DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 21(7), 839-846 (1995)

HEAT TREATMENT OF CHITOSAN FILMS

L Y Lim and Lucy S C Wan
Department of Pharmacy, National University of Singapore
10 Kent Ridge Crescent, Singapore 0511

ABSTRACT

Chitosan films, prepared from aqueous solutions of chitosan in 1% v/v acetic acid, were subjected to heat treatment in an oven at known temperatures for specific durations. The effect of heat treatment on the water resistance of the films was measured by the extent of swelling and dissolution of the films in aqueous media of different pH. The results were correlated to heat induced changes in the physical characteristics of the films. Heat treatment enhanced to a greater extent the water resistance of chitosan films in a medium of low pH than in one of high pH. The improved water resistance may be attributed to the formation of crosslinks and/or crystallites in the films. Both the temperature and duration of heat treatment influenced the degree of heat induced changes in the films.

INTRODUCTION

Chitosan is a deacetylated derivative of chitin, a polysaccharide found in abundance in the shells of marine crustaceans. Due to its good biocompatibility, chitosan has been formulated into microspheres[1], beads[2] and membranes[3], and used as a tablet excipient[4] to effect the controlled release of drugs. The solubility of chitosan in aqueous media of low pH is advantageous in that noxious organic solvents may not be required in formulation. Yet this same property can limit the effectiveness of a chitosan matrix to control the delivery of incorporated drugs. Chitosan and other aqueous soluble polymers eg sodium alginate[5], gelatin[6] and polyvinyl alcohol[7] have had their water resistance enhanced after interaction with formaldehyde or glutaraldehyde. These crosslinking agents are, however, toxic and residues in the final product may cause adverse reactions on skin and mucous membranes[8].

An alternative method to increase the water resistance of aqueous soluble polymers is to subject the polymers to heat treatment. In this study, the effect of heat treatment on the water resistance of chitosan films was investigated. The water resistance was measured in terms of aqueous solubility and extent of swelling of the films in different media, and correlated to heat-induced changes in the physical characteristics of the films.

MATERIALS

Chitosan (Tokyo Kasei Kogyo Co. Ltd.), potassium dihydrogen orthophosphate (AR, BDH Ltd.), sodium hydroxide (AR, BDH Ltd.) were used as supplied. Acetic acid and hydrochloric acid (GR) were from Merck.

METHOD

1. Film preparation

Weighed amounts of chitosan were dissolved in 1% v/v acetic acid to give 1% w/v solutions. The solutions were filtered and degassed to remove entrapped air bubbles. Twenty ml of solution were pipetted into plastic petri dishes having a diameter of 85 mm, and allowed to dry on a levelled surface for 16 hours in an oven at a temperature of 60°C. The dried films can easily be peeled off the petri dishes. The films were cut into discs measuring 30 mm in diameter and heat treated in an oven at 90°C, 120°C and 150°C for known periods ranging from 1 to 4 hrs. They were then stored in desiccators at ambient temperature for at least 24 hrs prior to analyses.

2. Determination of water resistance

Films were weighed (W_1) before being placed into 100 ml of dissolution medium in a covered conical flask maintained at 37°C. The dissolution media used were 0.1M HCl (pH 1.4), distilled water (pH 5.7) and pH 7.4 phosphate buffer (USP). After 24 hours of immersion, the film remnants were recovered, wiped of excess surface moisture using filter paper, and weighed (W_2). The swollen films were dried to constant weight in an oven at 60°C and reweighed (W_3). The extent of aqueous dissolution was calculated as the percent difference between the initial and final weights of the dried film. The capacity for water uptake was measured by a swelling index which was the ratio of (W_2 - W_3) and W_3. The experiments were performed in triplicates.

3. Film Characterisation

The chitosan films were characterised using IR spectroscopy (Philips PU 9624 FTIR), differential scanning calorimetry (Perkin Elmer DSC 4) and X-ray diffraction (Rigaku 2027). To obtain the IR spectra, thinner films were used and these were prepared from 0.25% w/v chitosan solutions in 1% v/v acetic acid. DSC thermograms were obtained by scanning 3mg film samples from 60°C to 340°C at a rate of 20°C/min. The X-ray crystallograms of film samples were taken at room temperature using a Ni-filtered CuKα radiation generated at 30kV and 5mA. The samples were scanned from 5 - 30° (2θ) at a rate of 4° (2θ)/min. Films were also measured for thickness using a Mitutoyo thickness gauge. The surface morphology of the films was viewed under a scanning electron microscope (SEM) (Joel JSM 5200) operating at 25°C with a beam voltage of 15kV. Samples for SEM were mounted on studs and coated for 120 s with a layer of gold using a sputter coater (Bio-Rad SC502).

