Gatifloxacin Nanoparticles for Ophthalmic Delivery

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ABSTRACT

Ocular efficacy of drugs being greatly influenced by the corneal contact time, the most promising approach to increase ocular bioavailability would be to increase pre-corneal residence time by using adequate drug delivery systems. Present research work aimed at developing the cationically modified PLGA nanoreservoir systems of Gatifloxacin for ocular delivery. A modified Emulsion-diffusion-evaporation technique was used to prepare PLGA nanoreservoir systems, stabilized by polyvinyl alcohol and chitosan. The prepared nanospheres were characterized for particle size and size distribution (polydispersity) by dynamic light scattering, zetapotential, drug content & encapsulation efficiency and in vitro drug release profile (phosphate buffer pH 7.4, 100 ml). Surface properties of the nanospheres were studied by TEM and AFM. The designed nanospheres have average particle size from 318-556 nm (polydispersity from 0.325-0.489) and zetapotential from 21-36 mV at pH 7.4.

Keywords: gatifloxacin, PLGA, nanoparticles, ocular delivery.

1 INTRODUCTION

Polymeric nanoparticles or submicroscopic colloidal carriers have been suggested to combine the ophthalmic prolonged action with the ease of the application of liquid eye drops [1]. Biodegradable colloidal nanoparticles of Poly (lactide-co-glycolide) (PLGA) have received considerable attention as a possible means of delivering drugs because of their favorable biocompatibility and easy resorbability through natural pathways [2]. Various methods reported for preparation of PLGA nanoparticles include: emulsion-evaporation [3], salting-out [4], nanoprecipitation [5], cross-flow filtration [6] or emulsion-diffusion [7]. However, improvements in the existing methods are still needed to overcome the difficulties in terms of obtaining reproducibility, particle size, and shape of nanospheres.

With the problem of increasing resistance to fluoroquinolones amongst bacterial ocular isolates, Gatifloxacin has been suggested as a promising novel broad-spectrum antibacterial agent. However, given by intravenous infusion or oral route, it leads to adverse effects associated with gastrointestinal tract and CNS [8]. Hence, the objective of this study was to develop bioadhesive chitosan coated PLGA nanoreservoir systems of Gatifloxacin for local delivery with reduced dose of the drug, to avoid the systemic side effects and hence to improve the therapeutic index of drug.

2 EXPERIMENTAL

2.1 Materials

[Poly (d,l-lactide-co-glycolide)] (PLGA, 75:25 lactide:glycolide), Purasorb–PDLG was received as a gift sample from PURAC Biochem, The Netherlands. The polymer Chitosan (CS) (Specifications: low molecular weight, viscosity of 1% w/v aqueous solution in 1% v/v acetic acid-130 cps, Deacetylation degree >80%) was a generous gift from India Sea Foods. Polyvinyl alcohol (PVA) was purchased from CDH Labs. Gatifloxacin was supplied by Lupin Labs Ltd., India. All other reagents and solvents used in the study were of analytical grade and used as received.

2.2 Preparation of Nanoparticles

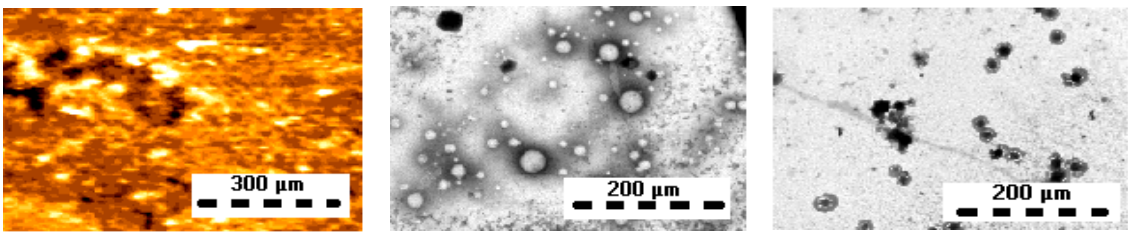
PLGA nanospheres were prepared by a modified Emulsion-Diffusion-Evaporation (EDE) technique [3]. Briefly; 100 mg of PLGA is dissolved in 10 ml of methylene chloride (organic phase) at room temperature. The organic phase is then added to an aqueous solution containing 20 mg gatifloxacin, PVA (0.25%, 0.50% or 1.0%) and chitosan (0.10%, 0.20% or 0.40%) in 10 ml of 2% v/v glacial acetic acid under stirring. The emulsion is stirred at room temperature for 1 h. followed by homogenization at 15,000 rpm for 10 min using Heidolph DIAX 900 homogeniser (Heidolph, Germany). To this emulsion, 20 ml of water is added under stirring resulting in nanoprecipitation and formation of chitosan-coated PLGA nanoreservoir systems of gatifloxacin. Stirring is continued on a water bath maintained at 40^0C to remove organic solvent. Nanospheres were finally collected by centrifugation at 18,000 rpm for 30 min at 4^0C (REMI, India).

