Available online at www.sciencedirect.com
ScienceDirect
Mendeleev Commun., 2009, **19**, 149–151
Mendeleev Communications

In situ formation of chitosan hydrogels with anionic polysaccharides

Yury A. Shchipunov,* Nadezhda A. Ivanova and Sergei A. Sarin

Institute of Chemistry, Far East Branch of the Russian Academy of Sciences, 690022 Vladivostok, Russian Federation. Fax: +7 4232 34 8353; e-mail: yas@ich.dvo.ru

DOI: 10.1016/j.mencom.2009.05.012

Solutions of various anionic polysaccharides were jellified through an *in situ*-forming polyelectrolyte complex with chitosan whose solid dispersion was dissolved by slow acidification of hydrolyzing chemical acidulant glucono-δ-lactone.

Chitosan, a partially de-acetylated derivative of chitin, which is the second abundant organic compound on the earth after cellulose, is considered as an extraordinary biopolymer of significant versatility and promise.[1–3] A severe restriction on its use is in its inability to form hydrogels.[4–6] Numerous derivatives produced by its chemical modification or chemical cross-linking have been used to overcome this problem, but this leads to a sacrifice of one of its main advantage being in its biocompatibility and distinctive biological activities.[7] Although chitosan has been extensively studied over the past four decades, during the last years there has been a surge in interest[3,8–12] for the jellification of its solutions with the retention of unique advantages. However, this is still a formidable challenge.

Chitosan is a linear biopolymer whose macromolecule is made primarily of β(1→4) linked D-glucosamine residues with a variable number of randomly located *N*-acetylglucosamine groups.[1,3] Its solubility depends on the pH of solution. As a polyelectrolyte, chitosan dissolves in mildly acidic media owing to the charging by amino groups accepting the proton. Where pH is near and above the chitosan pK_a value of 6.3, the amino groups dissociate that leads to the polysaccharide precipitation. The pH-dependent charging and solubility of chitosan were taken into account when we developed our jellification method.

The technique used to jellify solutions of charged polymers lies in the formation of polyelectrolyte complexes (PECs) by cationic and anionic counterparts as they are mixed together.[13,14] Its limitation for chitosan is in the PEC precipitation even in the presence of trace amounts of anionic substances.[4,15–17]

Here, we propose a new approach, in which chitosan precipitation is obviated by the slow acidification of solutions containing its solid dispersion and an anionic polysaccharide (Figure 1). The pH decrease *in situ* results in the slow charging of amino groups in chitosan macromolecules (Figure 2). The instant electrostatic interactions with anionic groups of its counterpart lead to a three-dimensional network formation from electrostatically cross-linked carbohydrate macromolecules.

The slowed-down acidification of solutions was performed by means of the chemical acidulant glucono-δ-lactone (GDL).

