MECHANISM OF FORMATION OF THERMOSENSITIVE CHITOSAN CHLORIDE GELS

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Abstract
In these studies, an attempt has been made to explain the mechanism of gel formation. The studies were carried out to develop the mechanism of chitosan gel formation in the presence of glycerol phosphate GP, taking into account suggestions presented in the literature. The analysis was carried out on the basis of a change in the gel structure after conditioning in water.

Key words: *thermosensitive gel, chitosan.*

1. Introduction

Thermosensitive structures are made from polymers which show temperature-dependent sol-gel transition in aqueous solutions. The transition from a viscous liquid to hydrogel is the result of a quick increase in viscosity at a temperature called the lower critical solution temperature (LCST) [1 - 3]. For biomedical applications, thermo-gelling injectable systems with an LCST around or below 37 °C would be ideal, as they would transform from a solution to a gel upon injection into a body cavity [4, 5].

Currently, there is much interest in hydrogel materials of natural origin, in particular chitosan, which is a product of chitin deacetylation [6]. Chitosan hydrogels, whose sol-gel transition takes place at natural temperatures of the human body, are formed mainly from chitosan salt solutions with low viscosity with the use of β-glycerol phosphate as a neutralising agent [7 - 13], but also from polyvinyl alcohol and sodium bicarbonate [14 - 16] by the enzymatic method with the use of urease and urea [17] and by using β-tricalcium phosphate [18].

The phase transition is related to the interactions between polymer chains and water molecules, and chain-chain interactions, and thus depends on both the degree of deacetylation and the molecular weight of the polymer. Below the phase transition temperature, the macromolecule is maintained in the solution owing to the solvation sphere. It is formed as a result of hydrogen bonds between hydrophilic fragments of the polymer chain and water molecules. An increase in temperature causes weakening of the polymer-water hydrogen bonds and strengthening of polymer-polymer hydrophobic interactions (dipole, hydrogen, van der Waals and other interactions). Consequently, this leads to a change in the chain structure into the form of a random coil and to phase separation. The change is shown as volume phase transition.

The mechanism of gel formation from chitosan salts in the presence of β-glycerophosphate is a subject of discussion in the literature. Cho [19] declares that the major driving force of chitosan gelation at high temperatures in the presence of β-GP are hydrophobic interactions and a decrease of solubility. Xueying Qiu [20] revealed that both hydrogen bonding and hydrophobic interactions within chitosan or between chitosan and *β*-glycerophosphate molecules are the main reasons for gel formation – firstly the formation of hydrogen bonds makes the hydrophobic sites more accessible, and then the synergistic hydrogen bonding and hydrophobic interactions lead to the final formation of the CS/*β*-GP gel network. Chenite [21] states that in the presence of glycerophosphate, the gel can be formed due to electrostatic repulsion and possible ionic interactions between $-NH_3^+$ groups in a chitosan molecule with $-O^-$ glycerophosphate [22].

On the other hand, Filion and Lavertu [21] suggest that the gel is formed as a result of the combination of protons from amino groups with glycerophosphate.

In these studies, an attempt has been made to explain the mechanism of gel formation. The studies were carried out to develop the mechanism of chitosan gel formation in the presence of glycerophosphate (GP), taking into account suggestions presented in the literature.

The analysis was carried out on the basis of a change in the gel structure after conditioning in water.

2 Materials and Methods

2.1. Preparation of chitosan hydrogels

Chitosan chloride solution was prepared by swelling 400 mg of chitosan (CH, chitosan from shrimp shells with low viscosity and degree of deacetylation ~79.5% SIGMA-ALDRICH®) in 16 ml of 0.1 M HCl. The solution was stirred (at slow rotations) until complete dissolution. Next, the sample was cooled to 4 °C. To this cooled sample, 2 g of β-glycerophosphate disodium salt pentahydrate (GP, SIGMA-ALDRICH®) dissolved in 2.5 ml of distilled water was added drop-wise under stirring in an ice bath. The final solution was mixed for another 20 min and stored at 4 °C for 12 h. Samples were stored at 37 °C for 24 h, to obtain a gel structure. Gel samples were prepared in the shape of cylinders with a diameter of 3 cm and height of 3 cm.

2.2. Methods

The structural properties of hydrogels after conditioning in water were studied. The conditioning in water was performed using an ERWEKA apparatus. The frequency of fluid mixing was fixed at 50 revolutions/min. The release process of GP was studied in 500 ml distilled water at pH 5 ± 0.5. The temperature of the released medium was kept constant at 37 °C during the whole release process.

