INFLUENCE OF CROSSLINKING PROCESS CONDITIONS ON MOLECULAR AND SUPERMOLECULAR STRUCTURE OF CHITOSAN HYDROGEL MEMBRANE

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Abstract
Unmodified (Ch) and ionically crosslinked with sodium tripolyphosphate (Ch/TPP) chitosan membranes were prepared. Various crosslinking conditions (pH, crosslinking time) were applied. Differences in membrane molecular structure was examined using FTIR spectroscopy. Scanning electron microscopy (SEM) coupled with Energy Dispersive X-Ray (EDX) Spectrometer as well as atomic force microscopy (AFM) were used for estimation of an effect of crosslinking conditions on supermolecular structure of chitosan membrane. Strong effect of pH of crosslinking TPP solution on crosslinking agent distribution inside the membranes and roughness of membrane surface was found. Differences in membrane morphology prior and after crosslinking results from differences in crosslinking density.

Key words: *crosslinking, chitosan, membrane, morphology, supermolecular stucture.*

1. Introduction

Recently considerable attention has been given to natural polymers, including also chitosan (Ch, ***Figure 1.a***). Chitosan in the form of capsules, beads, fibers or membranes found applications in many fields of life [1].

Because both chitosan powder as well as membranes, beads or fibers formed from this polymer are instable in acidic media, this polymer is modified using chemical or physical methods. One of the well known method of modification is crosslinking. Low- or highmolecular compounds, including ionic compounds, are used as crosslinking agents. Ionic crosslinking is known as one of simpler method of crosslinking and goes fast under mild conditions [2]. The crosslinking process has effect on numerous membrane properties, also on their sorption and transport properties. These properties are important with regard to the applications of membranes in different separation processes.
The aim of the presented work was synthesis of ionically crosslinked chitosan membranes using the low-molecular sodium tripolyphosphate (TPP) (***Figure 1.b***) as a crosslinking agent.

FTIR spectroscopy, scanning electron microscopy (SEM), energy dispersive X-Ray spectrometry (EDX) and atomic force microscopy (AFM) were used for estimation of effect of crosslinking process conditions on molecular and supermolecular structure of chitosan hydrogel membranes.

2. Materials and methods

2.1. Materials

Commercially available medium molecular weight chitosan (MMW-Ch) from crab shells (M_v = 730 kDa determined by viscometry [3, 4], degree of deacetylation DDA = 75.72 ± 3.82% determined by potentiometric titration method [3, 5]) and pentasodium tripolyphosphate (TPP) were purchased from Sigma Aldrich (Germany). Acetic acid (HAc), hydrochloric acid (HCl) and sodium hydroxide were analytical grade and were purchased from POCh (Poland). Potassium bromide (KBr) for spectroscopy was purchased from Merck (Germany).

2.2. Membrane preparation

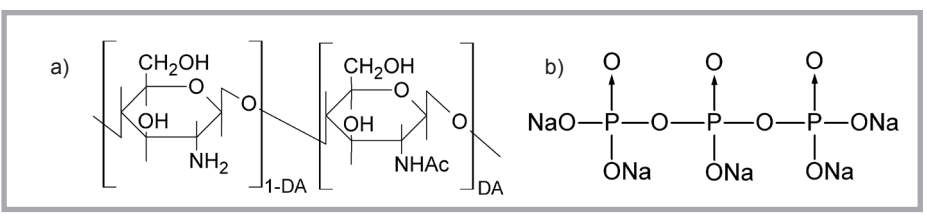

Figure 1. *Chemical structure of (a) chitosan (DA - degree of acetylation) and (b) pentasodium tripolyphosphate..*

Chitosan membranes (Ch) were prepared by casting solution and solvent evaporation technique. To obtain pure chitosan membranes 1 wt.% solution, prepared by dissolving chitosan powder in 2 wt.% HAc, was cast as film on clean glass plate, evaporated to dryness at 37 ºC and further dried under vacuum at 60 ºC.

