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Swelling Behavior of Carboxymethylcellulose Hydrogels in Relation to Cross-Linking, pH, and Charge Density

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ABSTRACT: Hydrogels of carboxymethylcellulose (CMC) with 2.5%, 25%, 50%, and 100% cross-linking were prepared. Cross-linking and stoichiometry was controlled by appropriate addition of CMPJ and determined by potentiometric titration. The cross-linked polymer was then sulfated using a sulfur trioxide−pyridine complex (SO_3−Py) in a heterogeneous mixture. Water uptake of the gels was studied in relation to cross-linking, pH, and sulfation. FT-IR spectra of the gels were recorded at different pHs to evidence the hydrogen bonds. Hydrogen bonds formation between the chains may explain the correlation found between water uptake properties and the chemical composition of the gels.

Introduction

Carboxymethylcellulose (CMC) is an ionic ether of the cellulose and its major commercial derivative.[1] It is a highly water-soluble anionic polysaccharide widely used in pharmaceuticals,[2] as an emulsifying agent, in cosmetics, and in foods.[3] In the biomedical field it is used to prevent the postoperative adherences[4] and epidural scarring.[5]

$X = H, CH_2COOH$
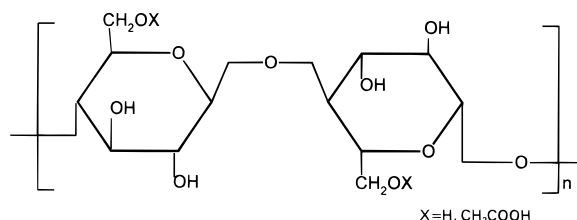
The chemical characteristics of CMC are well-known in the substitution range from $x = 0.25$ to 0.75 per disaccharide unit.[6−9] ^{13}C and ^{1}H NMR spectroscopy[10−12] and high-pH anion-exchange chromatography[13] show that the carboxymethyl group is generally distributed according to O2 ≥ O6 ≥ O3 within the AGU (anhydroglucase units). A potentiometric study of sodium salt of CMC with different degrees of carboxymethylation showed the cooperative influence of the carboxylic groups during dissociation.[14]

Many polysaccharides have been used in the form of hydrogels, but to our knowledge no gels made by chemical cross-linking of CMC chains have being reported in the literature. Novel synthetic routes recently explored in our laboratories[15] have been applied to the sodium salt of carboxymethylcellulose in order to obtain cross-linked CMC. Hydrogels carrying protonable groups are referred to as "intelligent materials" because they respond to external stimuli by shrinking or water uptake. The stimulus may be a change in pH, ionic strength, or temperature.[16−21]

This study deals with the synthesis and chemical characterization of carboxymethylcellulose-based hydrogels with different cross-linking degrees and their water uptake response to pH changes. A potentiometric

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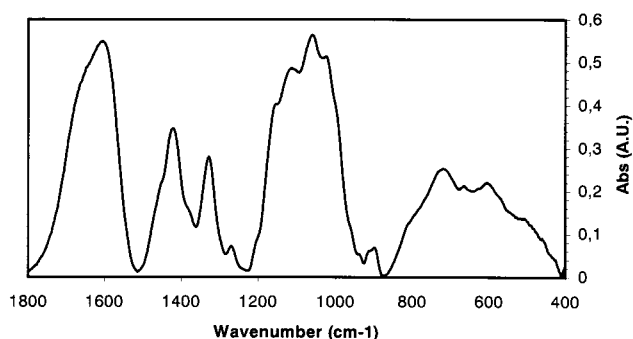
Figure 1. FT-IR spectrum of the commercial carboxymethylcellulose sodium salt (NaCMC).

method was used to determine free carboxyl groups and consequently the degree of cross-linking. The simplicity and speed of this method makes it suitable for studying the chemistry of the cross-linking reaction. The gel was sulfated to increase the number of negative charges responsible for water uptake.

Experimental Section

Materials. The sodium salt of carboxymethylcellulose (NaCMC, Viscosity 402 mPa·s in 2% w/v aqueous solution at 25 °C, carboxymethylation degree of 0.9 ± 0.1 per disaccharide unit, $M_w = 100\,000$) was supplied by Hercules Italia S.p.A. (Italy).

