Peroxide-Containing Chitosan Derivative for Hydrogel Synthesis

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Summary: Peroxide-containing chitosan was synthesized using a polymer-analogous reaction between chitosan and tert-butylperoxymethyl ester of maleic acid. Peroxide-containing chitosan macromolecules cross-link into a polymeric hydrogel network in the presence of 1-vinyl-2-pyrrolidone upon heating. Polymeric hydrogels are formed due to reactions of radical chain transfer and recombination. Reacting radical species are chitosan macroradicals and poly(vinylpyrrolidone) macroradicals formed by decomposition of peroxide-containing fragments. Synthesized hydrogels are pH-responsive and facilitate drug release.

Keywords: drug release by hydrogels; peroxide-containing chitosan; pH-responsive hydrogels

Introduction

Chitosan, poly-β-1,4-(2-desoxy-2-amino-D-glucose), is produced by deacetylation of chitin, the second most redundant natural polysaccharide after cellulose. Chitosan derivatives are widely used in a variety of biomedical applications, as they possess anti-radiation, antimicrobial, preservative properties, and biodegradability.

The fabrication of responsive (sensitive to pH, temperature and solvents) polymeric hydrogels is considered as an important challenge in biopolymer chemistry, for use in implant manufacture or the development of novel drug delivery systems based on hydrogels.[1]

In the literature, few publications report on the peroxide-containing derivatives of cellulose,[2] dextran,[3] and starch[4] synthesized by grafting peroxide-containing oligomers, or polymer-analogous reactions of polysaccharides. These derivatives are promising materials for various applications. Peroxide-containing polysaccharide fillers improve the physical and mechanical properties of composite materials,[2] as well as impart biodegradability[3] and hemocompatibility.[5]

In this work, we targeted the synthesis of peroxide-containing chitosan, using the polymer-analogous reaction between chitosan and tert-butylperoxymethyl ester of maleic acid. Our further goal was to fabricate polymeric hydrogels, using radical reactions between peroxidized chitosan and 1-vinyl-2-pyrrolidone.

Experimental Part

Materials

Chitosan (Chi) (Aldrich, molecular weight 53,200 g/mol, deacetylation degree 73%) was used as received. 1-Vinyl-2-pyrrolidone (VP) (Aldrich) was distilled under reduced pressure prior to use. Maleic anhydride (MA) (Aldrich) was purified using re-crystallization in trichloromethane. Tert-butylperoxymethanol (TBPM) was synthesized using the method described in Ref.[6] Ammonium peroxodisulfate (PSA) (Aldrich) was used as received. Sodium cefazoline (SC) (Arterium) was used as received.

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Synthesis

Tert-butylperoxymethyl ester of maleic acid (PM) was synthesized using acylation of TBPM with MA in the presence of catalyst.[7]

Peroxide-containing chitosan (Chi-PM) was synthesized using 1% w/w Chi (62×10^{-3} mol/l) and different PM concentrations in aqueous solution at 20 °C under stirring for 30 min.

Copolymerization of Chi-PM and VP to form a hydrogel network (Chi-PVP) was carried out using 1% w/w Chi-PM and different VP concentrations in aqueous solution at 60 °C. The residual VP monomer was extracted from the hydrogel network using aqueous solution of acetic acid (pH 2), followed by washing with distilled water.

Methods

The intrinsic viscosity, [η] of Chi (in aqueous solution of acetic acid, at constant 1:3 molar ratio of NH_2 groups of Chi to COOH groups of acetic acid during all measurements) was measured at 25 °C using an Ubbelohde viscometer.

The Chi viscosity average molecular weight ($M\upsilon$) was calculated using the Mark-Houwink equation:

$$[\eta] = K \cdot M\upsilon^{\alpha} \quad (1)$$

where $M\upsilon$ - viscosity average molecular weight of Chi, [η] - intrinsic viscosity of Chi.

