

CROSS-LINKING OF CHITOSAN WITH GLUTARALDEHYDE IN THE PRESENCE OF CITRIC ACID — A NEW GELLING SYSTEM*

Yu Fang** and Dao-dao Hu

Department of Chemistry, Shanxi Normal University, Xi'an 710062, China

Abstract The procedure for preparing a new type of uniform and porous chitosan gel from citric acid medium is described. Its swelling behavior in different media was compared with those of the gels prepared by other methods. The ultrastructure of the xerogel prepared from citric acid was characterized using electron microscopy (SEM).

Keywords Chitosan, Gels, Citric acid, Xerogel

INTRODUCTION

The preparation methods and some properties of chitosan gels prepared in aqueous phase by glutaraldehyde (Glu) crosslinking have been studied by a number of research groups^[1, 2, 3]. Generally, rigid gels were obtained by mixing Glu solution with chitosan acetate solution. This kind of gel does not dehydrate easily and occupies the total volume of the solutions from which they originate. Roberts and Taylor^[2] have found, from viscosity measurements, that the rate of gelation is proportional to the concentrations of both chitosan and Glu and to the temperature of the reaction medium. An increase in the concentration of acetic acid, however, inhibits the process. Addition of neutral electrolytes has also been found to inhibit the process.

The aim of the present study is to investigate how the acidity influences the gelation process, the properties and the structures of the chitosan gels. In this article, we report a new chitosan gelling system, together with a comparison of the swelling behaviors of the gels from this system with those from other acid media.

EXPERIMENTAL

Chemicals

Chitosan having an average degree of N-acetylation (DA) of approximately 40% was prepared by deacetylation of chitin (Aldrich) in NaOH solution. The crude chitosan was redissolved in 4 wt% acetic acid, filtered and precipitated with NaOH solution. The precipitate was washed with distilled water to pH 7 and vacuum dried. The degree of acetylation was estimated using FTIR and pH titration method^[4]. Glu, citric acid and other reagents were purchased from Aldrich, and used without further purification.

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** Corresponding author: Yu Fang (房喻), E-mail: yfang@snnu.edu.cn

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Preparation of Gels

The crosslinked chitosan gels were prepared by the following procedure: purified chitosan (1 g) was suspended in 40 mL of distilled water. To the suspension 5 mL of citric acid solution (2 mol/L) was added with stirring. The mixture was stirred for another 20 min to ensure that chitosan was dissolved completely. The solution was thoroughly degassed and a Glu solution (10 mL, diluted to 10 wt%) was added slowly with shaking. The mixture was agitated for another 5 min and then kept at room temperature. The other gels were prepared in a similar way.

Determination of the Gelation Rate

The gelation rates of the above gelling systems were determined by viscometry on a standard volume (6 mL) of solution using a Brookfield Viscometer (LVT model). The apparent viscosity was recorded at a spindle speed of 6rpm. The onset of gelation can be determined by plotting the apparent viscosity against time during formation of the gels.

Swelling Measurements

The gels obtained were removed from the vessel, sliced into disks (about 3mm thick), and soaked in methanol for several days (refreshed periodically). Swelling measurements were carried out by immersing the gel disks (in triplicate) in a given solvent under study. The attainment of equilibrium was monitored by periodically withdrawing the disks from the solvent and weighing them after removing excess water on the surface by lightly blotting it with a filter paper. The weight of each disk was individually measured in the way described until it reaches a constant value. The relative swelling ratio (R) was expressed as $R = W_s/W_o$, where W_s and W_o stand for the weight of the swollen gel and that of the original gel, respectively.

Preparation of Xerogels

The xerogels used for SEM measurements were prepared by rapidly freezing a cube (approx. 2 cm³) of the gels in liquid nitrogen (approx. -60°C) and lyophilized and further dried in vacuum at 60°C for 2 days. The SEM was performed using a JEOL 5200 electron microscope.

FTIR spectra of all samples pelletized with KBr were recorded using a Perkin Elmer 1600 Fourier transform infrared spectrometer.