RESULTS AND DISCUSSION

Films obtained from drying the solution of chitosan in acetic acid were transparent, flexible (even after desiccation) and had a tinge of yellow colour. SEM micrographs showed the films to be homogeneous in appearance with no apparent fissures or pores on either of their surfaces. Average thickness of the chitosan films was 37.82 ± 4.38 μm.

As has been reported[9,10], the solubility of chitosan films differed according to the dissolution medium used. The films were most soluble in 0.1M HCl and least soluble in pH 7.4 phosphate buffer. Whereas films immersed for 24 hours in the phosphate buffer retained their integrity, films immersed in the acid medium swelled considerably, softened and eventually dissolved within the same period. Weight differences prior to and after immersion showed that 30% of the chitosan films had dissolved in pH 7.4 phosphate buffer. Film remnants retrieved from the phosphate buffer medium had a swelling index of 3.58 ± 0.15, indicating a capacity to imbibe 3.5 times their weight of water. Immersion in distilled water caused the chitosan films to swell and disintegrate. Dissolution of the films was, however, incomplete after 24 hours of immersion. Small pieces of transparent gel remained in the medium but these were too soft to be retrieved for weight measurements. SEM micrographs of film remnants retrieved from the phosphate buffer medium were similar to those of films retrieved from the distilled water medium. The film remnants showed surface erosion, but pores were absent.

The difference in solubility of a chitosan film in the different media may be attributed to the ionisation of amino groups in the chitosan molecules[10]. In an acid medium, the protonation of amino groups increased charge density along the molecular chain, causing the chain to unfold and assume an elongated conformation. Swelling, followed by dissolution of the film were observed due to the solvation of molecular chains in the film. Protonation of the amino groups is not favoured in solutions of relatively higher pH. However, as the film was not neutralised upon formation, chitosan molecules in the film may still be protonated in distilled water because of the presence of acetic acid in the film. Considerable degrees of swelling and dissolution were therefore observed upon immersion of the chitosan film in distilled water. In the pH 7.4 phosphate buffer which contains sodium hydroxide, the chitosan molecules will shrink and become more compact following the neutralisation of the protonated amino groups. In addition, the phosphate buffer has a relatively high ionic strength which may cause the desolvation of chitosan molecules. For these reasons, swelling and dissolution of the chitosan film in the phosphate buffer medium were restricted.

Heat treatment for a duration of 1 hour reduced film solubility to different extent in the three media (figure 1). Heat treatment at 90°C markedly reduced the solubility of a chitosan film in distilled water but further increase in the temperature of treatment produced smaller reduction in solubility. Film solubility in the acid medium, as well as in the phosphate buffer medium, decreased proportionately with higher temperatures of heat treatment. However, the extent of decrease in solubility in the phosphate buffer was smaller than in the acid medium. Heat treatment also reduced the differences in film solubility in the three media. The untreated films had disparate solubilities, while films heat treated at 150°C dissolved to the same extent (15%) in the three media.

The effect of heat treatment for one hour on the swelling index of chitosan films is illustrated in figure 1. The capacity of the untreated films to imbibe water in the acid medium was consistently higher than in the other two media. Heat treatment reduced most significantly the swelling index of chitosan films in the acid medium, and an inverse linear relationship between swelling index and temperature of heat treatment was obtained. On the other hand, the swelling index of chitosan films in phosphate buffer was not affected by heat treatment. The swelling index of films in distilled water was reduced by heat treatment at 90°C, but further rises in the temperature of treatment did not produce an enhanced effect.