Two-component chitosan/pentasodium tripolyphosphate (Ch/TPP) membranes were prepared by immersing of pure chitosan membranes in aqueous 1.3% (w/v) tripolyphosphate solution for define time period (30, 60, 90 and 120 min). Applied crosslinking conditions were as follows: $T_{crosslink.}$ = 4 ºC, pH = 9.0 (initial pH) or 5.5 (initial solution acidified with HCl).

2.3. FTIR spectroscopy

FTIR analysis of pentasodium tripolyphosphate powder, chitosan and ionically crosslinked chitosan/pentasodium tripolyphosphate membranes was performed using two techniques. Transmission FTIR spectra of powdered samples in KBr disc form were recorded on Perkin-Elmer 2000 FTIR spectrometer, as described in details elsewhere [6]. Reflectance FTIR spectra (ATR-FTIR spectra) of the membranes were recorded on Mattson Genesis II FTIR spectrometer, equipped with ATR (Pike) attachment. ATR accessory contained a ZnSe crystal (refractive index n = 2.43). The infrared transmission and reflectance spectra were recorded in the range from 4000 to 600 cm^{-1} with a resolution 4 cm^{-1} and 100 scans.

2.4. Microscopic analysis

The surface and cross-section morphology of pure and modified, thoroughly dried chitosan membranes and the elemental composition of polymer samples before and after crosslinking of chitosan membranes were examined using a scanning electron microscope (SEM), LEO 1430 VP model (LEO Electron Microscopy Ltd.), coupled with Energy Dispersive X-Ray (EDX) Spectrometer, Quantax 200 with XFlash 4010 Detector (Brucker). Prior to the surface observation samples were mounted on metallic base using double-sided carbon tape. SEM imaging of the surface of uncoated and coated with gold-palladium (10 nm thickness) polymer membranes was performed with potentials of 20 kV achieving magnifications ranging from 100 to 10 000. The specimens for the SEM and EDX images of the cross-sections of the membranes were prepared by fracturing the films in liquid nitrogen. SEM-EDX analysis was performed analogously to the surface imaging method but without Au-Pd coating.

The surface imaging was also performed using atomic force microscope (AFM) (Multimode Scanning Probe Microscope Nanoscope IIIa, Digital Instruments) operating in tapping and phase imaging modes in air. Roughness of the surface of analyzed membranes was compared using the root mean square (R_q) values, calculated using Digital Instruments v.5.12r2 software and expressed as [**7**]:

$$R_q = \sqrt{\frac{\sum(Z_i - Z_{avg})^2}{N}}$$

where Z_{avg} is the average of the Z values (Z - difference between the highest and the lowest point) within the given area, Z_i is the current value, and N is the number of points within a given area.

In the EDX spectra of non-crosslinked and crosslinked chitosan membranes the peaks corresponding to such elements as carbon, nitrogen, oxygen and phosphorous were observed. The quantitative elemental analysis of studied polymer samples was carried out by EDX software and the percentage content of phosphorous and carbon and/or the P/C mass ratio in the membrane cross-sections was estimated. Because the crosslinking process does not influence the carbon content in membrane cross-section, hence this element was used as a reference.

3. Results and discussion

In ***Figure 2*** the transmission infrared spectra of powdered pentasodium tripolyphosphate (TPP), chitosan (Ch) and ionically crosslinked chitosan/pentasodium tripolyphosphate (synthesized by crosslinking in TPP solution of different pH values) are shown.

As we have discussed in details earlier [**6**], two absorption bands appearing in the spectrum of chitosan membrane crosslinked with TPP: (i) the band at 1635 cm^{-1}, assigned

