Research Article

Development and evaluation of floating microparticles for Levofloxacin as a gastroretentive carrier

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Abstract

Objective: In the present study an effort was initiated to prepare alginate floating microparticles for oral delivery of levofloxacin. **Methods:** Alginate floating microparticles were prepared by ionotropic gelation method with calcium carbonate ($CaCO_3$) 1% w/v in gelation medium used as gas-forming agent. Chitosan was used to improve the drug encapsulation efficiency. A number of physiochemical properties such as particle size, shape, in vitro buoyancy and drug release were compared with non-floating microparticles. **Conclusion:** Data signifies that gas bubbles gives rise to cavities in the particles which may be held responsible for enhanced *in vitro* buoyancy and rapid release of levofloxacin from floating microparticles.

Keywords: Levofloxacin, microparticles, chitosan, oral delivery

Introduction

Levofloxacin [(-) -9- oxacin [(-) -9- Fluoro-3-methyl-10-(4-methyl-1-piprazinyl)-7-oxo-2, 3-dihydro-7H-pyrido [1, 2, 3-de] luoro-3-methyl-10-(4-methyl-1-piprazinyl)-7-oxo-2, 3-dihydro-7H-pyrido [1, 2, 3-de] [1, 4]-benzoxazine-6-carboxylic acid], a second generation 1, 4]-benzoxazine-6-carboxylic acid], a second generation fluoroquinolone, possesses excellent activity against gram-positive, gram-negative and anaerobic bacteria (excellent activity against gram-positive, gram-negative and anaerobic bacteria (Davis and Bryson, 1994; North et al., 1998). As compared to others, fluoroquinolone resistance relates directly to human and veterinary usage and emerging bacterial resistance poses the single greatest threat to the future survival of fluoroquinolone drugs as an antibiotic class (Davis And Bryson, 1994; North et al., 1998). As compared to other fluoroquinolones, ofloxacin and ciprofloxacin it also has more pronounced bactericidal activity against organisms such as Pseudomonas, Enterobacteriaceae and Klebsiella (Klesel et al., 1995). The drug distributes well to target body tissues and fluids in the respiratory tract, skin, urine and prostrate and its uptake by cells makes it suitable for use against intracellular pathogens prostrate for use against intracellular pathogens (Langtry and Lamb, 1998). Levofloxacin is metabolized in the liver to demethyl-levofloxacin and levofloxacin and levofloxacin-N-oxide and excreted in urine (Langtry and Lamb, 1998). The pharmacokinetics of levofloxacin have been investigated in man (Chulavatnatol et al., 1999), calves (Dumka and Srivastava, 2006, 2007a, and 2007b; Dumka, 2007; Dumka and Srivastava, 2006, 2007a, and 2007b; Dumka, 2007; Dumka et al., 2008 Dumka et al., 2008) and guinea pigs (Edelstein et al., 1996). However, there is only meager information available on the pharmacokinetics of levofloxacin in buffalo species, except one report after intramuscular administration of levofloxacin in buffalo calves oxacin in buffalo calves (Ram et al., 2008).

Several dosage forms are being developed to improve therapeutic efficacy and reduce frequency of administration. The gastroretentive tablet and sustained-release pellet have been developed for once-daily administration (Chavanpatil et al., 2005, Zhang et al., 2012). In this study, levofloxacin-loaded Chitosan/Alginate microparticles were prepared and their physicochemical properties were evaluated in vitro.

Materials and methods

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Materials

Levofloxacin hydrochloride was obtained from Ranbaxy (P) Ltd New Delhi, India as a gift sample. Chitosan (Acylation 87%) and Sodium alginate was procured from Sigma Aldrich, Bangalore, India. Calcium chloride and sodium carbonate was purchased from Hi Media, Mumbai. All other chemicals were of analytical grade and bought from local supplier.