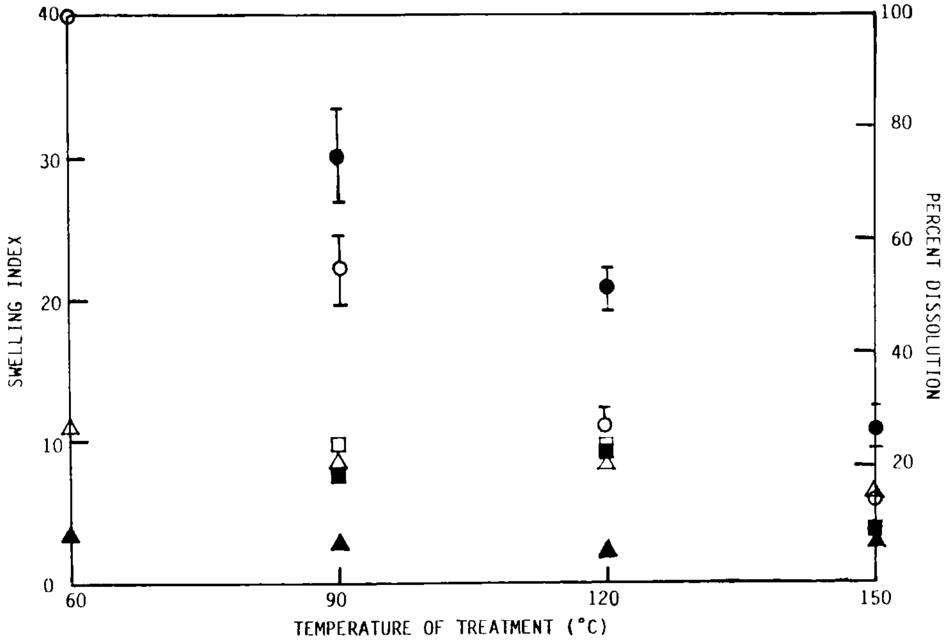

Figure 1
The effect of heat treatment at different temperatures on the dissolution and swelling index of chitosan films in different aqueous media, △ = pH 7.4 buffer, □ = distilled water, ○ = 0.1M HCl, open symbols = percent dissolution, closed symbols = swelling index.

Ionisation of amino groups in the chitosan molecules occurred in the acid and distilled water media, causing the unfolding of chitosan molecules and the subsequent swelling and dissolution of films. Heat treatment enhanced the water resistance of chitosan films in these two media. The pH 7.4 phosphate buffer did not encourage the unfolding of chitosan molecules and it was noted that heat treatment did not affect the degree of swelling of chitosan films in this medium. These observations indicate that heat induced changes in the chitosan molecules hindered the ability of the molecules to unfold upon ionisation in a suitable medium. Consequentially, the degrees of swelling and dissolution of the chitosan films were reduced. When the chitosan molecules were prevented from unfolding after treatment at a sufficiently high temperature, the chitosan films then showed similar degrees of water resistance in the three media of different pH.

An increase in the water resistance of an aqueous soluble polymer is usually caused by an increase in film crystallinity and/or the formation of crosslinks between the polymer molecules. Heat treatment did not change the microstructure of chitosan films as viewed under the SEM. But the yellow colouring of the films was intensified, with films treated at 150°C becoming yellowish brown. Such a colour change has been reported[11] after chitosan was reacted with glutaraldehyde, and has been attributed to the formation of interchain crosslinks. The implication is that heat treatment reduced the aqueous solubility of chitosan films by inducing the formation of crosslinks between chitosan molecules in the films.