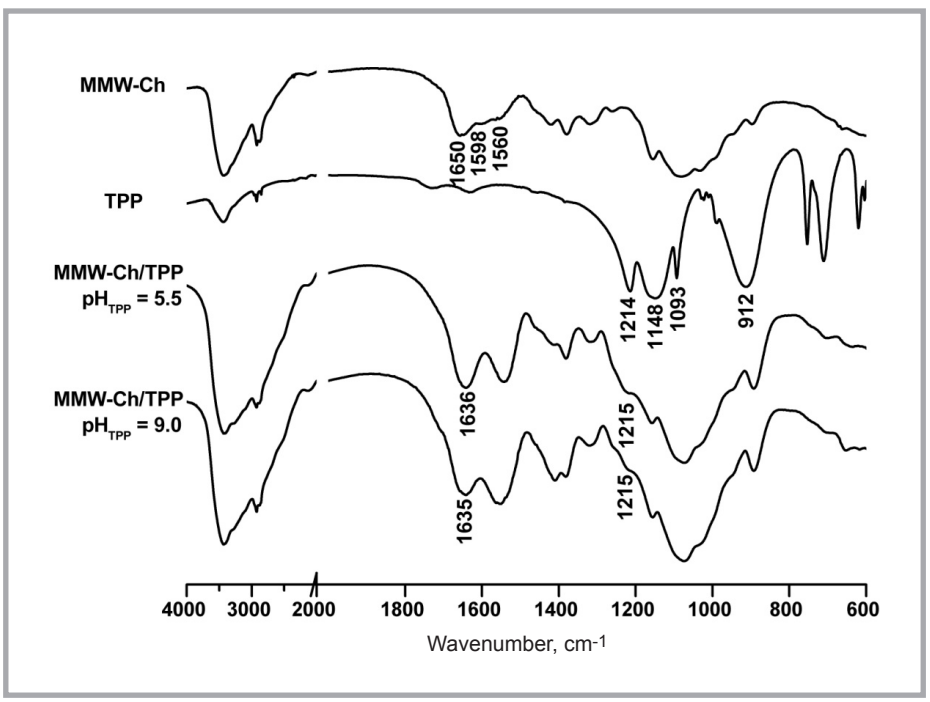

Figure 2. *FTIR spectra of chitosan, pentasodium tripolyphosphate and ionically crosslinked chitosan/pentasodium tripolyphosphate membranes.*

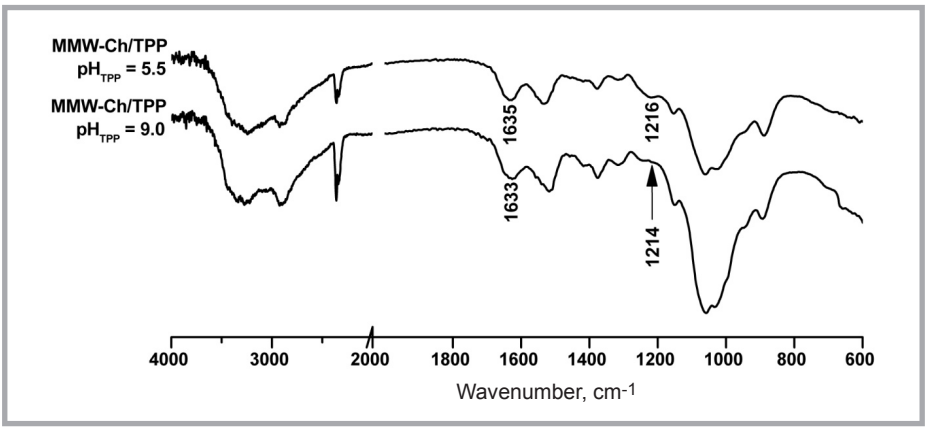

Figure 3. *FTIR-ATR spectra of chitosan membranes crosslinked with TPP at pH = 5.5 and pH = 9.0.*

to antisymmetric deformation N-H vibrations in NH_3^+ ion and (ii) the band at 1215 cm^{-1}, corresponding to antisymmetric stretching vibrations of PO_2^- groups, confirms the formation of ionic crosslinks between NH_3^+ groups of chitosan and tripolyphosphate ions [6, 8].

Because the applied crosslinking procedure, described in chapter 2.2, is based on the diffusion of tripolyphosphate into the pure chitosan membrane, the most visible changes in a membrane molecular structure should be observed on a membrane surface. For that reason FTIR-ATR spectra of chitosan membrane crosslinked with TPP at different pH of crosslinking TPP solution were recorded and compared (***Figure 3***).

