

FORMATION MECHANISM OF THERMOSENSITIVE CHITOSAN HYDROGELS CONTAINING URIDINE 5'-MONOPHOSPHATE DISODIUM SALT

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Abstract

In this study, the gelation mechanisms of two types of thermosensitive chitosan hydrogels containing uridine 5'-monophosphate disodium salt (UMP) as a sol-gel transition agent were investigated. These systems were prepared from chitosan lactate and chitosan chloride solutions and are characterised by the ability to transition from sol to gel at both low and high temperatures. The formation mechanisms of the above hydrogels were developed based on our previous studies and the analysis of FTIR spectra of the chitosan systems at 4°C and 37°C without and with the addition of uridine 5'-monophosphate disodium salt. It has been demonstrated that the sol-gel transition of chitosan colloids is a complex, multi-step process resulting from the coexistence of multiple interactions (electrostatic interactions, hydrogen bonds and hydrophobic interactions) that determine the final properties of the hydrogels.

Keywords: *chitosan, thermosensitive hydrogel, uridine 5'-monophosphate disodium salt, gelation mechanism*

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1. Introduction

Currently, the main interest of researchers in the field of chitosan hydrogels is thermosensitive hydrogels formed *in situ*. Such hydrogels remain in the sol form at room temperature, and, after heating to a temperature close to the physiological temperature of the human body (approximately 37°C), transform into a gel state. Much attention in the literature is paid to hydrogels obtained from chitosan chloride solution with β -glycerophosphate disodium salt (β -GP) used as a cross-linking and/or pH-regulating agent [1, 2].

The first thermosensitive hydrogel obtained from chitosan chloride solution using β -GP was developed in 2000 by Chenite *et al.* [2, 3]. That hydrogel was formed due to electrostatic repulsion between chitosan chains and ionic interactions between the $-\text{NH}_3^+$ group present in the chitosan chain and the O^- ions of the phosphate group.

Nevertheless, the exact mechanism of the sol-gel transition of chitosan systems has not been clearly explained for over two decades. Cho *et al.* [4] indicated that the driving force of gelation is the hydrophobic chitosan-solvent interaction, which strengthens the interactions between polymer chains. With the increase in temperature, the ionic strength of the chitosan- β -glycerophosphate solution increases, which results from the rise of β -GP anions ionisation. Consequently, the anions lead to a weakening of the electrostatic repulsion of chitosan amino groups through their screening, thus the hydrophobic interactions strengthen.

On the other hand, the concept formulated in publications of Filion *et al.* and Lavertu *et al.* [5, 6] stated that there is a significant decrease in the *pKa* of chitosan with rising temperature, whereas the temperature change does not affect the *pKa* of β -glycerophosphate. Due to the rise of the system temperature, the thermodynamically driven decrease of the *pKa* of the polymer leads to the transfer of a proton from the $-\text{NH}_3^+$ groups of chitosan to the negatively charged phosphate groups in β -GP. As a result, the polymer chains are neutralised – the electrostatic repulsive forces weaken, and the attractive forces dominate, facilitating the formation of a three-dimensional hydrogel network.

According to Qiu *et al.* [7], the hydrogel formation occurs in two stages. Firstly, hydrogen bonds are formed, which makes hydrophobic sites more exposed. In the second stage, a three-dimensional network is formed due to the synergistic action of hydrogen bonds and hydrophobic interactions.

Alternatively, the theory of Supper *et al.* [1] assumes that two different types of intermolecular interactions are responsible for the formation of hydrogel, i.e., hydrogen bonds and hydrophobic interactions. Hydrogen bonds enable the polyol fragments of glycerophosphate to form a protective hydration layer around the polymer chain, which is sensitive to temperature change. Therefore, the hydrogen bonds weaken upon temperature increase, leading to destruction of the protective layer, and thus stronger hydrophobic interactions could dominate, supporting the formation of a hydrogel.