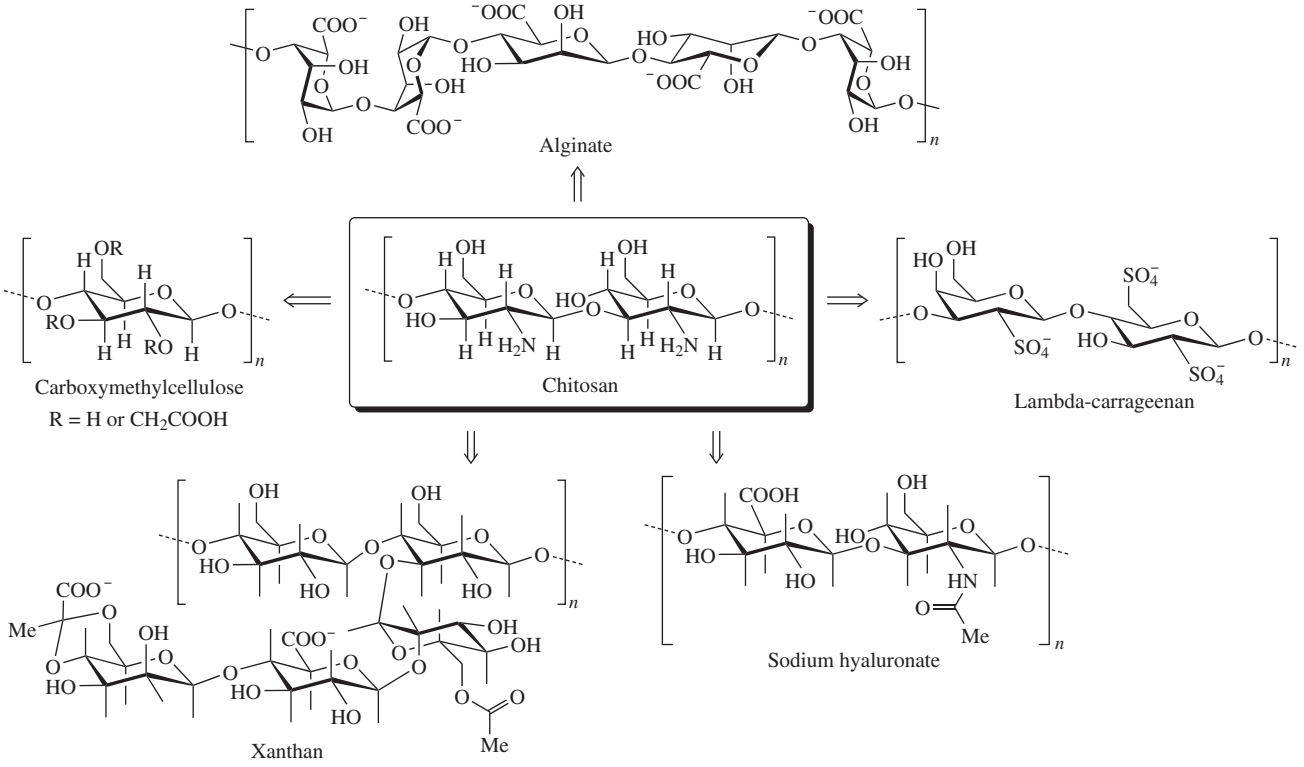

Figure 1 Structural formulae of polysaccharides.

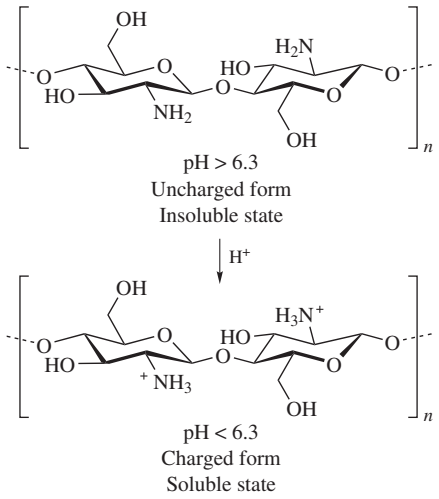

Figure 2 Molecular structure of chitosan in charged and uncharged forms caused by a change of pH.

The GDL hydrolyzes slowly into gluconic acid after the contact with water, thereby gradually reducing its pH. It was previously applied to jellify solutions of alginate by calcium carbonate[18,19] and milk or sodium caseinate that mimics bacterial fermentation.[20,21] We used a similar approach† to gradually charge a chitosan macromolecule, thus smoothly increasing the attractive electrostatic interactions with oppositely charged anionic polysaccharide.

Preliminary experiments were performed to test hydrogel formation by the gradual acidification of dispersed chitosan particles with alginate, carboxymethylcellulose, carrageenan, sodium hyaluronate and xanthan (Figure 1). The jellification took place in all the instances. Because these polysaccharides present the majority of industrially important anionic biopolymers, the jellification of their solutions allows us to consider the approach as a common one. It is our opinion that it can be extended to hydrogel formation by chitosan with DNA and proteins, as well as with synthetic negatively charged polymers.

On the visual observation of jellification processes with the above anionic polysaccharides, certain stages emerged. Once the GDL was added, no change in solution was revealed. In due course, the swelling of chitosan particles was seen. There was a simultaneous increase in the solution viscosity. With time the mixture got more and more sluggish and finally solidified. Meanwhile, the swollen particles, which became initially larger and then begun disintegrating, distributed over all the bulk of jellified solution.

