

## Stability of Chitosan Solutions for Potential Use in Ocular Drug Delivery

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### ABSTRACT

*In this study, the physicochemical properties of chitosan and its stability in solution for potential use as an ocular drug delivery vehicle were studied. The physicochemical properties of the chitosan used were characterized in terms of its moisture content, degree of deacetylation (DD) and viscosity-average molecular weight ( $\bar{M}_v$ ) and were found to be 13.5%, 94.0% and  $6.03 \times 10^5$ , respectively. Chitosan solutions of 0.1% and 0.3% w/v concentrations in 1% aqueous L(+)-lactic acid were prepared. Sterilization of the solutions by autoclaving at 121°C at 15 psi pressure for 15 mins resulted in rapid acid-catalysed hydrolytic chain scission of the chitosan which, in turn, resulted in a drastic reduction in solution viscosity. Thereafter, the solutions remained stable during storage at 30°C, slightly more so at 2-8°C, with only slow and relatively small further decreases in viscosity over a period of 60 days.*

*The main conclusion to be drawn from this study is that 0.1% and 0.3% w/v chitosan solutions may be of value as ocular drug delivery vehicles because of their low toxicity, good ocular tolerance and storage stability.*

**Key words:** Chitosan solution, Storage stability, Ocular drug delivery vehicle

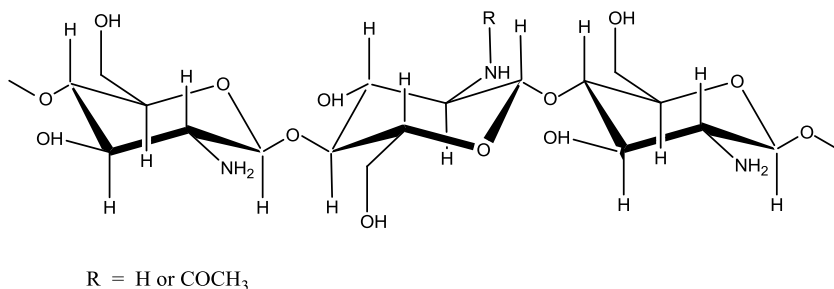
### INTRODUCTION

Chitosan has found widespread application in conventional pharmaceutical devices as a potential formulation excipient due to its suitable binding, disintegrating and tablet-coating properties (Singla and Chawla, 2001). The polymer has also been investigated as a potential adjuvant for swellable-controlled drug delivery systems. The use of chitosan in novel drug delivery as a mucoadhesive, in gene and peptide drug administration via the oral route, as well as its absorption-enhancing effect

has been explored by a number of researchers (Singla and Chawla, 2001). Chitosan is soluble in dilute acidic solutions wherein it becomes protonated. The positive charges on the protonated chitosan molecule enable it to interact with polyanions, a process that has been used to obtain complexes as well as micro and nanoparticulate drug delivery systems. Chitosan is a very promising biomaterial in ophthalmology because of its mucoadhesive and antimicrobial activity, as recently corroborated by the findings of Felt et al., (2000).

Chitosan is biodegradable, biocompatible and non-toxic. The chemical structure of chitosan is shown in Fig. 1. When used as a mucoadhesive material in drug delivery, the clearance of the drug is controlled by the mucus turnover rate which is much slower than the tear turnover rate. This prolonged retention of the drug formulation implies, for a drug with good permeability properties, an enhanced ocular drug bioavailability (Alonso and Sanchez, 2003). Consequently, chitosan is a very promising biomaterial in ophthalmology, not only because of the favourable biological properties mentioned above, but also because of its inherent biological activity which may also have an impact on ocular therapeutics.

The objectives of this present research were to evaluate the effects of sterilization by autoclaving and the subsequent storage stability (via intrinsic viscosity,  $[\eta]$ ) of chitosan solutions stored at 2-8°C and 30°C for 60 days. The method of preparation of the chitosan solutions was taken from the literature and modified accordingly (Leesawat et al., 2005).