Without denying the undoubted value of the theories cited above, it should be emphasised that they are generally single-threaded and focus mainly on discussing phenomena that accompany the chitosan chloride solutions. Since the chitosan system formation mechanism at low temperatures has not been addressed in the literature so far, it is worth considering. Therefore, this publication attempts to explain the sol-gel transition phenomenon in the low and high temperature ranges, as is the case with hydrogels containing uridine 5'-monophosphate disodium salt (UMP). This may initiate a discussion on the possibility of forming hydrogels containing disodium salts of other nucleotides.

2. Materials and Methods

2.1. Materials

Chitosan from crab shells (CH) (product no. 50494, CAS no. 9012-76-4), lactic acid (LA) (product no. L6661, CAS no. 50-21-5), hydrochloric acid (HCL) (product no. H1758, CAS no. 7647-01-0) and uridine 5'-monophosphate disodium salt (UMP) (product no. U6375, CAS no. 3387-36-8) were purchased from Sigma-Aldrich (Poznan, Poland). All chemical reagents were of analytical grade and were used as received.

2.2. Preparation of Biomaterials

Chitosan systems were prepared by physical blending with or without the addition of uridine 5'-monophosphate disodium salt. Samples were processed at 4°C and 37°C. The introduction of uridine 5'-monophosphate disodium salt to chitosan solutions resulted in an increase in the pH to approximately 7.

To obtain the systems without UMP, 2.5% (w/v) chitosan salt solutions were prepared by dissolving 0.4 g of CH in 16 mL of 0.1 mol/L LA or HCL. After thoroughly mixing, the samples were left at room temperature for 24 h. The prepared formulations were stored at 37°C or 4°C for about 48 h in the next step.

To obtain the systems with UMP, 2 g of UMP was dissolved in 2.5 mL of deionised water, and this solution was added gradually to the chitosan salt solution upon stirring. The samples were stored at room temperature for about 2 h. The prepared formulations were subsequently stored at 37°C or 4°C to complete their gelation.

Finally, the samples were frozen at -20°C and then lyophilised under a 0.63 mbar pressure and at -25°C for about 48 h using the Martin Christ Freeze Dryer ALPHA 2-4. Freezing is the basic form of preserving the porous structure in hydrogels. This method of sample preparation is well known and often described in the literature [8–10].

2.3. Methods

Fourier transform infrared (FTIR) spectra of the lyophilised hydrogels were obtained using a Nicolet™ iS50 FT-IR apparatus equipped with a monolithic diamond ATR crystal (Thermo Fisher Scientific Inc., Waltham, MA, USA). All spectra were recorded with 100 scans at a 4.0 cm⁻¹ resolution in the 4000 - 500 cm⁻¹ wavenumber range.

3. Results and Discussion

Based on the results of rheological measurements and NMR studies presented by us earlier [11], it can be assumed that a different driving force is responsible for the sol-gel transition of the chitosan systems with UMP at low temperature than that responsible for the gelation process at a temperature close to the physiological temperature of the human body. To formulate appropriate conclusions regarding the formation mechanisms of the hydrogels in both temperature regions, the FTIR spectra of samples at 4°C and 37°C without and with UMP were analysed. Figures 1 and 2 show the FTIR spectra of the systems obtained from the chitosan lactate solutions at 4°C and 37°C.