To study the possible effect of the viscosity of the chitosan solution on the particle size of chitosan-coated PLGA nanoparticles, different concentrations of the two hydrophilic polymers (CS and PVA), and their ratios were tried (from 1:0.625 to 1:10), which are presented in Table 1 with the observed particle size, polydispersity and zetapotential.

Table 1: Formulation of Chitosan-PLGA Nanoparticles, Effect of Concentration of CS & PVA and their Ratio.

PLGA (%)	Chitosan (CS) (%)	PVA (%)	CS : PVA Ratio	Particle Size (nm)*	Polydispersity Index (P.I.)*	Zetapotential (ζ) (mV)*	Enacpsulation Efficiency (%)*
1.0	0.10	0.25	1 : 2.50	318 ± 12	0.325 ± 0.03	+21 ± 1.1	92.58 ± 1.88
		0.50	1 : 5.00	341 ± 18	0.341 ± 0.06	+24 ± 0.7	88.69 ± 3.21
		1.00	1 : 10.0	323 ± 13	0.332 ± 0.02	+21 ± 2.1	73.42 ± 2.11
1.0	0.20	0.25	1 : 1.25	367 ± 21	0.329 ± 0.01	+26 ± 0.8	91.29 ± 2.03
		0.50	1 : 2.50	379 ± 17	0.356 ± 0.01	+27 ± 1.4	89.52 ± 1.64
		1.00	1 : 5.00	391 ± 22	0.413 ± 0.05	+27 ± 2.2	82.43 ± 1.98
1.0	0.40	0.25	1 : 0.625	436 ± 16	0.381 ± 0.04	+34 ± 1.6	93.31 ± 3.19
		0.50	1 : 1.25	511 ± 21	0.463 ± 0.02	+36 ± 1.9	89.73 ± 2.53
		1.00	1 : 2.50	556 ± 28	0.489 ± 0.01	+31 ± 1.8	73.24 ± 3.84

*Average of three experiments (n = 3) ± S.D.



1 (A) 1 (B) 1 (C)

Fig. 1: Chitosan-coated PLGA nanospheres of gatifloxacin (A) AFM image of PLGA nanospheres (B) TEM image at 0.10% chitosan concentration and (C) TEM image at 0.40% concentration

2.3 Characterisation of Nanoparticles

Particle size and size distribution (polydispersity index) study of the polymeric nanosuspensions was carried out in triplicate (n=3) at 25^0C by photon correlation spectroscopy (PCS) at detection angle of 90^0. Zetapotential (ζ) was measured by Zeecom in triplicate (n=3) at 25^0C in phosphate buffered saline, pH 7.4.

Encapsulation efficiency (%) was calculated from the ratio of amount of drug entrapped to amount of drug added during preparation of nanoparticles. Surface topography and three-dimensional organization of nanospheres was studied by Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM), respectively (Fig. 1). In vitro release profiles of the nanoparticles was studied by flow-through cell method in phosphate buffer (pH 7.4), 100 ml, and 25 rpm at $37^0 \pm 0.5^0$C.

3 RESULTS AND DISCUSSION

It was observed that upon increasing the concentration of either chitosan (0.10 to 0.40%) or PVA (0.25 to 1.00%) increases the polydispersity index (P.I.) of nanospheres with simultaneous increase in particle size (318 to 556 nm). This could be attributed to the higher viscosity of the aqueous solution of these polymers at higher concentrations. The encapsulation efficiency was found to be varying from 73.24% to 93.31% respectively and the least encapsulation efficiency was observed at the highest concentration of both CS and PVA polymers. In vitro release study showed a prolonged release of drug with $t_{50\%}$ of 13.20 h followed by a more gradual sustained release phase up to 48 hrs. TEM and AFM studies showed that the nanoparticles are spherical and monodispersed. Chitosan coating on the PLGA nanoreservoir systems of Gatifloxacin could also be seen clearly in TEM at 0.40% of chitosan.

4 CONCLUSION

Polymeric submicroscopic particles are better tolerated by the patient than larger particles in the conventional eye drop formulations and are shown to overcome the important mucosal barriers such as ocular, nasal and intestinal. The biodegradable and biocompatible and cationically charged chitosan-coated PLGA nanospheres of gatifloxacin, which can interact intimately with extraocular structures (negatively charged), would increase the concentration of the associated drug by increased penetration of nanospheres through corneal epithelium. These mucoadhesive nanospheres can be used as nanoreservoir systems for prolonged and controlled delivery of gatifloxacin thus increasing patient compliance and for improving therapeutic index.

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