The structure of gels was observed under a FEI QUANTA 200 F scanning electron microscope. Using this microscope, the elemental composition of the gel before and after conditioning in water was determined. Observations of the structure and determination of the elemental composition of gels were performed for the lyophilised samples.

The structural characteristics were based on the analysis of XRD and IR spectra.

The XRD analysis was used to identify structural changes in the analysed samples. Room temperature powder X-ray diffraction patterns were collected using a PANalytical X'Pert Pro MPD diffractometer in Bragg-Brentano reflecting geometry. Copper CuK_{α} radiation was applied from a sealed tube.

The FTIR spectra were obtained using a Bio-Rad apparatus in ATR-FTIR mode, in the range 4000 - 500 cm^{-1} with a resolution of 4 cm^{-1} and at 100 scans.

3. Results and discussion

The SEM image of chitosan thermosensitive gels after lyophilisation is shown in ***Figures 1.a*** (before conditioning in water) and ***1.b*** (after conditioning in water). ***Figure 2*** displays the FTIR spectrum of the lyophilised chitosan hydrogels immediately after gelation and after conditioning in distilled water. ***Figure 3*** shows the X-ray Diffraction (XRD) patterns of chitosan hydrogel after conditioning in water (samples after lyophilisation). Elemental composition of the gel after conditioning in water is given in ***Table 1***.

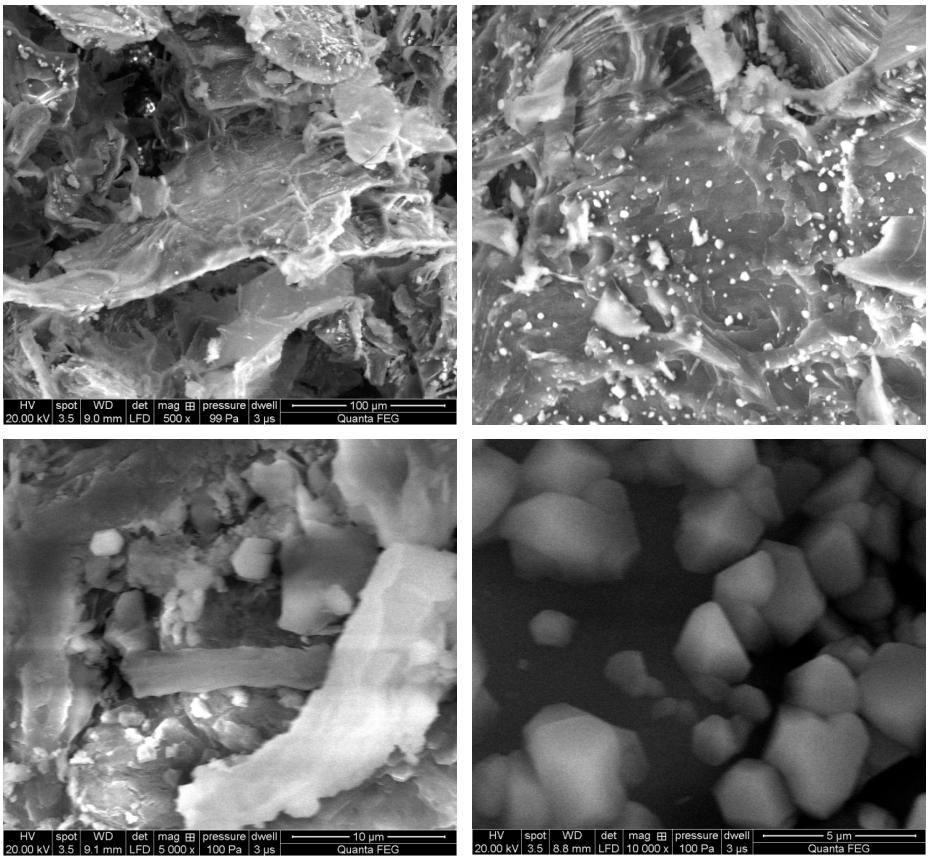

Figure 1.a. *SEM image of chitosan thermosensitive gels before conditioning in water.*

