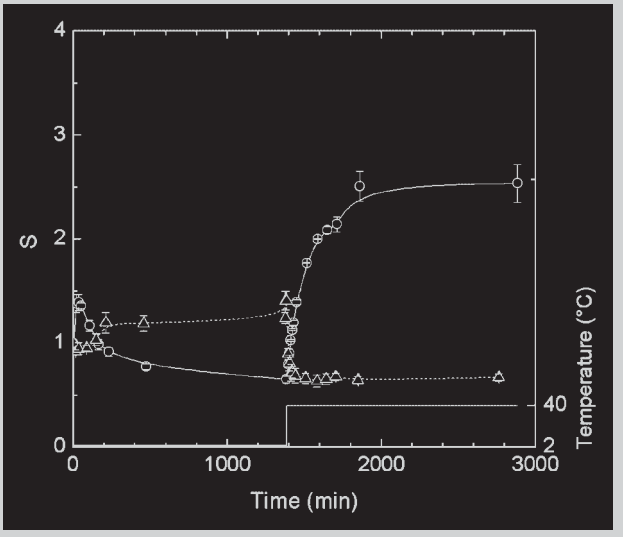
Full Paper: The ability to form a gel through the physical or chemical crosslinking of chitosan has been well documented. In an attempt to mimic biological systems, thermal and pH-sensitive chitosan cylindrical hydrogels were produced by a combination of physical and chemical crosslinking processes. To this end, chitosan hydrogels prepared from alkali chitin were molded in cylinders and, once washed, were further crosslinked with glutaraldehyde at stoichiometric ratios, R (= [–CH=O]/[–NH_2]), of 1.61 and 3.22×10^{-2}. Variation in swelling as a result of stepwise changes in temperature between 40 and 2 °C at pH values of 7.0, 7.6, and 8.0 revealed that the system responds in markedly different manners dependent upon the pH. At pH 7.0, cooling from 40 to 2 °C results in contraction of the gel network structure. While raising the temperature from 2 to 40 °C leads to a rapid swelling response (i.e., ca. a twofold increase in the amount of solvent uptake). Subsequent cooling to 2 °C is accompanied by a new contraction cycle. At pH ≥ 7.6 the temperature dependence of the swelling–contraction behavior is exactly the opposite of that observed at pH 7.0. Very similar trends were observed for the gels at both degrees of crosslinking. The swelling–shrinking behavior observed in gels of pH ≥ 7.6, is similar in kind to that of uncrosslinked gels and is interpreted in terms of a lower critical solution temperature (LCST) volume phase transition, driven by hydrophobic association, presumably involving residual acetyl groups in the chitin. The results at pH 7.0 suggest that the slight ionization of the $-NH_3^+$ groups leads to destruction of the hydrophobic hydration thus effectively reversing the negative thermal shrinking.



Evolution of the swelling ratio, *S*, as a function of time and temperature for crosslinked chitosan hydrogels. Circles represent *S* values recorded at pH 7.0 and triangles those at pH 7.6.

Effect of Chemical Crosslinking on the Swelling and Shrinking Properties of Thermal and pH-Responsive Chitosan Hydrogels

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Keywords: chitosan; crosslinking; hydrogels; swelling; thermosensitivity

Introduction

Hydrogels are hydrophilic three-dimensional polymer networks capable of absorbing a large volume of water or other biological fluid. The ability of polymer hydrogels to undergo a volume transition between swollen and collapsed phases as a function of their environment is one of the most remarkable and universal properties of these materials.[1] The phenomenon of gel volume transitions, which can be induced by temperature, pH, solvent composition, ionic

strength, electric field, light, stress, and the presence of specific chemical stimuli, is reversible and has prompted researchers to investigate gels as potential actuators, sensors, controllable membranes for separations, and modulators for the delivery of drugs.[2–6] These gels are called ''smart'', ''responsive'', or ''intelligent'' gels and can be tuned to change their physical properties, namely swelling behavior, permeability, or mechanical strength, in predictable and pronounced ways. These polymer systems are currently the subject of intensive study due to the great potential they bear for biomedical and bioengineering applications, namely, pulsatile drug release, molecular separation processes, diagnostics, cell culture, and bioreactions.[7–9]

Often, temperature sensitive hydrogels exhibit lower critical solution temperatures (LCST). Below the LCST, the gel is swollen, hydrated, and hydrophilic. Above the LCST, the gel becomes collapsed, dehydrated, and hydrophobic. Furthermore, its phase transition can be controlled by incorporating more hydrophilic or hydrophobic functional groups or monomers in their structure.[10] This principle has been used to prepare copolymers that exhibit temperature-sensitive swelling–deswelling changes over a limited pH range.[11] Well known gel systems exhibiting LCST include synthetic polymers such as poly-*N*-isopropylacrylamide (NIPA) (LCST at ~32 °C),[12] its copolymers and interpenetrated networks, *N*,*N*-diethylacrylamide, and poly(ethylene oxide)/poly(propylene oxide) block copolymers, among others. Most such polymer systems are of synthetic origin and very little work has been conducted on hydrogels from natural polymers.

