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Swelling studies of chitosan/cashew nut gum physical gels

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Abstract

Gels from chitosan/cashew nut gum (CH/CNG) were prepared with different chitosan to cashew nut gum ratio and their swelling behaviour was investigated as a function of pH and salt. Infrared and Carbon-13 nuclear magnetic resonance spectroscopy were used to elucidate the gel structure. Swelling in water diminishes sharply when the ratio CH/CNG increases. In the presence of Na^+ counterions the degree of swelling (Q) remains fairly constant, whereas in presence of Ca^{2+} counterions Q increases with the CH/CNG ratio. It was found that swelling is maximal at pH 2 and at pH 10, where the gel can absorb water up to 44 times its own weight. Chitosan amine and cashew nut carboxylate groups seem to play a major role in the swelling mechanism, being the polysaccharide gum the major factor responsible for water absorption.

Keywords: Cashew nut gum; Chitosan; Gel

1. Introduction

Natural polymers are very important materials not only because they are readily available and renewable but also because of their unique properties such as the ability to form gels. Among the natural polymers, polysaccharides play a very important role because of their capability of interaction with dispersed or dissolved species, thereby allowing them to perform vital biological functions in the association with hormones, lipids, proteins or other molecules. As a consequence, many applications are envisaged in the field of medical, pharmaceutical and chemistry industries (Crescenzi, 1994; Crescenzi, Dentini & Coviello, 1991; Miskiel & Pazur, 1991; Yalpani, 1988).

Chitosan (CH) is a macromolecule obtained from deacetylation of chitin, containing in its structure glucosamine and acetylglucosamine joined through β-D-(1 → 4) linkages. Chitosan has application in the cosmetic and medical industries (Sanford, 1990). Cashew nut gum (CNG) is an exudate from *Anarcardium ocidentale L.* trees, containing galactose in the main backbone linked by β-(1 → 3) bonds and branched chains joined through β-(1 → 6) linkages. The composition of CNG was found to be galactose (72%), arabinose (4.6%), rhamnose (3.2%), glucose (14%) and glucuronic acid (4.5%) (De Paula, Heatley & Budd, 1998). CNG has attracted much attention from academia due to its potential use in the chemical and pharmaceutical industries. It is a potential by-product of the Brazilian cashew nut industry, which is the second world producer, being responsible for the processing of 200,000 tones of cashew nut, with a turnover of around $150 million/year.

It is considered that a composite material combining both the gel and film forming ability and polycationic features of chitosan with the slightly acidic character of CNG would result in an interesting novel material with useful physico-chemical and mechanical properties. This work reports on the preparation of chitosan and CNG gels as well as on the investigation of the salt and pH effects on the swelling behaviour.

2. Experimental

2.1. Materials

Chitosan was supplied by Polymar Ind. Com. Imp. Ltd. (deacetylation degree 75%, $M_v = 1.8 \times 10^5$ g/mol). CNG (molar mass $= 1.1 \times 10^5$ g/mol) was collect as a natural exudates from native trees in Ceará-Brasil and purified as described by De Paula et al. (1998). Ground gum (5 g) was dissolved in distilled water (100 ml) and 0.5 g of NaCl was