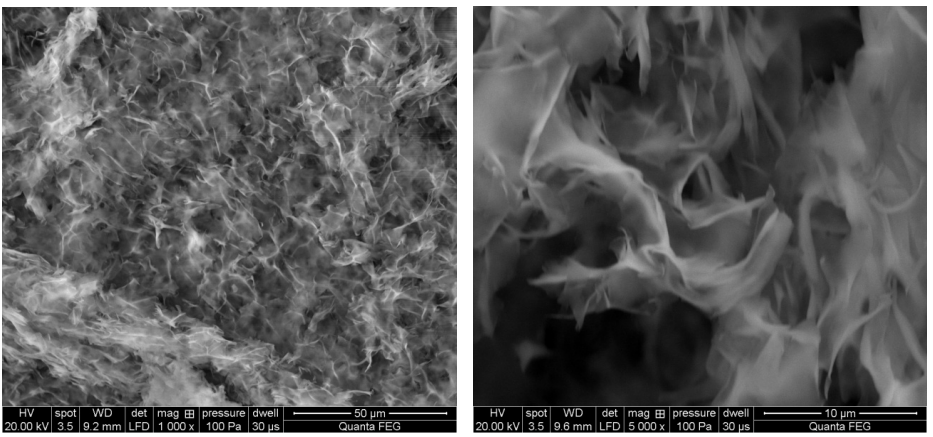

Figure 1.b. *SEM image of chitosan thermosensitive gels after conditioning in water.*

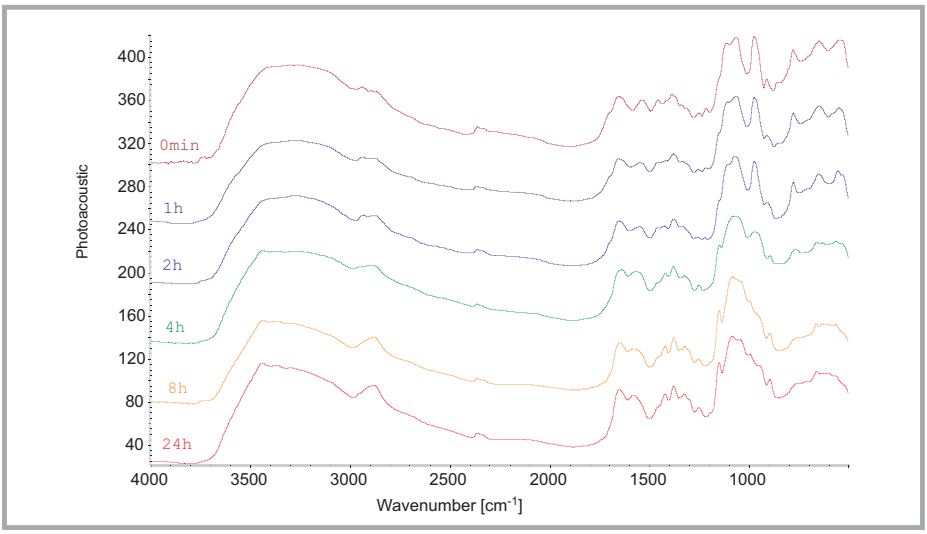

Figure 2. *FTIR spectrum of the lyophilised chitosan hydrogels immediately after gelation and after conditioning in distilled water.*

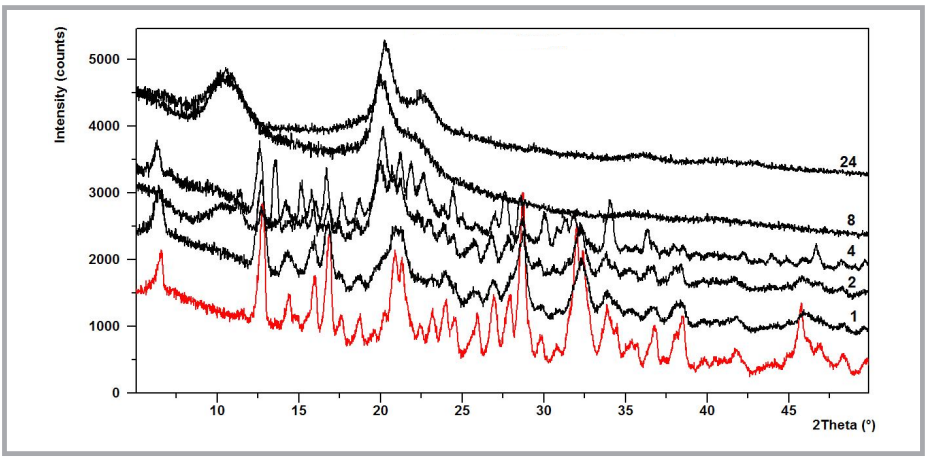

Figure 3. *X-ray Diffraction (XRD) patterns of chitosan hydrogel after conditioning in water.*

SEM pictures show a change in the gel structure after conditioning in water. Immediately after formation, pores in the structure are of the order of several μm and one can observe the precipitation of crystals in them. On the basis of the proposed model, it can be suggested that this is NaCl. After conditioning in water, the crystals are washed out, the structure becomes more compact and pores are of the order of 10 μm.

