ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
www.ijptonline.com
Research Article

DEVELOPMENT AND PROCESS OPTIMIZATION OF VARIABLES FOR PREPARATION OF NOVEL POLYMERIC NANOPARTICLES CONTAINING CROMOLYN SODIUM

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Received on 29-12-2012 *Accepted on 18-01-2013*

Abstract

The main purpose of this study is developed and drug loaded nanoparticles of azelastine hydrochloride, an antiallergic drug and optimization in terms of chemical properties, drug concentration, polymer concentration, cross-linking agent and stirring time. Nanoparticles of azelastine hydrochloride were fabricated using chitosan and sodium alginate as polymers by ionotropic pregelation method. Calcium chloride was also included in the formulation for pregelation of sodium alginate. Chitosan and sodium alginate suspension further crosslinked with calcium chloride. Different formulations of nanoparticles were prepared using different concentrations of chitosan, stirringtime of rotation and polymers to drug ratio in the nanoparticles. The average particle size ranged between 232.30 nm to 638.3 nm. Drug entrapment ranged between 70.00%-92.2%. The result indicated that the drug loaded nanoparticles of azelastine hydrochloride showed optimum particle size, maximum drug entrapment, zeta potential (>30)with drug polymer ratio 05:75, cross-linking agents 0.5 ml, stirring rate 800 rpm and stirring time 30 min.

Keywords: Nanoparticles, Chitosan, Sodium alginate, Calcium chloride, ionotropic pre gelation.

Introduction

All conventional dosage forms for ocular drug delivery that account for 90% of currently accessible ophthalmic formulations. Eye drops have excellent acceptance by patients but have major disadvantages such as low ocular bioavailability, pulse entry, high frequency of administration. An optimum ocular drug delivery system would be one which can be delivered in eye drop form with no blurring of vision or irritancy and reduced frequency of instillations.

Nanoparticles are colloidal drug carrier in the submicron range (10-1000nm). These carriers were evaluated for ophthalmic drug delivery [1].

Recent research has concentrated on biodegradable polymers of natural origin especially from the view point of cost, environmental concerns and safety [2]. In addition, the natural polymers are also suitable to use for ophthalmic drug delivery because of their mucoadhesive properties. Alginate is a biodegradable, biocompatible and mucoadhesive polymer having lot of applications in the drug delivery systems [3,4]. Chitosan and alginate are two biopolymers that have received much attention and have been extensively studied for such use [5,6,7]. There are many chitosan–poly anion complexes that have been investigated as drug delivery systems for drugs, proteins, DNA and other oligonucleotides, with encouraging results [8,9,10]. Among the various types of chitosan–poly anion complexes reported in the literature, the combination of chitosan and sodium alginate is considered to be the most interesting for colloidal carrier systems [11,12]. Hence it was selected as nanoparticulate carrier in the present study. Various approaches have been attempted to increase the corneal bioavailability and corneal residence time of drugs. Maximizing the corneal drug absorption and minimizing precorneal drug loss. Formulation of drug delivery systems that provide controlled and continuous delivery of ophthalmic drugs to the pre- and post-intraocular tissues.

Materials and Methods

Materials

Sodium alginate and chitosan was a gift sample from Signet Chemical Corporation Pvt. Ltd (India). Cromolyn sodium was a gift from Sun Pharmaceutical Ltd (India). Other materials were analytical grade chemicals.

Methods

Preparation of Sodium Alginate Nanoparticles

SA nanoparticles were prepared by the cation induced controlled gelification of SA polymer[13] 13. Blank nanoparticles were obtained upon the addition of calcium chloride (0.5 ml, 18mM) solution to 9.5ml of SA aqueous solution. Different concentrations of SA were used in order to determine the effect of SA concentration on particle size. Calcium chloride (0.5 ml, 18mM) was added to 9.5 ml of SA solution (0.06% w/v) provided nanoparticles with optimum size properties. Stabilizing agent was added to above mixture and stirred for 30 min. The final mixture was

kept at room temperature overnight for complete gelification. Drug loaded nanoparticles were recovered by ultracentrifugation at 30000g for 45 min and after decanting the supernatant, pellet was washed with distilled water[14].

Optimization of SA Nanoparticles:

Various formulation variables were optimized to prepare nanoparticle viz. polymer concentration and cross-linking agent concentrations. The effects of these variables on the particle size, shape, visual appearance, size distribution entrapment efficiency, zeta potential were studied. Process various variables that could affect the preparation and properties of final preparations were optimized i.e. conc. of Sodium alginate, Chitosan.