The chemical composition of the native polymer was checked by FT-IR spectroscopy (Figure 1). The absorption peaks at 1620 and 1400 cm^{-1} were attributed to COO^- stretching and bending, respectively. The broadness of these absorption bands was due to the large number of hydrogen bonds involving OH and COO^- groups. The peaks at 1450 and 1330 cm^{-1} were attributed to CH_2 and CH absorptions, respectively. The broad bands from 1200 to 1000 cm^{-1} were due to sugar ring absorption.[22]

From the titration curve for protonation and deprotonation of the carboxylic groups,[14] the degree of carboxymethylation of the sodium salt of CMC was found to be 1.0 ± 0.1, in agreement with the carboxymethylation degree declared by the producer.

N,N-Dimethylformamide (DMF), 2-chloro-1-methylpyridinium iodide (CMPJ), tetrabutylammonium hydroxide (TBA), 1,3-diaminopropane, triethylamine, sulfur trioxide−pyridine complex (SO_3−Py), ethanol, and all the other reagents were purchased from Fluka Chemie AG (Switzerland).

Table 1. Molar Concentration of the Reagents in Hydrogels Synthesis

hydrogel	tetrabutyl-ammonium salt of CMC (disaccharide unit), mol	chloromethyl-pyridine iodide, mol	1,3-diamino-propane, mol
gel CMC 2.5%	1.00×10^{-3}	5.00×10^{-5}	2.50×10^{-4}
gel CMC 25%	1.00×10^{-3}	5.00×10^{-4}	2.50×10^{-3}
gel CMC 50%	1.00×10^{-3}	1.00×10^{-3}	6.00×10^{-3}
gel CMC 100%	1.00×10^{-3}	2.00×10^{-3}	1.00×10^{-2}
gel CMC 100%	1.00×10^{-3}	6.00×10^{-3}	1.20×10^{-2}

Methods. a. Hydrogel Synthesis. Synthesis of the polysaccharide hydrogel is similar to that previously described for hyaluronic acid-based hydrogel.[15]

It is first necessary to convert the sodium salt to tetrabutylammonium (TBA) to make CMC soluble in DMF. Briefly, the procedure consists of sodium–hydrogen ion exchange of sodium CMC solution using Dowex 50WX8 resin (Fluka, Switzerland) to obtain the acid form of CMC. A 5% tetrabutylammonium hydroxide solution was then added up to a final pH of 8. The solution was then lyophilized.

The CMC–TBA was dissolved in dimethylformamide (DMF) under stirring and nitrogen flow, and the solution was kept at about 0 °C. The stoichiometric amount of chloromethylpyridine iodide (CMPJ) was added to the solution to activate 2.5%, 25%, and 50% of the carboxylate groups. (The quantities of the reagents are summarized in Table 1.) 100% cross-linking was carried out by adding the stoichiometric amount of CMPJ and a large excess. An excess of the cross-linking agent, namely 1,3-diaminopropane, was then added together with a small amount of triethylamine as catalyst. The gel formed immediately, but the process was completed, leaving the mixture under stirring for 3–4 h at room temperature. The gel was then washed several times with EtOH and water until no more solvents or reagents were found in the washing solution. The gel was then dried by lyophilization. Samples of gels were sulfated using a solution of sulfur trioxide–pyridine complex (SO_3–Py) in DMF, as previously described.[23]

b. Chemical Characterization. Potentiometric Titration. Potentiometric titrations were carried out in a thermostated glass cell at 25 °C, at a constant ionic strength of 0.1 M NaCl. The potentiometric titration of the native polysaccharide Na^+ salt was performed with a standard 0.1 M solution of sodium hydroxide after protonation of the carboxyl groups using a strong acid ion-exchange resin (Dowex 50WX8 resin, Fluka, Switzerland). The lyophilized gels were finely dispersed and added to the NaCl solution with a known amount of 0.1 M HCl. Then titration was performed with 0.1 M NaOH and back-titration with 0.1 M HCl. The default conditions for the experiment were a stabilization time of 60 min for the initial system and a delay time of 18 min between each addition of titrant.