Infrared spectra of gels before and after conditioning in water indicate changes in the gel structure. The broad band corresponding to the oscillation of O-H is observed in the range of

Table 1. *Elemental composition of gel after conditioning in water*

Thermogels-phosphorus	Na [% at.]	P [% at.]	Cl [% at.]	P/Na
0 min	7.589	4.543	1.37	0.59
1h	4.941	3.794	0.869	0.82
2h	5.134	3.513	0.359	0.85
4h	4.931	4.268	0.424	1.05
8h	0.477	0.794	0.093	1.70

wave number of 3600 - 3100 cm^{-1} in both cases. The asymmetric shape of this peak which is visible in the range of lower wave numbers indicates the presence of strong hydroxyl bonds and amine N-H groups in the structure. Asymmetry increases with an increase in the time of sample conditioning in water.

In the range of 2850 - 2950 cm^{-1}, the spectrum of chitosan has one asymmetric band at 2890 cm^{-1}. This band consists probably of two overlapping bands which represent the stretching vibrations in aliphatic groups ($–CH_2$ and $–CH_3$) that are characteristic of the pyranose ring of chitosan. The spectrum of hydrogel before conditioning in water is split into two distinct bands at 2890 and 2920 cm^{-1}. For the hydrogel, after conditioning in water, this band appears at 2890 cm^{-1} and has a minor shoulder.

The spectrum of chitosan shows a band at 1650 cm^{-1} that is assigned to the C=O stretch of the amide bond and at 1590 cm^{-1} that is assigned to the NH_2 group of chitosan. These bands indicate that chitosan is a partially deacetylated product of chitin. In this range of frequency, no significant changes were observed.

In the range of 1200 - 1500 cm^{-1}, the chitosan molecule showed four peaks at the bands at 1419, 1375, 1315 and 1264 cm^{-1}. The bands at 1419 and 1314 cm^{-1} are associated with oscillations characteristic of C-H bending of CH_2 groups. The band at 1375 cm^{-1} represents the C-O stretching of the primary alcoholic group $-CH_2-OH$. In this frequency range, also during sample conditioning in water, no changes were observed in general.

In the wave number range 800 - 1200 cm^{-1}, the FTIR spectrum of chitosan showed three bands at 1160, 1020 and 900 cm^{-1}. The wide band at 1155 - 1037 cm^{-1} represents the bridge C-O-C stretch of the glucosamine residues.

Before conditioning in water, the system has not only the bands characteristic of chitosan in 800 - 1200 cm^{-1} wave number range. However, two new bands characteristic of GP appear in this region, at 980 cm^{-1} and 1050 cm^{-1}, with a minor shoulder at 920 cm^{-1}; the band at 980 cm^{-1} is characteristic of GP and indicates aliphatic P-O-C stretching; the band at 1050 cm^{-1} is characteristic of the $-PO_4^{2-}$ group and the band at 920 cm^{-1} may indicate the presence of the $-HPO_4^{2-}$ group.

The FTIR spectra obtained for the systems conditioned in distilled water after 8 h only show peaks that are characteristic of chitosan molecules (at 1150, 1030 and 895 cm^{-1}).

The peaks connected with the presence of phosphorus in the system structures, P-O-C and $-PO_4^{2-}$, disappear.

The FTIR spectrum of chitosan has one broad band at 660 cm^{-1}, which is connected with vibrations of the O=C-N group. The hydrogel spectra display two bands in this range at 800 and 700 cm^{-1}. The band at 800 cm^{-1} is characteristic of GP (aliphatic stretching of P-O-C).

The FTIR spectrum of the system after gelation indicates characteristic bands for chitosan and glycerophosphate disodium salt. For the system conditioning in water for 8 h, only bands characteristic of the chitosan molecule are observed. The spectrum in the range of 1200 - 900 cm^{-1}, which is assigned to the saccharide structure, is similar to the spectrum of chitosan molecules. There are no additional bands and shifts observed.

Diffractograms presented in ***Figure 6*** confirm the suggestions resulting from the analysis of FTIR spectra. The diffractograms of thermogel directly after formation are characterised by a number of peaks most probably related to the presence of glycerophosphate.

Based on ***Figure 5***, it can be concluded that the structure of hydrogel, where GP is still present, is maintained for up to 4 h and is lost between 4-8 hours of conditioning and that after elution of GP after 24 h the gel structure is similar to that of chitosan.

