked chitosan [CLChs1] | 40 | 2 | 1 **:** 0.08 |
| Crosslinked chitosan [CLChs2] | 40 | 4 | 1 **:** 0.16 |

## 2.4 CHARACTERIZATION

The swelling behaviour of the crosslinked and non-crosslinked films was measured by swelling the films in media of different pHs at room temperature and in deionised water at 25, 35 and 45°C. Pre-weighed dry hydrogel films (approximately 0.05 g and 25 $mm^2$) were immersed in buffer solutions ranging from pH 2 to 9. The immersion time was 140 mins.

The films were withdrawn from the solutions at different time intervals and their wet weight were determined after first blotting with a filter paper followed by blowing with a stream of air to remove the surface water and immediately weighing the films. The swelling ratio was calculated using the equation

$$E_{sr}(\%) = ((Ws - W_d) / W_d) \times 100 \qquad (1)$$

where $E_{sr}$ is the water absorption (%wt) 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 from the following equation:

$$EWC\ (\%) = ((W_e - W_d) / W_e) \times 100 \qquad (2)$$

where $W_e$ represents the weights of the swollen state at equilibrium.

The states of water in the hydrogels was investigated by DSC in the temperature range of –50°C to 20°C with a heating rate of 5°C/min under a $N_2$ flow. The amounts of free water and bound water were calculated from the melting enthalapies using equation 3. This equation assumes that the heat of fusion of free water in the hydrogel ($Q_{endo}$) is the same as that in ice ($Q_f$, 79.9 cal /g):

$$W_b(\%) = W_t - (W_f + W_{fb}) = W_t - (Q_{endo} / Q_f) \times 100 \qquad (3)$$

where $W_b$ is the amount of bound water; $W_f$ and $W_{fb}$ are the amounts of free water and intermediate water, respectively; and $W_t$ is the equilibrium water content [EWC (%)].

Thermal analysis was performed on a Perkin Elmer Pyris 6 DSC. The sample mass ranged between 2-3 mg and scanned from 30 – 250°C at a heating rate of 10°C/min. The second scan was considered in calculating the glass transition temperature ($T_g$).

The thin hydrogel films were scanned on a Perkin Elemer Infra-Red Spectrometer Spectrum 1000. 32 scans at a resolution of 2 $cm^{-1}$ were obtained and stored.

# 3 RESULTS AND DISCUSSION

All swelling behavior is plotted on the average of three trials. The swelling kinetics and time dependent swelling behaviours of chitosan hydrogels in deionised water (pH 7) at 25, 35 and 45°C are given in Figure 1. The NCLChs sample had the highest swelling ratio, while CLChs2 had the lowest. The swelling ratio for NCLChs at 25°C was at its minimum and increased with temperature. Since the NCLChs was not crosslinked, the polymer chains were flexible and increase in temperature caused breaking of secondary interactions, creating more space for water within the matrix of the gel. NCLChs hydrogels reached equilibrium swelling in approximately 90 minutes. This indicates that NCLChs structure is becoming mechanically very fragile and easily fractured. However, the swelling ratio for CLChs1 and CLChs2 remained relatively unchanged at the three temperatures. Crosslinking in these gels reduced chain flexibility, and did not have the same effect as in the case of NCLChs gels. The swelling kinetics shows that CLChs1 and CLChs2 samples attained equilibrium in approximately 30 minutes at all temperatures.

The temperature dependent equilibrium swelling behaviour of the hydrogels in deionised water (pH 7) at a temperature range from 25 to 45°C is shown in Figure 2. As the temperature of the hydrogels in the swelling state increased, the swelling ratio of the hydrogel samples increased. All hydrogels exhibited a temperature responsive swelling behaviour due to the association/dissociation of hydrogen bonding between the amino groups within the chitosan chains. While the NCLChs sample showed pronounced increases in the equilibrium swelling ratio with increasing temperature, the increase in the equilibrium swelling ratio of the CLChs1 and CLChs2 samples were not so significant as the temperature increased. Dissociation of hydrogen bonds

between the amino groups of chitosan with increase in more free water could be present in the hydrogel network. Such relaxation of the polymer chains is restricted when the chains are linked by a crosslinker.