K and α (Eq. 1) were evaluated using Eq. 2 and 3:[8]

$$K = 1.64 \cdot 10^{-30} \cdot DA^{14.0} \quad (2)$$

$$a = -1.02 \cdot 10^{-2} \cdot DA + 1.82 \quad (3)$$

where DA - Chi deacetylation degree, %.

The deacetylation degree (DA) of Chi was determined using inverse pH-metric titration of primary amino groups.

The effect of a radical initiator on decomposition of Chi macromolecules was investigated by heating the Chi aqueous solution at 60 °C for 4 hours in the presence of different radical initiator (PM or PSA) at different concentrations. Afterwards, the Chi was precipitated using aqueous solution of ammonium hydroxide (pH 10), isolated by centrifugation, washed with distilled water and dried in a vacuum. The intrinsic viscosity of Chi samples after the heating in the presence of radical initiator was measured as described above.

To determine the Chi-PVP gel-fraction, the hydrogel samples were dried to constant weight after several extractions with aqueous solution of acetic acid (pH 2), followed by immersion in distilled water after final extraction.

Equilibrium swelling of Chi-PVP hydrogels in aqueous media at different pH was calculated using Eq. 4:[9]

$$Es = [(W_E\text{-}W_D)/W_D], \quad (4)$$

where Es - equilibrium swelling of Chi-PVP hydrogel, g/g, W_E and W_D - weight of the swollen and dried sample, g.

Drug loading by Chi-PVP hydrogels were monitored using an aqueous solution of SC (Figure 1). Dried samples of Chi-PVP hydrogel were immersed in an aqueous SC solution ($1.2 \cdot 10^{-3}$ mol/l) for 24 hours at pH 4 and dried to constant weight.

Rate of SC release from the Chi-PVP hydrogel samples to an aqueous medium was monitored at pH 5.5 by recording the absorption intensity at 272 nm using a Cary Varian 100 UV-Vis spectrophotometer.

The IR spectra of Chi and Chi-PM thin films were recorded using a Specord-M80 spectrophotometer in the range of 4000–500 cm^{-1}.

Results and Discussion

Interaction between primary amino groups located in deacetylated Chi fragments and carboxyl groups in PM results in Chi-PM formation (Scheme 1):

Figure 2 shows IR-spectra of Chi (before interaction with PM) and of Chi-PM. New absorbance bands are observed in the Chi-PM spectrum. The absorption band at 3060 cm^{-1} reveals the presence of a CH=CH group with a conjugated bond.

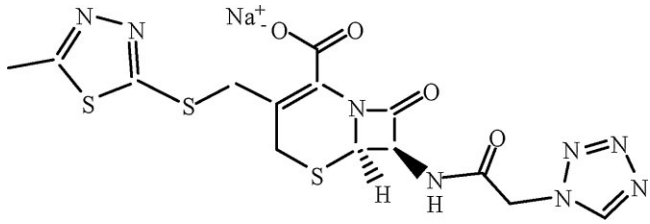
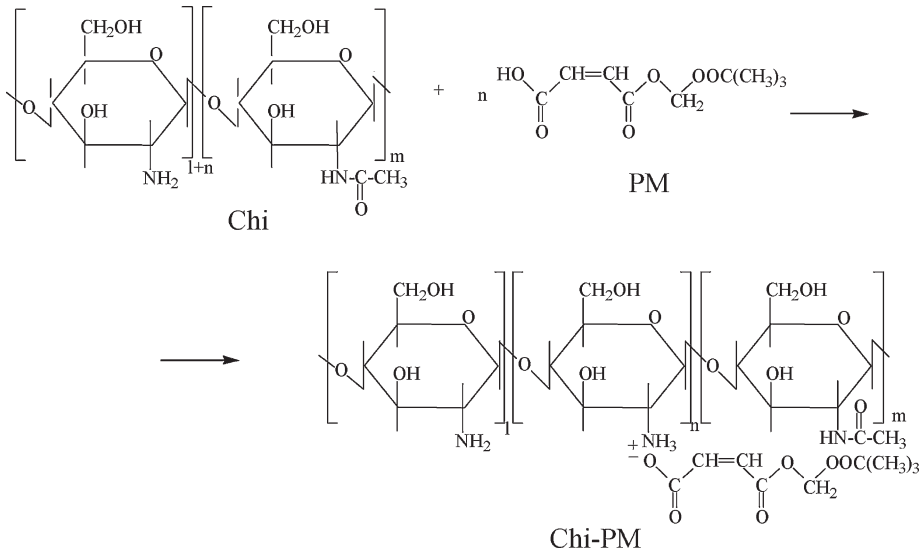


Figure 1.
Chemical structure of Sodium Cefazoline.



