Bionanocomposites formed by *in situ* charged chitosan with clay†

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Bionanocomposites are formed through the electrostatic interactions of exfoliated clay nanoparticles initially dispersed in an aqueous solution with chitosan macromolecules that are gradually charged in the course of a progressive pH decrease by chemical acidulating agent to exclude the phase separation.

Chitosan is a product of the de-acetylation of chitin, which is the second abundant organic compound on Earth after cellulose. Although this polysaccharide presents a huge renewal resource of biomass, of which annual production in nature reaches 100 billion tonnes, it is still almost unutilized, being a biopolymer of significant versatility and promise.[1–3] Clay is a natural mineral that is widely used for making various materials and in many industrial processes. Clay nanoparticles of smectite-type, such as montmorillonite and saponite, dispersed into polymers remarkably improve their material properties including strong reinforcing, increased heat resistance, decreased gas permeability and flammability.[4,5] Their effect is observed after exfoliation into negatively charged flat sheets of size 10 nm to microns and about 1 nm in thickness. The huge surface area of nanoparticles causes strong interactions with polymers and improvement of their properties at a concentration less than 3 vol%.[4,5] Bionanocomposites, which are formed by biopolymers with clays, possess all the mentioned values of materials, but they have also the advantages of biocompatibility and biodegradability.[6] Today, they are a subject of active research interest worldwide. Negatively charged clays can form bionanocomposites mainly with chitosan through strong electrostatic interaction[6,7] because it is the only cationic polysaccharide.[8] Nowadays, it is made by mixing their solutions, but this leads to precipitation.[7,9] This is typical of chitosan, which does not allow preparation of homogeneous and monolithic bionanocomposites.[8,10] Here we obviate this problem, caused by the association with oppositely charged clays, by gradual chitosan charging in the course of a progressive pH decrease by chemical acidulating agent in a solution of exfoliated clay in which the polysaccharide was dispersed initially as tiny uncharged particles. When the charging proceeds smoothly *in situ*, a three-dimensional network of nanosized fibrils is generated that provides a monolithic hydrogel formation without precipitation and its strong reinforcing. This technique extends areas of chitosan applications and has great potential for fabrication of hydrogels, biomaterials, bionanocomposites, drug-delivery systems, biosensors and biocatalysts.

The suggested methodology is based on a dependence of chitosan charging on the pH of solution. Its linear macromolecule, shown in Fig. 1A, consists mainly of β(1→4)

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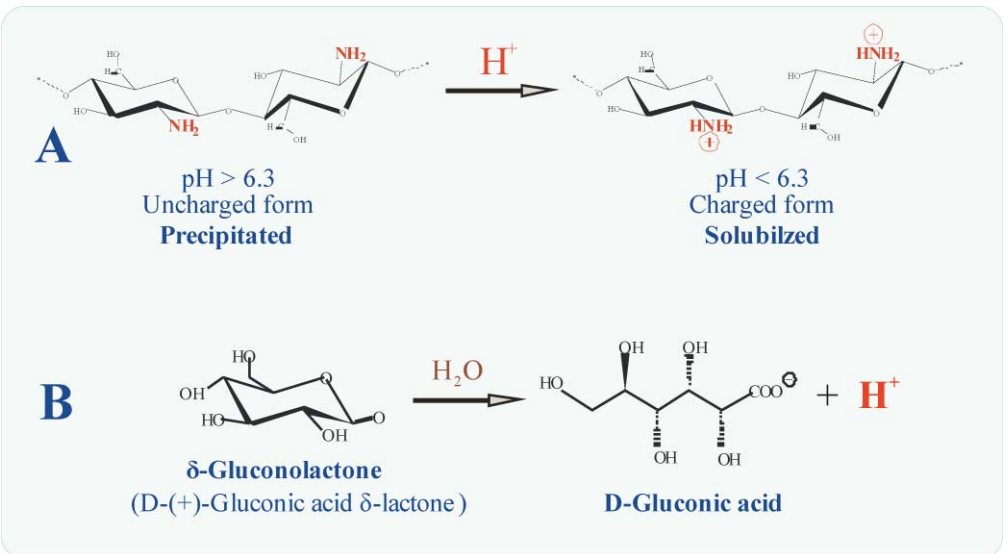