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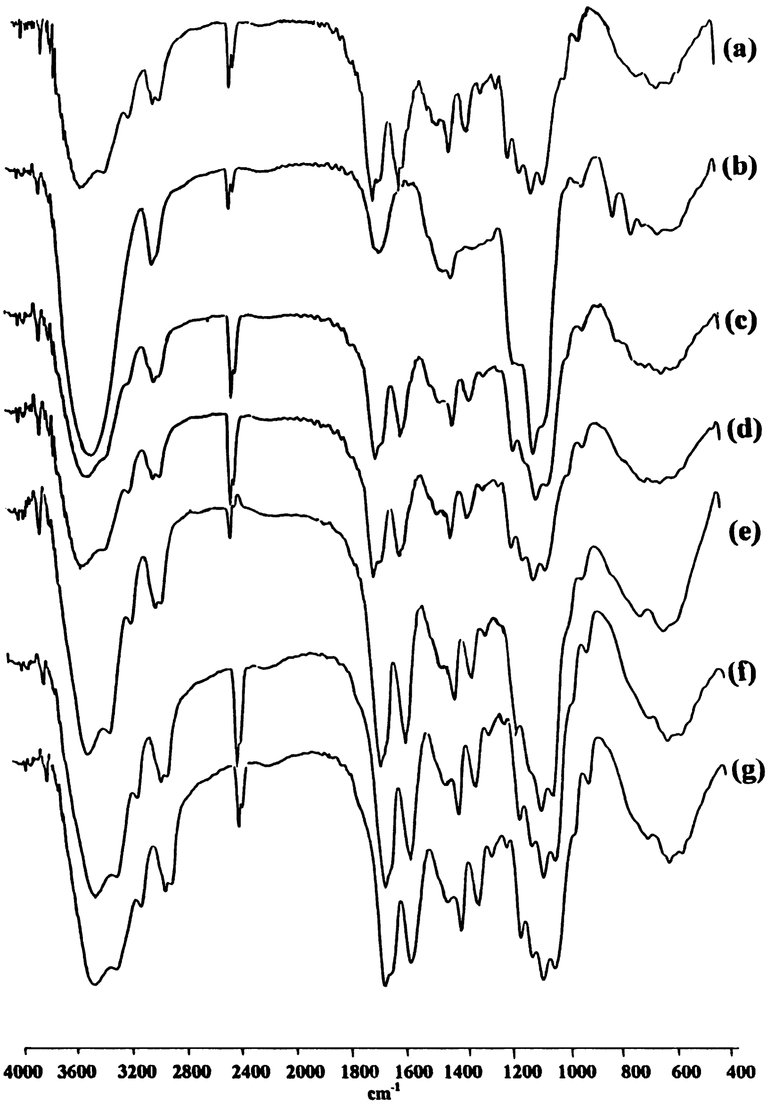

Fig. 1. FT-IR spectra of: (a) CH gel; (b) CNG; (c) CH/CNG 1:1; (d) CH/CNG 5:1 (e) CH/CNG 10:1; (f) CH/CNG 15:1 and (g) CH/CNG 20:1.

added. The solution pH was adjusted to 6.0. The solution was filtered and precipitated with ethanol AR. The gum was re-dissolved in water, dialysed against distilled water and re-precipited with ethanol (yield 70%). All the other reagents were analytical grade or equivalent and were used as purchased.

2.2. Procedure

The gels were prepared by a modification of Hirano's method (Hirano, Ohe & Ono, 1976). Briefly, chitosan (0.1 g) was dissolved in 5.0 ml of a 10% acetic acid solution by shaking at room temperature. Acetic anhydride (2.5 ml) was added and the mixture was left at room temperature overnight. The product were poured in 300 ml of methanol and stirred overnight. Freeze-drying of suspensions in distilled water yielded the product. For CH/CNG gel preparations, chitosan (140–260 mmol) was dissolved in 15 ml of a 1% acetic acid solution and added to a 5 ml CNG aqueous solution, the mixture being stirred until complete dissolution resulting in a 2.0% (w/w) final solution concentration. After filtration, an excess of acetic acid anhydride was added dropwise to the mixture, until gelation was complete. The gel was then exhaustively washed with distilled water, dialysed against water and freeze-dried. CH gels were prepared in the same way, however no CNG was added to the solution. The CH concentration was in the range 1.0–2.0% (w/w). The ratios of CH/CNG used for gel preparations were 1:1; 1:2; 5:1; 10:1; 15:1 and 20:1 (w/w).