Scheme 1.
Synthesis of Chi-PM.

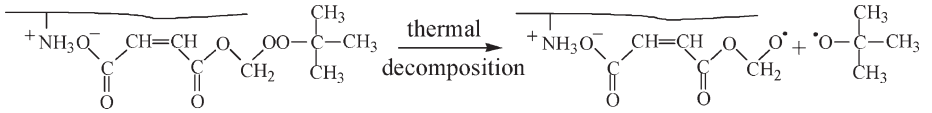
The doublet at 1380-1360 cm^{-1} and the single band at 1200 cm^{-1} are characteristic of tert-butyl residue.[10] Bands at 864 cm^{-1} and 888 cm^{-1} can be attributed to a tert-butoxyl fragment and an –OO– group, respectively.[11] All these bands indicate the presence of peroxide fragments in the Chi-PM macromolecule.

Absorbance bands at 1580 cm^{-1} and 1540 cm^{-1} in the spectrum of Chi-PM are typical of the carboxylate group (COO^-). The band at 1780 cm^{-1} can be attributed to valence vibrations of C=O ((νC=O)) in the carboxyl group. Broadening at 3000 cm^{-1} is characteristic for the ammonium (NH_3^+) group in a condensed state.[10] The appearance of carboxylate and ammonium groups provides strong evidence that a salt is formed when Chi interacts with PM.

Peroxide groups of Chi-PM in aqueous solution decompose upon heating at 60 °C. Decomposition results in formation of radicals (Scheme 2)[12] initiating polymerization.[13]

It is known that in presence of a radical initiator, Chi undergoes reactions of chain transfer and recombination.[14–18] Cross-linking of Chi macromolecules provides an opportunity to synthesize polymeric hydrogels based on Chi-containing copolymers.

It has been shown that the interaction of Chi and the radicals formed during PM thermolysis is not accompanied by degradation of the Chi chain. Data from Table 1



Scheme 2.
Thermal decomposition of peroxide groups in Chi-PM.

Table 1.
Intrinsic viscosity [η] of Chi samples after heating in the presence of peroxide initiator (PM or PSA) for 4 hours at 60 °C *

In the presence of PM			In the presence of PSA		
Sample	PM $\times 10^3$, mol/l,	[η] dL/g	Sample	PSA $\times 10^3$, mol/l,	[η] dL/g
Chi	0	19	Chi	0	19
Chi-1	1	27	Chi-4	2.2	2.38
Chi-2	2	35.1	Chi-5	4.4	1.64
Chi-3	3	51	Chi-6	11	0.9

*Concentration of Chi – 62×10^{-3}, mol/l.

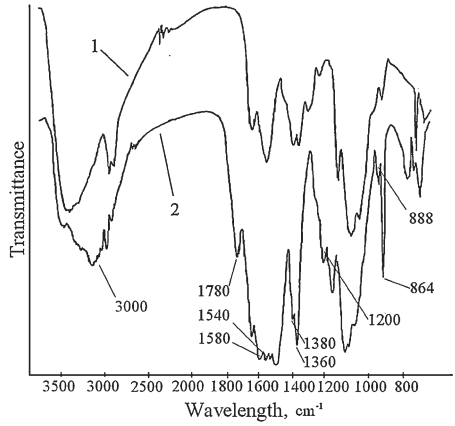


Figure 2.
IR-spectra of Chi (1) and Chi-PM (2).

reveal that the intrinsic viscosity of Chi increases after thermostating with PM, depending on quantity of peroxide fragments. We suggest that after heating of peroxide-containing Chi-PM, due to decomposition of peroxide groups, the following process occurs: branching of Chi macromolecules due to macroradical recombination. This fact is proven by the increase in intrinsic viscosity of the Chi (Table 1).