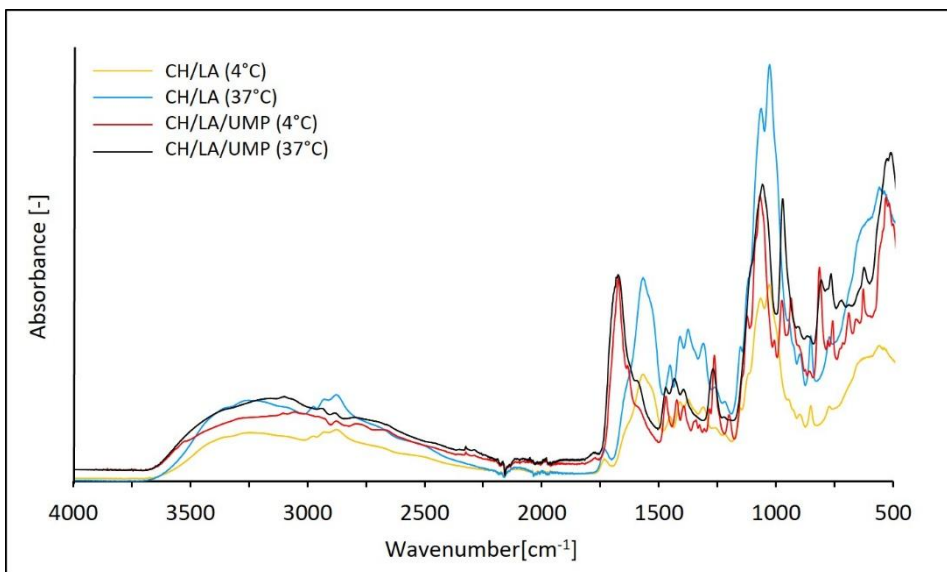


Figure 1. FTIR spectra of the systems obtained from the chitosan lactate solutions at 4°C and 37°C without and with UMP in the 4000 - 500 cm⁻¹ wavenumber range.

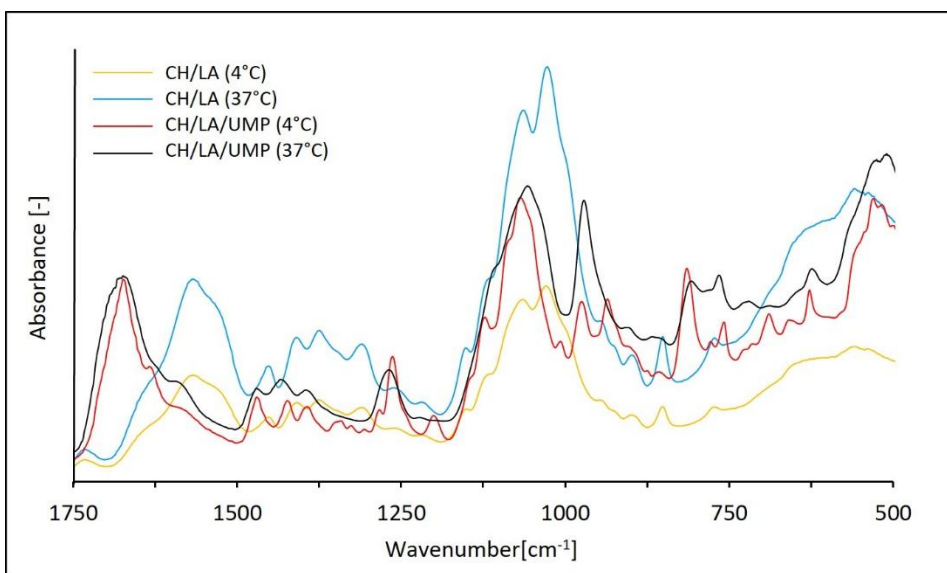


Figure 2. FTIR spectra of the systems obtained from the chitosan lactate solutions at 4°C and 37°C without and with UMP in the 1750 - 500 cm⁻¹ wavenumber range.

Based on the obtained FTIR spectra, it can be concluded that:

- the bands appearing in the spectra of the systems formed at 37°C are more intense than the corresponding signals observed for the samples solidified at 4°C; this increase in intensity may indicate enhanced molecular interactions or structural rearrangements at higher temperatures;