RESULTS AND DISCUSSION

Chitosan represents partially deacetylated chitin and is considered to be a random copolymer of N-acetyl-D-glucosamine and D-glucosamine^[5]. Therefore, it can be easily crosslinked by using bifunctional compounds containing amine and/or hydroxyl groups such as Glu^[1-3], acid anhydrides^[6], hexamethylene diisocyanate^[7], and bis(vinylsufonyl)arenes^[7] etc. It has been demonstrated that the physico-chemical properties and ultrastructures of the crosslinked chitosan gels are dependent upon the properties of the chitosan (i.e. DA, molecular weight etc.), the nature of the crosslinker, the degree of crosslinking and the type of solvent. In addition, acidity also has a pronounced effect upon the rate of gelation and the structures of the gels formed in chitosan systems.

Gelation Rates

The results obtained from viscosity measurements demonstrated that the onset of gelation in formic acid, acetic acid, tartaric acid and citric acid are 270, 13, 1850 and 420 min, respectively, for the systems with the same molar concentrations of ionizable hydrogen ions (0.5 mol/L), chitosan (15 mg/mL) and Glu (0.1 mol/L). As an example, Fig. 1 shows a plot of the relative viscosity *versus* time for the system containing acetic acid. The longer gelation time for the formic acid system than that for the acetic acid system may be understood by considering the fact that the former is a stronger acid (K_a , 1.77×10^{-4} , 25°C) than the latter (K_a , 1.75×10^{-5} , 25°C). A higher

proton concentration in the system leads to a reduction in the fraction of effective (free) amine groups, which are essential for the crosslinking reaction, and thereby a reduction in the rate of gelation. Clearly, the same arguments can be applied to the tartaric acid and citric acid systems. The first order ionisation constants of the two acids are 1.0×10^{-3} and 7.4×10^{-4} , respectively, which is in agreement with the order of gelation rate observed. It should be noted, however, that the gelation rate of the system containing citric acid is significantly lower than that of formic acid system even though the former acid is a stronger one. This observation may be explained by considering the fact that citric acid is a kind of multibasic acid and therefore, the ionic strength of the system containing citric acid should be greater than that of the system containing formic acid. The greater ionic strength will reduce the activity of protonated chitosan and thereby reduce the gelation rate.

It should also be noted that chitosan gels cannot be prepared from alanine solution. This is because alanine reacts very rapidly with Glu to produce a dark brown precipitate. Gels could also be prepared from inorganic acid media. However, the times to onset of gelation, are much longer than those measured in organic acid media. For example, the time of onset of gelation in HCl is at least 140 h.

Swelling Behaviors

The swelling behaviors of the gels obtained from different acid media in NaCl solution at pH 7 are similar. The swelling ratios decrease from approx. 3 to 1 with increasing salt concentration from nil to 8 wt%. Similarly, the gels prepared from different acid media have similar swelling behaviors over a wide pH range (0–14) accessed (Fig. 2). Each gel has two maximal swelling ratios within the pH range studied, which appears at pH values approx. 2 and 12, respectively, irrespective of the types of gels. The swelling of any of the gels at pH zero is negligible. Interestingly, at pH 14, all gels will contract rather than swell.

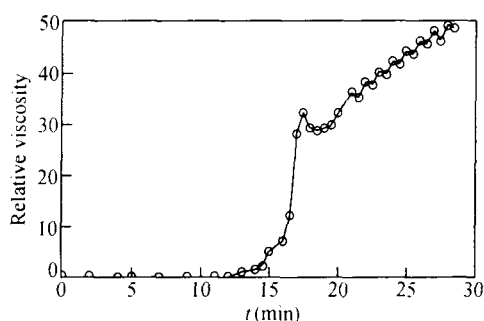


Fig. 1 Plot of apparent viscosity versus time during the formation of chitosan gels in acetic acid medium