Chitosan hydrogels can be obtained by various mechanisms of chemical or physical crosslinking such as covalent,[13,14] ionic,[15,16] hydrogen bonding,[17,18] or hydrophobic association.[19,20] Recently, chitin hydrogels prepared from alkali chitin[21] have been demonstrated to form as a result of a phase separation process dominated by hydrophobic association.[22,23] Alkali chitin solutions have LCST at ~30 °C.[22] In a preliminary work,[24] we have documented the thermal and pH-sensitive response of non-crosslinked chitosan hydrogels in the range of pH 7.3 to 12.0 and at rather high ionic strength ($I = 0.5$). Under these conditions little influence of the electric charge of residual $-NH_3^+$ groups of chitosan is expected, since the pH is above the range of the intrinsic pK_0 values found for chitosans under varying solvent conditions and measurement techniques (~6.1 to ~7.2).[25] In the present study, we aimed to investigate the swelling behavior of chitosan hydrogels crosslinked with glutaraldehyde with respect to temperature and pH. This system will allow us to understand the influence of ionized amino groups at lower pH values (~7.0) as well as the contribution of the increase in crosslinking density using the well-known chemical reaction of chitosan with glutaraldehyde.[26–29]

Experimental Part

Materials

Chitin (degree of acetylation, DA, ~79 mol-%) was a sample previously isolated from prawn (*Ploeticus mülleri*) shell waste and it was from the same processing batch as that used in previous studies.[24,30] All standard chemicals were USP grade, from Panreac (Barcelona, Spain). Glutaraldehyde 50 wt.-% Grade I solution was from Sigma–Aldrich Chemie GmbH (Steinheim, Germany) and was stored below 0 °C to ensure that it was free of α/β-unsaturations and self-polymerization products. Bi-distilled water was used throughout.

Preparation of Chitosan Hydrogels

Alkali chitin solution (1.6 wt.-%) made in cold (0 °C) aqueous NaOH (16 wt.-%) was prepared according with the protocol of Sannan et al.[21] A Teflon plate (70×70 mm^2) perforated with cylinders of well-defined dimensions ($\phi = 3$ mm, $l =$ 8.7 mm) was loaded with the alkali chitin solution and left to stand under vacuum at 25 °C for 72 h, a process that led to setting of turbid hydrogels. Once set, the gels were expelled from the plate by gentle pressure from a pipette into a water bath kept at 65 °C and under gentle stirring to remove excess NaOH. Several further cycles of changes of water and stirring at room temperature were needed to ensure the complete removal of NaOH from the gels. Uncrosslinked chitosan hydrogels were frozen by rapid immersion in liquid nitrogen and freeze dried.

Crosslinking of Chitosan Hydrogels

Twenty-five chitosan cylindrical hydrogels weighing ca. 1.02 g (2.6154 wt.-% solid matter) were reacted with 12.5 mL of glutaraldehyde solution (1×10^{-5} mol · L^{-1} or 2×10^{-5} mol · L^{-1} of glutaraldehyde in H_2O) at 40 °C in a vial placed in an incubation cabinet under constant stirring at 180 rpm for 72 h. The stoichiometric molar ratio of aldehyde to total amine groups, defined as R (= [–CH=O]/[–NH_2]), was 1.61×10^{-2} or 3.22×10^{-2}. In the calculation of R, it was assumed that one glutaraldehyde molecule reacted with two amine groups, resulting in two Schiff bases involving both aldehyde groups of the glutaraldehyde molecule and two chitosan units. Once the reaction time elapsed, the hydrogels were thoroughly washed with water. Crosslinked hydrogels were frozen by rapid immersion in liquid nitrogen and freeze dried.

Physicochemical Characterization

The degree of acetylation of chitosan hydrogels was determined by UV first derivative spectroscopy.[31] The intrinsic viscosity ([η]) was determined by capillary viscometry in 0.3 M acetic acid/0.2 M sodium acetate at 25 °C.[32] Sodium content was analyzed by inductively coupled plasma (ICP) emission spectroscopy on a Perkin–Elmer (Optima 4300 DV) spectrometer.