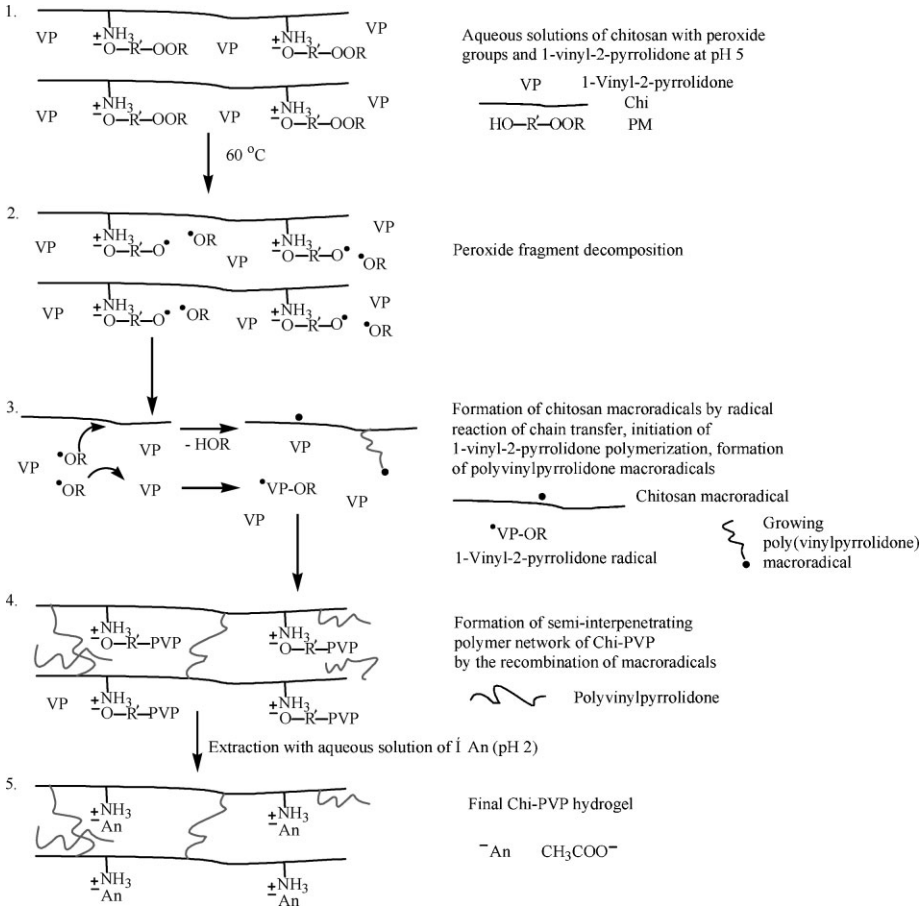
The advantage of using Chi-PM is that the Chi is not fragmented under the influence of radicals generated, whereas radicals generated by PSA lead to decomposition of Chi chain. Copolymerization of Chi with other monomers is commonly initiated by PSA.[19–21] This process is accompanied by degradation of Chi due to the cleavage of the C_1-C_4 bonds, making it difficult to obtain hydrogel.[22]

In our experiments, we fabricated Chi-based hydrogels in the presence of VP (Scheme 3).

Polymerization of VP has been initiated by PM-generated tert-butoxyl radicals and Chi macroradicals (Scheme 3 stages 2 and 3). The recombination reaction between macroradicals results in macromolecules cross-linking and the formation of a polymeric network (Scheme 3 stage 4). Depending on the reagents' composition, the gel-fraction of synthesized Chi-PVP hydrogels varies between 31 and 60%. It is our assumption that a semi-interpenetrating polymeric network is formed. It consists of covalently cross-linked Chi-PVP and physically bonded PVP macromolecules.[23]

Proposed convenient method of hydrogel synthesis, utilizing peroxides, foresees almost complete after-synthesis elimination of peroxide fragments from the content of final hydrogel structure.

