EQUILIBRIUM SWELLING STUDY OF CROSSLINKED CHITOSAN MEMBRANES IN WATER, BUFFER AND SALT SOLUTIONS

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Abstract

Physically crosslinked membranes were prepared by treating chitosan (Ch) with pentasodium tripolyphosphate (TPP) at different reaction conditions. The equilibrium swelling ratio, S_{eq}, of Ch/TPP membranes in water, buffer and salt solutions at 37 °C were determined. The experimental data indicated, that S_{eq} depended on pH of buffer solution and type and concentration of salt solution. Swelling capacity in water depended on the content of TPP ions in the membrane and crosslinking density.

Key words: *chitosan, membranes, crosslinking, equilibrium swelling*

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1. Introduction

Hydrogels are three-dimensional and hydrophilic polymer networks capable to swell in water or biological fluids and retaining a large amount of fluids in the swollen state (usually more than 20% of the total weight) [1]. The physical properties of hydrogels, including swelling, permeation, mechanical strength and surface characteristics can be modulated through their structural modifications. A variety of natural, modified natural and synthetic polymers are used to form hydrogels, including chitosan.

Chitosan, a copolymer composed of β-(1→4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose units (Fig. 1), is the most important derivative of chitin, a polysaccharide found in the exoskeleton of shellfish like shrimp and crab. It is obtained by deacetylation of chitin under alkaline conditions or by enzymatic hydrolysis in the presence of chitin deacetylase [2]. One of the most convenient and effective approach to modify the properties of chitosan hydrogels consists in its crosslinking [3]. The functional amino and hydroxyl groups on glucosamine units provide the reactive sites for crosslinking reaction. Various types of crosslinkers and crosslinking techniques are used. Depending on the nature of the crosslinker, the main interactions forming the network are covalent or ionic bonds. Most of the crosslinking agents used to obtain covalently crosslinked hydrogels may induce toxicity and thus the application of such materials is limited. To overcome this problem such non-toxic crosslinkers as genipine or TPP are used.

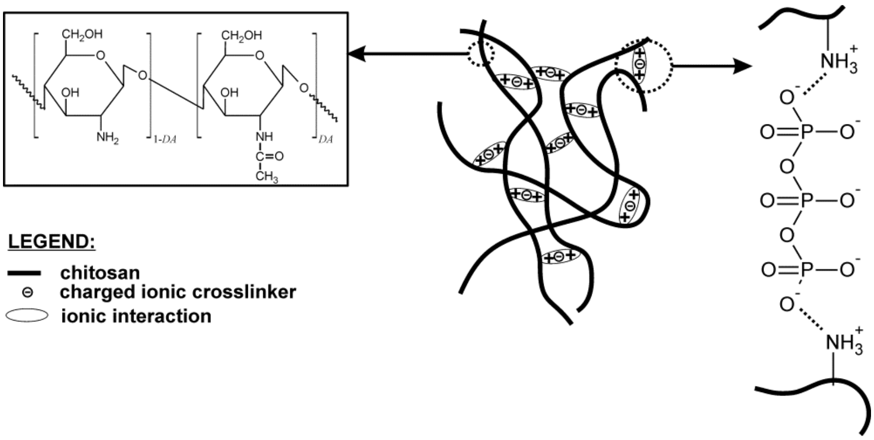

Figure 1. Chemical structure of chitosan and TPP-crosslinked chitosan.

Chitosan is one of the most promising polymers for the preparation of membranes for various uses [4]. However, chitosan membranes are soluble in acidic solutions. To improve mechanical and chemical resistance of chitosan membranes they are modified by different methods, including bulk and surface crosslinking. In our previous work we have used the crosslinking reaction of chitosan with sodium tripolyphosphate, (TPP), to obtain physically crosslinked (ionically crosslinked) chitosan membranes, (Ch/TPP), featuring improved mechanical and chemical stability in acidic media. We have studied the influence of crosslinking process conditions on molecular and supermolecular structure of chitosan membranes [5]. In the present work we have analysed an effect of crosslinking density on swelling ability of modified chitosan membranes in water, buffer and salt solutions.