Fig. 1 Structural formulae of (A) chitosan in charged and uncharged forms at various pH of aqueous solution and (B) acidulating agent glucono-δ-lactone and product of its hydrolysis.

linked D-glucosamine residues with a variable number of randomly located N-acetyl-glucosamine groups at the C-2 position.[1] An amino group is in a charged form when it accepts a proton. This happens when the pH is near or below the chitosan pK_a value of 6.3; otherwise the polysaccharide is uncharged because of the deprotonation. These two main forms are given in Fig. 1A. Chitosan charging makes it soluble in water due to the polyelectrolyte effect. When a solution becomes neutral or alkaline, this polysaccharide precipitates because of the transition into an insoluble form.[8,11] The pH-dependent charging and solubility of chitosan were used here to regulate its electrostatic interactions with nanosized clay sheets.

The essence of chitosan–clay bionanocomposite fabrication by the suggested approach is illustrated schematically by Fig. 2 (see also footnote‡). In the beginning (A), saponite taken as clay nanoparticles is dispersed in water to have nanosized sheets homogeneously distributed in the bulk. The second step B, consists of adding grinded fine particles of chitosan that are distributed homogeneously over the entire volume by vigorous stirring. To provide a pH decrease, a weighted amount of chemical acidulating agent glucono-δ-lactone (GDL) is dissolved (step C). Its contact with water triggers the slow GDL hydrolysis into gluconic acid (Fig. 1B). The acid thus generated results in gradual shift of solution pH into acidic region. This provided a progressive charging of chitosan that gives a smooth rise in the number of electrostatic linkages between carbohydrate macromolecules and the negatively charged surface of clay sheets. These physical cross-linkings led to a formation of a three-dimensional network, as shown below, that was responsible initially for an increase in the viscosity and then jellification of solutions.

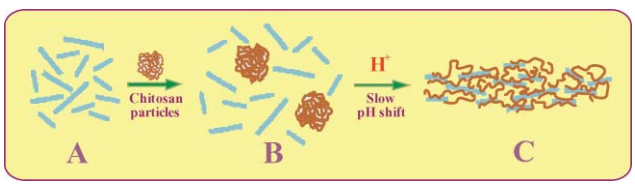

Fig. 2 Schematic presentation of main stages of formation of monolithic hydrogel by chitosan and clay.

A tentative quasiternary phase diagram of the chitosan–saponite–water system is shown in Fig. 3. A monolithic hydrogel (blue area) was generated as soon as critical concentrations of both the polysaccharide and clay were attained. It was observed that the amount of saponite was in some excess over that of the chitosan. Furthermore, the monolith formation was found if critical concentrations of both the components were reached. Otherwise, a phase separation took place. This manifested itself as a slow precipitation. A two-phase area is shown in light brown in the diagram. There is also a light-green area between one-phase and two-phase areas. This presents mixtures that can be considered as a metastable system. The formation of a hydrogel was not reproducible in this area. One could see also a slow precipitation or syneresis, *i.e.*, the slow hydrogel shrinkage accompanied by the separating out of a solution. A difference between the syneresis and the precipitation was in time. The former developed much slower than the latter. The

