

Physico-chemical studies of the gelation of chitosan in a hydroalcoholic medium

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Abstract

The formation of chitosan physical hydrogels without any external cross-linking agent was studied. This gelation took place in an acetic acid-water-propanediol solution. Static light scattering was used to detect the gel point and then, to study the gelation for different initial conditions. Thus, we investigated the influence of the degree of acetylation, the gelation temperature and the nature of the initial solvent. The variation of the solvent composition during gelation was determined from a simple weighting, and the ionisation state of the polymer at the gel point, by pH titrations. This work showed that it was possible to form a chitosan physical-hydrogel, whatever the degree of acetylation provided typical conditions were observed. The mechanism of gelation simply consisted in the modification of the hydrophilic/hydrophobic balance allowing the formation of both hydrophobic interactions and hydrogen bonding. Several parameters had an important role on this mechanism: 1—the apparent charge density of chitosan, modified by the degree of neutralisation, 2—the dielectric constant of the solvent, related to the composition of the medium, 3—the degree of acetylation, 4—temperature, playing a role on the interactions responsible for the physical cross-linking and the molecular mobility, and, 5—the molecular mobility depending on possible changes of conformation, steric hindrance and viscosity of the medium.

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1. Introduction

In recent years, a great attention has been paid to the study of biopolymer physical hydro-gels because of their interesting biological properties and consequently, for their potential applications in biomedical and pharmaceutical fields. Among the natural polymers, glycosaminoglycans, especially chitosan, constitute a very interesting family having the rare property of bioactivity. Chitosan is a natural polysaccharide produced from *N*-deacetylation of chitin under alkaline conditions [1]. Chitin occurs mainly in the cuticles of arthropods, the endoskeletons of cephalopods and fungi. Previous studies reported that chitosan is bioabsorbable [2] and

has a good biocompatibility [3]. In addition, it has been demonstrated to be haemostatic [4,5] and bacteriostatic [6]. Moreover, it plays an important role in the cell regulation and tissue regeneration [7–10]. Chitosan, as chitin, belongs to the family of the linear copolymers of (1 → 4)-2-amino-2-deoxy-β-D-glucan (GlcN) and (1 → 4)-2-acetamido-2-deoxy-β-D-glucan (GlcNAc). The DA, the degree of acetylation corresponding to the molar fraction of acetyl units within the polymer chains is a very important parameter. Then, it is generally considered that chitosan refers to polymers soluble in dilute acidic aqueous solutions and then to DAs below 60%.

Numerous papers can be found in the literature explaining how to produce chemical gels with chitosan, especially with aldehydes like scleraldehyde [11], glutaraldehyde [12], etc. Beside chemical gels, some physical hydrogels of chitosan were produced. We can mention the case of the reaction of chitosan with acids such as thioglycolic [13], acrylic [14] and oxalic acids [15]. We

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will also mention the formation of ionic cross-links by electrostatic interaction with metal ions such as molybdenum (VI) [16] or with other biopolymers (xanthan [17], carrageenan [18], etc.). Recent results reported on the thermal gelation of chitosan in the presence of salts of phosphoric acid derivatives [19]. The first true physical hydrogels based on this series of copolymers, without any use of other cross-linking agent than the polymer structure were obtained by Hirano et al., during the acylation of chitosan with various anhydrides in a hydro-alcoholic medium [20]. In the same time, Moore and Roberts studied the gelation of solutions of chitosan during the treatment with anhydrides [21]. The gelation process was proposed to be due to a progressive decrease in the solubility of the polymer molecules arising with the increasing extent of *N*-acylation. The results exhibited that in this particular system, gelation occurred when almost 70% of the amine groups were acylated and the degree of *N*-acylation required to initiate gelation decreased on increasing the polymer concentration. Vachoud and Domard extensively studied the formation of gels made by *N*-acetylation of chitosan with acetic anhydride in a solution containing an equivalent amount of water and 1,2-propanediol [22]. They showed that gelation was achieved for a critical value of the balance between hydrophilic and hydrophobic interactions depending on various external parameters. The mechanism of gelation was attributed to the formation of physical junctions due both to hydrophobic interactions involving *N*-acetyl groups and hydrogen bonding.

Nevertheless, the formation of true physical hydrogels of chitosan with no external cross-linking agent was never reported in the literature. Then, the aim of this work was to show that it was still possible to form a chitosan physical hydrogel, whatever the degree of acetylation, provided typical conditions were observed. In a first series of experiments, we studied the gelation of chitosan in a mixture water/1,2-propanediol, during the evaporation of the solution. The most important parameter allowing us to achieve the critical value of the balance between hydrophilic and hydrophobic interactions, responsible for gelation is the apparent charge density. This parameter is tightly related to inter-correlated parameters corresponding to DA, the degree of neutralisation, the ionic strength and the dielectric constant of the medium. DA has an influence on both hydrophobic interactions brought about by acetyl groups and hydrogen bonding, and modifies the ionic character of the amine functions, the intrinsic pK of which increases with DA [23]. DA also plays a role on the molecular organisation and mobility, partly responsible for the kinetics of gelation. Indeed, we showed that the stiffness of the chains is dependent on DA [24], and is partly at the origin of the kinetics aspect of the interactions and of their quantitative limits. Then, we

investigated the influence of different parameters such as the DA, the temperature and the composition of the initial solvent. In this study, the time to reach the gel point was determined by static light scattering and the change in the solvent composition at the gel point from a simple weighting. By pH titration, we evaluated the charge density of the polymer on this point.

2. Experimental section

2.1. Purification and acetylation of chitosan

The initial chitosan produced from squid pens was purchased from France Chitin (batch number 114) and was purified as follows. The polymer was dissolved at 0.5% (w/v) in a stoichiometric amount of aqueous acetic acid. After complete dissolution it was filtered successively on 3, 0.8 and 0.45 μm membranes (Millipore). Then, the solution was precipitated with dilute ammonia up to a constant pH of 9 and centrifuged. The precipitate was repeatedly rinsed with distilled deionised water and centrifuged until a neutral pH was achieved, then dispersed in water and freeze-dried. Samples of different DAs were prepared by reacylation of a highly deacetylated chitosan with acetic anhydride as reactive, in a water/alcohol solution [22]. Thus, an aqueous acetic acid solution of the starting chitosan was prepared at a concentration of about 1% (w/w), acetic acid was added to achieve the stoichiometric protonation of the $-\text{NH}_2$ sites. This solution was mixed with 1,2-propanediol to achieve a final polymer concentration of 0.5% (w/v). The acetylating reactive was constituted of a freshly prepared solution of acetic anhydride in a small amount of 1,2-propanediol [22]. This reactive was slowly added under stirring to the chitosan solution and the medium was let to stand for 3 h. The amount of acetic anhydride corresponded to the stoichiometric amount necessary to achieve a given degree of acetylation. The polymer was fully precipitated by addition of aqueous ammonia and repeatedly washed and centrifuged with distilled deionised water, then freeze-dried.

2.2. Characterisation of chitosans

After lyophilisation, the DAs of the different samples were characterised from $^1\text{H-NMR}$ spectra recorded on a Bruker 250 spectrometer (250 MHz) at 25°C. Thus, 10 mg of chitosan were solubilised in 1 g of D_2O containing 0.21% by weight of HCl. DA was then evaluated as proposed by Hirai et al. from the ratio of the area of the peaks of the methyl protons of the *N*-acetylglucosamine residues to those of all the H_2 to H_6 protons of both glucosamine and *N*-acetylglucosamine residues [25]. The DA of the initial chitosan was found to be close to 5.2%. $^1\text{H-NMR}$ also allowed us to check

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