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2. Materials and Methods

2.1. Materials

Commercially available chitosan (Ch) from crab shells of medium molecular weight and pentasodium tripolyphosphate (TPP, analytical grade) were purchased from Sigma-Aldrich (Germany). All other chemicals: acetic acid, hydrochloric acid, sodium hydroxide, sodium chloride, calcium chloride, aluminium chloride, sodium acetate and TRIS buffer were analytical grade and were purchased from POCh (Poland) or Sigma-Aldrich (Germany). Potassium bromide for spectroscopy was purchased from Merck (Germany).

2.2. Chitosan characterization

Degree of deacetylation, DDA, determined by means of potentiometric titration method [6], was found to be (75.72 ± 3.82)%. Chitosan viscosity average molecular weight, M_V, determined by viscosity analysis of chitosan solutions [7], was equal to (730 ± 16) kDa. The details of DDA and M_V determinations were described elsewhere [8].

2.3. Non-crosslinked and crosslinked chitosan membranes preparation

Modified chitosan membranes were prepared in two steps, as described elsewhere [5]. First, pure chitosan membranes (Ch) were prepared by solution casting and solvent evaporation technique. Chitosan solution of 1 % (w/v), prepared by dissolving chitosan powder in 2 % (w/v) acetic acid, was filtered, left over night, degassed, cast as film on a clean glass Petri dish and evaporated to dryness in an oven at 37 ºC. Then, membranes were immersed in 2 M sodium hydroxide solution for 5 min to remove the residual acid, thoroughly washed with deionised water and dried, at first in air and further under vacuum at 60°C. The membrane thickness was controlled by pouring a definite amount of chitosan solution on Petri dish with the same surface area and measured by a micrometer. Thickness of each Ch membrane sample was measured at five different points and averaged. Ch membranes of average thickness of 250 μm were used for crosslinking.

Membranes crosslinked with sodium tripolyphosphate, (Ch/TPP), were prepared by immersing Ch membrane (1cm×1cm pieces) in 1.3 % (w/v) TPP solution of different pH for define time period (0.5, 1 and 24 h). The applied crosslinking conditions were as follows: T=4°C, pH = 9.0 (pH of initial TPP solution), 7.0 and 5.5 (initial TPP solution acidified with HCl). Ch/TPP membranes, designed as $Ch/TPP_{pH=5.5}$, $Ch/TPP_{pH=7.0}$ and $Ch/TPP_{pH=9.0}$, were then thoroughly washed with deionised water and dried, first at air and then under vacuum at 60 °C. All prepared chitosan membranes were stored in desiccator.

2.4. Swelling measurements

The swelling ratio after hydrogel membrane had swollen to equilibrium, S_{eq} , was measured by the gravimetric method. The preweighed, completely dried membrane sample was immersed in swelling medium (water, buffer or saline solution) at temperature 37 ºC for 24 h, which was checked to be sufficient to reach an equilibrium state. Then membrane was taken out, wiped quickly with filter paper and weighed. S_{eq}, expressed as the percentage of water in the membrane at equilibrium, was calculated using the following equation:

$$S_{eq} = \frac{W_s - W_d}{W_d} \cdot 100\% \qquad (1)$$

where W_s is the weight of the swollen membrane and W_d is the weight of the dry membrane.

To obtain a mean value of S_{eq} all experiments were performed at least three times for each membrane. Standard deviations were less than 5%.
In swelling experiments different buffered solutions of constant ionic strength and NaCl, $CaCl_2$ and $AlCl_3$ solutions of different concentration were used. The following buffer solutions were prepared: hydrochloric acid solutions (pH 1.0, 1.5, 2.0), 10 mM acetic acid-sodium acetate solutions (pH 3.5, 4.5 and 5.5) and 10 mM Tris buffered solutions (pH 6.9, 7.4, 8.5 and 9.5). The ionic strength of the mentioned buffered solutions was carefully adjusted to 0.145 M by adding appropriate amount of sodium chloride.

3. Results and Discussion

3.1. Membrane swelling in buffer solutions

Ch and Ch/TPP hydrogel membranes belong to a class of ionic hydrogel membranes. As discussed earlier by Peppas et al. [9, 10], Berger et al. [3] and Baipai and Singh [11], the equilibrium swelling of ionic hydrogels is a function of the network structure, crosslinking degree, hydrophilicity and ionization of the functional groups. The swelling behaviour of covalently and ionically crosslinked hydrogels depends also on degree of crosslinking and nature of the crosslinking agent [10, 12]. Moreover, ionic strength and pH can also affect the swelling of ionic hydrogels [9].

