OCULAR NANO DRUG DELIVERY SYSTEM OF OFLOXACIN

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ABSTRACT

Ocular nano-delivery can be one of the approaches of increasing the ocular bioavailability from the topical delivery based on the concept of controlled and sustained delivery of drug from the formulation by retarding the release of drug. The present work is based on the development of ocular nanodelivery of Ofloxacin for conjunctivitis that is expected to be sustained releases and having a broad spectrum in-vitro activity against Gram-negative and Gram-positive microorganism. Cross-Ionic method was chosen for development of drug loaded nanoparticles with different drug-polymer combination and entrapment efficiency was calculated. Nanoparticles were characterized and drug entrapment and In-vitro release studies occurs.

KEYWORDS: Ocular, Nano-Delivery, Ofloxacin, Cross-Ionic, In-vitro

Topical delivery of eye drops into the lower cul-de-sac is the most common method for the administration of therapeutic agents in the treatment of ocular diseases and in diagnostics. However, Bioavailability problem arising due to the extensive drug loss from the precorneal lachrymal fluid by solution drainage, lachrymation, and nonproductive absorption by the conjunctiva (Lee and Robinson, 1979). Sultana et al., (2006) and Mark A. Babizhayev et al., (2009). Hence, the development of ofloxacin loaded nanoparticles for conjunctivitis that is expected to be sustained releases and retained for longer period of time (Herrero-Vanrell R,2001).

Ofloxacin was selected as drug of choice because of its broad spectrum in-vitro activity against both Gram-negative and Gram-positive microorganism, short biological half-life in the eye, It is well tolerated in the eye tissue i.e., free from irritation and having well established therapeutic activity among other group of antibiotics such as aminoglycoside (gentamycin, tobramycin etc.) and chloramphenicol etc.

In the present research work chitosan have been selected as polymers for the formulation development.The Deacetylated (hydrolyzed) form of chitin is known as chitosan. Chitin and chitosan are polysaccharides that support numerous living organisms.

Chitin is a linear polysaccharide consisting of (1-4)-linked 2-acetamido-2-deoxy-β –D-glucopyranose. The main commercial sources of chitin are the shell wastes of shrimp, crab, lobster, krill, and squid etc. The isolation of chitin includes two steps: demineralization with hydrochloric acid (HCl) and de-proteiniation with aqueous NaOH (sodium hydroxide). These operations are mainly empiric and vary with the differently mineralized shells, seasons, and presence of different crustaceans in the catch. And hence, isolated chitin is a highly ordered copolymer of 2-acetamido-2-deoxy-β-D-glucose and 2-amino-2-deoxy-β-D-glucose. As a point of difference from other abundant polysaccharides, chitin contains nitrogen.6 Chitin is insoluble in pure water but the Deacetylated form (chitosan) can be dissolved in acetic acid.

Chitosan is a linear polysaccharide consisting of (1-4)-linked 2-amino-2-deoxy-b-D-glucopyranose. The presence of a prevailing number of 2-amino- 2-deoxyglucose units in a chitosan permits bringing the polymer into solution by salt formation. The following salts, among others, are water soluble: formate, acetate, lactate, malate, citrate, glyoxylate, pyruvate, glycolate, and ascorbate (M.N.V Ravi kumar et. al., 2004). Chitosan has unique properties of bioadhesion, absorption enhancement by increasing the residence time of dosage forms at mucosal sites, inhibiting proteolytic enzymes, and increasing the permeability of various drugs across mucosal membranes. Chitosan is degraded by the microbial flora that is present in the colon; as a result, chitosan is a good candidate for site-specific drug delivery.

MATERIALS AND METHODS

Drug sample of ofloxacin I.P was obtained from Combitic Global Caplet. The assay of the sample was characterized in the laboratory as per the I.P for description, solubility, melting point, pH, loss on drying and spectral analysis and compared with company