The titration data were collected by a Crison MicropH-2002 equipped with a combined electrode (model 6.0204.000) used with an automatic Crison microburet (model 3031) connected to a PC.

c. Determination of Gel Sulfation. The number of sulfate groups (equiv of SO_3^- groups/g of dry CMC gel) was determined by the "toluidine blue" method:[24,25] an exact amount of dry CMC gel was weighed and immersed in an exact volume of toluidine blue solution, the absorbance (630 nm) of which had been measured previously. The decrease in absorption, due to violet complex formation between the toluidine agent and the sulfated groups of the polysaccharide gel, was recorded by a spectrophotometer (Pharmacia, Biochrom 4060), and the number of sulfate groups in the CMC gel samples was calculated using a calibration curve. The calibration was obtained with aqueous solution of sulfated hyaluronic acid ($HyalS_{3.5}$), the sulfation of which (3.5 sulfate groups per disaccharide unit) was determined by elemental analysis and HPLC.[26]

d. FTIR-ATR Analysis. ATR spectra of the samples in dry form were recorded on a Bio-Rad FTS 6000 between 4000 and 750 cm^{-1}. A horizontal ATR accessory with a 45E zinc-selenide crystal was used. A MCT detector was used, and the apparatus was purged with nitrogen. Typically 50 scans at a resolution of 1.0 cm^{-1} were averaged. The frequency scale was internally calibrated with a helium–neon reference laser to an accuracy of 0.01 cm^{-1}.

e. Water Uptake Measurements. Established amounts (the starting weight W_d was obtained after lyophilization of the gels) of cross-linked dry polymers were enclosed in small bags of hydrophobic water-permeable net (Nylon) and immersed for 24 h at room temperature in 50 mL of distilled water or buffer solution. At regular intervals the bags was removed from the solvent, its surface was pressed gently with tissue paper to remove the excess solvent on the surface, weighed, and then returned to the medium. This process of water uptake and weighting was continued until the gels attained a constant final weight (W_s).

$$\text{water uptake} = \frac{W_s - W_d}{W_d} \times 100$$

Water uptake measurements were performed at different pHs in the following solutions: pH 2, 6.5 mL of 0.2 M HCl mixed with 25 mL of 0.2 M KCl; pH 7.4, PBS (phosphate buffer solution); pH 9, 50 mL of 0.1 M TRIS with 5.7 mL of 0.1 M HCl.
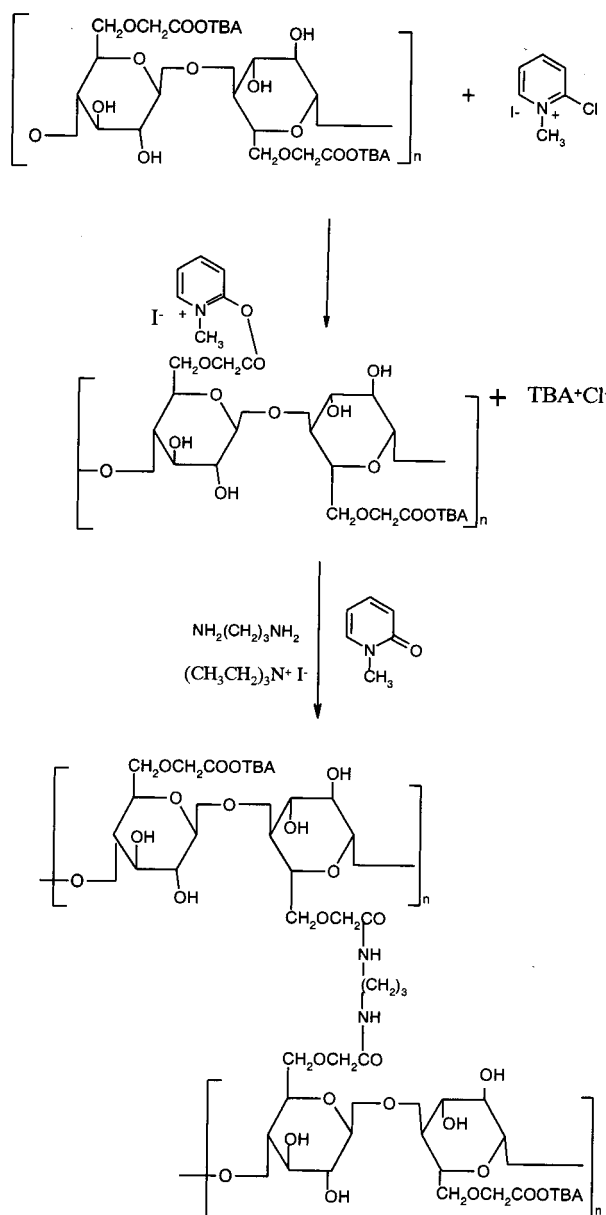
Results and Discussion

