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A Novel Chitosan-Based Gel System

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Abstract

Previous methods for obtaining chitosan gels are briefly reviewed, as is the recently reported solvent system for chitosan – aqueous sodium formaldehyde bisulphite. The use of chitosan dissolved in this solvent to form gels at room temperature on mixing with aqueous solutions of ammonium alginate is described, together with some of the properties of the gels formed.

Introduction

Polymer gels may be subdivided into two classes: chemical gels, in which bonds, usually covalent bonds, are formed linking specific groups on the polymer chains, and physical gels in which the junction points holding chains together are formed by the binding together of chain segments without the formation of specific bonds.

Chitosan, because of the presence of both amine and hydroxyl groups along the chain, is very suitable for the preparation of chemical gels and indeed the first chitosan-based gel was of this type. Its preparation was reported by Broussignac (1968) and involved the reaction between chitosan and formaldehyde, which is able to act as a bifunctional reagent. Since then there have been a number of papers dealing with the formation of chitosan gels by reaction with aldehydes, mainly using the dialdehyde glutaraldehyde (Roberts and Taylor, 1989). Other methods of forming chitosan-based chemical gels include reaction with epichlorohydrin (Choi and Ahn, 1990), complex formation with metal ions such as Mo(VI) (Draget *et al.*, 1992), and most recently tyrosinase-mediated reaction between chitosan amine groups and cresol (Kumar *et al.*, 2000).

Most crosslinking agents used are low molecular weight, highly reactive di- or polyfunctional molecules and possess two major disadvantages because of their high reactivity. First is the difficulty in ensuring uniform distribution throughout the viscous polymer solution before reaction occurs. Second is the difficulty in removing unreacted, hydrolysed or partially reacted reagent and this is particularly important if the resultant gels are to be used in biomedical applications. For these two reasons physical gels are of particular interest for chitosan applications.

The first chitosan-based physical gel was reported by Hirano and Yamaguchi (1976). These were non-reversible *N*-acetylchitosan gels formed by homogeneous re-*N*-acetylation of chitosan dissolved in an aqueous acetic acid/methanol medium containing acetic anhydride as the acetylating agent. The methanol was required in order to suppress any *O*-acetylation as Hirano and Ohe (1975) had previously shown that homogeneous re-acetylation in aqueous acetic acid

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alone gave *N,O*-acetylated products having an *O*-acetyl DS of 1.36. The *N*-acetyl-chitosan gels formed in the presence of methanol were rigid, transparent, colourless and infusible, underwent syneresis, and were soluble only in formic acid. *N*-acylchitosan gels were also obtained by homogeneous *N*-acylation of chitosan in aqueous acetic acid/methanol using acyl anhydrides up to dodecanoic anhydride. Higher homologues than dodecanoic anhydride, although giving rise to *N*-acylchitosans, formed precipitates rather than gels (Hirano and Yamaguchi, 1976).

These 'Hirano' gels are the most studied of any chitosan-based gel systems. Although high acetic anhydride:amine group ratios were used initially, it was soon found that a ratio as low as 1.7:1 was sufficient to give a rigid gel (Hirano and Yamaguchi, 1976; Moore and Roberts, 1980a). The importance of other variables has been studied by determining their effects on the rate of gelation, as monitored by a rotating cylinder viscometer (Moore and Roberts, 1980a). The rate of gelation was found to increase with increase in chitosan concentration, acyl anhydride concentration and temperature, and to decrease with increase in the molecular weight of the acyl anhydride. Furthermore the level of *N*-acylation at which gelation occurs was found to decrease with increase in the molecular weight of the acyl anhydride, as was the Energy of Activation of *N*-acylation (Moore and Roberts 1980b).

Although the use of alternative co-solvents to methanol had been briefly studied earlier (Moore and Roberts, 1980a), no serious investigation on this aspect was carried out until the late 1990s, when Vachoud *et al.* (1997; 1998; 2000) studied the formation of *N*-acetylchitosan gels in an aqueous acetic acid/1,2-propanediol medium. The behaviour of gel formation was found (Vachoud *et al.*, 1997) to be similar to that previously found for gelation with methanol as co-solvent. A detailed study of syneresis of the gels when immersed in various solutions was carried out (Vachoud *et al.*, 1998) and in addition a study of different transport phenomena in these gels was carried out (Vachoud *et al.*, 2000).