Ch and $Ch/TPP_{pH=5.5}$, $Ch/TPP_{pH=7.0}$, $Ch/TPP_{pH=9.0}$ hydrogel membranes differ both in their molecular structure and thus hydrophilic/hydrophobic properties, content of ionizable groups and degree of ionic crosslinking, as well as in supermolecular structure (degree of crystallinity). All membranes are semicrystalline polymers, but crystallinity of the Ch membrane decreased after crosslinking with TPP [5]. Thus, it could be expected some differences in swelling ability of non-crosslinked and TPP-crosslinked membranes.

Our earlier FTIR studies of Ch/TPP membranes [5] confirmed the presence of TPP in the membranes and indicated the formation of ionic crosslinks between protonated amino groups of chitosan and tripolyphosphate anions, as presented in Fig. 1. We have also observed the influence of reaction conditions (pH of reaction solution and time of crosslinking) on the content of TPP ions and/or crosslinking density of characterized membranes. We have found that for any studied crosslinking time the content of TPP ions and thus crosslinking density in the membranes decreased in the following order: $Ch/TPP_{pH=5.5} > Ch/TPP_{pH=7.0} > Ch/TPP_{pH=9.0}$.

The swelling of the chitosan hydrogels depends mainly on the protonation of the amino groups in the polymer chain, and the ionic crosslinking density in the network. In ionically crosslinked hydrogels the crosslinking density is modified by external conditions, mainly the pH of swelling medium [3, 13, 14]. pH of swelling medium influences global charge densities of chitosan and crosslinker, (TPP), which directly determines the crosslinking density, interactions and degree of swelling. The swelling characteristics of TPP-crosslinked chitosan membranes at different pH values are presented in Figs 2A-D. For all crosslinked membranes values of S_{eq} are strongly dependent on pH of swelling medium and they are high at low values of pH and decrease with increasing pH of buffer solution. Moreover S_{eq} depends on crosslinking conditions (Fig. 2D) and in acidic media decreased in series: $Ch/TPP_{pH=9.0} > Ch/TPP_{pH=7.0} > Ch/TPP_{pH=5.5}$.

Two main factors are responsible for differences in S_{eq} values with pH: changes in crosslinking density and protonation of free amino groups of chitosan. The changes in crosslinking density are a consequence of variation in the charge number of tripolyphosphate ions and the degree of ionization of chitosan. Both these factors can lead to disruption or formation of some TPP-chitosan ionic crosslinks. According to our calculations (Fig. 3) with the decrease of pH of solution from neutral to acidic the charge number of TPP decreases and thus crosslinking density slightly decreases.

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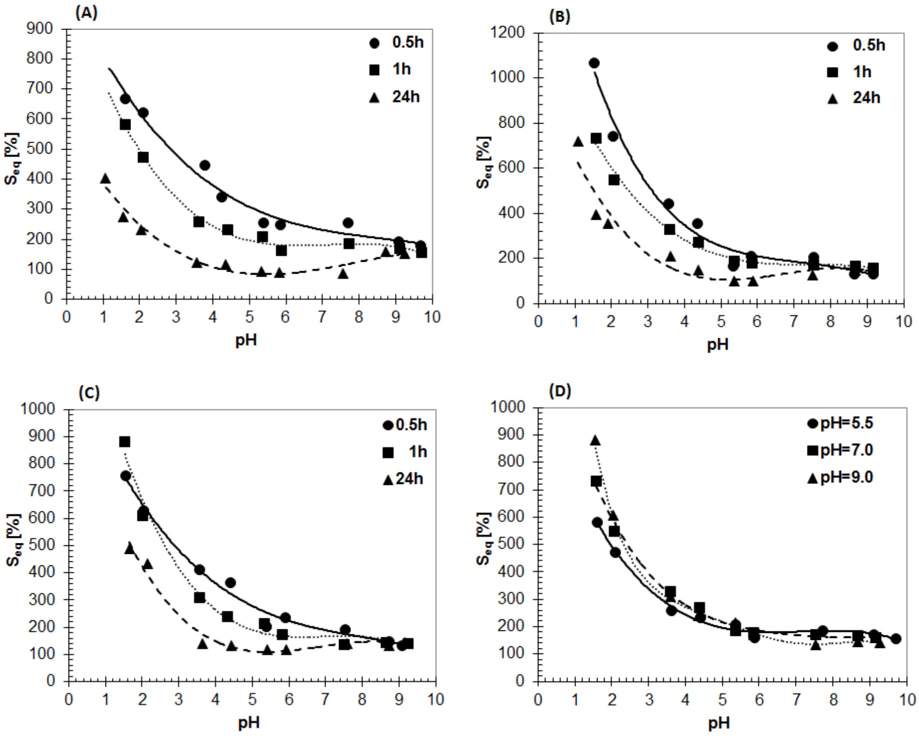