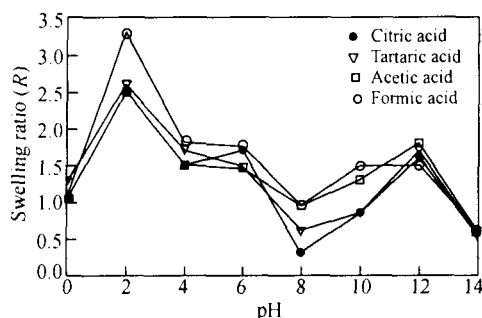


Fig. 2 Variation of the swelling ratio of chitosan gels as a function of pH

It is known that a swelling equilibrium of a polyelectrolyte network is determined by a balance of attractive forces and repulsive forces. At low ionic strength, the concentration of bonded charges and accompanying counterions within the gels exceed the concentration of salts in the external solution, a large ion swelling pressure causes the gel to expand, and thereby decreases the concentration of counterions within the gel. As the external ion concentration increases, the swelling ability of the gels continuously decreases until the mobile ion concentrations within and surrounding the gel become approximately equal. If the ionic strength of the medium is greater than that within the gel, shrinkage of the gel will occur. This is because a negative osmotic pressure makes the gel dehydrate in order to decrease the ionic strength of the medium. The effect of pH upon the swelling behaviors of the gels is rather complex and might be a combination of ionic

strength and acid-base interactions. The acid-base interaction will alter the charge density of a gel and the partition of relevant ions simultaneously. These changes must shift the swelling-shrinking equilibrium to one side or another.

Based upon the above discussion, the observation of the two "unusual" maximal swelling ratios of the gels at pH 2 and 12 may also be rationalized. For example, for a gel system equilibrated at pH 7, an initial decrease in pH will protonate some of the amine groups within the chitosan gel, leading to an increase in counterion concentration and an increase in repulsion between the protonated amine groups within the gel. Both effects will make the gel expand. Further increase in acid concentration will increase the ionic strength of the medium due to the saturation of the protonation. This increase will inhibit the swelling of the gel and even make the gel shrink. Therefore, it is not surprising that there is a maximum swelling pH for each system at pH lower than 7. A similar argument can be applied to the high pH region.

Conversely, the swelling behavior of the gels in a mixture of water and ethanol is largely dependent upon the type of counterions of a given gel (Fig. 3). For example, the swelling ratio of a gel of smaller and simpler counterions in a given water-ethanol mixture is always greater than those obtained from multibasic acid media. Furthermore, the swelling ratios of all gels decrease with increasing ethanol content in the mixture. These outcomes may result from the dependence of the dielectric constant of the mixture upon ethanol concentration. It is known that an increase in ethanol concentration leads to a decrease in the dielectric constant of the mixture, and thereby a decrease in the solubilizing ability of the mixture for polyelectrolyte. Therefore, any increase in ethanol concentration will inhibit the counterions of the gels diffusing into the surrounding medium, resulting in a reduction of the swelling ratio. Gels of larger and multicharged counterions are more sensitive to changes in the polarity of the surrounding medium. Therefore, the swelling ratio of this kind of gels are strongly affected by the introduction of ethanol.

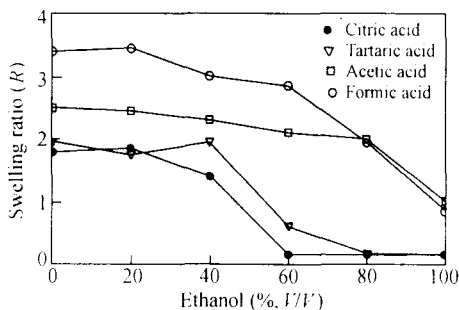


Fig. 3 Variation of the swelling ratio of chitosan gels as a function of aqueous-ethanol composition

It is interesting to note that the swelling ratios of the gels formed in the citric and tartaric acid systems decrease sharply as the concentration of ethanol increases from 40% to 60% in volume. These transitions in gel volume and weight may be useful for separation purposes^[8-10].