Gel Synthesis. Scheme 1 shows gel synthesis. Cross-linking was determined by appropriate addition of CMPJ. The carboxylate groups of carboxymethylcellulose were activated by nucleophilic attack on the carboxylate ion by CMPJ with release of TBA^+Cl^-.[15] The reaction temperature was kept at 0 °C because a higher temperature led to the formation of an unstable gelatinous network that could block the primary cross-linking reaction. The carbonyl group of the intermediate undergoes nucleophilic attack by the diamine eliminating 1-methyl-2-pyridone. The reaction was catalyzed by a small amount of triethylamine, as hydrogen iodide captor. The product of the reaction is gelatinous even in organic solvent.

Four gels with different cross-linking (2.5%, 25%, 50%, and 100%) were synthesized. Cross-linking corresponded to the percentage of carboxylate groups involved in the cross-linking process (see Gel Characterization). The consistency of the gels increased with cross-linking from a 2.5% to 100%.

The equivalent SO_3^-/g found in sulfated CMC gels was low (10^{-6} equiv of SO_3^-/g of product), considering the numbers of OH groups in the macromolecule, and was similar to that of sulfated hyaluronic acid (HyalS) gels.[15] This behavior may be related to the availability of OH groups in the gel, compared with that of the free polysaccharide, due to the fact that the reaction occurs in heterogeneous phase and in organic solvent in which the product does not swell. Furthermore, sulfation decreased with increasing the cross-linking of the gels.
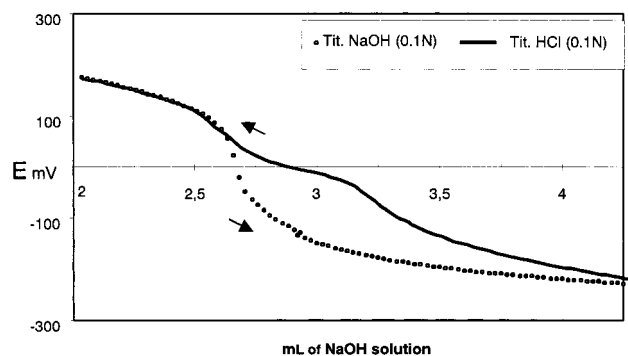
Gel Characterization. Potentiometric Titration. Cross-linking determined by potentiometric titration was in good agreement with that calculated from the amount of CMPJ added. During titration, since the 2.5% gel did not maintain the gel structure, it was not possible to confirm the degree of cross-linking. The theoretically 25% gel was found to be 24 ± 2%, and the 50% gel was 46 ± 2%. The potentiometric titration also
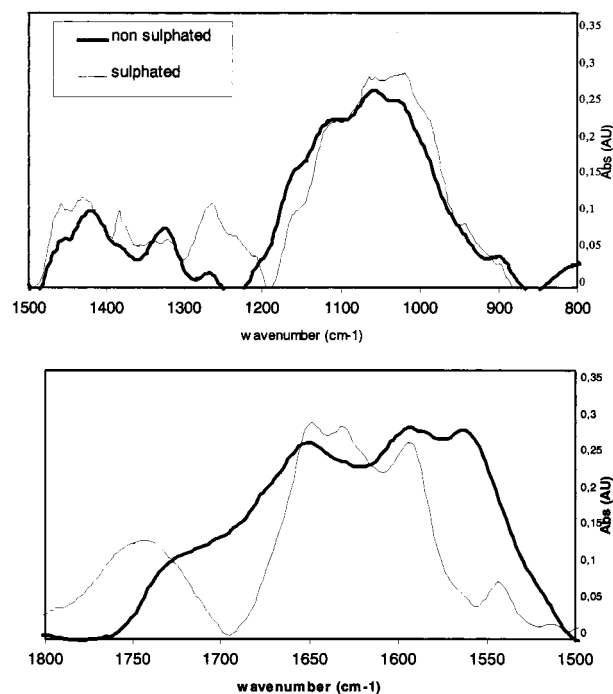
Scheme 1

Figure 2. Potentiometric titration of a fine dispersion of 50% cross-linked gel with 0.1 N HCl or 0.1 N NaOH. (The titration occurs in the heterogeneous phase.) The volume reported in the *x*-axis refers to the titration with 0.1 N NaOH solution. The volume increase in the opposite way during the titration with 0.1 N HCl solution.