Figure 2. Equilibrium swelling ratio as a function of buffer pH for chitosan membranes crosslinked at different pH and time: pH_{TPP}=5.5 (A), pH_{TPP}=7.0 (B), pH_{TPP}=9.0 (C) and t = 1h (D).

As a result it can lead to higher membrane swelling. Similar phenomenon takes place in basic media where decrease in crosslinking density results from low degree of ionization of Ch. Protonation of “free”, unreacted amino groups of chitosan, i.e. groups not engaged in formation of ionic crosslinks, has also a great impact on membrane swelling. In acidic solutions (pH<4) almost all amino groups of chitosan are protonated (Fig. 3) and thus S_{eq} is high. In general, swelling is favoured by the $-NH_2$ groups protonation and repulsion of charged chitosan chains. If pH of swelling solution increases, the degree of ionization of Ch decreases and induces a decrease of swelling. At high values of pH almost all amino groups are neutralised (at pH 9 chitosan is practically completely deprotonated) and S_{eq} is very low. In such solutions hydrogel swelling is mainly controlled by the content of hydrophilic groups of non-protonated chitosan. Thus, the relationship between swelling and pH of swelling medium observed in Fig. 2 is a result of these both effects.

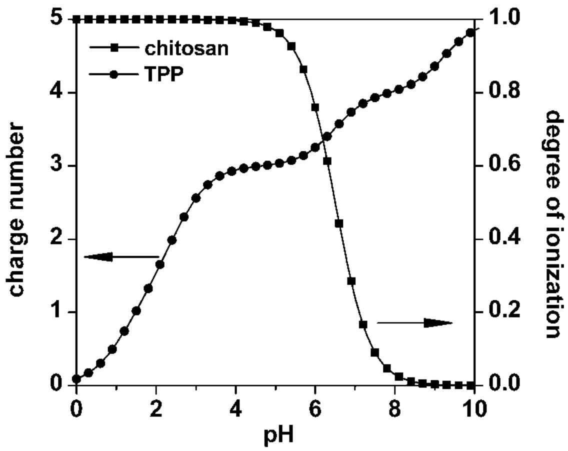

Figure 3. Charge number of tripolyphosphate ion and degree of ionization of chitosan as a function of pH.

3.2. Membrane swelling in water

The swelling behaviour of chitosan hydrogel membranes crosslinked at pH=5.5 and at time t = 0.5, 1 and 24 h is compared in Fig. 4. It can be seen, that the membrane with the lowest crosslinking time presented the highest hydrophilicity and equilibrium water content decreased with an increase of crosslinking time. The decrease in water content can be attributed to increase of the content of phosphate groups ionically bounded to protonated amino groups of chitosan.