A New Chitosan-Based Gel

The aqueous sodium formaldehyde bisulphite solvent system for chitosan

The author recently reported a new solvent system for chitosan (Roberts, 2001; 2004). The solvent system is aqueous sodium formaldehyde bisulphite ($HOCH_2SO_3Na$) formed by the reversible addition reaction between formaldehyde and sodium metabisulphite:

$$2CH_2O + Na_2S_2O_5 + H_2O \leftrightarrow 2\ HOCH_2SO_3Na$$

The amine groups on the chitosan chains react with the sodium formaldehyde bisulphite, forming the condensation product sodium *N*-(sulphonomethyl) chitosan:

$$Chit\text{-}NH_2 + HOCH_2SO_3Na \leftrightarrow Chit\text{-}NHCH_2SO_3Na + H_2O$$

which is water-soluble and gives smooth-flowing solutions. This reaction is reversible and the chitosan can be regenerated if required. The solvent system is non-degradative, unlike aqueous acid solvent systems, and solutions of chitosan prepared using sodium formaldehyde bisulphite are stable and do not undergo chain hydrolysis even on standing for prolonged periods.

There are several routes for the preparation of sodium *N*-(sulphonomethyl) chitosan.

a) Direct formation by stirring solid chitosan in water containing sodium formaldehyde bisulphite; typically 1 g of chitosan in 100 ml water containing 1 g $HOCH_2SO_3Na$, will give a solution within 6-8 hours, although low molecular weight chitosan will dissolve up more rapidly.

b) Sodium *N*-(sulphonomethyl) chitosan may be isolated as a stable solid by precipitation on addition of a solution, prepared as described above in (a), to acetone or other water-miscible non-solvent. The precipitate, after washing with aqueous acetone, may be dried and stored until required, when it may be dissolved by addition to water at neutral pH. The dry powder has a shelf life in excess of 3 years when stored at room temperature.

c) Solid sodium *N*-(sulphonomethyl) chitosan may also be prepared directly by reacting chitosan

with sodium formaldehyde bisulphite under heterogeneous conditions, typically by stirring chitosan particles in 60% (v/v) aqueous methanol containing sodium formaldehyde bisulphite, followed by rinsing with aqueous methanol to remove excess sodium formaldehyde bisulphite, and drying.

The composition-dependence of solubility in aqueous sodium formaldehyde bisulphite is similar to that in aqueous acetic acid and like the latter depends on the method of preparation. Chitosans prepared by heterogeneous deacetylation give good solutions in the solvent system up to a maximum F_A value of 0.40-0.45, whereas for homogeneously prepared *N*-acetylchitosans, the upper limit of F_A values giving good solutions is 0.70-0.75. Reaction of almost 100% of the available primary amine groups is required for solubility, regardless of a given sample's F_A value, and there is no evidence that disubstitution of the amine groups occurs although it is theoretically possible.

Gel formation with solutions of sodium *N*-(sulphonomethyl) chitosan

As *N*-(sulphonomethyl)chitosan is an anionic polyelectrolyte it is compatible in solution with other anionic polyelectrolytes such as sodium alginate, carrageenan and hyaluronic acid and blends of chitosan with these other natural polyelectrolytes would be expected to show beneficial synergistic effects as biomaterials (Roberts, 2001; 2004). Films cast from *N*-(sulphonomethyl)chitosan/anionic polyelectrolyte mixtures are clear and visually uniform, indicating their homogeneity.

Although *N*-(sulphonomethyl) chitosan has been found to give stable, homogeneous solutions with a wide range of anionic polyelectrolytes this is not the case when it is mixed with ammonium alginate. In this case the initially homogeneous solution gradually forms a firm gel on standing for 24-48 hours at room temperature in a sealed container. No gel is formed under these conditions by a solution of either polymer in the absence of the other polymer. The nature and properties of these gels are currently being studied but some preliminary results are available.

Table 1. Effect of overall composition on the gelation behaviour of homogeneous blends of 2% solutions of N-(sulphonomethyl) chitosan) Solution A) and ammonium alginate (Solution B)

Ratio of A-B	Gelation behaviour
1:9	Did not gel
2:8	Gel v. soft
3:7	Gel soft
5:5	Gel rigid
7:3	Gel rigid
8:2	Gel soft
9:1	Gel* v. soft

* Formed a very soft, easily deformable gel after standing for a further 24 hours at 25°C

Effect of composition

Solutions (2% w/v) of both *N*-(sulphonomethyl) chitosan and ammonium alginate were prepared and mixed together in different proportions and the effect of the blend solution composition on the gelation behaviour noted after standing at 25°C for 24 hours.