Swelling Studies

Swelling experiments were conducted in the following way. Each hydrogel (triplicates) was immersed in a plastic vial

containing a buffer solution of varying pH between 7.0–8.0 (citric acid/Na_2HPO_4) and adjusted to constant ionic strength ($I = 0.5$) with KCl. The vials were immersed in a circulating water bath equilibrated to the desired temperature. The approach to equilibrium was monitored by periodically withdrawing the gel cylinders from the solvent and weighing after removal of the excess surface solvent by light blotting with a filter paper. The weight of the cylinders was monitored individually in the way described until the hydrated weight reached a constant value. After equilibration at one temperature, the gels were then re-equilibrated at a different temperature. The relative swelling ratio (S) was determined gravimetrically and represented the gel water uptake with respect to the equilibrium initial state once the network is fully hydrated by Equation (1).

$$S = w_h/w_i \quad (1)$$

Where w_h is the weight of the hydrated gel, and w_i is the equilibrium weight of gel after initial swelling. Measurements were conducted in triplicate.

Scanning Electron Microscopy

The cross-sectional surface morphology of freeze-dried hydrogels was determined using scanning electron microscopy (JEOL, JSM 6400). Hydrogels were mounted on a brass mount and sputtered with Au/Pd in a Balzers SCD 004 Sputter Coater.

Results and Discussion

Physicochemical Characteristics

Uncrosslinked chitosan hydrogels had the following characteristics: degree of acetylation, DA, 27.5 mol-%; intrinsic viscosity, $[\eta]$, ~695.0 $dL \cdot g^{-1}$, and sodium content ~949.9 ppm. These values were very much within the limits observed in chitosan hydrogels prepared from alkali chitin under varying conditions.[24] It is well established that alkali chitin undergoes deacetylation under homogeneous conditions when the reaction is conducted below 40 °C for prolonged periods of time, typically above 100 h.[33] This reaction is pseudo first-order when NaOH is in excess as in this case.[34]

Crosslinking Reaction

The reaction of chitosan with glutaraldehyde was performed in an almost neutral medium (pH ~ 7.05) and left to proceed for 72 h, conditions that ought to favor that it proceeded to completion. It is known that a low pH of the reactive medium interferes negatively in the course of the glutaraldehyde–NH_2 reaction and the best condition of reactivity is connected to neutral or basic media.[34–36] The reaction between chitosan amino groups and glutaraldehyde in the present system, though, is highly unlikely to proceed homogeneously as in previously documented studies where chitosan chains reacted with glutaraldehyde in the fully disordered random coil conformation. In this case, chitosan chains can be conceived to be highly constrained into a macroscopic physical gel network. Hence, the kinetics of the crosslinking reaction is bound to be limited both by the restricted mobility of chitosan species and by the radial diffusion of glutaraldehyde itself from the surface of the gel inwards. Therefore, the absolute stoichiometry of the reaction cannot be determined and the R values calculated must be considered cautiously. Cross-sectional scanning electron micrographs of crosslinked gels are consistent with this suggestion as shown below.

Swelling Studies

In a preceding study,[24] we have demonstrated that solvent uptake of freeze-dried uncrosslinked chitosan hydrogels shows Fickian diffusion kinetics at 25 °C and negative reversible thermal response in the range of 2 to 25 °C (i.e., increasing swelling levels with decreasing temperature). Such changes seem to depend closely on pH and become more pronounced in the vicinity of pH ~ 7.6. In these systems, it was very difficult to address the behavior of gels at pH ≤ 7.3. Indeed, at pH 7.3, the gels became too soft to be handled upon changing from 25 to 2 °C.[24]

Figure 1 shows the results for the swelling response of the uncrosslinked chitosan hydrogels used in the present study when left to equilibrate in a buffer of pH 7.0 at 40 °C. Notice that the experimental error grows with time because of the gradual difficulty faced in handling the gels as they became increasingly swollen and soft, indicating that in the absence of covalent crosslinks the network became gradually dissolved. No further measurements at pH 7.0 and varying temperature were possible. The value of