**Figure 1.** Chemical structure of chitosan.

## MATERIALS AND METHODS

### Materials

Chitosan prepared from chitin (squid type) was purchased from Ta Ming Enterprises Co., Ltd., Thailand. L(+)-lactic acid (min. assay 88%) was purchased from Carlo Erba.

### Determination of moisture content

The moisture content (% by weight) of the chitosan was determined by heating at 60°C to constant weight in a vacuum oven and calculating the weight loss. The moisture content was then obtained from:

$$\text{Moisture Content} = \frac{\text{initial weight} - \text{dry weight}}{\text{initial weight}} \times 100\% \quad (1)$$

Due to its nature as a hydrogel, chitosan absorbs a significant amount of moisture when exposed to air. This absorption continues until the chitosan reaches its equilibrium water content (EWC) according to the ambient conditions (temperature, pressure, relative humidity).

### Determination of degree of deacetylation

The degree of deacetylation, DD, of the chitosan was determined by a chemical titration method following the procedure described by Hayes and Davies (1978). Pre-dried chitosan was dissolved in 10% aqueous acetic acid and chitosan hydrochloride precipitated by dropwise addition of hydrochloric acid. From the titration of 10 ml of a solution of a known weight of the hydrochloride dissolved in 100 ml of distilled water with 0.1 M sodium hydroxide solution, the DD of the original chitosan was calculated from :

$$\text{DD} = \frac{(C \times V_1 \times V_2 \times \text{MW} \times 100)}{(1000 \times V_3 \times W)} \% \quad (2)$$

where C is the exact molar concentration of the sodium hydroxide ( $\approx 0.1$ ),  $V_1$  is the volume of sodium hydroxide (ml),  $V_2$  is the made-up volume of the chitosan hydrochloride solution in ml (=100), MW is the molecular weight of chitosan hydrochloride (=197.5),  $V_3$  is the volume of the chitosan hydrochloride solution in ml (=10) and W is weight of chitosan hydrochloride dissolved in  $V_2$  (g).

### Determination of viscosity-average molecular weight ( $\bar{M}$ )

The chitosan was further characterized by determining its viscosity-average molecular weight ( $\bar{M}$ ) by dilute-solution viscometry, using a Schott-Gerate AVS 300 Automatic Viscosity Measuring System. The solvent used was an aqueous solution containing 0.2 M acetic acid, 0.1 M sodium chloride and 4 M urea. A series of chitosan solutions were prepared with concentrations of 0.025, 0.050, 0.075 and 0.100 g/dl. For flow-time measurements, 15 ml of each solution were accurately pipetted into a Ubbelohde-type viscometer, clamped vertically in a constant temperature water bath at  $25.0 \pm 0.1^\circ\text{C}$ . At least 15 minutes were allowed for temperature equilibration before flow-time measurements were made. The value of  $\bar{M}$  was calculated from equation (3) (Rathke and Hudson, 1994):

$$[\eta] = 8.93 \times 10^{-4} \bar{M}^{0.71} \text{ dl/g} \quad (3)$$

where  $[\eta]$  is the intrinsic viscosity of the chitosan in units of dl/g.

### Preparation of chitosan solutions for storage stability studies

The method of preparation of the chitosan solutions was taken from the literature and modified accordingly (Leesawat et al., 2005). Chitosan 1% w/v was dissolved in 1% aqueous L(+)-lactic acid at room temperature with magnetic stirring. It was then diluted to 0.1% and 0.3% w/v using Feldman's ophthalmic buffer pH 7.3 and 7.7, respectively, and sterilized by autoclaving at 121°C and 15 psi for 15 mins. The osmolalities of these 0.1% and 0.3% chitosan solutions were determined by an Osmomat 030. The stability of the chitosan solutions was investigated in terms of their pH and intrinsic viscosity ( $[\eta]$ ) changes during storage at 2-8°C and 30°C.

