

# Characterization of the interaction between chitosan and inorganic sodium phosphates by means of rheological and optical microscopy studies

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## ABSTRACT

The physicochemical and rheological properties of chitosan and two different inorganic sodium phosphate dispersions ( $\text{NaH}_2\text{PO}_4$  and  $\text{Na}_3\text{PO}_4$ ) were investigated in order to elucidate the role of different factors, such as ratios between polymer and sodium inorganic phosphates, different pHs and storage stability, on the gelling properties of chitosan. This was deemed opportune since physico-chemical characterizations of chitosan in the literature often appear incomplete and questionable. We also compared the elastic modulus values of the different chitosan/inorganic phosphate systems and examined their behaviour through optical microscopy analyses.

The most efficient formulations that showed a thermogelling capacity with a significant gel transition behaviour after 24 h were the  $\text{NaH}_2\text{PO}_4$ /chitosan and  $\text{Na}_3\text{PO}_4$ /chitosan systems at ratio 2 and pH 7.0.

These results confirmed the importance of the pH value and ratio selection for the final systems.

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## 1. Introduction

Stimuli-responsive polymers have been proposed for a wide range of applications (Bajpai, Shukla, Bhanu, & Kankane, 2008; Jeong & Gutowska, 2002; Osada & Gong, 1993) and in the last decade a great interest was deserved to those polymers able to modify their physical state from sol to gel as the temperature changes (Ruel-Gariépy & Leroux, 2004; Schmaljohann, 2006).

Chitosan, an amino polysaccharide obtained by alkaline deacetylation of chitin, is a biocompatible and biodegradable pH-dependent cationic polymer that has been proposed as suitable material for many purposes in a wide range of medical, pharmaceutical and food applications (Dash, Chiellini, Ottenbrite, & Chiellini, 2011; Illum, 1998; Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Ravi Kumar, 2000; Shahidi, Arachchi, & Jeon, 1999).

Solubility of chitosan in aqueous solutions is attained via protonation of its amine groups in acidic environments. Its limited water solubility is well known ( $\text{pK}_a$  6.3); moreover, the neutralization of its amino groups leads to the formation of a hydrated gel-like chitosan precipitate. In fact, the deprotonation of the amino groups present in its backbone leads to the removal of repulsive inter-chain electrostatic forces, allowing hydrogen bonding and hydrophobic interactions between chains (Goycoolea et al., 2007).

In addition, its ability to interact with different salts, anionic molecules or crosslinkers to form hydrogel for pharmaceutical and

biomedical applications has been well reviewed by various authors (Berger et al., 2004; Bhattarai, Gunn, & Zhang, 2010; Liu, Tang, Wang, & Guo, 2011; Ruel-Gariépy, Chenite, Chaput, Guirguis, & Leroux, 2000).

In the last decade, from the first works reported by Chenite and colleagues (Chenite et al., 2000; Chenite, Buschmann, Wang, Chaput, & Kandani, 2001; Ruel-Gariépy et al., 2000), the use of chitosan as a hydrogel able to undergo sol–gel transition has attracted the attention of many research groups (Cho, Heuzey, Bégin, & Carreau, 2005; Chung, Bae, Park, Lee, & Park, 2005; Crompton et al., 2005; Kempe et al., 2008; Zan, Chen, Jiang, Lin, & Ding, 2006; Zhou et al., 2008).

Different strategies were developed to obtain chitosan hydrogels and rheological characterization has been proposed to elucidate the influence of the different parameters involved. Various organic and inorganic phosphates, for example  $\beta$ -glycerophosphate ( $\beta$ -GP), potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ), dipotassium hydrogen phosphate ( $\text{K}_2\text{PO}_4$ ), tripotassium phosphate ( $\text{K}_3\text{PO}_4$ ), ammonium hydrogen phosphate (AHP), sodium tripolyphosphate (TPP) and dibasic sodium phosphate ( $\text{Na}_2\text{HPO}_4$ ), have been employed as gelling agents (Chenite et al., 2000, 2001; Cho et al., 2005; Li & Huang, 2012; Li et al., 2010, 2011; Nair, Starnes, Ko, & Laurencin, 2007; Ta, Han, Larson, Dass, & Dunstan, 2009).

Studies indicate that the most used phosphate,  $\beta$ -glycerophosphate, allows better solvation of chitosan at physiological pH (from 6.3 to 7.4) and improves thermogelling (Berger et al., 2005; Chenite et al., 2001; Cho, Heuzey, Bégin & Carreau, 2006).

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Moreover, tests with the different inorganic phosphate salts showed that the proper pH value was fundamental for achieving thermogelification of the chitosan solution (Li et al., 2010; Nair et al., 2007; Ta et al., 2009). The use of weak base phosphates is decisive for avoiding chitosan precipitation during the initial phase of sample preparation, and improves the gelling ability of the final systems.

The aim of this study was to definitively clarify the role of different physicochemical parameters in chitosan thermo-hydrogel systems. We used two different sodium inorganic phosphate salts ( $\text{NaH}_2\text{PO}_4$ , a weak base with a  $\text{pK}_a$  of 7.2, and  $\text{Na}_3\text{PO}_4$ , a stronger base with a  $\text{pK}_b$  of 2.23) as the gelling agent. Moreover, we evaluated the influence of different pHs (from 5.0 to 7.0) and phosphate/chitosan ratios (0.5, 1.25 and 2), as well as the storage stability of the hydrogel systems.

The rheological data obtained was corroborated by optical microscope analyses, which made it possible to confirm and clarify the thermogelling ability of the system.