Figure 3. FT-IR spectrum of 50% cross-linked sulfated ($-$) and nonsulfated ($—$) gels at pH = 2, from 1500 to 800 cm^{-1} (top) and from 1800 to 1500 cm^{-1} (bottom).

revealed that the maximum cross-linking of 100% CMC was 89 ± 2% when a concentration of CMPJ equimolar to that of COO$^-$ was added, whereas it was possible to increase cross-linking up to 94 ± 2% using an excess of CMPJ. In this paper the 100% gel refers to the latter compound.

The two inflection points (Figure 2) from which we determined cross-linking were only evident when the hydrogel was titrated with a strong acid. The titration of the gels in the acidic form with NaOH produced only one jump due to the neutralization of excess acid in solution, even though the lag time of acquisition was increased. The difficulty of titrating the carboxylic acid groups of the gel with NaOH may be because in acid medium gel structure is devoid of charge, which makes it extremely compact, as demonstrated for the CMC polysaccharide in the free form,[14] because of the presence of several hydrogen bonds. It is difficult for OH$^-$ ions to penetrate the bulk of the gel to dissociate the COOH groups. At basic pH the carboxylic groups are completely dissociated, and the network is more open because of electrostatic repulsion between COO$^-$ groups.

This situation appears to be favorable for the protonation of the carboxylate groups.

Figure 3 shows the spectra of native and sulfated 25% CMC gel. Evidence of sulfation is observed between 1300 and 1000 cm^{-1} (Figure 3, top). The shoulder at 1250 cm^{-1} and the increase in intensity at 1020 and 1000 cm^{-1} are due to the S=O and SO$_3^-$ stretching frequencies, respectively. The region from 1800 to 1500 cm^{-1} (Figure 3, bottom) remained unaltered, evidencing that sulfation reaction did not destroy or alter the cross-linking of the CMC chains.

Water Uptake Measurements. A relationship between degree of cross-linking and water uptake in distilled water was found (Figure 4). Water uptake decreased with increasing cross-linking. Polysaccharide chains with fewer cross-links are freer in the gel network, and the fixed negative charges, i.e., COO$^-$ moieties, cause chain stiffening and uptake of large amounts of water. The influence of the negative charges on water uptake was well documented by the sulfated
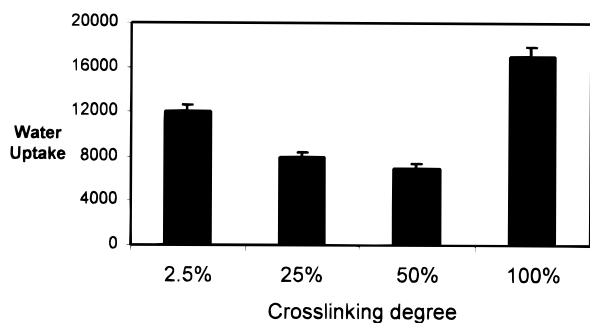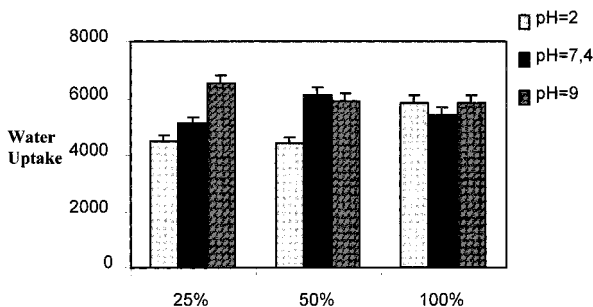
Figure 4. Water uptake in water of CMC gels as a function of cross-linking.
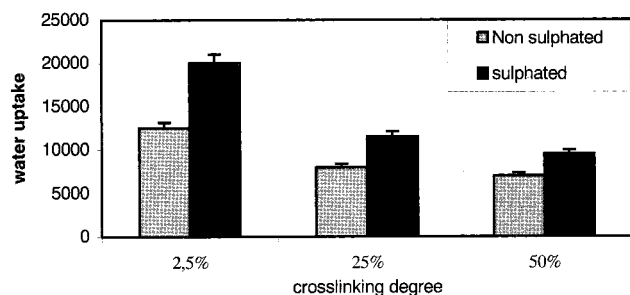