FTIR Analysis

FTIR spectroscopy has been successfully used for exploring the differences in molecular conformations, crystal packing and hydrogen bonding arrangements for different solidstate forms of an organic compound. The infrared spectra of cromolyn sodium, blank nanoparticles and cromolyn sodium loaded nanoparticles were recorded on a Jasco FT/IR 5300 spectrophotometer by potassium bromide (KBr) pellet method.

Differential Scanning Calorimetry Analysis

Differential scanning calorimetry (DSC) analysis was used to characterize the thermal behavior and any possibleinteraction between SA and cromolyn sodium. DSC thermograms were obtained using a Perkin-Elmer Pyris 1 DSC, equipped with Intracooler 2P cooling accessory. In the present study DSC scans were recorded from 0 to 300 ºC at the rate of10ºC/min.

Morphological characterization of nanoparticles

Transmission electron microscopy (TEM) was used to examine the morphology of the nanoparticles prepared inthis study. TEM micrographs were obtained with a Philips CM-200 (Philips, Netherland) transmission electron microscope. The surface topography of AZL nanoparticles were studied using dried pellets obtained after ultracentrifugation of nanoparticles. Pellets were coated using JEOL Ion sputter with thin layer of gold. Images were recorded on Jeol JSM-840 (Jeol Ltd, Tokyo, Japan) scanning electron microscope.

X-RAY DIFFRACTION STUDIES

X-ray pattern of cromolyn sodium, blank nanoparticles and drug loaded nanoparticles were recorded using D8 Advance X-ray diffractometer (Bruker, Germany). The sample were irradiated with Ni filtered 2.2 KW Cu Anode, Dermic X-ray tube equipped with a sample holder with zero background and PMMA & Lynux Eye detector. The samples were step scanned between 0-70^0 at 2θ Scale.

Measurement of Entrapment Efficiency

For the determination of the entrapment efficiency, the nanoparticles were first separated from the aqueous suspension medium by ultracentrifugation at 30000μ*g* for 45 min. The amount of free cromolyn sodium in supernatant was measured by validated UV spectrophotometric method at 238 nm. The cromolyn sodium entrapment efficiency (EE) of nanoparticles was determined in triplicate and calculated as indicated below [15].

Result and discussion

The preparation of CS–ALG nanoreservoir systems, based on an ionotropic gelation process, involves mixing the two aqueous phases at room temperature. Size and size distribution of SA nanoparticles depend largely on concentration of polymer and calcium chloride [16,17]. Hence SA nanoparticles were prepared by varying concentration of SA solutions and calcium chloride and their effect on the particle size was investigated. The effect of SA concentration on Particle size has been shown in Table **1**.

Measurement of Particle Size and Zeta Potential

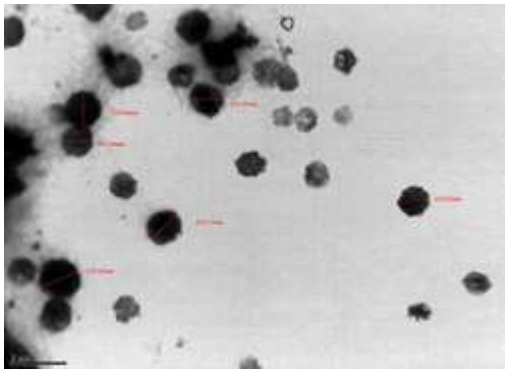
Nanoparticle size was determined by photon correlation spectroscopy (PCS), using Beckman Coulter N5 plus Submicron Particle Size (Coulter Corporation, U.S.A.). Dilution of sample is a necessary step in PCS measurements to avoid multi-scattering phenomena which arises due to droplet interactions. To ensure light scattering intensity within instrument's sensitivity range, the samples were diluted with distilled water previously filtered through 0.45 micron membrane filter. For the determination of electrophoretic mobility nanoparticle samples were diluted with 0.1 mm KCl and placed in the electrophoretic cell, where a potential of 150 mV was established and zetapotential was measured in triplicate by Zetasizer Nano-ZS, UK.

Table-1: Effect of various stabilizing agents on particle size of SA Nanoparticles.