The swelling rate of synthesized polymeric hydrogels depends on pH and copolymer composition (Figure 3) indicating that synthesized hydrogels are pH-sensitive. At lower pH, it is expected that Chi fragments with amino groups are protonated and solvated. Positively-charged Chi chains thus repel each other, resulting in polymer network volume enlargement (swelling). The degree of



Scheme 3.
Principal schematic for Chi-PVP hydrogel synthesis.

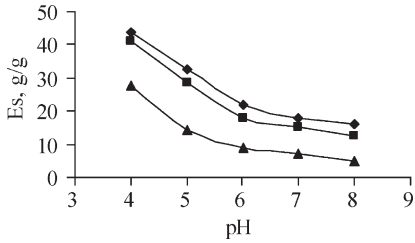


Figure 3.
Equilibrium swelling of Chi-PVP hydrogels vs. pH. Synthetic conditions: [Chi] = $62 \cdot 10^{-3}$ mol/l; [VP] = $930 \cdot 10^{-3}$ mol/l; [PM]: ◆ – $1.0 \cdot 10^{-3}$ mol/l, ■ – $2.0 \cdot 10^{-3}$ mol/l, ▲ – $3.0 \cdot 10^{-3}$ mol/l.

ionization of basic amino groups in Chi fragments changes with pH. When pH decreases, the number of ionized amino groups increases and swelling becomes more pronounced, due to stronger electrostatic repulsion between charged Chi fragments. When pH increases, the degree of ionization decreases as well, as does the swelling, and the hydrogel network transforms into a more collapsed form.[24]

Our first experiments on loading and release of SC as a model drug substance show that the loading capacity of Chi-PVP hydrogels varies between 80 and 120 mg of SC per 1 g of dry hydrogel (Figure 4a). The release of SC from the hydrogel samples varies between 22 and 28% within the first

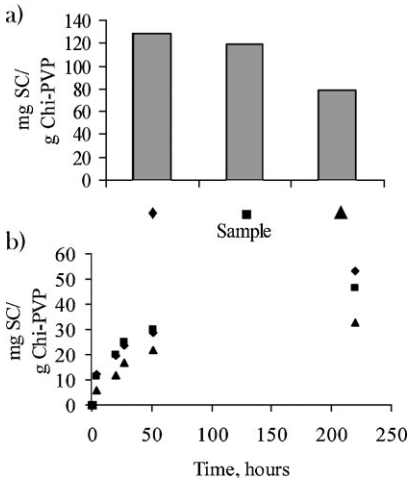


Figure 4.
Equilibrium SC loading (a) and release (b) by Chi-PVP hydrogels. Synthetic conditions: [Chi] = $62 \cdot 10^{-3}$ mol/l; [VP] = $930 \cdot 10^{-3}$ mol/l; [PM]: ◆ – $1.0 \cdot 10^{-3}$ mol/l, ■ – $2.0 \cdot 10^{-3}$ mol/l, ▲ – $3.0 \cdot 10^{-3}$ mol/l.

50 hours of the experiment, and between 33 and 54% within 220 hours of the experiment (Figure 4b).

Conclusion

Peroxide-containing chitosan with a controllable number of primary-tertiary peroxide groups was synthesized from chitosan and tert-butylperoxymethyl ester of maleic acid. Peroxide-containing chitosan macromolecules cross-link into a polymeric network in the presence of 1-vinyl-2-pyrrolidone at 60 °C. Polymeric hydrogels are formed due to reactions of radical chain transfer and recombination. Reacting radical species are chitosan macroradicals and poly(vinylpyrrolidone) macroradicals formed by decomposition of peroxide-containing fragments. The synthesized hydrogels are pH-sensitive and facilitate drug release.

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