Figure 5. Water uptake of the 50% cross-linked sulfated and nonsulfated gel.

Figure 6. Water uptake of CMC gels as a function of cross-linking at different pHs.

gels (Figure 5). Water uptake of these compounds was in fact greater than that of nonsulfated ones because the greater number of negative charges in the sulfated gels magnifies the polysaccharide chains repulsion, thus enlarging its meshes and promoting the water uptake.[27]

To understand the anomalous high water uptake of the 100% cross-linked hydrogel, the water uptake of hydrogels with 25%, 50%, and 100% cross-linking was measured at different pHs (Figure 6). These values are lower than water uptake values measured in water because ionic strength reduces the water uptake degree. An increase in water uptake, together with pH increasing, was observed for the two hydrogels containing free COO- moieties (25% and 50%), but the trend was different. The 100% cross-linked gel did not show pH dependence. The 25% hydrogel showed a very small increase in water uptake value from pH 2 to pH 7.4 and a jump from pH 7.4 to pH 9, whereas the 50% hydrogel showed a jump from pH 2 to pH 7.4 and plateau to pH 9. Hydrogen bonds in CMC hydrogels affect pH-dependent water uptake. As illustrated in Figure 6, the 25% and 50% cross-linked hydrogels had the same water uptake at acidic pH. Occupation of COO$^-$ by protonation or involvement in cross-links gave these gels the same water absorption and water uptake degree behavior. These carboxylic acid groups should become ionized at
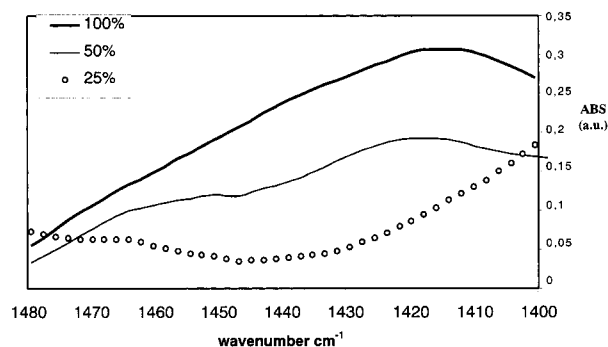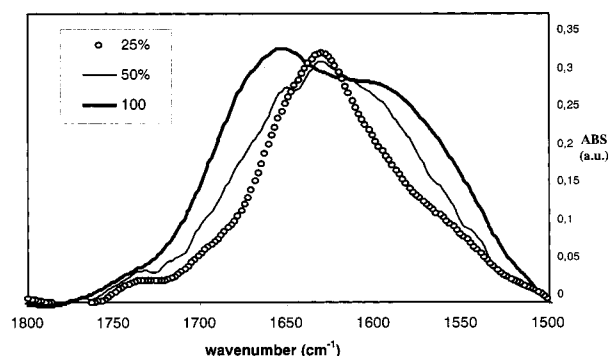
Figure 7. FT-IR spectra of CMC gel with 25%, 50%, and 100% cross-linking, from 1800 to 1500 cm^{-1} (top) and from 1500 to 1400 cm^{-1} (bottom).

pH 7.4 since the pK_a of the carboxylic acid in the polysaccharide is ≈3.2.[14] At pH 7.4 the hydrogen bonds would be broken, electrostatic repulsion would arise between macromolecules, and water would be taken up. The effect was only evident in the 50% cross-linked hydrogel, which has fewer hydrogen bonds than the 25% hydrogel. A complete water uptake and ionization of the 25% gel are obtained at higher pH values (pH 9). In the 25% hydrogel, in fact, the larger content of carboxylic groups determines a higher number of hydrogen bonds and a very hard H-bond network to break for which a pH of 9 is needed. The 50% hydrogel forms fewer hydrogen bonds, and the response to changes in pH is immediate.