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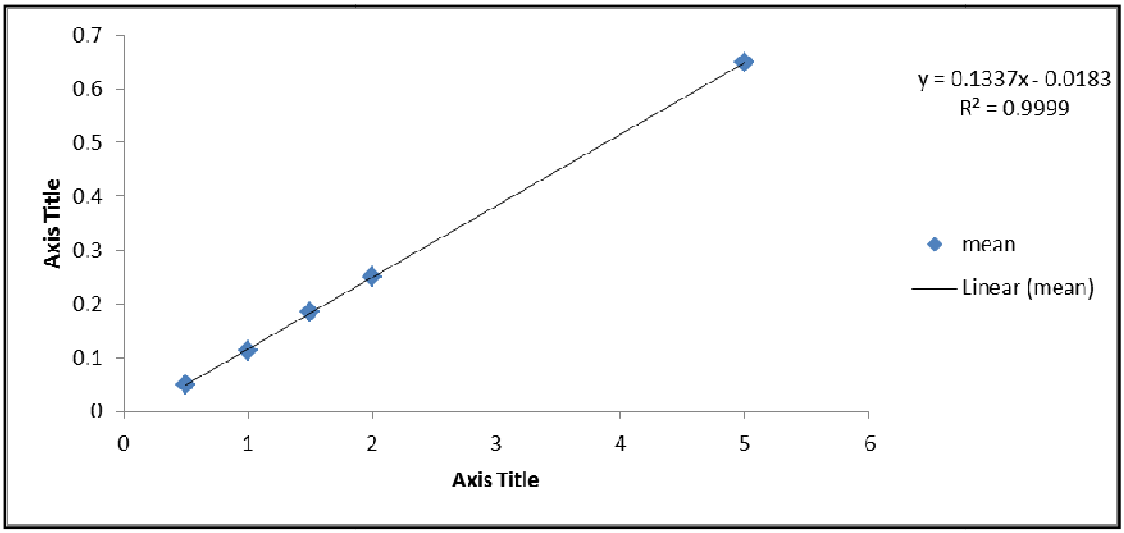
norms. The melting point was found to be 271.80C, which was in the melting range of 270-275.0C. Loss on drying was also found as per pharmacopeial standards. The U.V. spectrum of the drug in the distilled water exhibited a λmax 278 nm and it was as per I.P specifications.



Ionic Cross-Linking Method for Chitosan Polymer

Cross-links are bonds that link one polymer chain to another. They can be covalent bonds or ionic bonds. The aqueous solution of cross-linker is added in the aqueous continuous phase containing polymer and drug solution. The solution is then subjected to the homogenization & kept for overnight

Method

Polymer chitosan was accurately weighed and dissolved in 40 ml of 0.2% acetic acid. The accurately weighed drug is allowed to solubilize into above chitosan solution. The cross linker tripolyphosphate (TPP) (20 ml) was added drop wise into above solution while stirred magnetically at 2500 rpm at room temperature and left it for 24 hrs. for crosslinking. The resulting nanoparticles thus obtained by centrifuged at 15000 rpm. The nanoparticles was separated from supernatant and washed with distilled water and then freeze dried to obtained dry nanoparticles. Various formulae for placebo nanoparticles were prepared by varying the proportions of TPP. Three concentration of TPP was varied between 0.1-0.2%. The different formulae are shown in table.

Table No.1: Different formulae of medicated chitosan nanoparticles

In Gredients	Formulation Code		
	S-1	S-2	S-3
Drug (% w/v)	0.3	0.3	0.3
Tripolyphophate (TPP) (% w/v)	0.1	0.15	0.2
Chitosan (% w/v)	0.4	0.4	0.4
Distelled Water (ml)	40	40	40

Characterizations of medicated nanoparticles

The nanoparticles were prepared by cross-ionic method and evaluated as:

Tindal Effect

The sample of nanoparticles was suspended in water in the vial and inanother vial simple distilled water was taken. A red laser light was allowed to pass through the vial/sample from upper side.

Drug Loading

To separate the free drug in supernatant from the nanoparticles, thenanoparticle suspension was centrifused at 15000 rpm for 2 hr. thesupernatant was removed and nanoparticles sediments were washed twicewith distilled water and allow removing the unabsorbed drugs. Thenanoparticles obtained were dried using freeze dryer, weighed and thenanoparticles yield is calculated.

$$\textbf{Nanoparticle recover}\ (\%) = \frac{\text{Amount of nanoparticles recovered}}{\text{Amount of (Polymer + Drug + Excipients)}} \times 100$$

The amount of drug in nanoparticle could be determined using twomethods:

• The amount of non-entrapped drug (free drug) was measured inthe clear supernatant using UV spectrometry

$$\text{Amount of drug in nanoparticle} = \text{Total azmt of drug used} - \text{Amt. of drug in supernatant}$$

In-vitro Release Studies

The freeze dried chitosan and ethyl cellulose nanoparticles were accuratelyweighed containing 1.5 mg ofloxacin and placed in a beaker containing 50 mldistilled water which is continuously stirred by magnetic stirrer at 50 r.p.m.samples are collected at different time interval and analysed U.Vspectrophtometrically.