For the swelling experiments, samples (circa 1.0×10^{-2} g) were placed in 10 ml test tubes and swollen in about 5.0 ml of water, 0.1 M NaCl, $CaCl_2$, or in buffer solution pH 2, 3, 5, 7, 8, 9, 10. Buffers were prepared using potassium hydrogen phatalate and hydrochloric acid or sodium borate and sodium hydroxide. Ionic strengths were

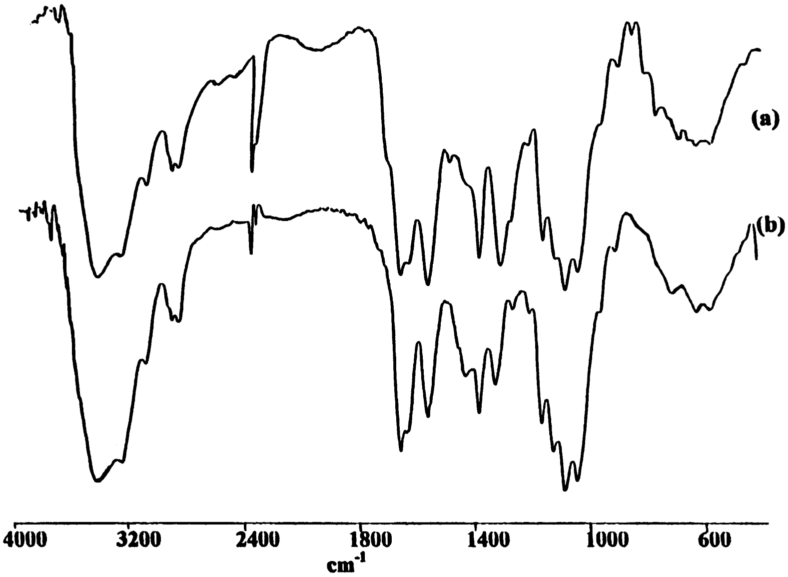

Fig. 2. FT-IR spectra of CH/CNG 1:1 at (a) pH = 3 and (b) pH = 9.

0.056 M (pH 2, 8, and 10); 0.084 M (pH 9) and 0.173 M (pH 3, 5, 7) (Lide, 1992). Solutions were exchanged after 24 h. After reaching equilibrium (circa 48 h) the gels were dried and accuratly weighed. All samples were ran in duplicate. The degree of swelling (*Q*) was calculated from the ratio of the mass of water in the gel to the mass of dried gel. (Skorikova, Kalyuzhnaya, Vikhoreva, Gal'braikh, Kotova, Ageev et al., 1996; Yao, Liu, Cheng, Lu, Tu & da Silva, 1996). The effect of ionic strength on the degree of swelling was determined in NaCl 0.056, 0.084 and 0.173 M.

Infrared spectroscopy of dried gels was carried out in a Perkin Elmer Spectrum 1000 instrument, using KBr pellets.

^{13}C Solid-State Nuclear Magnetic Ressonance spectroscopy of the dried gels was carried out using a 300 BRUKER instrument, operating at 75 MHz. The cross-polarisation pulse sequence was used for all the samples, which were spun at the magic angle at 4.5 kHz. A contact time of 1 ms and a pulse repetition time of 3 s were used. An average of 1100 scans were accumulated for each sample.

3. Results and discussion

3.1. Gel formation

CH/CNG gels are likely to be formed by the following mechanism:

$$\text{CNG–COO}^- + \text{CH–NH}_3^+ \rightarrow \text{CNG–COO}^- + \text{H}_3\text{N–CH}$$

This kind of polyeletrolyte has been identified in other chitosan complexes with substances such as pectin (Yao et al., 1996), α-keratose (Park, Lee & Há, 1996), hyaluronic acid and chondroitin sulphate (Denuziere, Ferrier & Domard, 1996), carboxymethyl cellulose (Peniche-Covas & Argüelles-Monal, 1995), xanthan (Chu, Kumagal & Nakamura, 1996), poly(acrylic acid) (Skorikova et al., 1996; Agree, Kotova, Skorikova & Zezin, 1996), poly(ethylene glycol) (Lee, Kim & Lee, 2000). It has been demonstrated that strong electrostatic interactions take place between the $-\text{NH}_3^+$ groups of chitosan and the $-\text{COO}^-$ groups of the above mentioned compounds. In our laboratory we found that these complexes are able to absorb water and form gels, which are quite stable, whatever the pH. Furthermore, the resulting chitin-like gel structure imparts a additional stability to the complex network. The complexes are not disrupted even after they have been freeze-dried and moreover, they actually behave like hydrogels, whereby water is absorbed to a limited extend, without leading to gel dissolution.