This water uptake mechanism governed by hydrogen bonds is confirmed by the behavior of the 100% cross-linked gel which did not show pH dependence but swelled as much as the maximum observed for the 25% and 50%. Its water uptake particularly in acidic medium was greater than that of the other two gels because occupation of all COO$^-$ by cross-links and consequently their not involvement in hydrogen bond formation.

FT-IR Characterization. Figure 7 (top) shows the spectral regions (1800−1500 cm^{-1}) of CMC hydrogels with 25%, 50%, and 100% cross-linking where the largest variations are observed. The increase in absorption at 1650 and 1560 cm^{-1} is due to amide I (C=O stretching) and amide II (NH deformation), respectively, and the decrease in absorption at 1600 cm^{-1}, assigned to carboxylate stretching, may be related to the increase in cross-linking. In Figure 7 (bottom) (1500−1400 cm^{-1}) the band at 1440 cm^{-1} attributed to CH stretching of cross-links increases with increasing cross-linking.

The spectra of gels at different pHs elucidated the role of hydrogen bonds in gel water uptake.

At pH 2 (Figure 8) the 25% and 50% cross-linked gels showed the characteristic absorption at 1720 cm^{-1} due
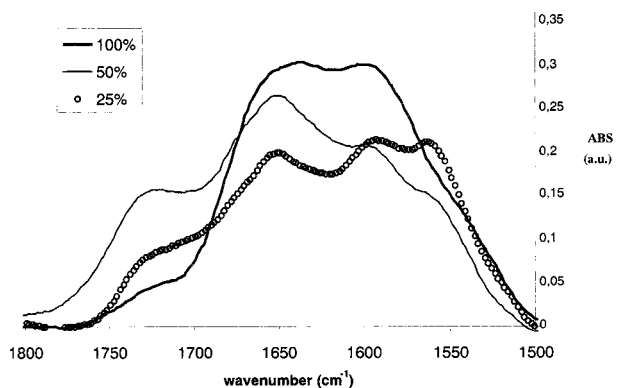
Figure 8. FT-IR spectra of gels with 25%, 50%, and 100% cross-linking at pH 2.
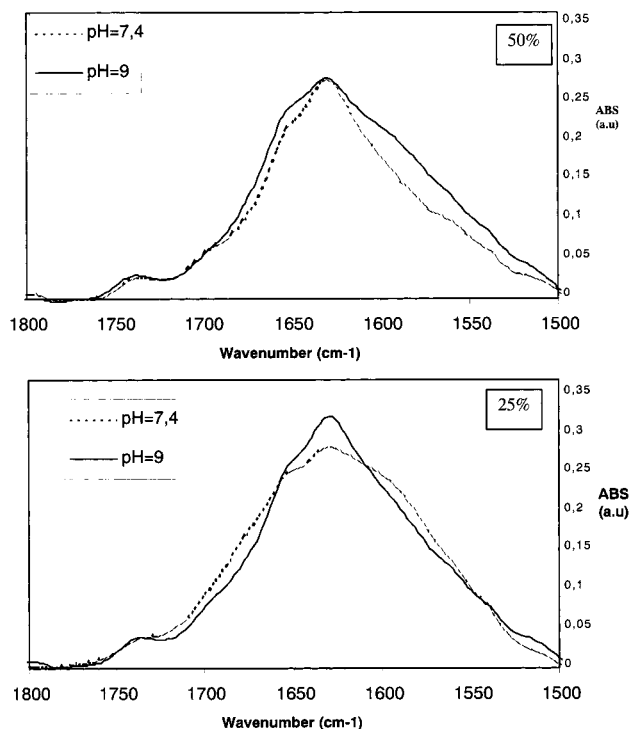
Figure 9. FT-IR spectra of 50% (upper) and 25% (lower) cross-linked gels at pH 7.4 and 9.