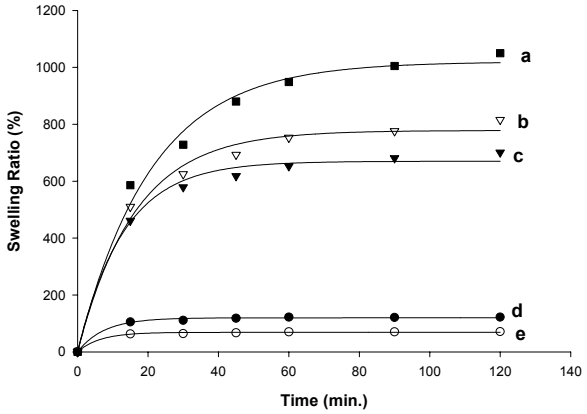



**Figure 1.** Swelling kinetics of chitosan hydrogels at varoius temperatures at pH 7. **a** NCLChs at 45°C **b** NCLChs at 35°C **c** NCLChs at 25°C **d** CLChs1 at 25°C **e** CLChs2 at 25°C

To observe the response of the chitosan hydrogels when exposed to different pH conditions, the hydrogels were allowed to swell to equilibrium in an aqueous medium of pH 2, 4, 7 and 9 at 25$^{o}$C. The effects of pH on the swelling behaviour are summarized in Figure 3. It can be seen that the hydrogels swelled the most in acidic pH as compared with basic pH.

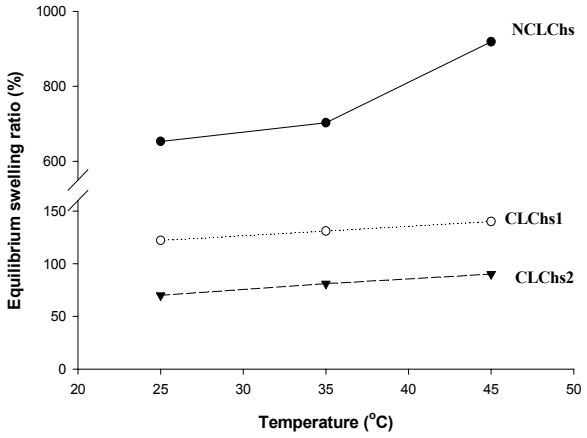



**Figure 2.** Equilibrium swelling ratio of chitosan hydrogels at various temperatures at pH 7

All the hydrogels swelled the maximum at low pH. NCLChs swelled the most followed by CLChs1 and CLChs2. Swelling ratio decreased as the pH increased. For CLChs1 and CLChs2 the decrease was gradual over the entire pH range but for NCLChs there was no significant change untill pH 4 but decreased drastically above pH 4.

At low pH, protonation of the amino groups of chitosan take place. This leads to repulsion in the polymer chains, temperature allowed relaxation of the polymer chains, thus dissociation of secondary interactions such as intramolecular hydrogen bonding, allowing more water into the gel network. As the pH increased, amino groups become deprotonated, repulsion in the polymer chains receded allowing shrinking. NCLChs gel showed intense swelling and shrinking compared to CLChs1 and CLChs2. Crosslinking resulted in the gel structure becoming more compact which did not allow significant swelling and shrinking.

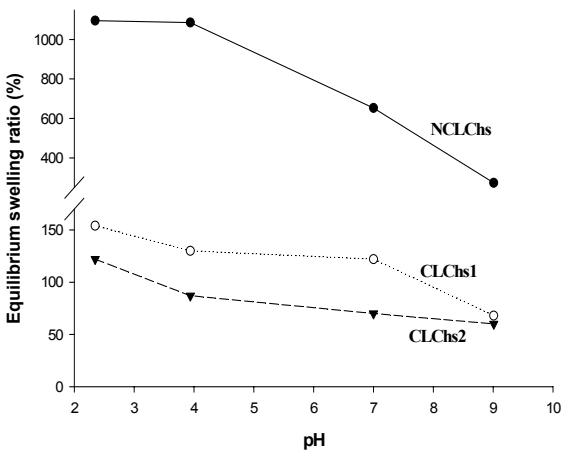



**Figure 3.** Equilibrium swelling ratio of chitosan hydrogels in various pHs buffer solution at 25°C

There are three types of water in hydrogels which are referred to as free water, intermediate water and bound water. Free water is freezing water and shows a melting endotherm. The water molecules do not form hydrogen bonds with the polymer molecules and shows greater degree of mobility. Intermediate water are water molecules that interact with the polymer molecules and has a melting endotherm < 0 $^{0}$C. Bound water or non-freezing water refers to water molecules that are hydrogen bonded to the polymer chains, are immobilized and show no melting peak. Bound water were calculated by taking the ratio of the endothermic peak of the water-swollen hydrogel to the melting endothermic peak of heat of fusion for pure water. Free water is expressed as the difference between total water and bound water. EWC values, free water and bound water contents are calculated and listed in Table 2.