3.2. IR spectra analysis

A standard IR spectrum of CH/CNG gels can be seen in Fig. 1. The main absorption bands fall into three regions: from 1557 to 1561 cm^{-1} due amino groups of chitosan, from 1640 to 1656 cm^{-1}, owing to overlapping of signals from CNG carboxylate and CH acetals groups; and from 1100 to 1152 cm^{-1} due to methylene hydroxyl groups of both CH and CNG.

The degree of acetylation (DA) of CH/CNG and CH gels was determined by IR spectroscopy method as proposed by Moore and Roberts (1980), whereby the percentage of the amine group acetylated is given by: $A_{1655}/A_{3450} \times 100/1.33$, where A_{1655} and A_{3450} are the absorbance of amine I and hydroxyl band respectively. The 1.33 value is the ratio for a fully *N*-acetylated chitosan. An average DA of 47.6 ± 2.4 for CH/CNG gels was found for all prepared samples.

In order to elucidate the assignment of the overlapped bands, freeze-dried samples at pH 3 and 9 were analysed. IR spectra shown in Fig. 2 reveals that for gel at pH 3 there

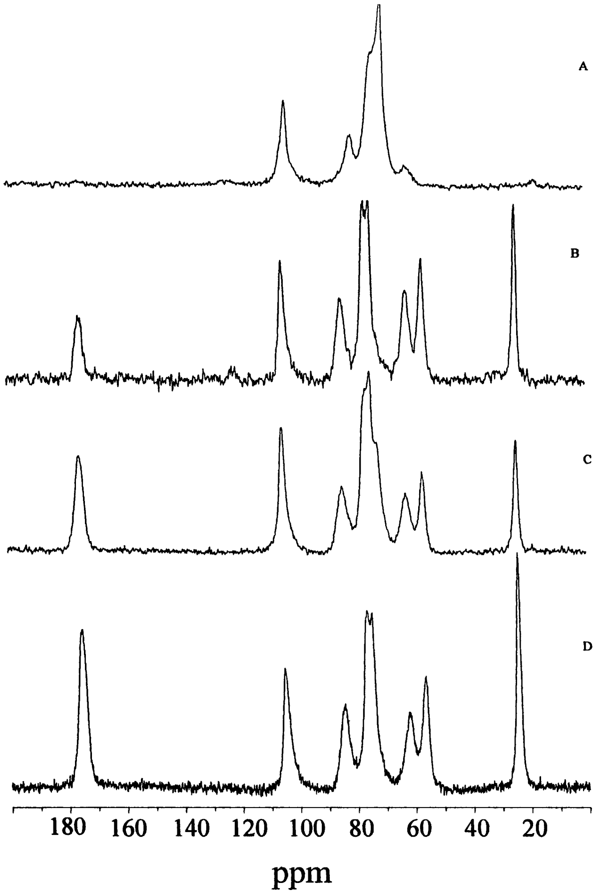

Fig. 3. ^{13}C solid-state NMR spectra of (a) CNG; (b) CH gel; (c) CH/CNG 1:1; and (d) CH/CNG 10:1.

occurred an increase in the intensity of the band at 1562 cm^{-1} due to NH_3^+ of chitosan. This seems to be in good agreement with the increase of H^+ species at low pH. The disappearance of the band at 1421 cm^{-1} due to carboxylate groups can be also seen that is likely to be explained by the fact that in acidic medium CNG carboxylate groups seem to be partially converted to carboxyl groups, whereby a shoulder at 1740 cm^{-1} is now noticeable. IR analyses of freeze-dried gel at pH 9 resulted in very similar spectrum to those obtained for samples in water (pH 6.0). This seems to indicate that CH amino groups are kept in the protonated form, even though in alkaline medium.