It can be seen some differences in the intensity of the absorption band assigned to antisymmetric stretching vibrations of PO_2^- group in the spectra of chitosan membranes crosslinked with TPP at pH 5.5 and 9.0. The intensity of the PO_2^- band increases as pH of TPP solution decreases. It seems to indicate on differences in TPP content on membrane surface and thus differences in crosslinking density. Using TPP solution of pH_{TPP} = 5.5 led to the formation of Ch/TPP membranes with the higher crosslinking density.

Data on the phosphorous content in the cross-sections of membranes were also obtained from SEM-EDX spectra analysis. In the EDX spectrum of non-modified chitosan membranes the peaks corresponding to carbon, nitrogen and oxygen were observed, but in the spectra of TPP crosslinked membranes an additional peak of phosphorous appeared. The P/C mass ratio in the five points of Ch/TPP membrane cross-sections is presented in ***Figure 4***. Values of membrane cross-section equal 0 and 1 refer to membrane surfaces and value of 0.5 refers to cross-section centre.

As can be seen, the P/C mass ratio for Ch/TPP membrane crosslinked with TPP at pH_{TPP} = 9.0 reaches highest value near membrane surface and markedly decreases toward the membrane centre. However, the P/C mass ratio for Ch/TPP membrane crosslinked with TPP at pH_{TPP} = 5.5 is practically constant and higher, even near membrane surface, than P/C mass ratio for Ch/TPP (pH_{TPP} = 9.0) membrane. Analogous results were observed by

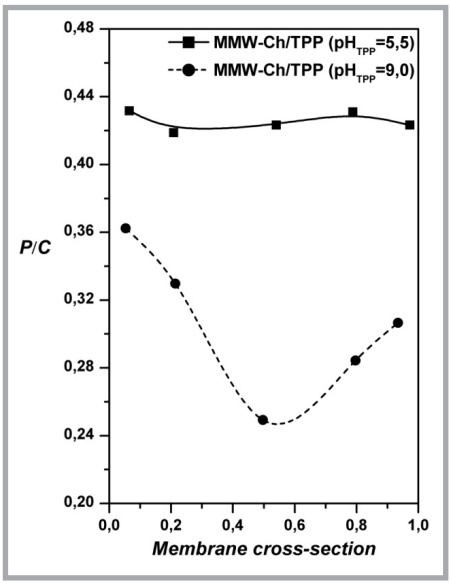

Figure 4. *P/C mass ratio in the cross-sections of Ch/TPP membranes (values of 0 and 1 on X-axis correspond to membrane surfaces).*

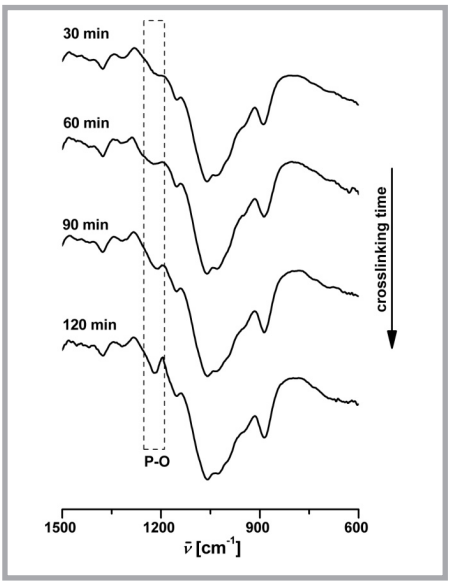

Figure 5. *Effect of crosslinking time on molecular structure of ionically crosslinked Ch/TPP membranes.*

Mi and coworkers [**9**] for chitosan beads prepared by dropping chitosan solution into TPP solution of different pH.