RESULTS AND CONCLUSION

When a light passes through the sample it showsa path of light that's shows the presence of colloidal particles in nano-range in the solution figure 2 & 3. The drug entrapment efficiency of chitosan nanoparticleswas found in the range of 54-56% as shown in the table no.2. The In-Vitro release data ofchitosannanoparticles are shown intable no.3, respectively and the cumulative percent of drug release areshown in fig no.4, respectively.

Figure 2: Tindal effect in Distilled water

Figure 3: Tindal Effect in Nano-particle diepersion

Table no. 2: Drug entrapment efficiency of different polymer

S.No.	Polymer	Absorbance (at $\lambda_{max.}$ =278 nm)	Concentration of Drug in Nanoparticles (mg)	Percentage Drug Entrpped (%)
1	Chitosan	0.465	108.96	54.6

Table no. 3:In-Vitro release of drug from shrimp cell's chitosan nanoparticles:

Time	Mean absorbance	Amount of drug released	Amount of drug remaining	Cumulative % of drug release
0.083	0.07	0.0981	1.2518	7.267
0.25	0.109	0.1867	1.1632	13.831
0.5	0.246	0.4964	0.8535	35.103
1	0.298	0.6138	0.7361	45.473
2	0.356	0.7449	0.605	56.125
3	0.446	0.9486	0.4013	70.268
4	0.493	1.0548	0.2951	78.139
6	0.538	1.1574	0.1925	85.736
8	0.571	1.2332	0.1167	89.12

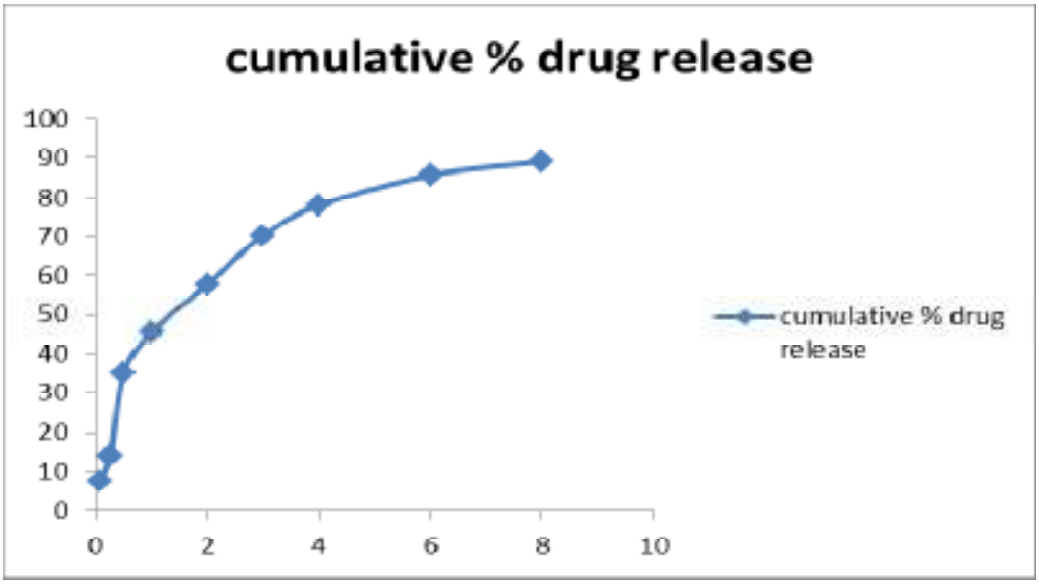


Figure 4: The cumulative percent of drug release

DISCUSSION

The objective of this work was to evaluate and develop a ophthalmic nanodelivery system of ofloxacin. The nanoparticles of chitosan were prepared by Cross-ionic method. Due to solubilization of polymers Chitosan in dilute acetic acid, the cross-ionicmethod was chosen.The nanoparticles obtained were characterized by various means like tindal effect, particle size, drug entrapment efficiency and in-vitro drug release studies. The characterization of nanoparticles shows the results which were within the acceptance limits. The Tindal effect shows the presence of nanoparticles in the solution, and the particle size of nanoparticles lies within the range of 420-550 nm. The drug entrapment efficiency shown by the polymers are in the range of 45-55%. In-vitro release studies show 88-91% drug released within 8hrs. The releasing of drug from the nanoparticles at different time intervals was evaluated by the cumulative percentage of drug release plots of different polymers. In the present investigation, we established that ofloxacin loaded chitosan nanoparticles have great potential to increase the bioavailability of a drug and reduce the elimination of drug due to physiological parameters. Hence, these nanoparticles can be given in the treatment of eye infection as nano-delivery system.

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