3.3. Solid-state C-13 nuclear magnetic resonance spectroscopy

Fig. 3 shows the C-13 CP/MAS spectra for CH/CNG freeze-dried gels. Peak assignments are based on literature data (De Paula et al., 1998; Le Dung, Milas, Rinaudo & Desbrières, 1994; Ottøy, Varum & Smidrød, 1996; Sashiwa & Shigemasa, 1999; Sieval, Thanou, Kotzi, Verhoef, Brussee & Junginger, 1998). Although a fairly good resolution can be achieved for CNG in solution, (De Paula et al., 1998) that is not the case for solid samples. In CNG spectrum (Fig. 3a) there is a broad overlapping region at 70.6 ppm which is assigned to the C-2, C-4 and C-5 carbons of the pyranosic ring. The peaks at 62.3 and 81.0 ppm can be assigned to C-6 and C-3 of galactose in CNG. Anomeric carbon resonance was observed at 104.0 ppm. A low intensity peak at 17.3 ppm is due to CH_3 of rhamose. The resonance due to C=O of glucuronic acid residues also present in CNG was not detected in the solid ^{13}C-NMR. CH gel solid ^{13}C-NMR spectrum (Fig. 3b) presents peaks at 24.0 and 175.3 ppm assigned to CH_3 and C=O of acetyl groups of re-acetylated chitosan. Resonances at 105.0; 56.4; 74.5; 84.6; 76.3 and 62.0 ppm can be inferred as being signals from carbons C-1 to C-6 of chitosan. Spectra of CH/CNG 1:1 and 1:10 gels are also showed in Fig. 3. The shoulder at 71.93 ppm (spectrum C) clearly indicates the presence of CNG for the CH/CNG 1:1 sample (De Paula et al., 1998). It should be noticed that CH carbons do exhibit resonances in the region 70.0–73.0 ppm. CH/CNG 10:1 spectrum is very similar to acetylated CH (CH gel) whereby no resonance from CNG carbons could be detected. In addition to that changes in the ratio of relative intensity of peaks due to CH–C2 (56.3 ppm) and the peak at 62.0 ppm (due C6 of CH and CNG) could be noticed. The ratios were 1.4; 1.36 and 1.30 for CH gel, CH/CNG 10:1 and CH/CNG 1:1 respectively. This indicates a increase in the peak at 62 ppm, when CNG is progressively added.

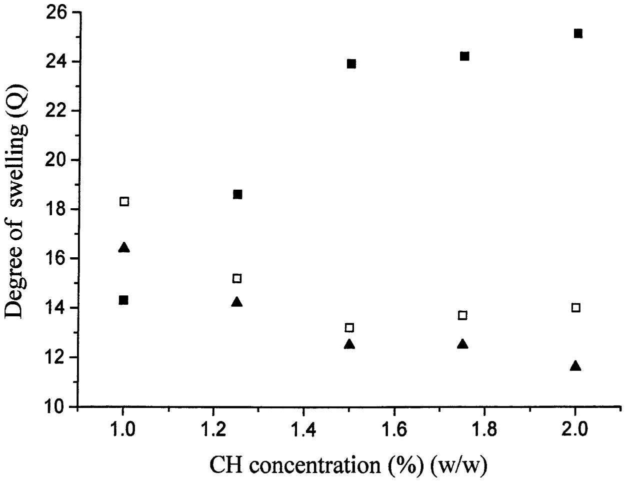

Fig. 4. Degree of swelling as function of CH gel concentration in (■) water; (□) 0.1 M NaCl solution and (▲) 0.1 M $CaCl_2$ solution.

3.4. Swelling studies

3.4.1. Salt effect

Fig. 4 shows the swelling of CH gels prepared at different concentrations in water, NaCl and $CaCl_2$ aqueous solutions, as a function of the initial CH concentration in gel preparation. It can be seen that *Q* in water increases with CH concentration and the system becomes saturated for CH concentrations larger than 2%. On the other hand, by adding

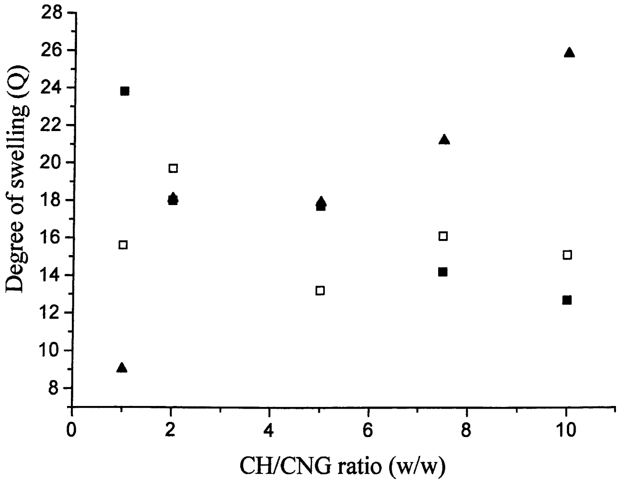

Fig. 5. Degree of swelling as function of CH/CNG ratio in (■) water; (□) 0.1 M NaCl solution and (▲) 0.1 M $CaCl_2$ solution.

