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Contributed Paper

Artificial Tear Formulation from Chitosan

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ABSTRACT

The main objective of this study was to produce an artificial tear formulation incorporating chitosan. The variable factors were concentration of chitosan, type of dissolving vehicle, effect of buffer system and sterilization and stability of the preparation. Furthermore, the optimal formulation was also compared with a commercial product. The viscosity of the artificial tear correlated directly with the concentration of chitosan. The acid that was suitable for use as a vehicle was lactic acid. The appropriate buffer system was borate buffer. Sterilization by autoclaving reduced the viscosity of the artificial tear. The optimum formulation consisted of 0.1% w/v chitosan in lactic acid with controlled pH using borate buffer. The pH of this formulation was 5.97. No irritation was detected during testing in rabbit's eyes. This artificial tear fluid adhered to eyes longer than the commercial product. The physical properties of the formulation did not change under the stability test. The final product was a clear solution which could be stored at room temperature.

Keywords : artificial tear, chitosan, dry eye syndrome.

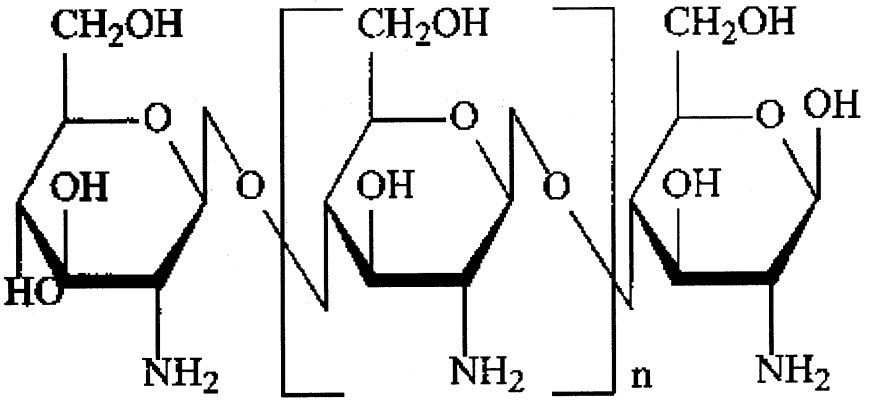
1. INTRODUCTION

Dry eye syndrome is the decline of the quality or quantity of tears bathing the eye. The patient experiences constant pain from eye irritation and a sandy or gritty sensation that, if untreated, can lead to scarring or ulceration of the cornea, and thus loss of vision.

There are many different causes of dry eye syndrome. The most common cause of dry eye is aging. As we get older, we produce fewer tears and evaporation increases. Other common causes include contact lens wear, sun, wind, dry air, reading, computer use and certain medications. Dry eye is seen with certain diseases such as Sjögren's syndrome as well.

In many cases, dry eye results from disorders of the various glands which work together to produce normal tears. Tears themselves are a complex combination of substances which form three layers on the eye [1]. The very thin outer layer contains lipids from the meibomian glands in the eyelid, to reduce evaporation. The lacrimal glands produce the middle watery layer that maintains the salinity and the acidity of the tears at proper levels. This middle layer also carries antibodies and other immune defense agents to defend the eye against infection. The inner mucous layer helps the tear film "stick" to the cornea and stay intact.

Dry eye is typically treated by applying artificial tears and ointments [2]. Chitosan, a cationic biopolymer (Figure 1), has been shown to have both good ocular compatibility and the ability to interact with the negative charge of mucin on the conjunctiva and cornea. Furthermore, chitosan has several



Chitosan

Figure 1. Chemical structure of chitosan.

favorable biological properties such as biodegradability and biocompatibility [3]. A sustained precorneal contact time of artificial tear containing chitosan is predicted, not only based on its ability to increase viscosity but also because of its mucoadhesive properties [4].

2. MATERIALS AND METHODS

2.1 Materials and Animals Testing

Chitosan was purchased from Taming Enterprises Co., Ltd., Thailand and was used without further modification. The 10% fluorescence dye was purchased from the Maharaj Hospital, Chiang Mai, Thailand. Six albino rabbits were used throughout the irritation test and ocular residence time study.

2.2 Formulation of Artificial Tear

The concentration of chitosan was varied from 0.1-1% w/v in 1% aqueous acetic acid and in 1% aqueous lactic acid. The pHs of the formulations were adjusted using acetate, phosphate and borate buffers. The optimal formulation was prepared by dissolving 0.1% w/v of chitosan in 1% aqueous lactic acid and was adjusted to pH 6 with the borate buffer while maintaining a final concentration of 0.1% w/v of chitosan. The formulation was sterilized by autoclaving at 121 °C for 20 mins (Autoclave Model KT30LD, Alp Co., Ltd., Japan) and was filtered through a 0.2 μm membrane filter. Physicochemical properties, such as the final pH (pH Meter Model EX-20, Horiba, Japan), osmolarity and viscosity (Brookfield Model DV-III Programmable Rheometer, U.S.A.) were evaluated.