Variation in TPP content and crosslinking density in Ch/TPP membranes obtained at various pH conditions results from differences in chitosan and TPP ionization degree. The intrinsic pK_a of chitosan is about 6.3 [**10**] and in basic medium it is only slightly ionized. TPP, being a salt of a weak polyprotic acid, hydrolyzes in water. Because of this in TPP water solution co-exist OH^- and TPP ions ($P_3O_{10}^{5-}$, $HP_3O_{10}^{4-}$ and $H_2P_3O_{10}^{3-}$ and $H_3P_3O_{10}^{2-}$ and $H_4P_3O_{10}^{-}$). Taking into account K_a values of polyphosphoric acid decreasing from K_{a1} to K_{a5} in a quantity of several orders if magnitude [11], it can be supposed that the concentrations of $HP_3O_{10}^{4-}$ and $H_2P_3O_{10}^{3-}$ and $H_3P_3O_{10}^{2-}$ and $H_4P_3O_{10}^{-}$ are rather small. In TPP water solution (pH = 9) all these anions can competitively interact with protonated amine groups of chitosan: TPP ions in crosslinking reaction and OH^- ions in deprotonation process. If pH of TPP solution decreases, then the number of OH^- ions decreases and the number of $-NH_3^+$ ions increases. In acidic solution only TPP ions are present in solution. These ions diffuse into chitosan membrane and interact with $-NH_3^+$ binding sites of chitosan. TPP can form either intermolecular or intramolecular ionic bonds with chitosan. Differences in ionization process and crosslinking mechanism, discussed above, explain both the variation in molecular structure of membranes prepared in different pH conditions as well as differences in membranes crosslinking density.

Figure 5 presents the FTIR-ATR spectra of chitosan membranes crosslinked in TPP solution of pH_{TPP} = 5.5 and at different time. It could be seen that intensity of the PO_2^-

Table 1. *Roughness measurements (in nm) with tapping mode AFM for 10x10 μm² scans of Ch/TPP membranes.*

Membrane	Ch	Ch/TPP (pH_{TPP} = 5.5)	Ch/TPP (pH_{TPP} = 9.0)
Roughness (R_q), nm	3.56 ± 0.26	23.71 ± 1.98	4.86 ± 0.26

band increases with increasing crosslinking time. This result suggests that both TPP content and crosslinking density increase with time of membrane modification.

Effect of ionic modification conditions on membrane morphology can be discussed using scanning electron microscopy images. Scanning electron micrograph of the surface of chitosan membrane crosslinked with TPP at pH=9.0 revealed that the composition of membrane is homogeneous (lack of differences in color). Light points visible on image of Ch/TPP (pH_{TPP} = 5.5) surface indicated on zones with an average molar mass of elements higher than a molar mass of carbon. Some differences in the membrane surface structures was also observed on images of Au-Pd coated samples. The surface of Ch and Ch/TPP (pH_{TPP} = 9.0) is smooth. On Ch/TPP (pH_{TPP} = 5.5) surface numerous lumps are visible. Variation in membrane surface morphology discussed above results probably from higher crosslinking density of Ch/TPP (pH_{TPP} = 5.5) membrane and stay in good agreement with our FTIR results and results presented earlier by Shu and co-workers [12] for citrate crosslinked chitosan films. Cross-sectional SEM images indicated that both unmodified as well as modified chitosan membranes are dense.

The observed differences in surface morphology of Ch membranes crosslinked with TPP at pH 5.5 and 9.0 confirm also results of AFM analysis. The roughness measurements are given in ***Table 1***.

From the presented results it appears that R_q value of chitosan membrane modified with TPP solution of pH = 9.0 is only slightly higher than roughness of unmodified chitosan membrane, but it is much higher in the case of the membrane crosslinked with TPP solution of pH = 5.5. Roughness of Ch/TPP (pH_{TPP} = 5.5) membrane increases a few times.

4. Conclusions

Chitosan membrane can be easily modified by treating it with low-molecular tripolyphosphate solution. Modification leads to the formation of ionic crosslinks between protonated amine groups of chitosan and dissociated tripolyphosphate. The applied conditions of the crosslinking process strongly affect both the molecular as well as the supermolecular structure of chitosan membrane. The lower TPP solution pH and longer crosslinking time leads to Ch/TPP membrane of higher crosslinking density. Depending on pH of crosslinking solution membranes differ in surface composition and roughness. All synthesized membranes exhibit dense structure without visible pores.

5. References

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