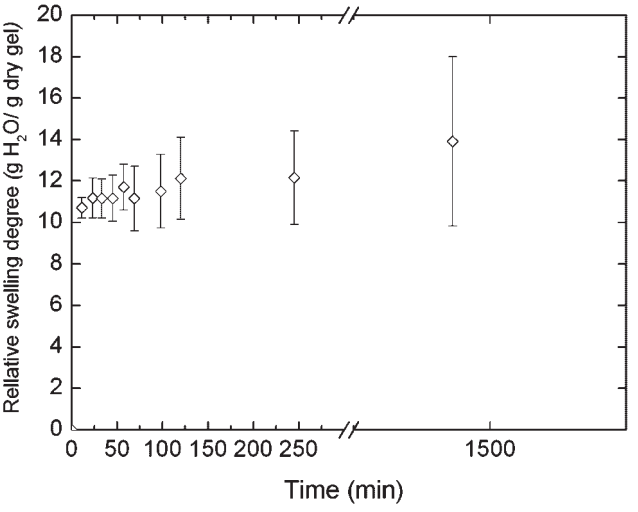

Figure 1. Time evolution of relative swelling degree (expressed as grams of water per gram of dry hydrogel) for uncrosslinked chitosan hydrogels at pH 7.0 and 40 °C. Mean average and error bar values are from triplicate analyses.

the intrinsic pK of chitosan (pK_0, i.e., the pK value extrapolated to zero charge) has been established between ~6.1 to ~7.2,[25] depending on the chitosan and measuring conditions. Hence at pH $\leq$ 7.3, as the pK_0 value is approached, the polymer becomes more protonated and more soluble, thus effectively favoring the osmotic flow of solvent into the gel network. Indeed, the neutralization behavior of chitosan solutions is a central characteristic which determines its solubility.[20] In addition, it has been shown that homogeneous, partially deacetylated, regenerated chitin is able to dissolve in water under a narrow range of DA (44–55 mol-%), while heterogeneously deacetylated samples of similar DA do not.[37] This has been ascribed to differences in crystalline order between both types of deacetylated products.[37,38] Contribution from both these mechanisms may be responsible for the high extent of swelling and eventual solubilization of chitosan hydrogels at pH $\leq$ 7.3 in the absence permanent crosslinks. Figure 2 shows the results of the evolution in swelling of uncrosslinked chitosan hydrogels that were initially equilibrated at 40 °C at pH 7.6 (Figure 2a) and 8.0 (Figure 2b) then changed to, and further left to equilibrate, at 2 °C. Gels at pH 7.6 experienced an increase in their equilibrium swelling coefficient, S, from 1.0 to ~1.3, while at pH 8.0 only a slight change in the swelling ratio is observed as a result of an identical temperature program. This behavior agrees qualitatively well with previous observations in uncrosslinked chitosan gels subjected to stepwise changes in temperature from 25 to 2 °C and from 2 to 25 °C.[24] It is also the typical behavior of polymer gels that exhibit LCST.[39]

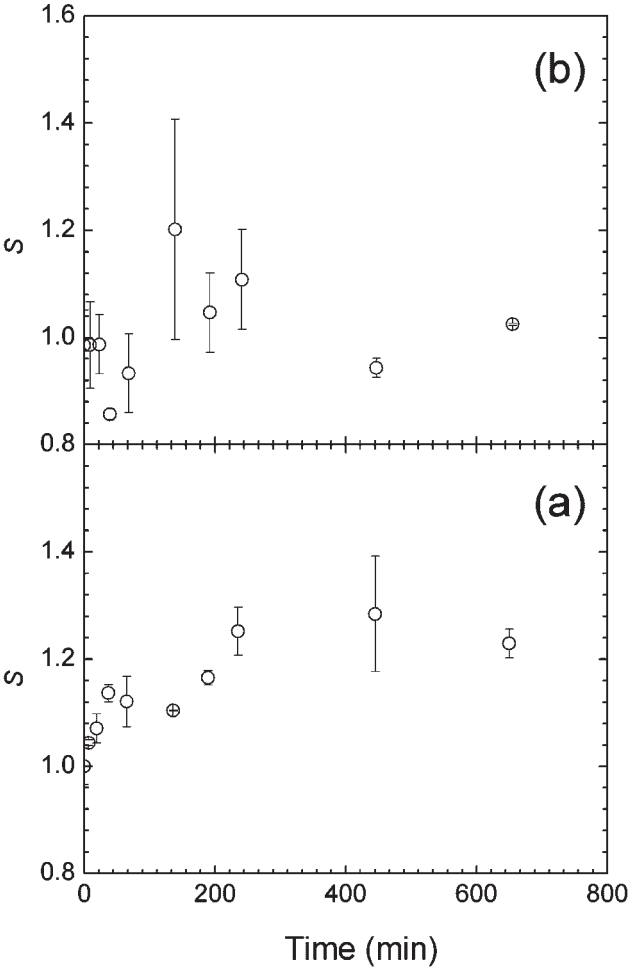

Figure 2. Evolution of swelling ratio, S, for uncrosslinked chitosan hydrogels previously equilibrated at 40 °C and subjected to a change of temperature (40 to 2 °C) at: a) pH 7.6 at and b) pH 8.0. Mean average and error bar values are from triplicate analyses.