Conc. of Sodium Alginate	Particle size	Visual appearance
1.0 %	--	Gel
0.5 %	--	Gel
0.4 %	--	Gel
0.3 %	--	Gel
0.2 %	--	Gel
0.1 %	870.5±1.2 nm	Viscous solution
0.075 %	335.3±3.1 nm	Viscous solution
0.05 %	**240.3±2.1 nm**	**Viscous solution**

When blank Sodium Alginate nanoparticles have prepared by varying the concentration of Sodium alginate solution from 0.5 % w/v to 0.05 %w/v, the solutions containing 0.05 and 0.06 % w/w SA concentration resulted in particles in nanosize range whereas at higher concentration gel was formed upon storage. The instability of nanoparticle formulation therefore polycationic polymer (chitosan) was evaluated for stabilization of nanoparticles. Effect of various stabilizing agents on Particle size of SA nanoparticles has been shown in Table 2,3,4,5.

In the study of polymer (Sodium alginate) ratio, Sodium alginate concentration was effected the particle size from 240.3-870.5 nm and visual appearance have viscous solution.



Figur-1: TEM of Cromolyn sodium loaded SA nanoparticles.

The drug concentration of chitosan also effect the particle size, zeta potential, drug entrapment and visual appearance with respect from 245-450 nm, 28.54-31.12 mv and viscous solution and 90.40-68.45 % respectively. The same

parameters were affected with using the different concentration of cross-linking agent 240-650 nm and 90.40%-68.45%.

Table no. 4. Indicates that the effect of stirring time on the particle size and drug entrapment. An increase in stirring time, the particle size of nanoparticles was reduced from 600.3-242.3 nm and entrapment of drug content was increases from 70.0-92.20% (figure 2).

The morphology of the nanoparticles was analyzed by transmission electron microscopy (Figure1), and it was observed thatthe particles were separate individual spheres in the size range 245-450 nm, with dense, solid structures. Similar features have been observed previously for nanoparticles composed of sodium alginate-chitosan. The sizes of the nanoparticles measured by TEM were smaller than the sizes obtained using the dynamic light scattering (DLS) technique.

This apparent discrepancy between the two results can be explained by the dehydration of the SA/CS hydrogel nanoparticles during sample preparation [18].

Table-2: Effect of stabilizing agents on particle size.

Sr. no.	Conc. of SA	Conc. of stabilizers	Particle size (nm)	Zeta potential (mV)	Visual appearance
1	0.05 %	Poloxamer (1% w/v)	450.0±2.6 nm	28.52	Viscous solution
2	0.05 %	Tween 80 (0.1% w/v)	355.1±5.1 nm	32.50	Viscous solution
3	**0.05 %**	**Chitosan 0.06**	**245.0±4.0 nm**	**31.12**	**Viscous solution**

Table-3: Effect of Drug Concentration on the drug entrapment.

Sr.no	Drug (% w/v)	Conc. Sodium alginate (% w/v)	Drug entrapment (%)
1	**0.05**	**0.05**	**90.20**
2	0.05	0.15	79.23
3	0.05	0.20	72.34
4	0.05	0.25	70.00

Table-4: Effect of cross linking agent concentration on particle size and size distribution of nanoparticles.

Sr.no	Volume of cross linking agent (CaCl2)	Average particle size (nm)	Drug Entrapment (%)
1	**0.5 ml**	**232.3±5.6 nm**	**90.20**
2	0.1 ml	355.3±5.1 nm	79.23
3	0.15 ml	568.4±4.5 nm	72.34
4	0.2 ml	653.3±5.6 nm	70.00

Table 5: Effect of stirring time on the partcle size and size distribution of nanoparticle.

Sr.no	Stirring time (min)	Average particle size (nm)	Drug entrapment (%)
1	15	242.3±5.6 nm	89.45
2	**30**	**450.±3.1 nm**	**92.20**
3	45	555.4±4.5 nm	71.34
4	60	600.3±5.6 nm	70.00

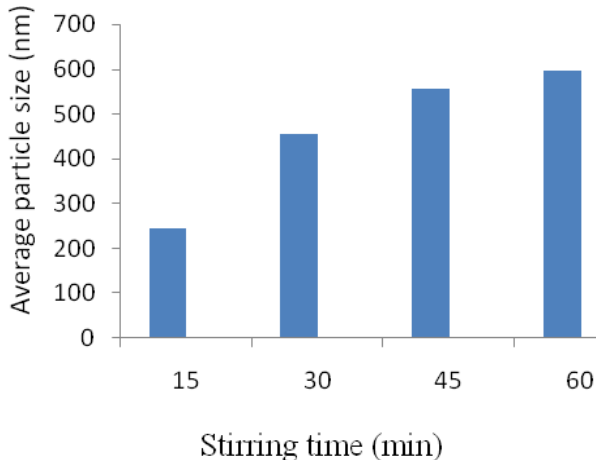


Figure-2: Effect of stirring time on the particle size.