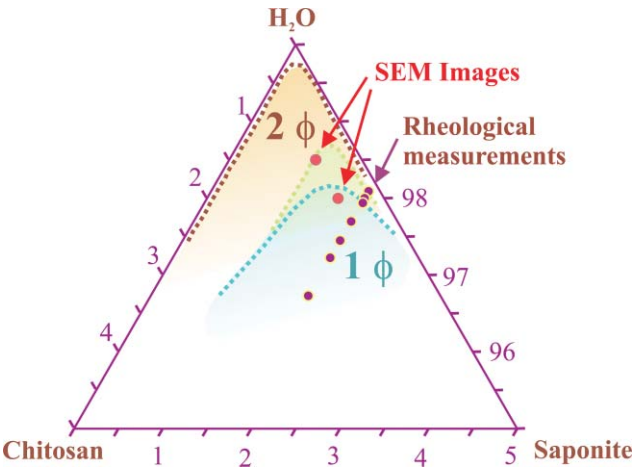

Fig. 3 Tentative quasiternary phase diagram for a system of chitosan–saponite–water prepared by an approach developed in this article. A one-phase region is shown in blue, the two-phase region, in which a precipitation was observed, in light brown and metastable region, in which a precipitation or syneresis was found, in light green. Points present the compositions of samples used to perform rheological measurements (Fig. 4) and take SEM images (Fig. 5). Further details in text.

phase separation in the case of syneresis could be seen continuing from a few days to a few weeks depending on the composition.

The addition of acidulating agent triggered processes led to a gradual change of mechanical properties of solutions. Initially, an increase of the viscosity was observed, that became obvious after some lag period. Then it accelerated in the course of time. The mixture got more and more sluggish and finally solidified. Solidified mixtures were robust and slightly elastic. The change in the mechanical properties of saponite solution after dissolving the chitosan may be seen in Fig. 4. There are dependencies of the zero-shear viscosity (η_o) and plateau modulus (G_o), which were measured in accordance with the method given in ref. 12 and detailed in the ESI.† The hydrogels examined by the rheology are traced by points in the phase diagram in Fig. 3. As can be seen from the plots in Fig. 4, the chitosan caused a steep growth of both η_o and G_o at low concentrations of around 0.1 wt%. The increase of zero-shear viscosity and plateau modulus are as much as three and four orders of magnitude, respectively. As follows from the examination of frequency dependencies of shear storage and loss moduli (shown in the ESI†), there was a transition from a viscoelastic solution with the Maxwell behaviour to a real hydrogel with the solid-like behaviour. Thereafter, an increase of the chitosan concentration resulted in a modest effect on the rheological parameters.

To clarify a reason for the jellification and the steep increase in the zero-shear viscosity and plateau modulus, morphological studies on prepared hydrogels were performed by means of the scanning electron microscopy. A set of pictures taken at three magnifications may be seen in Fig. 5. They give an impression about the bionanocomposite morphology at various length scales. The images concern chitosan–saponite aerogels prepared from initially fabricated hydrogels by supercritical drying. The two presented samples contained the same amount of chitosan (0.5 wt%) and various amounts of saponite (1.0 (Fig. 5A) and 1.5 wt% (Fig. 5B)). These samples are marked by points in

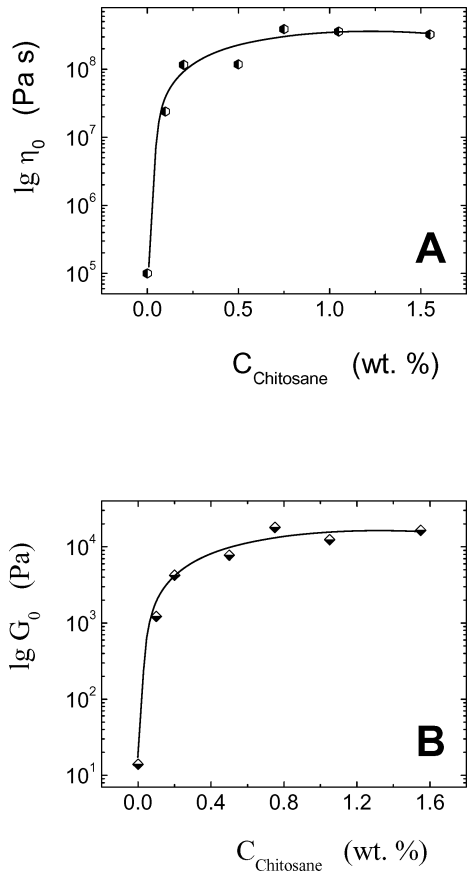

Fig. 4 The zero-shear viscosity (A) and plateau modulus (B) *vs.* the concentration of added chitosan into a solution containing 1.7 wt% of initially dispersed saponite. The method used to find the rheological parameters is described in the ESI.†