Na^+ and Ca^{2+} counterions to the system, Q decreases with increasing CH concentration. It can also be depicted from Fig. 4 that CH gels swell more in NaCl than in $CaCl_2$ solution. Both facts seem to indicate that a screening of electrostatic repulsion takes place with increasing ionic strength.

Fig. 5 presents the swelling behaviour of CH/CNG gels as function of CNG content. It reveals that the swelling in water diminish sharply when the ratio of CH/CNG increases. It can be inferred that CNG plays a major role in the swelling process, mainly due to its high hydrophilic character, where the highest Q value is obtained for sample containing equal amount of CH and CNG. The salt effect on the swelling of CH/CNG gels can also be observed in Fig. 5. In Na^+ solutions Q remains fairly constant, whereas in presence of Ca^{2+} counterions Q increases with the CH/CNG ratio. It has been shown (Paula & Rodrigues, 1995) that calcium exhibit a higher shielding effect that sodium counterions, when added to CNG solutions, resulting ultimately in molecule contraction. However, upon being added to a mixture of CH and CNG, the calcium counterions seem to promote the expansion of the CNG molecule and also to favour the intramolecular interactions, resulting in an overall positive contribution to the gel network stabilisation. This system is now able to retain more water inside and therefore shows higher swelling.

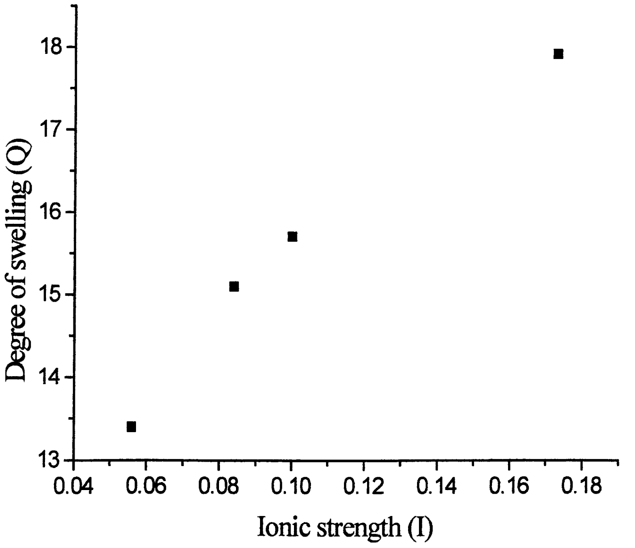

Fig. 6. Degree of swelling as a function of ionic strength for CH/CNG 10:1 gel.

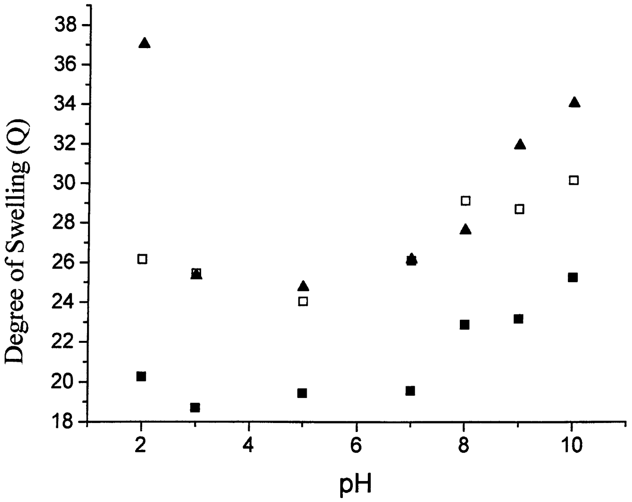

Fig. 7. Degree of swelling as a function of pH: (■) 2% CH gel; (□) CH/CNG 1:1 and (▲) CH/CNG 1:2.