## 2. Experimental

### 2.1. Materials

Chitosan in its free amine form (cat. no. 448869, molecular weight 50–190 kDa, viscosity 20–300 cps determined by Brookfield method, 1% solution in 1% acetic acid, and degree of deacetylation of 75–85%), monosodium phosphate ( $\text{NaH}_2\text{PO}_4$ ), and trisodium phosphate ( $\text{Na}_3\text{PO}_4$ ) were purchased from Sigma Aldrich. Deionized water was produced with a laboratory deionizer (Osmo Lab UPW 2, Gamma 3, Italy).

### 2.2. Preparation of chitosan solution

2% (w/w) chitosan stock solution was prepared in a 0.1 M hydrochloric acid water solution, under constant magnetic stirring overnight at room temperature. The resultant solution was refrigerated at 4 °C until investigation.

### 2.3. Preparation of chitosan/inorganic phosphate dispersions

Depending on the chitosan/inorganic phosphate ratio, a certain amount of inorganic phosphate ( $\text{NaH}_2\text{PO}_4$  [0.7 M] or  $\text{Na}_3\text{PO}_4$  [0.7 M]) solution was added dropwise to chitosan stock solution under magnetic stirring. The chitosan/inorganic phosphate systems were prepared at three different pHs (5.0, 6.0 and 7.0) by adding HCl (0.1 M) or NaOH (0.3 M) aqueous solutions. Before to start the analyses the pH values were well determined, however we found a decreasing in the pH values when the temperature were increased during the rheological tests. So far we decide to prepared the sample at a slightly higher pH values before to run the rheological tests.

The final chitosan concentration was 1.4% (w/w), while the inorganic phosphate/chitosan ratios were 0.5, 1.25 and 2. These ratios refer to equivalent amino groups estimated on the chitosan backbone.

The resultant dispersion systems were stored under refrigeration at 4 °C for later use.

### 2.4. Rheological characterization

Rheological analyses were performed in triplicate using a stress control rheometer (Stress-Tech, Reologica) equipped with a 4/40 cone-plate geometry (cone diameter was 40 mm, cone angle was 4°) operating in the oscillation mode. The gap was 150  $\mu\text{m}$ . Samples were analysed in triplicate at 1 day, 3 and 7 days from the preparation.

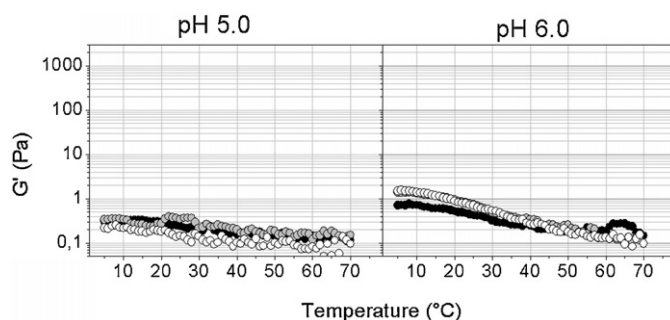


Fig. 1. Temperature sweep of chitosan dispersions (1.4%, w/w) at pH 5.0 and 6.0 ((●) 1 day, (●) 3 days, (○) 7 days).

The analyses were carried out as follows: in the oscillation stress sweep test, the samples were exposed at 25 °C and stress was increased at a constant frequency (1 Hz) in the range of stresses between 0.05 and 10 Pa. This test determines the linear viscoelastic regime (LVR) of the sample, and therefore the consequent choice of the stress value to use in the other oscillation tests.

In the temperature sweep test, the chitosan and chitosan/inorganic phosphate systems were analysed at constant frequency (1 Hz), at a stress of 1 Pa and in the range of temperature from 0 to 70 °C at a rate of 1 °C/min. Evaporation of samples was prevented by using a silicon oil sealing.

### 2.5. Optical microscope analyses

An optical microscope (MT9000 polarizing microscope, Meiji Techno Co. Ltd., Japan) equipped with a 3 megapixels Cmos camera (Invenio 3S, DeltaPix) was used to collect images of chitosan/inorganic phosphate systems at different stages.

## 3. Results

### 3.1. Rheological data analyses

Preliminary rheological analyses were performed on chitosan solutions prepared at different pHs. Both chitosan samples (pH 5.0 and 6.0) showed the same appearance and rheological properties (Fig. 1). On the contrary, it was impossible to prepare a chitosan solution at pH 7.0 because after pH 6.0 the chitosan solution started to aggregate inducing a phase separation.

No evident phenomena were observed when the temperature was increased for both samples, even if pH 6.0 has been reported to be more sensitive to temperature variation, as confirmed by a slight decrease of the  $G'$  modulus in our study. Moreover, during the seven day stability tests, both chitosan solutions appeared stable and did not show any significant change in the rheological properties.

The chitosan/ $\text{NaH}_2\text{PO}_4$  systems at pH 5.0, independently of the ratio, showed a behaviour similar to that of chitosan solution at the same pH (Fig. 2). In fact, they were stable and their behaviour was independent of the different temperatures applied. Moreover, the  $G'$  value was practically the same as that of the chitosan solution.

On the contrary, increasing the pH to 6.0 caused the systems to appear more opalescent, though without evident formation of aggregates, and modified the rheological behaviour for all the three ratios analysed. This was accompanied by an increase in the starting value of  $G'$  compared to the value at pH 5.0. However, at pH 6.0, a different  $G'$ /temperature trend was observed for the three inorganic phosphate/chitosan ratios. At a ratio of 0.5, a decrease in  $G'$  values at increased temperature were observed, confirmed for all seven days of the stability test (the three curves are superimposed). However, at a ratio of 1.25, we had a slight increase in  $G'$  at about 65 °C for the first and third days, a trend that disappeared after seven days,

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