The results show that gel formation occurs over a wide range of compositions, with the gels formed becoming softer as the composition moves to either extreme of composition (Table 2). The gels have good long-term stability and have shown little or no syneresis on storing at room temperature in closed containers for over 6 months.

Table 2. Effect of ammonium alginate/sodium alginate ratio on the gelation behaviour of homogeneous blends of *N*-(sulphonomethyl) chitosan and ammonium alginate/ sodium alginate mixtures.

Proportion of *N*-(sulphonomethyl) chitosan	50	50	50
Proportion of ammonium alginate	50	25	10
Proportion of sodium alginate	0	25	40
Gelation behaviour	Firm gel	Soft gel	No gel

Another series was prepared in which a solution of *N*-(sulphonomethyl)chitosan was added to

equal volumes of mixtures of ammonium alginate and sodium alginate, all solutions being 2% (w/v).

Stability of the gel

A gel was prepared as described above using equal quantities of 2% (w/v) *N*-(sulphonomethyl) chitosan and 2% (w/v) ammonium alginate. Sample portions were cut from this and steeped in a number of aqueous solutions to determine the stability of the gel. The results are given in Table 3.

Table 3. Stability of the gel on immersion for 168 hours in aqueous solutions.

Solution	Gel weight gain (+) or weight loss (-) on steeping	Appearance of gel on steeping
Distilled water	+89.9%	Soft and sticky but still handleable
0.1 M acetic acid	-22.7%	Firm, easy to handle, not sticky
0.1 M NaOH	- 74.5%	Complete loss of shape, very soft and sticky
0.1 M $NaHCO_3$	- 12.5%	Firm, easy to handle, not sticky
0.1 M $HOCH_2SO_3Na$	- 19.4%	Soft→firm, relatively easy to handle, not sticky

The gel formed is stable to water, mild acid or alkali and a fresh solution of the original solvent system. It is not stable however to sodium hydroxide solution, breaking down with high weight loss. This could be due to decomposition of the alginate itself through an alkali-induced β-elimination reaction rather than simple dissolution of the gel.

The gels are stable to heat and can be autoclaved at 115°C for 15 minutes without any noticeable effect on their structure.

Dehydration/Rehydration

A solution of *N*-(sulphonomethyl) chitosan and ammonium alginate (1:1; total polymer concentration 2% (w/v)) was allowed to stand for 48 hours at room temperature in a sealed tube. The firm, resilient gel was removed from the tube, placed on a glass plate and let stand in air with the weight monitored at intervals. After about 20 hours it was allowed to rehydrate in water for up to 10 days and its weight again monitored at intervals.

Table 4. Dehydration of a *N*-(sulphonomethyl) chitosan/ ammonium alginate gel

Time of dehydration/hours	0.0	2.5	5.5	20.5
Weight of gel/grams	56.5	55.0	53.4	47.1
Water content of gel/%	4900	4770	4625	4070

There was no evidence of syneresis as at no time was there any liquid water observable on the surface of the gel during the dehydration process. The gel was then immersed in distilled water at 25 °C, removed after various periods of time, surface water removed by patting with paper tissues, and weighed. Table 5 shows that the water content of the gel rises considerably on immersion in water but passes through a maximum on prolonged immersion. The initial increase in water content may be attributed to the osmotic effect of the high concentration of non-diffusible $ChitNHCH_2SO_3^-$ and $AlgCOO^-$ ions within the gel. The reason for the observed decrease is not so obvious but may be due to gradual aggregation of the polymer chains, leading to a more compact structure although the chemical composition remains constant. Alternatively the decrease may be due to a gradual hydrolysis of the *N*-(sulphonomethyl) groups so that segments of the water-soluble *N*-(sulphonomethyl) chitosan are gradually converted back to water-insoluble chitosan, which again would lead to aggregation and compaction of the structure together with a reduction in the hydrophilic character because of loss of $ChitNHCH_2SO_3Na$ groups.

Another gel produced in the form of an approximately 3 mm thick sheet was used to examine the effect of neutral electrolyte solutions on the water content. Portions of the sheet were weighed then steeped in NaCl solutions over the concentration range 0.1 M to 0.5 M for 24 hours at 25°C, removed and patted dry of surface liquid using paper tissues,

and re-weighed. A fourth sample was treated similarly using distilled water as a control. All the gel samples were firmer after the steeping process.