Crosslinked chitosan hydrogels dilate to different extents depending on the temperature and pH. Figure 3 shows changes in the swelling ratio, S, of chitosan crosslinked hydrogels at the three investigated pH values and for the two levels of crosslinking density. The dried hydrogels were initially allowed to uptake solvent at 40 °C until reaching steady weight values, and subsequently subjected to a stepwise change in temperature to 2 and further to 40 °C. In Figure 3a, 3b, and 3c, are plotted the results recorded for crosslinked hydrogels of R equal to 1.61×10^{-2} at pH 7.0, 7.6, and 8.0, respectively. At pH 7.0 (Figure 3a), a positive thermal swelling response is clearly observed, that is, deswelling occurs on cooling and swelling increases on heating. Notice that as soon as the temperature of the gels changed from 40 to 2 °C a slight sudden increase in S was observed before deswelling set in. At 2 °C the gels deswelled and equilibrated at $S \sim 0.79$. In turn, when placed back at 40 °C the gels swelled rapidly reaching a maximum value of ~2.90, followed by a reduction in S as clearly appreciated in Figure 3a. This may be the consequence of partial dissolution of the network at the low degree of crosslinking, and this decreasing trend was not present in the gels with a greater degree of crosslinking, as is further discussed below (cf. Figure 3d). It is worth pointing out that the ionic strength, I, used in the buffer solutions at the various pHs was rather high ($I = 0.5$). This should act in favor of the solvation of the gel network due to its effect on the Donnan potential, thus effectively favoring the penetration of solvent into the chitosan network.

The origin of the initial sudden increase in S as the temperature is lowered (Figure 3a and 3d) cannot presently be accounted for. It might be related to a sudden tendency to hydration of the network when subjected to 2 °C, due to the effect of low temperature in physical junctions mediated by hydrophobic interactions. As soon as the network becomes fully hydrated after this initial swelling, the elastic pressure exerted by the covalently crosslinked network on the solvent dominates and hence the gel shrinks.

On shifting to pH 7.6 (Figure 3b), the temperature-dependent positive swelling response is inverted with respect to that at pH 7.0, that is, swelling levels off with decreasing temperature while heating leads to expulsion of