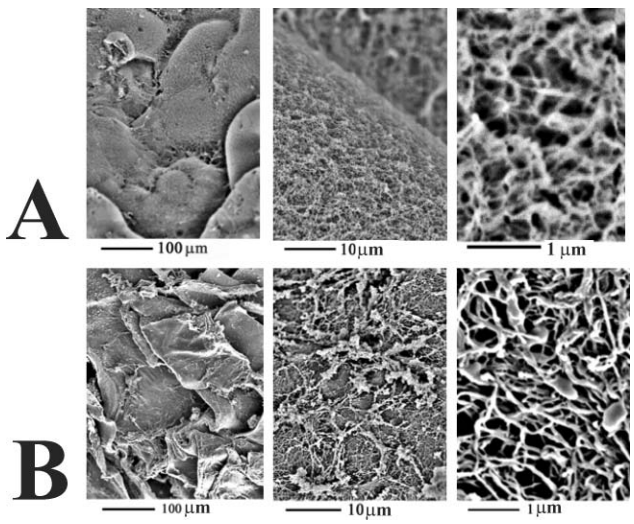

Fig. 5 SEM micrographs of aerogels prepared by the supercritical drying of initial hydrogels consisting of 0.5 wt% chitosan and 1.0 (A) or 1.5 wt% saponite (B). Each sample is presented by three pictures taken at various magnifications.

the phase diagram (Fig. 3). By examining pictures on the left taken at smaller magnification, one can find some differences in the morphology. Where the concentration of saponite was taken lower in the course of preparation (Fig. 5A), there is a roundish stones morphology, whereas at larger clay amount (Fig. 5B) there are sheets, some of which are jammed. The fine morphology can be seen in pictures at the right made at larger magnification. There is a network-like morphology in both the cases that consists of cross-linked fibrils. One may find also particles connected by fibrils. They are larger in size in the sample with increased amount of saponite. The fibrils are also thicker than that in hydrogel containing smaller amount of clay nanoparticles. They are not uniform. As is obvious from Fig. 6, taken at much larger magnification, the fibrils contain swellings and thinnings.

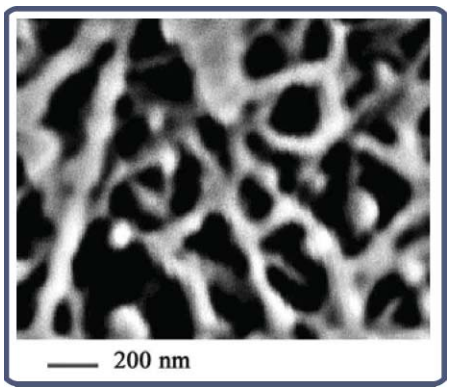

Fig. 6 SEM micrographs of a sample prepared as described in the legend of Fig. 5. Hydrogel was fabricated by taken 0.5 wt% chitosan and 1.5 wt% saponite.

The network-like morphology clarifies the reason for the jellification and strong reinforcing of saponote solutions by *in situ* charged chitosan. Its build-up is due to electrostatic interactions oppositely charged sheet nanoparticles and carbohydrate macromolecules. This was demonstrated in sufficient publications (see, *e. g.*, ref. 6,13). The electrostatic association of chitosan with saponite bears similarity to the polyelectrolyte complex formation by oppositely charged counterparts.[5] An anionic polyelectrolyte is substituted here by clay nanoparticles that bring numerous charged groups on their surface.