- for all samples, without and with UMP, the band occurring in the wavenumber range of 3600 - 3000 cm^{-1} (the broad, asymmetric band corresponding to the O–H stretching) is distorted compared to the band observed in the chitosan spectrum;
- a C=O band at 1730 cm^{-1} characteristic for the systems without UMP is shifted towards higher wavenumbers (1770 cm^{-1}) for samples with UMP; this may indicate the occurrence of complexes formed as a result of ionic interactions between the carboxyl groups of lactic acid and the amino groups of chitosan [12];
- for both systems with UMP, an intense peak at 1688 cm^{-1} (stretching vibrations of the carbonyl group $>\text{C}(2)=\text{O}$ of uracil) is noted, which is not present in the spectra of samples without UMP;
- in the spectra of systems without UMP, bands at the wavenumbers of 1565, 1525, 1215, 1020 and 853 cm^{-1} are observed:
 - the band at 1565 cm^{-1} indicates the existence of interactions between the $-\text{NH}_3^+$ groups of chitosan and the $-\text{COO}^-$ groups of lactic acid, which is the evidence that lactic acid not only plays the role of a physical cross-linker, but also chemically binds with chitosan via an amide bond; the lactic acid molecule is relatively small, and its $-\text{COO}^-$ groups do not constitute a spatial limitation; moreover, the corresponding amino groups of chitosan are not shielded by large molecules, thanks to which they can interact with lactic acid;
 - the band at 1525 cm^{-1} indicates that lactic acid causes protonation of the amino groups of chitosan;
 - the band at 1215 cm^{-1} corresponds to the stretching vibrations of the C–O bond of the secondary alcohol of lactic acid [13];
 - the band at 1020 cm^{-1} is typical for chitosan and is attributed to stretching vibrations of the C–O bond in the C–O–C oxygen bridge and in the $-\text{CH}_2(\text{OH})$ group;
 - the band at 853 cm^{-1} is characteristic for the $-\text{O}-\text{CH}-\text{CH}_3$ group of lactic acid [14];
- in the range of 1500 - 500 cm^{-1} of the spectrum of the CH/LA/UMP (4°C) system, there are bands characteristic for UMP, at the following wavenumbers [15–17]:
 - 1347 and 1326 cm^{-1} (twisting vibrations of the $-\text{CH}_2-$ group);
 - 1284 cm^{-1} (stretching vibrations of the N(1)–C(2)–N(3) moiety of the uracil ring, the C–C (ribose), the C–O (ribose) and the C(1')–N(1) moiety);
 - 1203 cm^{-1} (stretching vibrations of the uracil ring and bending vibrations of the C–H (uracil));
 - 1125 cm^{-1} (stretching vibrations of the C(4')–C(5')–O moiety and symmetric stretching vibrations of the $-\text{PO}_2^-$ group);
 - 1005 cm^{-1} (stretching vibrations of the uracil ring);
 - 936 cm^{-1} (symmetric stretching vibrations of the $-\text{PO}_3^{2-}$ group);
 - 688 cm^{-1} (bending vibrations of the C–C–O moiety (ribose) and bending vibrations of the uracil ring);
 - 657 cm^{-1} (bending vibrations of the C–C–O moiety (ribose) and the $>\text{C}=\text{O}$ (uracil));the above signals are not recorded for the CH/LA/UMP (37°C) system;
- there are also other bands associated with the presence of UMP in the spectra of both variants of the systems with UMP, which have been discussed in detail by us previously [18–20].

Based on the FTIR spectra interpretation, a hypothetical formation mechanism of the thermosensitive hydrogels prepared from the chitosan lactate solutions with uridine 5'-monophosphate disodium salt was proposed, which, in simplified terms, occurs according to the scheme shown in Figure 3.

Formation mechanism of thermosensitive chitosan hydrogels containing uridine 5'-monophosphate disodium salt