FTIR Analysis

As indicated in the IR spectra characteristic peaks of cromolyn sodium such as 1599 (NH Bending), 1653(C=N & C=C stretching) and 1732 (C=O stretching) cm-1 are prominently masked in the cromolyn loaded nanoparticles. Change in peak intensity is indicator of chemical and physical changes taking place through ionic gelation and nanoparticles formation process hence it ensured that there was probable interaction between the drug, the SA:CS matrix and $CaCl_2$ at nanoparticles formation and drug entrapment stage; resulting in successful sustained release formulation (Fig. 3).

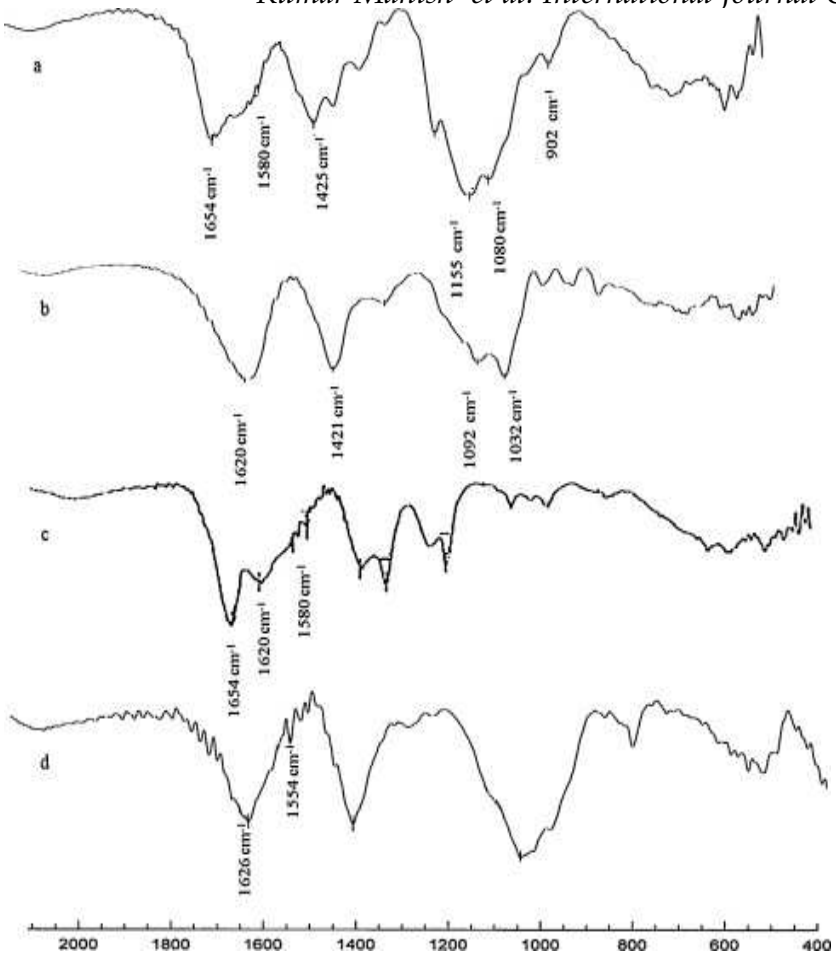


Fig. 3. Fourier transform infrared spectroscopy analysis of the cromolyn sodium, polymers and nanoparticles. The samples were (a) Sodium alginate; (b) Chitosan; (c) cromolyn sodium; (d) cromolyn sodium -loaded AG/CS nanoparticles.

Differential scanning calorimetry thermograms

In figure.4a, a narrow endothermic peak can be seen at 230 ◦C, due to fusion of drug. In Fig. 4b and c, broad endothermic peaks can be seen at 62 and 68 ◦C, for the physical mixture of the alginate and chitosan polymers, and for the SA/CS nanoparticles. The thermogram for the alginate/chitosan physical mixture shows a broader endothermic peak at 62 ◦C, which probably reflects overlap of the two separate endothermic peaks resulting from the individual contributions of the alginate and chitosan polymers. Fig. 4d shows the results for the physical mixture of SA/CS nanoparticles and cromolyn sodium, with an intense broad endothermic peak at 230 ◦C resulting from the combination of the peaks generated by the drug and the alginate and chitosan polymers present in the nanoparticle structure.