to C=O vibration of the protonated carboxylic groups involved in hydrogen bond formation. (The free protonated carboxylic groups have an absorption band at 1740 cm^{-1}.) The band at 1650 cm-1 attributed to amide I (C=O stretching) of the cross-link showed a shoulder shifted about 20 cm-1 lower and splitting of amide II band into two components: at 1590 and 1560 cm^{-1} which indicate strong H bonds. This feature was clearly evident in the 25% cross-linked gel (Figure 8) where the relative intensity of the "free" and "hydrogen-bonded" bands of amide II were the same. This means that half the NH groups were involved in hydrogen bonds, mainly with the carboxylic groups, the intensity of which shifted from 1740 to 1720 cm^{-1}. With 50% cross-linking (Figure 8), the number of hydrogen bonds decreased as revealed by the decrease in intensity of the amide II component at 1560 cm^{-1} ,which became a shoulder of the 1590 cm^{-1} component. This absorption completely disappeared in the 100% cross-linked gel (Figure 8).

As pH was increased (Figure 9) to 7.4, the absorption due to the protonated carboxylic group disappeared, and a peak due to COO⁻ groups was observed at 1628 cm^{-1}
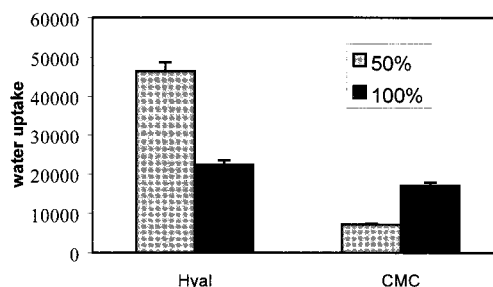
Figure 10. Water uptake in water of HYAL and CMC gels with 50% and 100% cross-linking.

in 25% and 50% cross-linked gels. The number of NH groups involved in hydrogen bonds decreased with respect to pH 2, as revealed by the decrease in intensity of the amide II band at 1560 cm^{-1} that became a shoulder in the 25% gel and completely disappeared in the 50% cross-linked gel. This trend suggests that the 25% cross-linked gel still had a significant number of hydrogen bonds at this pH, whereas the 50% cross-linked gel had very few.

When pH was increased to 9 (Figure 9), the NH absorption at 1560 cm^{-1} also completely disappeared in the 25% cross-linked gel, indicating breaking of hydrogen bonds and complete ionization of COOH groups. The spectra of the 100% cross-linked gel did not show pH dependence.

The introduction of sulfated groups caused a more drastic reduction in hydrogen-bond formation in the gels. For example, 50% cross-linked sulfated gel at pH = 2 (Figure 3, bottom) shows absorption at 1740 cm^{-1}, due to a "free" C=O vibration of protonated carboxylic groups, and a very low-intensity band due to hydrogen-bonded NH groups at 1560 cm^{-1}. This feature can be explained by the fact that at pH 2 the sulfated groups were still ionized and induced a strong electrostatic repulsion between the polymer chains, breaking the hydrogen bonds.

Conclusions

The different water uptake behavior of carboxymethylcellulose-based hydrogels with 2.5%, 25%, 50%, and 100% cross-linking was governed by H-bond formation. The 100% cross-linked gel swelled most, and water uptake was independent of pH. Water uptake of the others was pH-dependent. Carboxylic groups had two principal effects on water uptake: (1) when fully protonated (COOH), they formed several hydrogen bonds which reduced the water uptake of the gels; (2) when ionized (COO⁻), they produced electrostatic repulsion which opened the network and increased water uptake of the gels.

The synergistic effect of negative charges and hydrogen bonds explains water uptake behavior in the intermediate pH range.

Sulfation of these hydrogels introduced further negative charges, opening the network, as demonstrated by the increase in water uptake. Sulfation reduced the number of hydrogen bonds and increased water uptake because the extra negative charges caused electrostatic repulsion between chains.

The influence of H bonds and water uptake on the hydrophilic groups can be observed by comparing CMC-based hydrogels with the corresponding Hyal-based hydrogels[15] (Figure 10). The latter showed greater water uptake, presumably due to the larger mesh of the Hyal-

based hydrogel which had a carboxylic group for every disaccharide unit and thus cross-linking every two saccharide rings. This reduced the number of hydrogen bonds, and the acetyl amide groups increased the hydrophilicity of the polysaccharide, since they are more hydrophilic than OH groups.

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