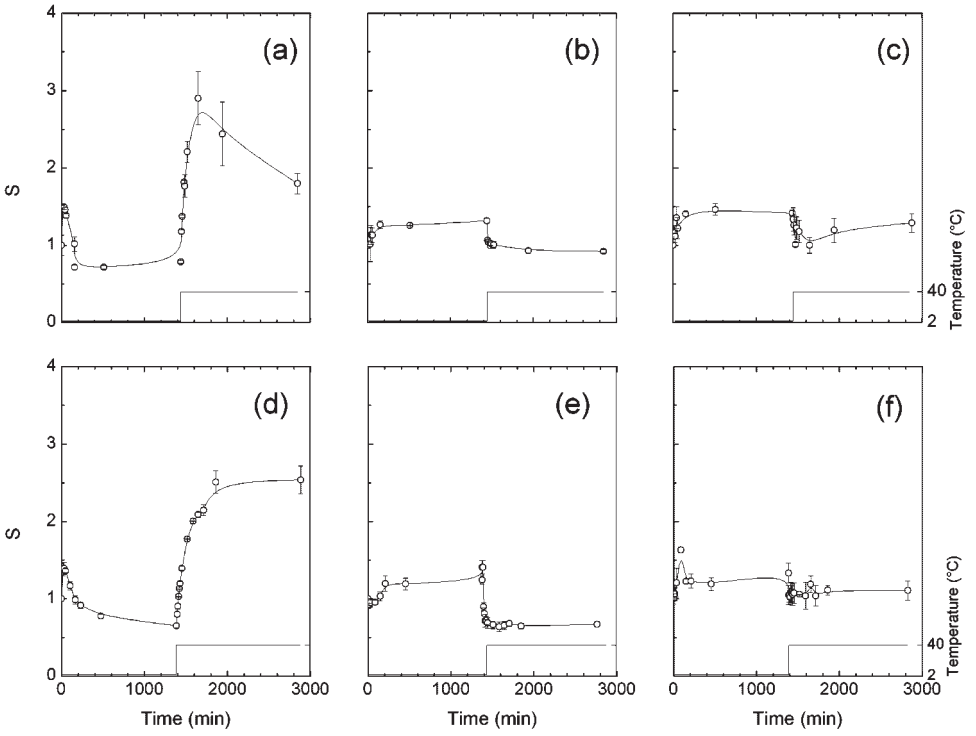

Figure 3. Evolution of the swelling ratio, S, as a function of time and temperature at various pH values: pH 7.0 (a and d); 7.6 (b and e) and 8.0 (c and f) for chitosan hydrogels crosslinked with glutaraldehyde at stoichiometric ratios, R (= [–CH=O]/[–NH_2]), of: 1.61×10^{-2} (a, b, and c) and 3.22×10^{-2} (d, e, and f). Stepwise changes in temperature are indicated by solid lines (right hand side scale). Mean average and error bar values are from triplicate analyses.

solvent from the gel network and hence shrinking. Similar trends are also clearly evident at pH 8.0 (Figure 3c). This behavior evident at 2 °C is comparable to that of uncrosslinked hydrogels at pH 7.6 or 8.0 (Figure 2). In turn, Figure 3d–3f show the temporal evolution of chitosan hydrogels crosslinked at $R = 3.22 \times 10^{-2}$. Much the same qualitative response to temperature as that for the gels of $R = 1.61 \times 10^{-2}$ persisted in the hydrogels with $R = 3.22 \times 10^{-2}$ at the same pH. At pH 7.0 (Figure 3d), however, the time needed to reach equilibrium seemed to be longer than

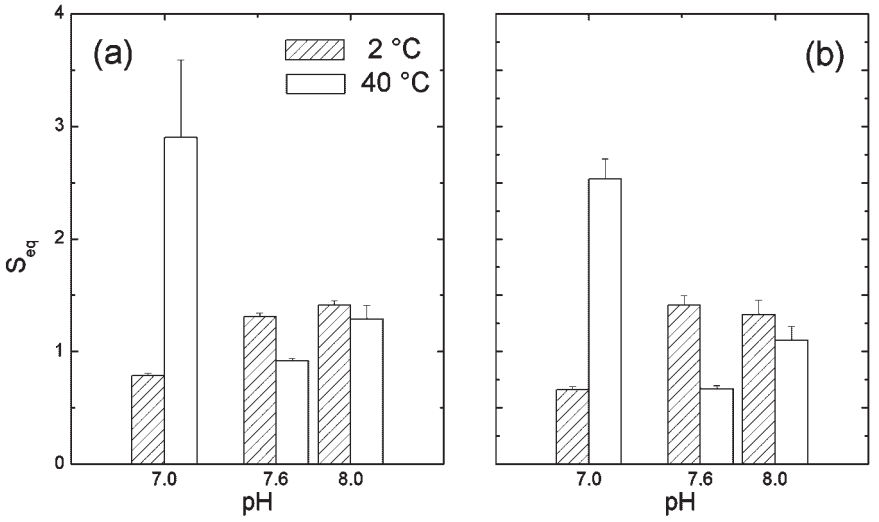

Figure 4. Comparison of equilibrium swelling ratio (S_{eq}) of chitosan crosslinked hydrogels of varying stoichiometry, R (= [–CH=O]/[–NH_2]) of: (a) 1.61×10^{-2} and (b) 3.22×10^{-2} as a function of pH and temperature (as shown in label). Mean average and error bar values are from triplicate analyses.

in the less crosslinked hydrogels. Besides, no maximum followed by a decrease in S values is observed at 40 °C, but a monotonic increase up to the equilibrium state is clearly appreciated throughout the swelling process. The relative variation between the equilibrium states at 2 and 40 °C at pH 7.0 is somewhat smaller than that of the less crosslinked systems, as one would expect from a more rigid and densely crosslinked network. At pH 7.6 (Figure 3e), swelling responds to the temperature stepwise variation from 2 to 40 °C in an opposite manner to that observed at pH 7.0. Interestingly enough, under these conditions, the overall magnitude of the change in volume between the equilibrium states at 2 and 40 °C exceeds that of the less crosslinked networks (cf. Figure 3b). At pH 8.0 (Figure 3f), only marginal changes of the same trend as those seen at pH 7.6 are observed. The hydrogels kept their swelling behavior on cooling and heating cycles, for at least 7 d (results not shown). A possible explanation for the positive thermal response would be that the ionization of part of the amine groups of chitosan at pH 7.0 yields the Donnan swelling osmotic flow because of the counterions within the gel, thus effectively favoring the permeation of solvent into the network. In copolymer gels of NIPA and sodium styrenesulfonate it has been suggested that the introduction of charges destroy the hydrophobic hydration and the LCST is bound to shift to greater temperature.[40,41] It is also evident that as the network crosslinking density increases, with the exception of the gels at pH 7.6, the magnitude of the differences between collapsed and swollen equilibrium states is less pronounced.