The total free water and bound water in the hydrogels were measured from DSC melting enthalpies of the swollen hydrogel and calculated using equation 3.

**Table 2.** Water states of crosslinked and non-crosslinked chitosan hydrogels calculated by DSC analysis

| Sample | EWC (%) | Total free water (%) | Bound water (%) |
|---|---|---|---|
| NCLChs | 65.6 | 45.8 | 19.8 |
| CLChs1 | 44.4 | 19.8 | 24.6 |
| CLChs2 | 34.3 | 10.1 | 24.2 |

NCLChs shows the highest EWC and free water content followed by CLChs1 and CLChs2. This result confirms that the hydrogels structure becomes increasingly compact with increasing concentration of glutaraldehyde i.e. increasing crosslinking.

Thermal analysis to determine the $T_g$ of the hydrogels was not very easily detectable. However, after repeated scans the $T_g$ for NCLChs was detected between 195-205$^oC$, for CLChs1 between 155-160$^oC$ and for CLChs2 between 90-105$^oC$. As the ratio of crosslinking increased, the $T_g$ decreased.

Figure 4 shows the absorbance spectra of the crosslinked hydrogels in the region of 1800 to 1000 $cm^{-1}$. The peaks at 1650 and 1550 $cm^{-1}$ are of the C = O and $NH_2$ groups respectively (Cerri *et al.* 1996). Previous studies (Monteiro and Airoldi, 1999) on the molecular interaction between chitosan and glutaraldehyde have shown that the amino groups of the chitosan are involved in the crossinking with the glutaraldehyde. Our results clearly show a reduction of the peak at 1550$cm^{-1}$ which is due to the $NH_2$ group being bonded during crosslinking.

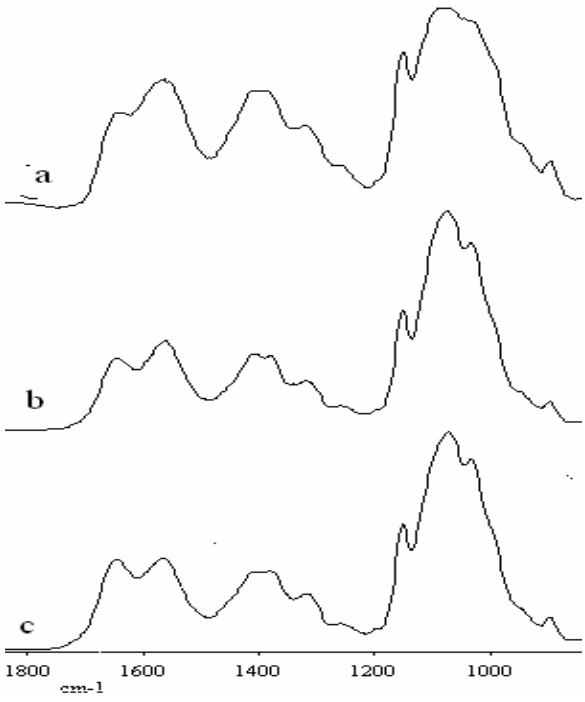



**Figure 4.** FT-IR absorbance spectra of **a.** NCLChs, **b.** CLChs1 and **c.** CLChs2

# 4 CONCLUSION

Chitosan was crosslinked with varying concentrations of glutaraldehyde to give hydrogels with different degrees of crosslinking. The prepared hydrogels were characterized by FT-IR and DSC. The swelling behaviours at different temperatures and pH and the amounts of free and bound water were also investigated. NCLChs showed maximum swelling ratio at all temperatures and pH. The swelling ratio decreased with increasing degree of crosslinking. NCLChs2 showed the lowest EWC and free water content followed by CLChs1 and NCLChs with the highest. These results show that CLChs1 and CLChs2 have a more compact structure in comparison with NCLChs.

FT-IR results showed the decrease in the peak intensity at 1550$cm^{-1}$ due to the amino groups of chitosan involved in the formation of bonds during crosslinking. The $T_g$ of the hydrogels appeared to decrease with increasing crosslinking.

# ACKNOWLEDGEMENTS

The financial support of The University of the South Pacific Research Committee and The Australian Regional Development Scholarship (ARDS) are greatly appreciated.

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