The following changes were very slow but obvious. First, there was a further disintegration of chitosan particles; instead, tiny species of different shape varying from spherical to fibrillar could be found under small magnification. Fibrillar-like species were most commonly observed. They were cross-linked and arranged into a three-dimensional network.

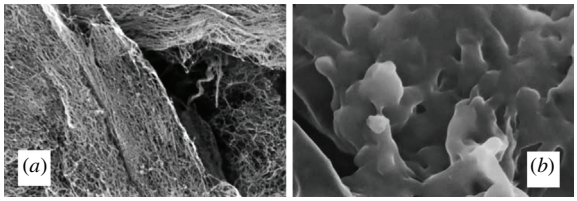

Figure 3 SEM micrographs of aerogels prepared by supercritical drying from initial hydrogels consisting of (*a*) 0.5 wt% chitosan and 1.0 wt% xanthan, (*b*) 0.75 wt% chitosan and 0.55 wt% alginate.

The initial observations on morphological differences were confirmed by means of scanning electron microscopy (SEM). The SEM pictures of two samples are given in Figure 3. One concerns a PEC of chitosan with xanthan, another, with alginate. As seen, the former has a fibrillar-like morphology (A), the latter, plate-like one (B). Note that the fibrils are assembled together into bundles. The initial chitosan particles of ~0.2 mm prepared by mechanical breakdown were shapeless. This means that their solubilization by slow acidification was followed by self-organization together with anionic polysaccharide into fibrils owing to the PEC formation through the electrostatic interactions. Our preliminary study revealed a gradual morphological change with varying the polysaccharide concentration. This provides an opportunity to manipulate the structure and mechanical properties of thus fabricated hydrogels. A preliminary examination of mechanical properties demonstrated that samples with fibrillar morphology were mechanically stronger than that with plate-like one.

Note that the *in situ* jellification resulted in monolithic as-formed hydrogels. When they left staying, the syneresis could be developed. The hydrogel shrinkage accompanying by the solvent separation took usually place where one of the oppositely charged polysaccharides was in excess. It is of interest that there was no phase separation in the vicinity of stoichiometry of opposite charges in the carbohydrate macromolecules. This differs from that observed for PEC hydrogels prepared by common procedures.[22,23] The stoichiometric complexation, in which the complete neutralization of charges takes place, results in the PEC precipitation owing to their increased hydrophobicity.[13] The difference of our approach lies in the slow acidification. This provides instant complexation in spatially separated points, in which the charged amino groups are generated. The cross-linking through the electrostatic interactions held the macromolecules from the precipitation, providing the self-organization of carbohydrate macromolecules into a three-dimensional network. When the electrostatic interactions are numerous, as with the charge stoichiometry, the merged chains are stiff enough to make the network structure stable with time; otherwise, there is a realignment leading to the shrinkage and syneresis.

Our belief is that the developed approach enhances the capabilities of chitosan in material chemistry, facilitating the fabrication of materials with novel structure and properties. Its advantages are the simple procedure and biocompatibility of hydrogels that consist of components with no chemical modification and cross-linking. Therefore, there are strong reasons for believing that chitosan retained its virtues, that is, biocompatibility, low toxicity and distinctive biological activities. In addition, the complexation with anionic polysaccharides, which have their own merits, could introduce novel good features. One would expect that the materials thus fabricated may have wide applications in many fields including medicine, pharmacy, cosmetics, food industry, biotechnology and bioengineering, where biocompatibility is the focus of attention.