Table 5. Rehydration of a *N*-(sulphonomethyl) chitosan/ ammonium alginate gel

Time of rehydration/hours	0.0	0.25	7.0	64	112	360
Weight of gel/grams	47.1	52.2	61.7	83.9	79.0	45.4
Water content of gel/%	4070	4520	5360	7325	6890	3920

The presence of a non-interacting neutral electrolyte would be expect to cause a reduction in water content by reducing segment coil expansion within the gel, thereby reducing the space available for water within the gel. Thus although an increase in water content on immersion in water was again observed, this was almost exactly compensated for by the effect of the lowest electrolyte concentration, 0.1 M NaCl. The effect of NaCl increases with increase in concentration, so that with both 0.25 M and 0.5 M NaCl the overall result is a reduction in the water content of the gel, the reduction being greater for the higher NaCl concentration.

Table 6. The effect of NaCl on the swelling of a *N*-(sulphonomethyl) chitosan/ammonium alginate gel: Initial water content of gel = 5010%; steeping time = 24 hours.

NaCl concentration	0.0	0.1 M	0.25 M	0.5 M
Water content of gel/%	6150	5030	4810	4690

Conclusions

Although *N*-(sulphonomethyl) chitosan, formed by interaction between chitosan and an aqueous solution of sodium formaldehyde bisulphite, is compatible with ammonium alginate, the initial homogeneous solution gradually forms a non-reversible gel on standing at room temperature. Gel formation takes from 24 to 48 hours. Gelation occurs over the range of *N*-(sulphonomethyl) chitosan: ammonium alginate ratios of 9:1 to 2:8 with the gels becoming softer as the composition is moved towards either extreme.

The gels formed are stable towards dilute acid, dilute alkali and aqueous sodium formaldehyde bisulphite, but are attacked by 0.1 M NaOH solution. They are stable towards heat and can be safely autoclaved under normal conditions.

The gels have been found to swell initially on immersion in water but prolonged steeping times causes a reversal in this effect and a reduction in the extent of swelling as measured by water content. The effect of a neutral electrolyte (NaCl) is to oppose this initial swelling, and with a sufficiently high NaCl concentration (> ~ 0.1M) the water content is reduced.

References

Broussignac, P. 1968. Chitosan: a natural polymer not well known by the industry. *Chim. Ind. Génie Chim.* **99** : 1241-1247.

Choi, K. S. and Ahn, H. S. 1990. A study of synthesis of crosslinked chitosan phosphate and adsorption characteristics of metallic ions. *Pollimo.* ***14(5)*** *: 516-526.*

Draget, K. I., Vårum, K. M., Moen, E., Gynnil, H. and Smidsrød, O. 1992. Chitosan crosslinked with Mo(VI) polyoxyanions: a new gelling system. *Biomaterials.* **13(9)** : 635-638.

Hirano, S. and Ohe, Y. 1975. Chitosan gels: a novel molecular aggregation of chitosan in acidic solutions on a facile acylation. *Agr. Biol. Chem.* **39** : 1337-1338.

Hirano, S. and Yamaguchi, R. 1976. N-Acetylchitosan gel : a polyhydrate of chitin. *Biopolym.* **15** : 1685-1691.

Moore, G. K. and Roberts, G. A. F., 1980a. Chitosan gels:1. Study of reaction variables. *Int. J. Biol. Macromol.* **2** : 73-77.

Moore, G. K. and Roberts, G. A. F. 1980b. Chitosan gels:2. Mechanism of gelation. *Int. J. Biol. Macromol.* **2** : 78-80.

Roberts, G. A. F. 2001. Chitosan condensation products, their preparation and their uses. *PCT WO 01/55220 A1.*

Roberts, G. A. F. 2004. A novel solvent system for chitosan. Presented at EUCHIS '04, Poznan, Poland, September 2004: to be published.

Roberts, G. A. F. and Taylor, K. 1989. Chitosan gels. 3) The formation of gels by reaction of chitosan with glutaraldehyde. *Makromol. Chem.* **190** : 951-960.

Vachoud, L., Zydowiez, N. and Domard, A. 1997. Formation and characterisation of a physical chitin gel. *Carbohydr. Res.* **302** : 169-177.

Vachoud, L., Zydowiez, N. and Domard, A. 1998. *Advances in Chitin Science: Syneresis in chitin gels.* (eds.) Domard, A., Roberts, G.A.F. and Vårum, K.M. Vol. II, Lyon, Jacques André: 484-491.

Vachoud, L., Zydowiez, N. and Domard, A. 2000. *Advances in Chitin Science: Transport phenomena in chitin gels.* (eds.) Peter, M.G., Domard, A. and Muzzarelli, R.A.A. Vol. IV, Potsdam, Universität Potsdam: 98-103.