## RESULTS

The physicochemical properties of the chitosan which were characterized, namely: moisture content, degree of deacetylation (DD) and viscosity-average molecular weight ( $\bar{M}$ ) were found to be 13.50%, 94.0% and  $6.03 \times 10^5$ , respectively. The storage stability of the chitosan solution was studied at two different temperatures: ambient (Asia) temperature (30°C) and refrigeration temperature (2-8°C) (Prakongpan, 2540). Stability was monitored in terms of intrinsic viscosity which, in turn, reflected changes in the chitosan molecular weight.

The decreases in intrinsic viscosity,  $[\eta]$ , of the 0.1% and 0.3% w/v chitosan solutions with sterilization and storage time at two different temperatures are shown in Figs. 2 and 3. The decreases are seen to be biphasic with an initial rapid sterilization phase, followed by a much slower storage phase. As the results show, the effect of storage is relatively small compared with the effect of sterilization. The effect of storage time only is seen more clearly in Figs. 4 and 5 on expanded scales. The intrinsic viscosity does decrease further on storage but only relatively slowly and with little difference between the two temperature regimens of 2-8°C and 30°C. The effect of increasing the solution concentration from 0.1% to 0.3% w/v is mainly to increase the solution viscosity for practical purposes (e.g., prolonged ocular retention time) rather than to influence storage stability. Finally, the pH values of the 0.1% and 0.3% w/v solutions fluctuated between 5.10-5.40 and 3.91-4.19, respectively (Table 1), with no obvious trend with storage time.

## DISCUSSION

Chitosan is well known to undergo acid-catalysed hydrolytic chain scission of its glucosidic linkages in dilute acid solution. This chain scission, which occurs at random points along the chain, results in a rapid molecular weight decrease with a corresponding rapid decrease in solution viscosity. For a random chain scission process in which there is an equal probability of chain scission occurring at any glucosidic linkage along the chain, it has been shown that, for most cellulose derivatives in dilute acid solution, the decrease in average molecular weight  $\bar{M}$  with time  $t$  can be fitted approximately to the second-order rate equation (4) (Haward, 1950), i.e.,

$$\frac{1}{\overline{M}_t} - \frac{1}{\overline{M}_0} = kt \quad (4)$$

where  $\overline{M}$  average molecular weight at time t  
 $\overline{M}$  initial average molecular weight at t=0  
 k rate constant for chain scission which is a function of temperature and acid catalyst concentration  
 i.e.,  $k = f(T, [\text{Acid}])$

To a good approximation, the viscosity-average molecular weight,  $\overline{M}$ , from viscometry of chitosan in dilute aqueous acid solution can be considered to be directly proportional to the intrinsic viscosity,  $[\eta]$ , of the solution (i.e.,  $\overline{M} \propto [\eta]$ ). This is because the value of the exponent 'a' in the Mark-Houwink Equation (5) is approximately equal to 1 for chitosan in dilute acid solution. Therefore, since K is a constant, the equation below

$$[\eta] = K\overline{M}^a \quad (5)$$

$$\text{approximates to } [\eta] = K\overline{M} \quad (6)$$

$$\text{hence } [\eta] \propto \overline{M} \quad (7)$$

Therefore, combining equations (4) and (6) gives

$$\frac{1}{[\eta]} - \frac{1}{[\eta]_0} = kt \quad (8)$$

which implies that a plot of  $(1/[\eta]_t - 1/[\eta]_0)$  against time t should yield a linear graph of slope k'.

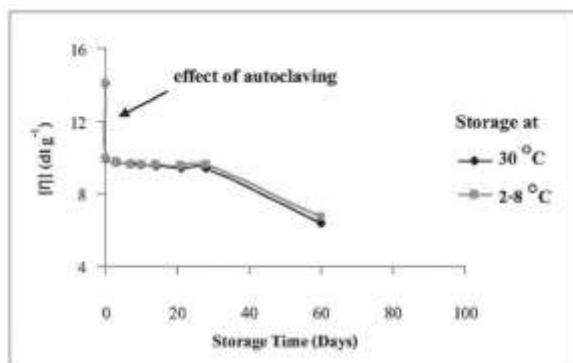
In this work, the values of  $[\eta]$  and  $[\eta]_0$  were estimated, again to a good approximation, from a single solution concentration via the Solomon-Ciuta One-Point Equation (9):