† Chitosan (*ca.* 300 kDa), alginate (35–40 kDa), xanthan (*ca.* 1000 kDa) and glucono-δ-lactone (GDL) were purchased from Fluka, carboxymethylcellulose (*ca.* 700 kDa), from Clariant (Germany). Lambda-carrageenan (1024 kDa, high degree of purification) was a gift from Hercules Copenhagen A/S (Denmark), bacterial sodium hyaluronate (*ca.* 1000 kDa), from Shiseido (Japan). To prepare a hydrogel, fine solid particles (*ca.* 0.2 mm) of chitosan were dispersed in an aqueous solution of one of the mentioned anionic polysaccharides at pH 6–7 and ambient conditions. Thereafter a batch of GDL was introduced into this solution that was vigorously agitated by a magnetic stirrer to dissolve the GDL and distribute the chitosan dispersion uniformly over the height. After a lapse of half to few hours, which depends on the acidulant amount and anionic polysaccharide, an increase in the solution viscosity was observed. Just as the change was mentioned, so it accelerated increasingly with time, accomplishing with a transition into a gel state. Their transformation lasted 10–15 h.

References

1 G. A. F. Roberts, *Chitin Chemistry*, MacMillan Education, Basingstoke, Hampshire, UK, 1992.
2 E. Khor, *Curr. Opin. Solid State Mat. Sci*., 2002, **6**, 313.
3 M. Rinaudo, *Prog. Polym. Sci*., 2006, **31**, 603.
4 E. V. Shumilina and Yu. A. Shchipunov, *Kolloid. Zh.*, 2002, **64**, 413 [*Colloid J.* (*Engl. Transl.*), 2002, **64**, 372].
5 K. Y. Lee and D. J. Mooney, *Chem. Rev.*, 2001, **101**, 1869.
6 D. K. Singh and A. R. Ray, *J. Macromol. Sci., Rev. Macromol*., 2000, **C40**, 69.
7 J. Berger, M. Reist, J. M. Mayer, O. Felt, N. A. Peppas and R. Gurny, *Eur. J. Pharm. Biopharm*., 2004, **57**, 19.
8 J. Berger, M. Reist, J. M. Mayer, O. Felt and R. Gurny, *Eur. J. Pharm. Biopharm*., 2004, **57**, 35.
9 K. Kurita, *Marine Biotechnol*., 2006, **8**, 203.
10 K. Y. Lee, *Macromol. Res*., 2007, **15**, 195.
11 H. T. Ta, C. R. Dass and D. E. Dunstan, *J. Control. Release*, 2008, **126**, 205.
12 J. F. Mano, G. A. Silva, H. S. Azevedo, P. B. Malafaya, R. A. Sousa, S. S. Silva, L. F. Boesel, J. M. Oliveira, T. C. Santos, A. P. Marques, N. M. Neves and R. L. Reis, *J. Royal Soc. Interface*, 2007, **4**, 999.
13 A. B. Zezin and V. A. Kabanov, *Usp. Khim.*, 1982, **51**, 1447 (*Russ. Chem. Rev.*, 1982, **51**, 833).
14 E. Tsuchida and S. Takeoka, *Adv. Polym. Sci*., 1992, **45**, 1.
15 A. Hugerth, N. Caramlelham and L. O. Sundelof, *Carbohydr. Polym*., 1997, **34**, 149.
16 J. K. F. Suh and H. W. T. Matthew, *Biomaterials*, 2000, **21**, 2589.
17 A. Drogoz, L. David, C. Rochas, A. Domard and T. Delair, *Langmuir*, 2007, **23**, 10950.
18 K. I. Draget, K. Ostgaard and O. Smidsrod, *Carbohydr. Polym*., 1991, **14**, 159.
19 Yu. A. Shchipunov, E. L. Koneva and I. V. Postnova, *Polym. Sci. A*., 2002, **44**, 758.
20 J. Chen and E. Dickinson, *Int. Dairy J*., 2000, **10**, 541.
21 J. A. Lucey, T. van Vliet, K. Grolle, T. Geurts and P. Walstra, *Int. Dairy J*., 1997, **7**, 381.
22 Yu. A. Shchipunov and I. V. Postnova, *Zh. Fiz. Khim.*, 2001, **75**, 1795 (*Russ. J. Phys. Chem*., 2001, **75**, 1641).
23 Yu. A. Shchipunov and I. V. Postnova, *Polym. Sci. A*., 2006, **48**, 171.

Received: 17th October 2008; Com. 08/3230