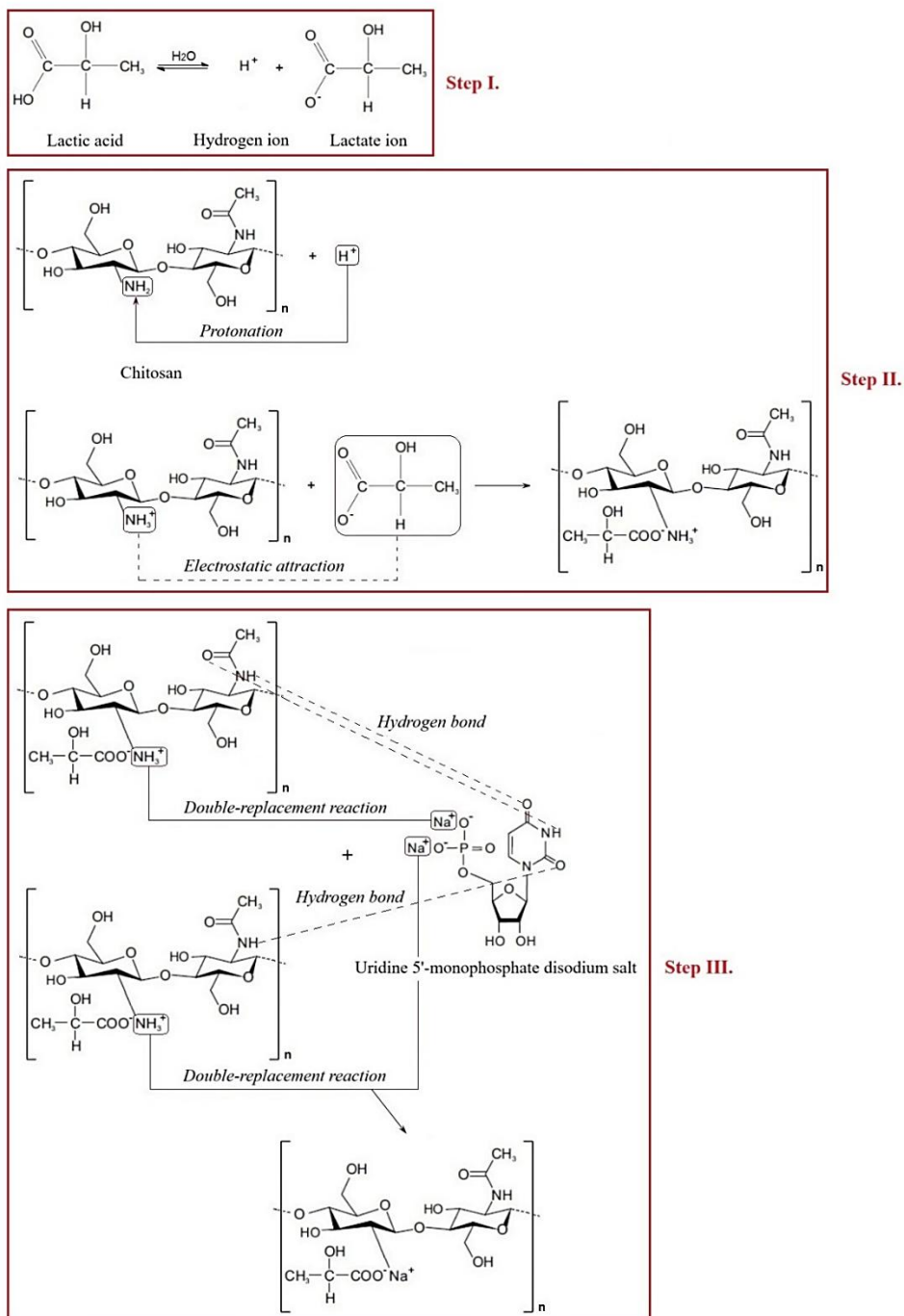


Figure 3. Formation mechanism of the CH/LA/UMP hydrogel.