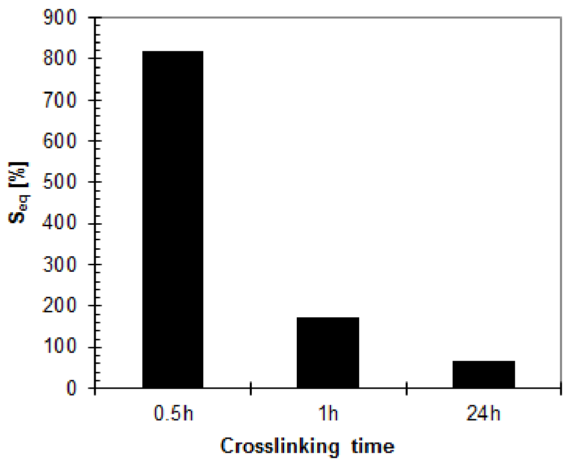

Figure 4. Swelling of $Ch/TPP_{pH=5.5}$ membrane in water

3.3. Membrane swelling in salt solutions

In these series of experiments the swelling capacity of Ch/TPP membranes was measured in NaCl, $CaCl_2$ and $AlCl_3$ solutions of different concentrations. Fig. 5 shows the equilibrium swelling ratio of the Ch/TPP hydrogels in different electrolyte solutions with various concentrations. The effect of salt concentration or ionic strength and cation type can be observed. Values of S_{eq} decrease with sodium chloride and aluminium chloride solution concentration. Similar dependencies were observed earlier by Norouzi et al. [15] for cationic hydrogels based on acrylamide. In the case of calcium chloride the relation S_{eq}=f(c_{salt}) is opposite and an increase in salt concentration within the range 0.05-0.30 M causes increase of degree of swelling. It is well known, that TPP ion binds strongly to metal cations, including calcium ions [16]. As a result, in $CaCl_2$ solutions degree of crosslinking diminishes and electrostatic interactions between positively charged amino groups increases resulting in gel swelling.

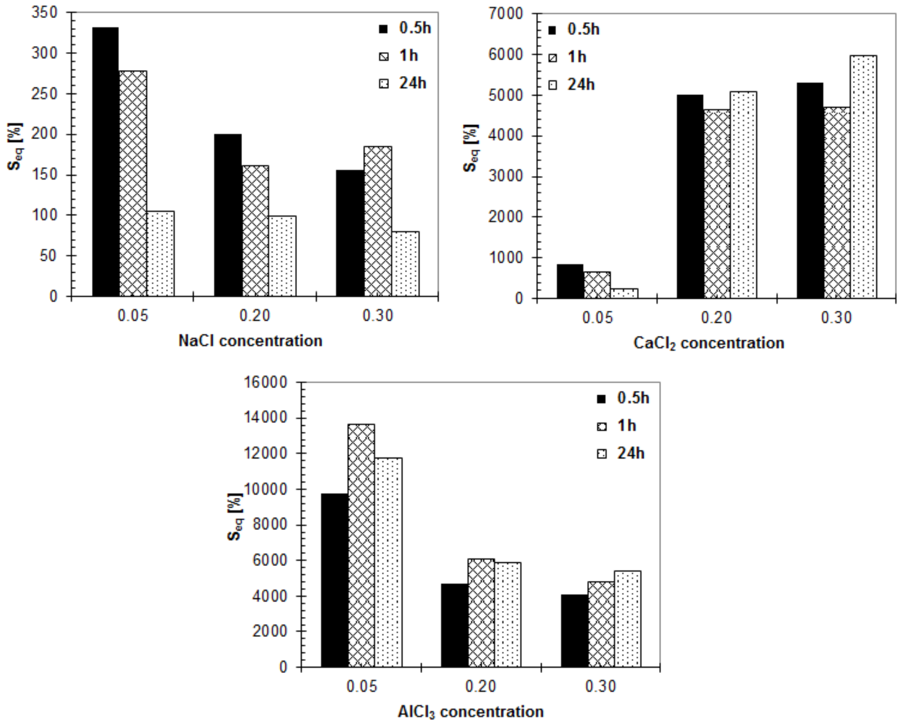

Figure 5. Equilibrium swelling ratio of $Ch/TPP_{pH=5.5}$ membrane in different salt solutions.

4. Conclusions

Modified hydrogel membranes were prepared by physical crosslinking of chitosan with sodium tripolyphosphate at different reaction conditions (different pH of TPP solution and different time of crosslinking). The swelling behaviour of Ch/TPP membranes in water, buffer and salt solutions were studied. It has been found, that equilibrium water content was dependent on the content of TPP ions in the membrane and crosslinling density. The swelling capacity of Ch/TPP membranes in buffer solutions was strongly dependent on pH of swelling medium. S_{eq} was high at low values of pH and decreased with increase of pH of buffer solution. Swelling behaviour of membranes in NaCl, $CaCl_2$ and $AlCl_3$ solutions of different concentration was dependent on cation type and salt concentration. Values of S_{eq} decreased with NaCl and $AlCl_3$ solution concentration but increased in the case of $CaCl_2$.

5. References

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