$$[\eta] = [2(\eta_{sp} - \ln \eta_{rel})]^{1/2} / C \quad (9)$$

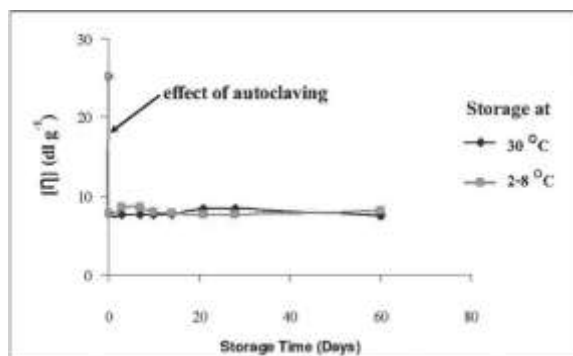
where  $\eta_{sp}$  is the specific viscosity,  $\eta_{rel}$  is the relative viscosity, and C is the concentration of the solution; in this case, C = 0.1 % or 0.3 % w/v (g dl<sup>-1</sup>). The variations in  $[\eta]$  with sterilization by autoclaving and storage time (days) at 30°C and 2-8°C are compared in Figs. 2 and 3. As the results clearly show, the main decrease in  $[\eta]$  is brought about by autoclaving during which the solutions are subjected to high temperature (121°C). The combination of high temperature and the presence of acid causes rapid hydrolytic degradation of the chitosan in solution, even during only a short period of time (15 mins). Further degradation then occurs during storage, although much more slowly and to a much lesser extent. The lower storage temperature of 2-8°C marginally increases storage stability (Figs. 4 and 5), although this effect is overshadowed in Figs. 2 and 3 by the much greater effect of autoclaving.

The pH values of the 0.1% and 0.3% chitosan solutions stored at 2-8°C and 30°C were in the range of 5.10-5.40 and 3.91-4.19, respectively (Table 1). The pH range 3.5-10.5 is usually tolerable by the human eye (Lund, 1994). The pH values

of the 0.3% chitosan solution were slightly lower than those of the 0.1% chitosan solution. At the same time, the osmolalities of the 0.1% and 0.3% solutions were 267 and 193 mOsmol/kg, respectively. The osmolality which can be tolerated by the eye is 160-670 mOsmol/kg (Charlton and Dalla, 1998).



**Figure 2.** Variations in intrinsic viscosity,  $[\eta]$ , of the 0.1% w/v chitosan solutions with autoclaving and storage time at different temperatures.

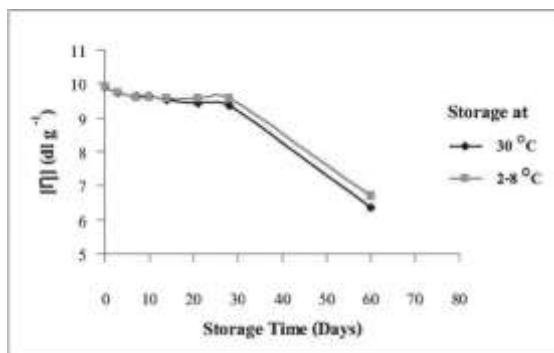


**Figure 3.** Variations in intrinsic viscosity,  $[\eta]$ , of the 0.3% w/v chitosan solutions with autoclaving and subsequent storage time at different temperatures.