The fibrillar structural organization of chitosan–saponite complexes (Fig. 5 and 6), in our opinion, is evidence of their spatial orientation. It is reasonable to relate it to clay nanoparticles. Their flat sheets in the size range of tens of nanometres are the main factor determining if there is orientation or not. The chitosan plays a secondary role. Its macromolecule is flexible and significantly shorter than clay nanoparticles. It can serve as a bridging linker between neighbouring sheets. At the initial stage of charging, when the number of charged groups in chitosan is minimal, its macromolecule can form electrostatic interactions with various nanoparticles through charged segments that are spatially separated. As new charged groups appear between these segments in a carbohydrate chain, they will cause the sheets to approach because of a shorter distance between the charges. Long particles come within short distance of each other if they align either. This is shown schematically in Fig. 2C. As a result of the saponite alignment, fibrils are formed.

In summary, we have demonstrated a new method to prepare bionanocomposites by combining nanosized clay particles with chitosan. There are a few fundamental advantages of our technique over current methods. (i) *Simplicity*. The bionanocomposites are prepared by the mixing of components in aqueous solutions. High temperatures and pressures are not needed as in the current melt-mix technology used to distribute homogeneously clay nanoparticles in polymers.[4,5] (ii) *Homogeneity*. The gradual charging of uniformly distributed chitosan leads to progressive cross-linking of neighbouring clay nanoparticles that retain their initial homogeneity in thus formed material. The common practice to prepare bionanocomposites by mixing of chitosan and clay solutions resulted in the precipitation as a heterogeneous mixture.[6,7,13] This is still a formidable challenge to jellify chitosan solutions.[8] Its inability to form hydrogels imposes a severe restriction on chitosan applications. Chemical modifications or chemical cross-linking have been used to overcome this problem (see, *e.g.*, ref. 8,11), but this leads to a sacrifice of one of its main advantages, *i.e.* biocompatibility and distinctive biological activities. (iii) *Biocompatibility*. Our method is not based on chemical modification or cross-linking. The jellification is provided by electrostatic interactions between oppositely charged clay nanoparticles and carbohydrate macromolecules. The chemical acidulating agent glucono-δ-lactone, which is used to charge the chitosan, hydrolyzes into biocompatible gluconic acid. The developed bionanocomposites do not include toxic components. In our opinion, the developed technique is very promising for versatile applications including hydrogels, biomaterials, bionanocomposites, drug-delivery systems, biosensors and biocatalysts.

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Notes and references

‡ Chitosan (*ca.* 300 kDa) and glucono-δ-lactone were purchased from Fluka, synthetic clay SKS-20 (Saponite) was provided by Clariant. Hydrogels were prepared by initially dispersing a weighted amount of saponite in aqueous solution with pH 6–7 by using a magnetic stirrer. Then, a fraction of grinded chitosan particles of size *ca.* 0.2 mm, which was sifted through separating sieves, was dispersed in a saponite solution. Thereafter, a batch of GDL was introduced into this solution that was vigorously mechanically agitated by a magnetic stirrer to dissolve the GDL and reach a homogeneous distribution of all the components of mixture. After a lapse of a half to a few hours, which depends on the acidulating agent concentration as well as chitosan and clay amounts, an increase in the solution viscosity was observed. After that, it accelerated increasingly with time, finishing with a transition into a gel state. Samples for SEM observations were made in the form of aerogels by exchanging first the water for acetone and then the acetone for liquid CO_2, which was removed at supercritical conditions. A fresh surface of the aerogels obtained by cutting was covered by a platinum layer. SEM pictures were taken by using a scanning electron microscope EVO 40 (Carl Zeiss). Rheological measurements were performed with a Rotovisco RT 20 (Haake) stress-controlled rheometer. Standard cells with plate-and-plate geometry were used. Oscillation and creep regimes were applied to find rheological parameters as shown in the ESI.† All the measurements were carried out at 25 ± 0.05 °C.

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