When chitosan is dissolved in an aqueous dissociated lactic acid (step I), the amine groups of the polymer undergo protonation. Thus, chitosan exhibits the polycationic character, and the -NH_3^+ groups can electrostatically attract lactate anions, leading to the formation

of chitosan lactate (step II). This may suggest that chitosan is cross-linked with lactic acid. After introducing UMP into the chitosan lactate solution (step III), a double-displacement reaction occurs, resulting in sodium lactate grafted onto chitosan. Since the UMP molecule contains a pyrimidine base (uracil), which has two oxygen atoms that are hydrogen bond acceptors and an N–H donor [21], UMP probably has an "innate" ability to form the hydrogen bonds. For this reason, it is assumed that the driving force of the gelation process at low temperatures (according to the rheological tests $\leq 6.4^\circ\text{C}$ [11]) are hydrogen bonds formed between the hydrogen atom of the N–H group belonging to the acetamide group of chitosan and the oxygen atom of the carbonyl group $>\text{C}(2)=\text{O}$ or $>\text{C}(4)=\text{O}$ of uracil. In addition, a hydrogen bond can be formed between the hydrogen atom of the N(3)–H group of uracil and the oxygen atom of the $>\text{C}=\text{O}$ group contained in the acetamide group of the polymer. A possible stabilisation of the system due to the formation of intermolecular π -stacking interactions is also worth noting. This assumption is dictated by the fact that uracil, whose planar ring is composed of six carbon atoms, resembles the structure of the aromatic molecule benzene or pyridine. It should be emphasised, however, that the aromaticity of uracil is not entirely unambiguous [22].

On the other hand, considering the process of spatial structure formation of the CH/LA/UMP system at high temperature (according to the rheological tests $\geq 37.3^\circ\text{C}$ [11]), it can be assumed that the main driving force of the sol-gel transition are hydrophobic interactions occurring between successive chitosan chains.

The FTIR spectra of the systems obtained from the chitosan chloride solutions at 4°C and 37°C without and with UMP are illustrated in Figures 4 and 5.

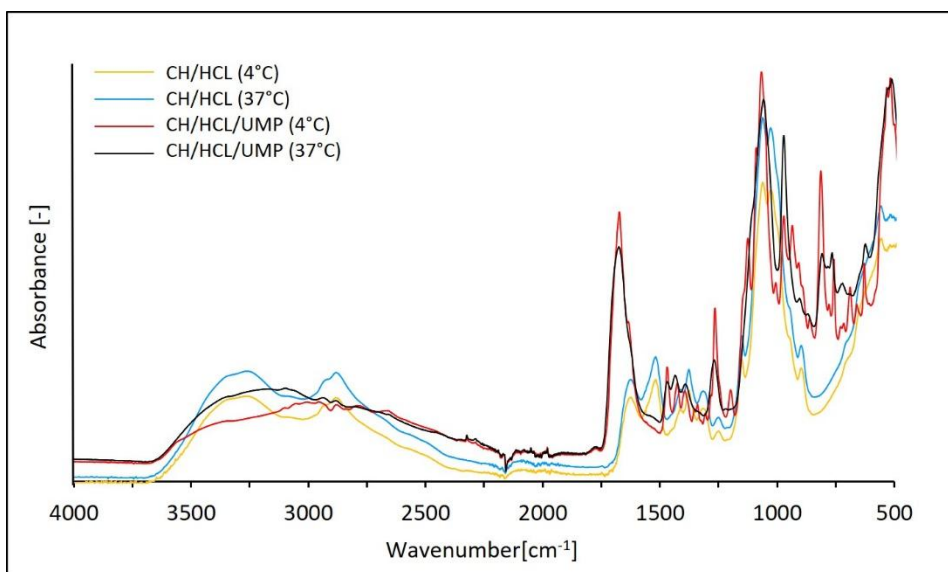


Figure 4. FTIR spectra of the systems obtained from the chitosan chloride solutions at 4°C and 37°C without and with UMP in the $4000 - 500 \text{ cm}^{-1}$ wavenumber range.