A number of maxima present in the diffractograms of thermogels containing phosphate is the evidence of higher ordering of the structure and higher crystallinity.

Elemental analysis confirms the presence of phosphorus in the structure of gels made from chitosan chloride for some time (8 h). The results show that the amount of phosphorus is almost constant in the first few hours (4 h), while the quantity of sodium and chlorine is reduced. The presence of Na^+ from phosphate and Cl^- from hydrochloric acid causes that after the lyophilisation of precipitated crystals, most probably of NaCl, can be observed in the structure. Atomic percentage of P and Na in the hydrogel structure is at the same level. Hence, it can be assumed that 50% of Na^+ neutralises one O^- group with glycerophosphate and through the second O^- group glycerophosphate is combined by electrostatic forces with the NH^{3+} group from the chitosan molecule. However, the electrostatic forces are weak and after sufficiently long conditioning, both P and Na are reduced in the structure and the elemental composition remains the same as for pure chitosan. Thus, Lavertu's theory is supported by phosphorus elution from the structure, while the theory presented by Chenite is backed up by the fact that the elution takes place after some time only, which suggests electrostatic interactions. Moreover, sodium from glycerophosphate can form sodium hydroxide with free OH groups. This suggestion is supported by an increase in the mechanical strength of thermosensitive chitosan gels. This requires further research.

Based on the above analysis, the following processes induced by the presence of GP in chitosan salt are proposed (***Figure 4***).

- GP dissociates,
- hydrolyses phosphate ions,

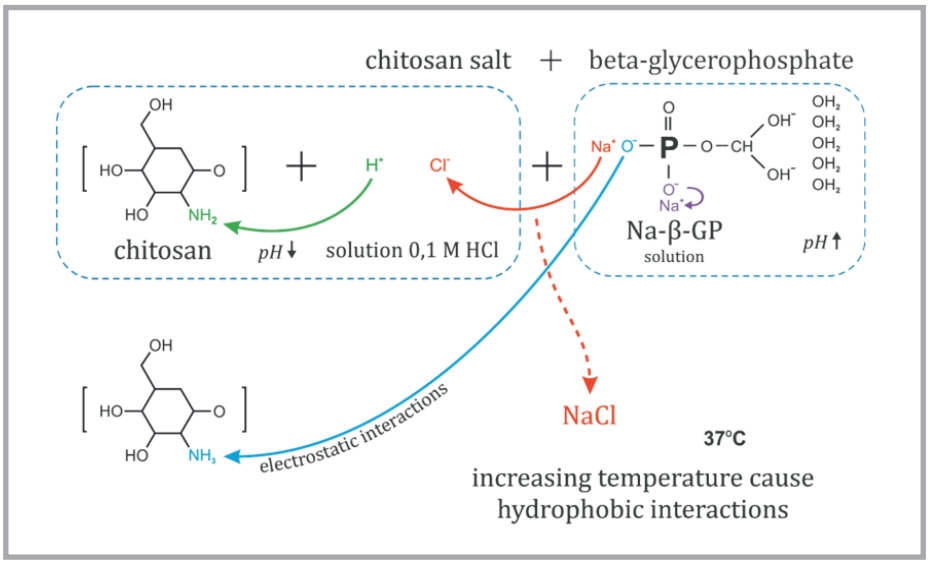

Figure 4. *Processes induced by the presence of GP in chitosan chloride.*

- this causes a pH increase in the chitosan solution,
- at low temperatures, there is electrostatic interaction between the negatively charged (residues) phosphate molecules from glycerophosphate and positively charged amino groups,
- GP also supports the hydration of chitosan molecules and the interaction of chitosan molecules with water,
- these processes protect chitosan against immediate separation,
- between polymer molecules, there are physical and hydrogen bonds, however weak,
- under the influence of temperature, electrostatic interaction still exists between the negatively charged (residues) molecules of phosphate from glycerophosphate and positively charged amino groups,
- water molecules protecting the polymer are removed by glycerine residues and new stronger hydrogen bonds are formed between hydrophobic groups, which causes the precipitation of polymer.

4. Conclusions

An attempt was made to explain the mechanism of gel formation on the basis of structural studies of thermosensitive chitosan gels conditioned in water. The results obtained confirm the theories of Cho and Chenite, but the interpretation suggested by Lavertu is supported by the removal of glycerophosphate from the structure. However, it is still debatable whether this is due to the combination of protons from amino groups with glycerophosphate or whether it is removed as a result of disconnection of sodium.

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6. References

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