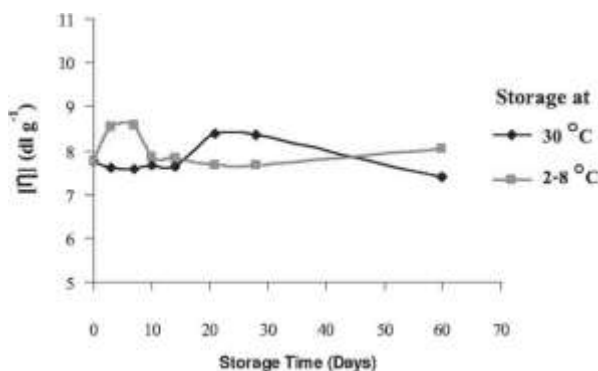
## CONCLUSION

The hydrolytic degradation of chitosan in aqueous acid solution is well documented (Biskup et al., 2007). The fact that this hydrolytic degradation results in random chain scission means that the molecular weight of the chitosan decreases very rapidly. Furthermore, the rate of hydrolysis increases with both acid concentration and temperature (Varum et al., 2001).

In this work, the chitosan solutions were prepared in a dilute (1% v/v) aqueous solution of a weak acid (L-lactic acid) and stored at moderate (30°C) or low (2-8°C) temperature. Under these conditions, the rate of hydrolysis of the chitosan in solution would be expected to be relatively slow over a period of days, if not weeks. However, chitosan solutions for use in ocular drug delivery need to be sterilized



**Figure 4.** Variations in intrinsic viscosity,  $[\eta]$ , of the 0.1% w/v chitosan solutions with storage time only at different temperatures.



**Figure 5.** Variations in intrinsic viscosity,  $[\eta]$ , of the 0.3% w/v chitosan solutions with storage time only at different temperatures.

**Table 1.** Variations in pH of the 0.1% and 0.3% w/v chitosan solutions with storage time at different temperatures.

| Day | pH ( $\pm 0.01$ )      |      |                        |      |
|-----|------------------------|------|------------------------|------|
|     | Chitosan solution 0.1% |      | Chitosan solution 0.3% |      |
|     | 2-8°C                  | 30°C | 2-8°C                  | 30°C |
| 0   | 5.17                   | 5.17 | 3.91                   | 3.91 |
| 3   | 5.18                   | 5.12 | 4.12                   | 4.16 |
| 7   | 5.20                   | 5.10 | 4.06                   | 4.02 |
| 10  | 5.27                   | 5.23 | 4.11                   | 4.08 |
| 14  | 5.30                   | 5.20 | 4.13                   | 4.11 |
| 21  | 5.26                   | 5.21 | 4.19                   | 4.13 |
| 28  | 5.27                   | 5.21 | 4.12                   | 4.08 |
| 60  | 5.40                   | 5.13 | 4.11                   | 3.98 |

before use with the recommended method, i.e., being autoclaved at 121°C at 15 psi pressure for 15 mins. By subjecting the solution to this high temperature, even for such a short time, it is sufficient to cause the chitosan to hydrolyse rapidly with a resultant drastic reduction in molecular weight. This effect has been observed here as the large reductions in intrinsic viscosity,  $[\eta]$ , in Figs. 2 and 3. Consequently, because the chitosan had already been degraded to such a large extent by autoclaving, the subsequent effects of storage at 2-8°C and 30°C were both small and slow in comparison. Autoclaving, necessary though it is in order to sterilize the solution, is a highly-degradative process in terms of the chitosan molecular weight. While this does not adversely affect the sterility of the solution, it does drastically reduce its viscosity.

In conclusion, these results need to be viewed within the wider context of this work which is to examine the potential use of these chitosan solutions as vehicles for ocular drug delivery. Bearing in mind the unavoidable effect of autoclaving, the main considerations are (a) whether or not the residual solution viscosity after autoclaving is still sufficient for ocular retention and (b) how stable the solutions are on storage. In answer to these questions, the results of this work have shown that (a) if the initial molecular weight and solution concentration of the chitosan are high enough, the residual solution viscosity after autoclaving is both sufficient and adjustable for practical use and (b) the autoclaved chitosan solutions can be stored safely for extended periods of up to 60 days at 30°C; storage at 2-8°C improves storage stability still further, as would be expected, but only marginally so. On the basis of these results, it is concluded that chitosan solutions in dilute aqueous L-lactic acid have considerable potential for use as ocular drug delivery vehicles. Further work is continuing in order to develop this potential.

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