When swelling data are related to the weight of the initial equilibrium weight, it allows decoupling of the initial uptake of solvent by the dry gel through capillary forces, from the fundamental thermodynamic parameters related to volume changes, namely, goodness of solvent, Donnan potential, density of crosslinks, length and rigidity of elastically active segments in the gel network.

Figure 4 summarizes results of the degree of equilibrium swelling, S_{eq}, for the chitosan crosslinked gels at 2 and 20 °C at the two different degrees of glutaraldehyde crosslinking. The difference in S_{eq} as a result of the temperature change was more pronounced for hydrogels with $R = 1.61 \times 10^{-2}$ (Figure 4a) than for those of $R = 3.22 \times 10^{-2}$ (Figure 4b), particularly at pH 7.0. This greater swelling capacity of the hydrogels with lower crosslinking density is the expected effect from the lower number of fixed covalent knots in the gel network of the less crosslinked gels, which effectively exerts lower retractile elastic pressure upon the solvent and hence allows more dilation. However, at pH 7.6, the differences in swelling seem to be more pronounced for the more crosslinked hydrogels (Figure 4b), particularly for the deswelling behavior observed at 40 °C ($S_{eq} \sim 0.67$ at $R = 3.22 \times 10^{-2}$ versus $S_{eq} \sim 0.92$ at $R = 1.61 \times 10^{-2}$). These effects could be a consequence of greater hydrophobic hydration in the gels at pH 7.6, thus effectively favoring greater interchain association in the gel with $R = 3.22 \times 10^{-2}$ system as previously noted in gels of 60% acetylated chitosan crosslinked with glutaraldehyde.[27]

The observed pH- and temperature-sensitivity in the crosslinked chitosan gel system could be interpreted as a direct consequence of a breakdown in the delicate balance between hydrophilic versus hydrophobic forces mediated by variations in the degree of dissociation of $-NH_3^+$ and ionic species of phosphate with pH and by association of residual acetyl groups. At pH 7.0, hydrogen bonding must play a significant role in maintaining association at low temperature, and their disruption at higher temperatures and dissociation of polymer–polymer complexes leads to positive thermosensitivity. At pH 7.6, hydrophobic association becomes dominant and the system has negative thermosensitivity. A related example of this type of gel comprises a random copolymer of monomers that are pH-sensitive (acrylic acid, AA) and temperature sensitive (*N*-isopropyl acrylamide, NIPA). For a composition of less than 10 mol-% AA, the gel exhibits a cloud point at pH 7.4. For higher AA content the LCST behavior disappears, because the AA components (which are ionized at pH 7.4) convey sufficient solubility to offset the aggregation of the hydrophobic temperature-sensitive components. In further studies, a graft copolymer composed of side-chain of temperature-sensitive NIPA grafted onto a pH sensitive backbone polymer of polyA, shows constant LCST independent of pH and AA content.[41]

Scanning Electron Microscopy Studies

The morphology and ultrastructure of the crosslinked hydrogels is shown in Figure 5. Cross-sectional views of the surface of the hydrogel cylinders of $R = 1.61 \times 10^{-2}$ and 3.22×10^{-2} equilibrated in water at 25 °C are shown in Figure 5a and 5b, respectively. It can clearly be appreciated in both micrographs that a gradient in the size of and density of the microporous structure is set up from the surface of the gel inwards. In the hydrogels with a greater degree of crosslinking (Figure 5b), the depth of the section of lower porosity is undoubtedly thicker (∼200 μm) than that in the hydrogels of $R = 1.61 \times 10^{-2}$ (∼60 μm). These micrographs strongly support the suggestion that the reaction of glutaraldehyde with the preformed chitosan network proceeded heterogeneously from the surface of the gel inwards. Figure 5c and 5d show micrographs of the equilibrium swelling state of the hydrogels of $R = 1.61 \times 10^{-2}$ at 2 and 40 °C, respectively, at pH 7.0. It is evident that at 2 °C, the pores appear smaller and more collapsed than those in the gel equilibrated at 40 °C. This is in close correspondence with the results of the swelling experiments. At pH 7.6, the differences in pore size and overall microstructure between the swollen