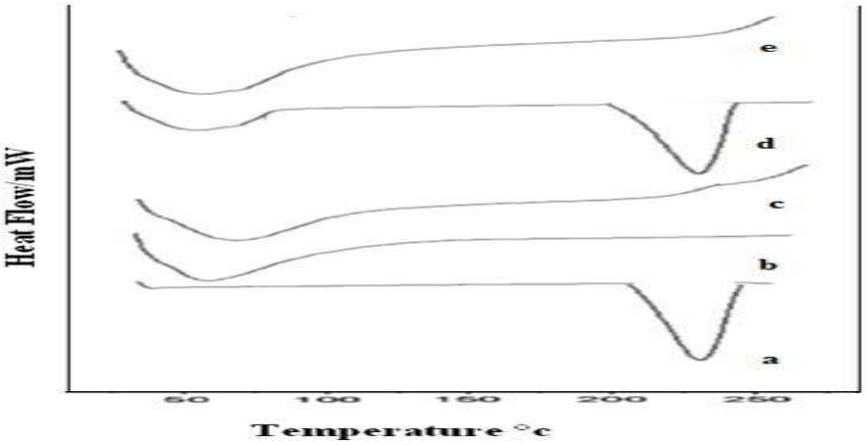


Figure 4 DSC thermogram for drug, polymers and nanoparticles. The samples were (a) cromolyn sodium; (b) SA/CS physical mixture; (c) SA/CS nanoparticles; (d) SA/CS nanoparticles + cromolyn sodium physical mixture; (e) cromolyn sodium-loaded AG/CS nanoparticles.

X-Ray diffraction studies (X-RD)

X-ray diffraction study of cromolyn sodium show peaks at 16.24^0, 23.24^0, 20.48^0, 24.46^0, 25.35^0, 30.14^0 and 34.61^0 but these peaks were not observed in the cromolyn sodium loaded nanoparticles. These results indicated that drug particles were dispersed in polymeric matrix (Figure 5).

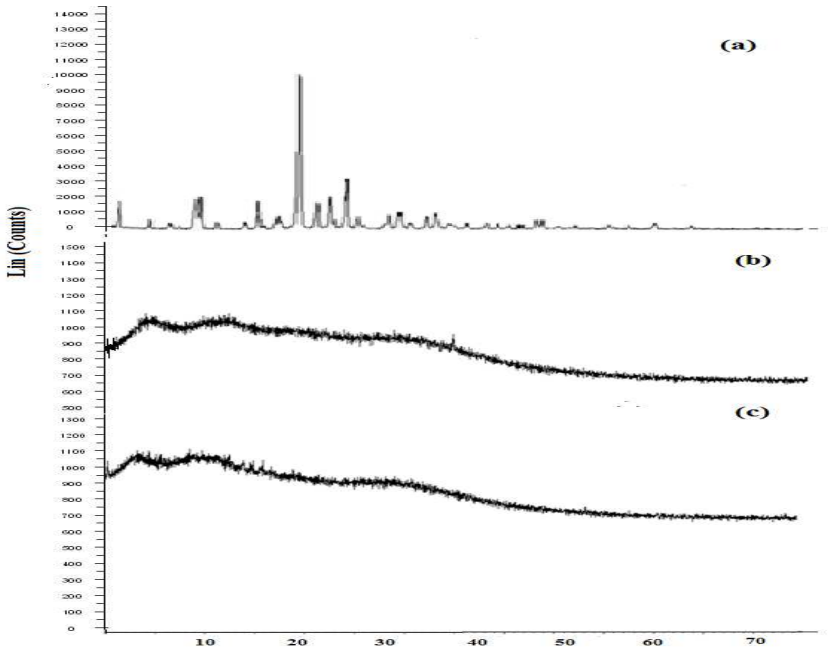


Figure-5: XRD of a) cromolyn sodium b) blank nanoparticles c) Drug loaded nanoparticles.

Conclusion

In the present study, the potential of CS–SA nanoparticles as drug carriers for ocular delivery was investigated. Cromolyn sodium antiallergic agent used in the treatment of ocular infections, was successfully formulated in the form of CS–SA nanoparticulate system with optimum particle size, zeta potential (>30) maximum entrapment of drug contents.

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