3.4.2. Ionic strength effect

In order to investigate the effect of ionic strength on the degree of swelling of CH/CNG gels, sample with CH/CNG ratio 10:1 was submitted to swelling at same ionic strength observed for buffer solutions in the pH effect experiment.

Fig. 6 shows that Q values increase with increasing ionic strength. This kind of behaviour has been observed for other polyelectrolyte complexes (Brøndsted & Kopecek, 1992) and was attributed to the effect of interruption of ionic forces in the gel.

3.4.3. pH effect

It is shown in Fig. 7 that by adding CNG to CH there is an overall increase in the swelling degree in the pH range 2–10. This is in good agreement with the literature, where it has been reported that adding a more hydrophilic polymer to chitosan increases the water absorption (Lee et al., 2000). On the other hand, it is also likely that CH molecules exist in a new conformation due to binding of NH_3^+ and COO^- species which also contributes to swelling. Swelling is maximal at pH 2 and pH 10, and reaches minimum values in pH around 5. At low pH a high water absorption can be explained by the fact that chitosan amine groups are protonated (NH_3^+) which favours chain expansion. In addition to that, CNG carboxyl groups in alkaline medium are present in the carboxylated form (see IR data), which also favours chain elongation.

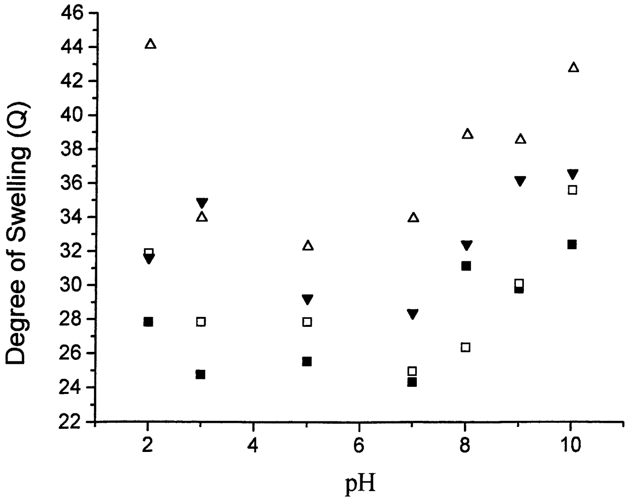

Fig. 8. Degree of swelling as a function of pH: (■) CH/CNG 5:1; (▲) CH/CNG 10:1; (□) CH/CNG 15:1; (▼) CH/CNG 20:1.

Fig. 8 shows the swelling as a function of the CH content, expressed as the CH/CNG ratio. It can be seen that swelling increases with the CH content (samples CH/CNG = 5 : 1; 10:1; 15:1; 20:1) whereby the highest swelling degree was achieved by CH/CNG = 10 : 1 sample. This seems to indicate to an optimal charge distribution for the CH and CNG chains; on the other hand, CH/CNG = 15 : 1 and 20:1 samples are likely to exhibit charge surplus, which leads to a shielding effect that promotes chain aggregation and ultimately less water absorption.

Finally, it can be concluded that addition of CNG to the CH gel increases the equilibrium degree of swelling and water capacity of the gel in all pH range. The highest values for *Q* were observed at extreme pH (2 and 10). As pH 2 and 10 have the lowest ionic strength it was expected that *Q* also reaches minimum values (see Section 3.4.2). Therefore the *Q* trends can be ascribed to pH, for CH/CNG polyelectrolyte complexes. Comparison of *Q* at pH 2 for CH gel and CH/CNG 1:2 sample resulted in an increase of 82.3% in the water absorption. The minimum *Q* value was found at pH 3 for CH gel and at pH 5 for CH/CNG 1:1 and 1:2 samples. Upon increasing the CH/CNG ratio (from 5:1 to 20:1) *Q* augments proportionally, whereby its maximal value is achieved for CH/CNG 10:1 sample (44.1%).

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