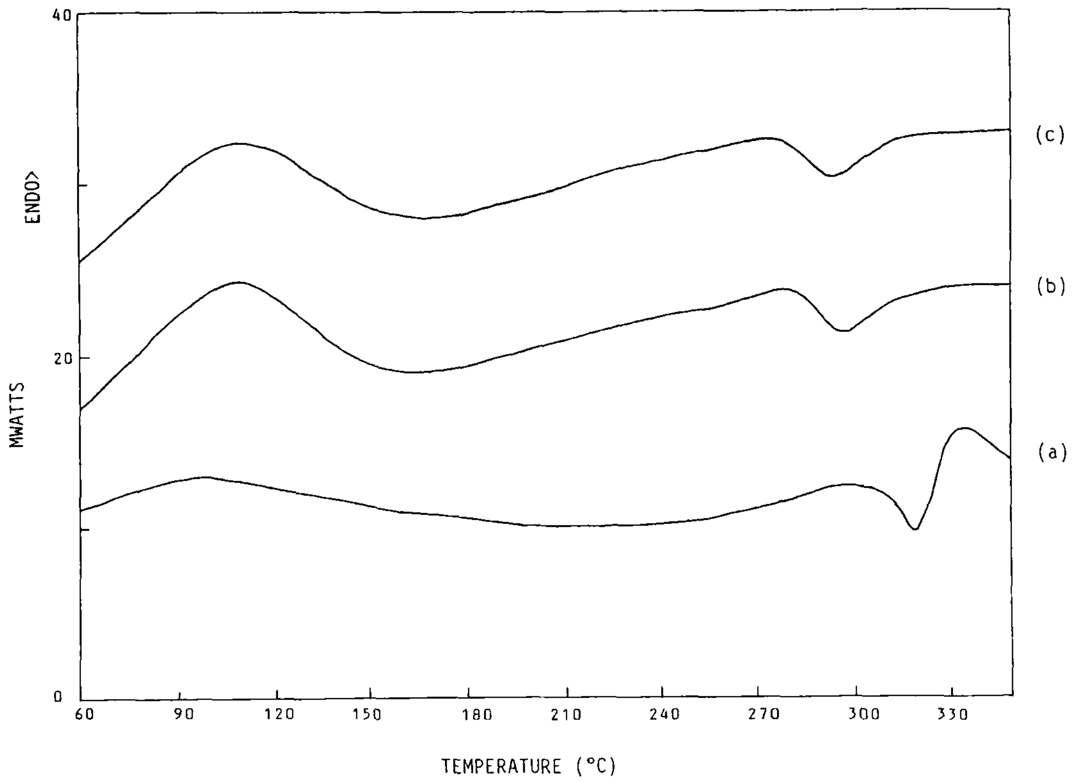

Figure 2
DSC thermograms of (a) chitosan flakes; (b) chitosan film prior to, and (c) after heat treatment at 150°C for 1 hour.

DSC thermograms of chitosan flakes, and of films prior to and after heat treatment at 150ºC for 1 hour are shown in figure 2. The exotherm observed at 315°C and 290°C respectively for the flakes and the films indicates polymer decomposition[12,13]. Processing of the chitosan flakes into films caused degradation to occur at a lower temperature. Heat treatment, on the other hand, did not affect the temperature of degradation of the films. The degree of deacetylation of the chitosan molecules was therefore unchanged by heat treatment[13]. The endotherm with a peak temperature near 100°C represents the energy required to vaporise water present in the film samples. Chitosan molecules have been reported to have a strong affinity for water molecules[13]. In this study, films subjected to a vacuum of 10^{-2} torr for 24 hours at ambient temperature still exhibited the endotherm. Chitosan films had a higher affinity for water compared to the flakes, probably because chitosan molecules in the films were protonated, rendering the films more hydrophilic than the flakes. Heat treated films had the same ability to bind water molecules as the untreated films, which may account for the films retaining their flexibility even upon heat treatment for 4 hours at 150°C.