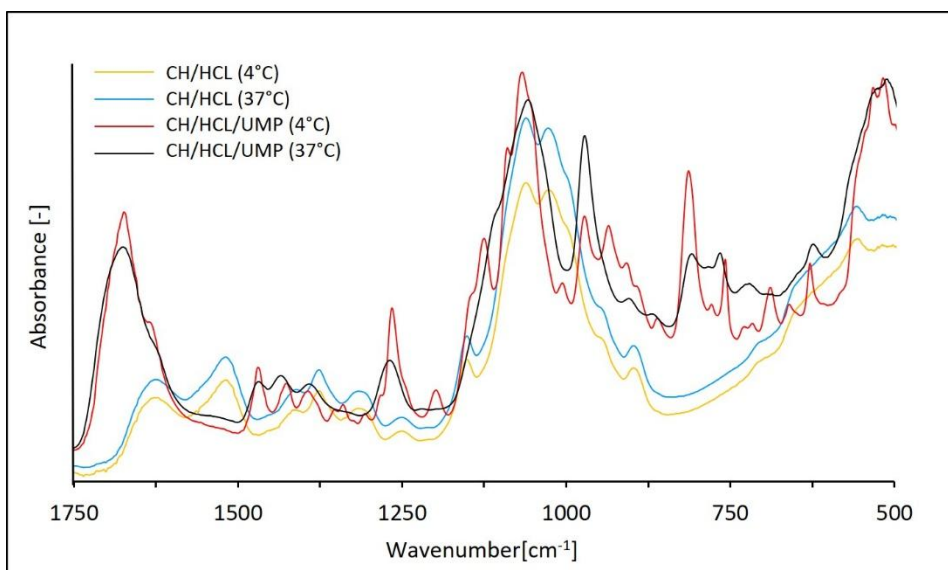


Figure 5. FTIR spectra of the systems obtained from the chitosan chloride solutions at 4°C and 37°C without and with UMP in the 1750 - 500 cm^{-1} wavenumber range.

Observations derived from the FTIR spectra analysis of chitosan systems given in Figures 4 and 5 are as follows:

- in the spectra of the samples with UMP, for both temperatures, the broad band in the range of 3600 - 3000 cm^{-1} is shifted towards lower wavenumbers compared to the analogous signal occurring in the spectra of the systems without UMP, and it is less intense;
- for the systems with UMP, there is an intense band at 1688 cm^{-1} , originating from the stretching vibrations of the $>\text{C}(2)=\text{O}$ group of uracil, which is not detected in the spectra of the samples without UMP;
- the amide I and II bands, present at 1625 and 1515 cm^{-1} , respectively, in the spectra of the samples without UMP, are not observed for the systems with UMP; this is caused by the overlapping of both bands with a very intense bands at 1630 and 1688 cm^{-1} ,
- in the range of 1500 - 500 cm^{-1} in the spectrum of the CH/HCL/UMP (4°C) system, the peaks typical for UMP are observed at 1342, 1205, 1122, 1008, 934, 688 and 655 cm^{-1} wavenumbers; these signals are not found for the CH/HCL/UMP (37°C) system;
- in the above range of wavenumbers, in the spectra of both variants of the systems with UMP, there are also several other bands associated with the presence of UMP, which have been discussed in detail by us previously [18–20].

The analysis of the FTIR spectra allowed us to propose a mechanism of the thermosensitive hydrogels formation, encompassing chitosan chloride solutions with uridine 5'-monophosphate disodium salt (Figure 6). Similarly to the process of chitosan dissolution in lactic acid, the use of an aqueous solution of hydrochloric acid, which is present in the dissociated form (step I), also results in the protonation of chitosan amine groups (step II). Chitosan in a protonated form is highly hydrophilic – the $-\text{NH}_3^+$ groups are surrounded by water molecules bound by hydrogen bonds. Adding UMP to the chitosan chloride solution (step III) causes, at low temperatures (according to the

rheological tests $\leq 10.0^{\circ}\text{C}$ [11]), electrostatic attraction between the $-\text{NH}_3^+$ groups of the polymer and the O^- ions present in the negatively charged phosphate group. At the same time, an ionic crystal lattice of sodium chloride is probably formed. The formation of NaCl is suggested based on the publication by Modrzejewska *et al.* [23]. This researcher presents SEM images illustrating changes in the structure of hydrogels obtained from the chitosan chloride solutions with β -glycerophosphate disodium salt pentahydrate after conditioning in water. It was demonstrated that pores in their structure are immediately of the order of several μm after the hydrogel formation, and the crystals (mostly of NaCl) precipitation is visible. On the other hand, after conditioning in water, the crystals dissolved or were washed out, causing the creation of a more compact structure.