2.3 Irritation Evaluation

Each formulation was evaluated in 6 rabbits by means of an eye irritation test according to TIS 162-1998 (Thai Industrial Standard: Shampoo Irritation Test). The results were evaluated from abnormalities on the cornea, iris and conjunctiva after 24, 48, 72 hrs and 7 days. The scale ranged from 0 for no effect to 4 for distinct irritation.

2.4 Ocular Retention Time Study

In vivo precorneal drainage of each formulation was assessed by visual inspection with the aid of black light fluorescence. After application of 25 μl of fluorescence-labeled solution onto one eye of the rabbit, the test was conducted until fluorescence on the eye could no longer be detected. The rabbit was kept in a restraining box. In this way, the optimal formulation and the commercial artificial tear fluid were compared on 6 albino rabbits free of any ocular damage.

2.5 Stability Test

The optimal products were kept at room temperature (approximately 30 °C), in a refrigerator (4-8 °C) and at 45 °C. The appearance and pH of the products were determined at intervals of 15, 30 and 60 days.

2.6 Antimicrobial Efficacy

Solutions containing 0.1-1.0% w/v of chitosan were evaluated *in vitro* for antibacterial efficacy against *S. aureas*, *E. coli* and *P. aeruginosa* by using the usual dilution technique.

3. Results and Discussion

3.1 Physico-chemical Properties of the Formulation

The solvents used for chitosan were acetic acid and lactic acid. Due to the pungent odor of acetic acid, lactic acid was preferred. Three buffer systems were employed to control the pH of the formulation. The borate buffer was found to be compatible with the formulation but the acetate and phosphate buffers caused precipitation to occur. An increase in concentration of the chitosan increased the viscosity of the solution. Chitosan exhibits a sol-gel transition at pH 6.5, its pKa [5]. The higher concentrations of chitosan in solution were avoided since they can cause blurred vision and discomfort. Consequently, the 0.1% w/v solution concentration was chosen for further evaluation.

Thus, the optimal formulation was prepared by dissolving 0.1% w/v of chitosan in lactic acid, adjusted to pH 6 with borate buffer, and the final concentration re-adjusted back to 0.1% w/v.

3.2 Irritation Test

Irritation evaluation using the TIS162-1998 Method showed that the optimal formulation was very well tolerated, as shown in Table 1. All scores from the test were zero, according to the standard scale.

3.3 Ocular Retention Time Study

The ocular retention time study with fluorescent staining demonstrated that the optimal formulation adhered to the precorneal area longer than the commercial product which had a 3-fold higher concentration, as shown in Table 2.

Table 1. Irritation test results from the TIS162-1998 Method.

Point of inspection	Characteristics of the irritation	Scale of effect on sample number					
		No.1	No.2	No.3	No.4	No.5	No.6
Cornea	Level of opacity	0	0	0	0	0	0
	Area of opacity	0	0	0	0	0	0
Iris	Response to light	0	0	0	0	0	0
Conjunctiva	Redness	0	0	0	0	0	0
	Chemosis	0	0	0	0	0	0
	Discharge	0	0	0	0	0	0

Table 2. Ocular retention time study.

Ocular retention time (mins)		
Optimal formulation	Commercial product	Control (Normal Saline Solution)
15.90	13.57	3.24

3.4 Antibacterial Activity

This study showed that chitosan exerts an excellent bactericidal effect against one pathogenic strain, *S. Aureus*. The minimal inhibitory concentration value of chitosan was 0.1% w/v for *S. Aureus* (Table 3). The chitosan solution had no antibacterial activity against either *E. coli* or *P. aeruginosa*.

3.5 Effect of Sterilization on Physical Properties of the Formulation

Autoclaving reduced the viscosity of the chitosan formulation significantly. The combination of heat and pressure from autoclaving degraded the chitosan, resulting in a decrease in its molecular weight. The reduction in viscosity was not proportional to the concentration of the chitosan in the formulation, as seen in Figure 2.

Table 3. Antibacterial activity of chitosan solution.