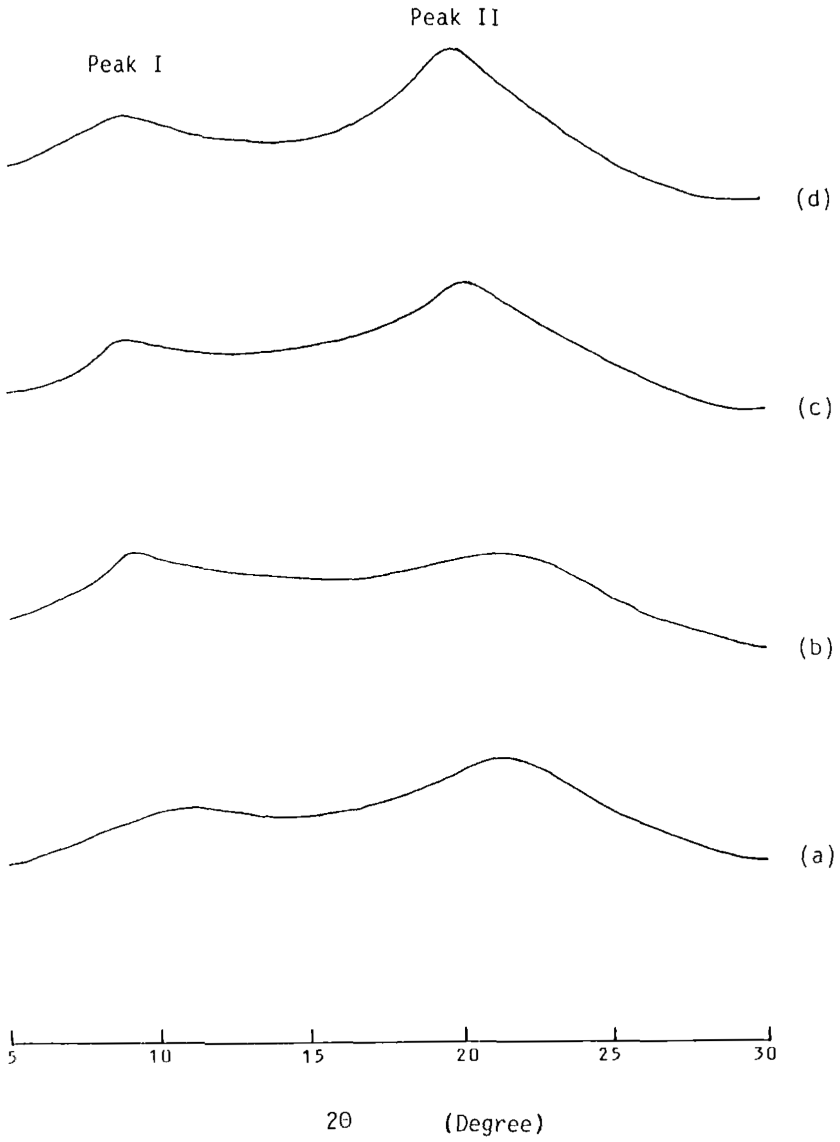

Figure 3
X-ray diffractograms of chitosan films - (a) untreated film; films treated for 1 hour at (b) 90°C, (c) 120°C and (d) 150°C.

The absence of other endotherms in the DSC thermograms, beside the broad endotherm at 100°C, implied that the chitosan films were amorphous. This was confirmed by X-ray diffraction patterns which showed two diffuse peaks (figure 3). Peak I has been attributed to the presence of an anhydrous crystal[14]. Heat treatment at increasing temperature enhanced the crystallinity of the anhydrous form, which may reduce the solubility of chitosan in its usual solvents e.g. an acid aqueous medium. Heat treatment also produced a more definite peak II which shifted to lower values of 2θ.

The IR spectra of a chitosan film before and after heat treatment at 150°C for 1 hour were similar except for a sharper peak at $1652 cm^{-1}$ after the heat treatment. In both spectra, there was a strong band at $1560 cm^{-1}$ for amide II (-NH-) and an absorption at $1652 cm^{-1}$ for amide I (-C=O)[15].

The duration of heat treatment affected the water resistance of chitosan films. For films treated at 90°C, the percent dissolution in pH 7.4 phosphate buffer was lowered from 21% to 16% when the duration of treatment was increased from 1 to 2 hours. A further prolongation of the duration to 4 hours did not produced an added effect. Similar results were obtained for films treated at 120°C and at 150°C. Films treated for 2 hours at 90°C and 120°C dissolved to a similar extent in the buffer medium as films heated for 1 hour at 150°C. It may therefore be feasible to use a lower temperature, but a longer duration of heat treatment to achieve the same degree of water resistance for the chitosan films.

CONCLUSION

Heat treatment enhances the water resistance of chitosan films by inducing the formation of crosslinks between chitosan molecules and/or the formation of crystallites in the films. The water resistance of the films in a medium of low pH is increased to a greater extent than that in a medium of higher pH. Both the temperature and duration of heat treatment influence the degree of enhancement in water resistance of the chitosan films.

ACKNOWLEDGEMENTS

The authors would like to thank Professor K. Yamamoto, Drs. T. Oguchi and E. Yonemochi of Chiba University, Japan, for their help with the X-ray diffraction work; and Ms Ng Guat Peng for her technical assistance.

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