Compositions and Structures of the Gels

The FTIR spectra of the purified chitosan, the gels from acetic acid medium and sodium acetate are shown in Fig. 4. It can be clearly seen that the spectra of the gels (Figs. 4b and 4c) are different from that of the chitosan (Fig. 4a), and some of the absorption peaks characteristic for the salt appear. Compared with the spectra of chitosan, the amide II peak at 1560 cm^{-1} , and the peak at 1408 cm^{-1} in the spectra of the gels are increased and broadened. In view of the spectrum of sodium acetate (Fig. 4c), the reasons for these spectral changes can be attributed to the introduction of acetate anions. Similar phenomenon can be seen in gels from other acid media. This result

indicates clearly that the gels prepared from different acid systems have different counterions, which is in fact the structural basis of the different swelling abilities of the gels in aqueous-ethanol.

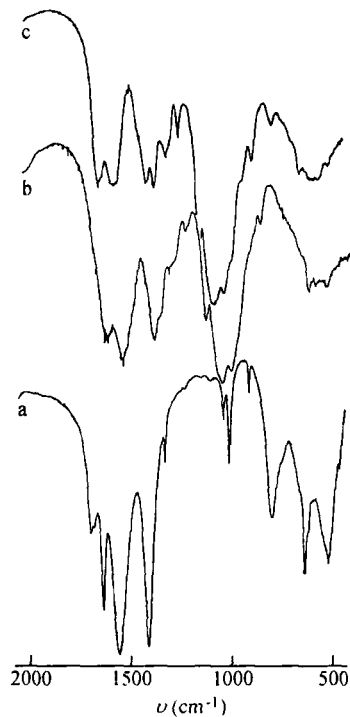


Fig. 4 FTIR spectra of a) chitosan, b) Glu crosslinked chitosan obtained from acetic acid medium and c) from sodium acetate

The ultrastructure of the Glu crosslinked chitosan gels prepared in different acid media was examined by SEM observation (Fig. 5a). The scanning photomicrograph of the xerogel prepared from formic acid shows that the gels assume an irregular structure. The partially broken pores present might be due to the formation of ice crystals on freezing and the cobweb-like fine structure could be the undissolved chitosan. Clearly, the photomicrograph of the xerogel from acetic acid medium (Fig. 5b) is different from that of the gel from formic acid medium. The gels adopt a uniform and cloud-like ultrastructure. The pores in this xerogel might be due to trapped air bubbles which should be extracted completely. The ultrastructure of the gel from tartaric acid medium is uniform and fibre-like (Fig. 5c). The “fibers” align themselves in the same direction, but the thickness of the fibres is different. The xerogel from citric acid adopt a very special ultrastructure (Fig. 5d). It is highly porous. The total volume of the pores occupies nearly 50% of the whole xerogel, and the diameters of the pores range from less than 10 μm to 25 μm . The broken part of the xerogels might be caused by the formation of ice crystals on freezing. It is presumed that some chitosan xerogels prepared from citric acid medium may find some potential applications.

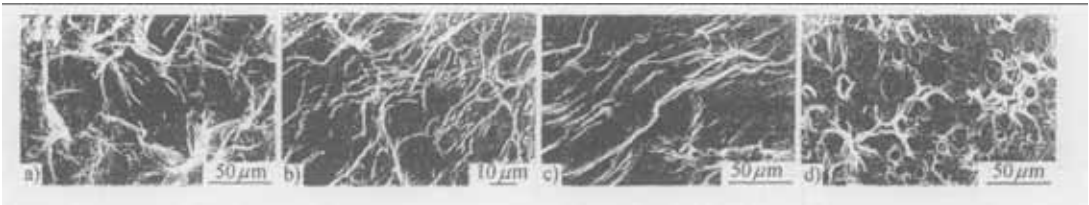


Fig. 5 General surface view of the crosslinked chitosan xerogels prepared from a) formic acid, b) acetic acid, c) tartaric acid and d) citric acid media.

CONCLUSIONS

Acidity influences the gelation of chitosan + Glu system. Generally speaking, the stronger the acid the slower the gelation process. The type of counterions of a chitosan gel has little effect upon its swelling behavior in aqueous phase. However, swelling of gels having multiple-charged counterions is more strongly affected by the introduction of ethanol into aqueous medium than those having simple counterions. The ultrastructure of the gels from citric acid medium is quite different from the others. It adopts a relatively uniform and porous structure.

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