Concentration (%) w/v	Inhibition zone size (mm)		
	S. aureus	*E.Coli*	*P. aeruginosa*
0.1	20	no	no
0.2	18	no	no
0.3	22	no	no
0.4	21	no	no
0.5	21	no	no
0.6	20	no	no
0.7	18	no	no
0.8	18	no	no
0.8	20	no	no
1.0	19	no	no

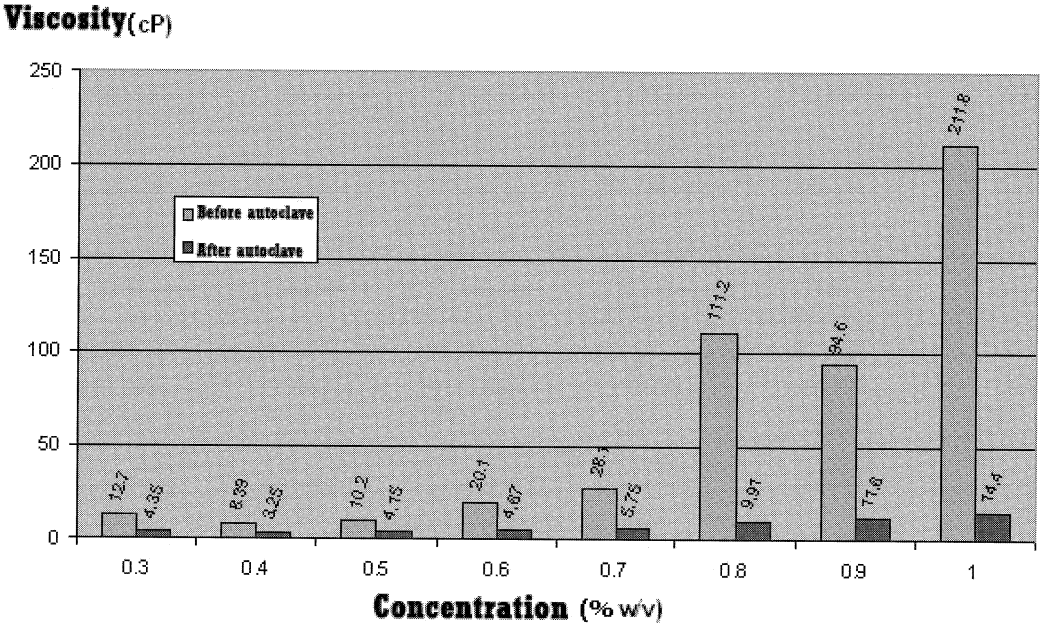


Figure 2. Effect of moist heat sterilization on the viscosity of the chitosan solution.

3.6 Stability Study

The stability study showed that the optimal formulation was stable over the temperature range 4-45 °C (as seen in Table 4). This observation suggests that the product can be stored safely at room temperature.

Table 4. Stability of the optimal formulation.

Temperature	Time (days)	Appearance	pH (±SD)
Room temperature	60	Clear and colorless solution	5.794 (± 0.047)
4 °C	15	Clear and colorless solution	5.832 (± 0.009)
	30	Clear and colorless solution	5.867 (± 0.025)
	60	Clear and colorless solution	5.912 (± 0.035)
45 °C	15	Clear and colorless solution	5.795 (± 0.026)
	30	Clear and colorless solution	5.818 (± 0.013)
	60	Clear and colorless solution	5.727 (± 0.013)

4. CONCLUSIONS

It can be concluded that the optimal formulation consisted of a 0.1% w/v solution of chitosan in 1% aqueous lactic acid, adjusted to pH 6 with borate buffer. The optimal product was colorless, clear and had an osmolarity close to that of natural tear fluid. The physical stability of the product appeared to be excellent, thus indicating that it could be kept on a shelf at room temperature. Chitosan, at a concentration of as low as 0.1% w/v, can exert some antibacterial activity. On comparing the optimal formulation with a commercial formulation containing hydroxypropylmethyl cellulose 0.3%, the following advantages were noted :

- Chitosan adhered to mucin on the cornea longer than the commercial product which had a higher concentration. The low viscosity exhibited by the chitosan solution facilitates its application and, therefore, allows for more accurate and reproducible administration. It will probably not create a sensation of blurred vision commonly associated with the use of hydrogels.
- The antibacterial properties of chitosan suggest that it may be prepared in unit-dose, giving the added advantage of avoiding the need for preservative while, at the same time, reducing the risk of infection in patients. Moreover, the antibacterial effect of chitosan is particularly interesting in the case of dry eye since it may have a synergic effect with endogenous antibacterial agents, such as lysozyme or betalysin.

It is therefore concluded from the results of this work that chitosan is a promising biopolymer for the treatment of dry eye due to its good antibacterial efficacy as well as its retention capacity on the cornea.

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