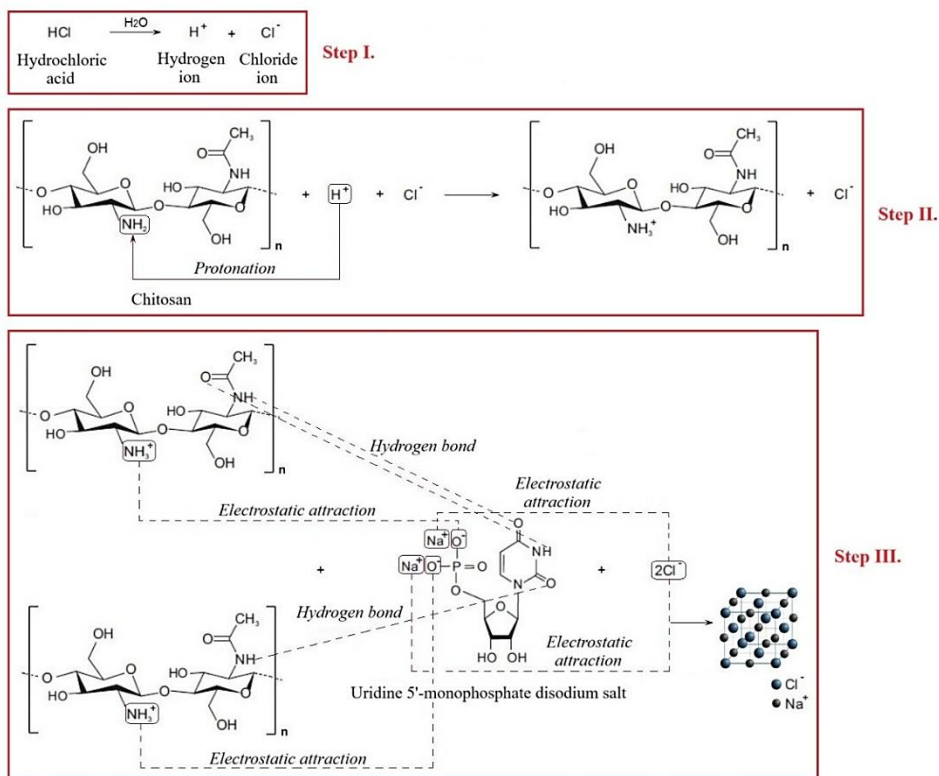


Figure 6. Formation mechanism of the CH/HCL/UMP hydrogel.

In addition, the analysed system is stabilised by intermolecular hydrogen bonds, which result from the unique properties of uracil. Therefore, ionic and hydrogen bonds are assumed to be responsible for physical gelation occurring at low temperatures.

After heating to intermediate temperatures, electrostatic interactions tend to diminish, and the system undergoes a transition to the sol state. Further heating above 41.2°C [11] results in the formation of a different type of physical hydrogel formed by hydrophobic interactions.

4. Conclusions

The complexity of the gelation process of the thermosensitive chitosan systems containing uridine 5'-monophosphate disodium salt has been demonstrated. The mechanism assumes the coexistence of various interactions, such as electrostatic forces, hydrogen bonds and hydrophobic interactions. All of these interactions determine the final properties of hydrogels.

It should also be emphasised that thermally induced gel-sol-gel transitions are fully reversible. Cooling the gel in an II state at a high temperature range to a lower temperature to induce the gel I state causes sequential gel-sol-gel transitions, which proceed in the reverse order compared to the heating of the sample [11]. This phenomenon, therefore, reflects the dynamic nature of typical physical hydrogels.

5. Acknowledgements

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