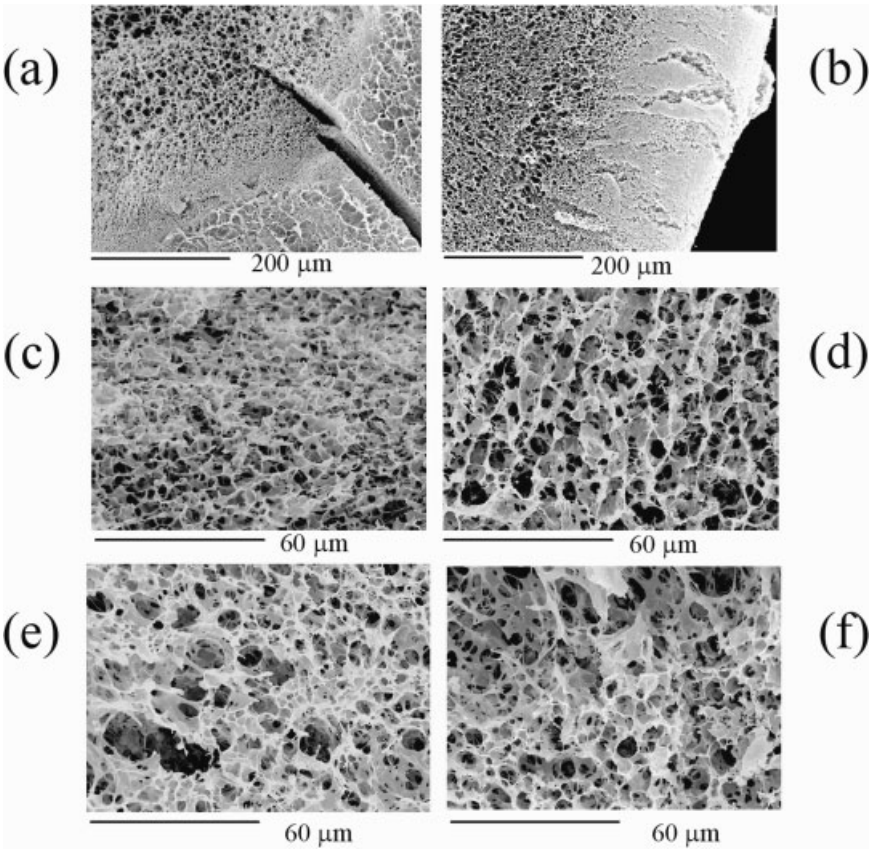

Figure 5. Cross-sectional scanning electron micrographs of freeze-dried chitosan hydrogels of varying degree of crosslinking equilibrated at different conditions: a) $R = 1.61 \times 10^{-2}$ in water at 25 °C; b) $R = 3.22 \times 10^{-2}$ in water at 25 °C; c) $R = 1.61 \times 10^{-2}$ in buffer pH 7.0 at 2 °C; d) $R = 1.61 \times 10^{-2}$ in buffer pH 7.0 at 40 °C; e) $R = 1.61 \times 10^{-2}$ in buffer pH 7.6 at 2 °C; and f) $R = 1.61 \times 10^{-2}$ in buffer pH 7.6 at 40 °C.

(Figure 5e) and collapsed state (Figure 5f) of the gel networks are not so evident.

Conclusion

Crosslinking did bring about a stabilization of chitosan hydrogels at pH < 7.3. The swelling–shrinking behavior observed in chitosan crosslinked hydrogels at pH 7.6 is similar to that previously documented for uncrosslinked chitosan hydrogels, though the differences in the equilibrium swelling ratios for the collapsed and the swollen states were much greater in the crosslinked networks. The behavior of the system at pH 7.6 can be interpreted in terms of lower critical solution temperature (LCST) phase transitions found in alkali chitin and driven by hydrophobic association, presumably involving residual acetyl groups in chitin.[26] Alkali chitin has a LCST value at ~30 °C, and it is likely that this value is greatly shifted with the degree of acetylation and of protonation of chitosan. The presence of greater amounts of ionized $-NH_3^+$ groups at pH 7.0 than at 7.6, even when small, could account for the drastic difference in swelling behavior for such a narrow change in pH. Accordingly, one may hypothesize that there must be a pH value lying somewhat between 7.0 and 7.6, such that the swelling response of the crosslinked chitosan network will be insensitive to temperature, due to the exact match between hydrophilic and hydrophobic forces, hence balancing the positive and the negative thermosensitivity of the system. This hypothesis is currently being investigated experimentally.

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