Preparation of floating microparticles

The floating microparticles were formulated by emulsion solvent diffusion method (Soppimath et al., 2001). Alginate was dissolved in distilled water at a concentration of 3% (w/v), the solution was blended thoroughly after the addition of levofloxacin (0.75% w/v) and calcium carbonate 1.5 %(w/v). The gelation medium was prepared by dissolving calcium chloride ($CaCl_2$) at 2% w/v and chitosan in 1% (w/v) concentrations in 2% (v/v) glacial acetic acid. The homogenous alginate solution was extruded using a 21G syringe needle into the gelation medium stirred at 1500 rpm with double blade propeller. The space between the edge of the needle and the surface of the gelation medium was about 10 cm. The gel microparticles produced were left in the solution with constant stirring for different time (10, 20, 30 min) at room temperature. After the collection of microparticles, they were washed twice with distilled water and oven-dried successively (40°C). The microparticles of levofloxacin which were prepared using the same method but without gas forming agent were employed for comparative study.

Characterization of microparticles

Size and shape of microparticles

The size of microparticles was determined using microscope (Olympus, India) fitted with an ocular micrometer and stage micrometer. Scanning electron microscopy (SEM) (Jeol JSM-1600, Tokyo, Japan) was carried out to characterize the surface of the formed microparticles. Microparticles were mounted directly onto sample stub and coated with gold film (~200 nm) under reduced pressure (0.133 Pa).

***In vitro* buoyancy**

The drug content and floating nature of prepared microparticles was determined by previous mentioned method (Jain SK et al., 2005). In brief, microparticles (300 mg) were spread over the surface of a USP XXIV dissolution apparatus filled with 900 ml of 0.1 N hydrochloric acid containing 0.02% Tween 80. The medium was agitated with a paddle rotating at 100 rpm for 12 h. The floating and the settled portions of microparticles were recovered separately. The microparticles were dried and weighed. Buoyancy percentage was determined as the ratio of the mass of the microparticles that remained floating and the total mass of the microparticles (Sriamornsak et al., 2005).

Encapsulation efficiency (EE)

To find out the incorporation efficiency floating microparticles were dissolved in a minimum amount of dichloromethane and the drug was extracted into a suitable aqueous media (0.1 N hydrochloric acid) by evaporating dichloromethane. The solution was screened through 0.45 µ membranes, diluted suitably and analyzed for drug content spectrophotometrically at 287.17 nm using 0.1 N hydrochloric acid as blank.

***In vitro* drug release studies**

The drug release was studied using a USP XXIV dissolution apparatus at 100 rpm in 0.1N hydrochloric acid as dissolution medium (900 ml) maintained at 37±1°C. A sample (10 ml) of the solution was taken out from the dissolution apparatus hourly and the samples were replaced with fresh dissolution medium. The samples were screened through a 0.45 µ membrane filter and diluted to a suitable concentration with 0.1 N hydrochloric acid. Absorbance of these solutions was measured at 287.8 nm using a UV/Vis double-beam spectrophotometer (UV-1800, Shimadzu). Cumulative percentage drug release was calculated using an equation obtained from a standard curve

To evaluate the mechanism of drug release from the microparticles the *in vitro* dissolution data were fixed to zero order, first order, Higuchi release model, Hixson and Crowell powder dissolution method and Korsmeyer and Peppas model (Jain et al., 2006).

Results and discussion

To enhance the levofloxacin loading in alginate microparticles, chitosan was dissolved in gelation medium to avoid the diffusion of the levofloxacin. When alginate solution is dropped into the gelation medium which is composed of $CaCl_2$ and chitosan in acetic acid glacial solution, the Ca^{+2} ions diffuse into the core of the drop of alginate and form the gel matrix through ionotropic gelation. At the same time, cationic polymer chitosan present in the gelation media also crosslinks alginate molecules through electrostatic interactions between negatively charged –COO− groups of alginate and positively charged $–NH_3$ + groups of chitosan. Alginate–chitosan complex clog up the large pore of Ca–Alg gel matrix and form a polyelectrolyte complex membrane on the surface of the microparticles and thereby decrease the permeability of the microparticles. Thus, the diffusion of levofloxacin is efficaciously prevented during the gelation (Bajpai et al., 2006).

The mean particle size of microsphere formulations comprising sodium carbonate was measured at 490 ±

70µm. The particle size of microparticles formulation without $NaCO_3$ was found to be 378 ± 19 µm.

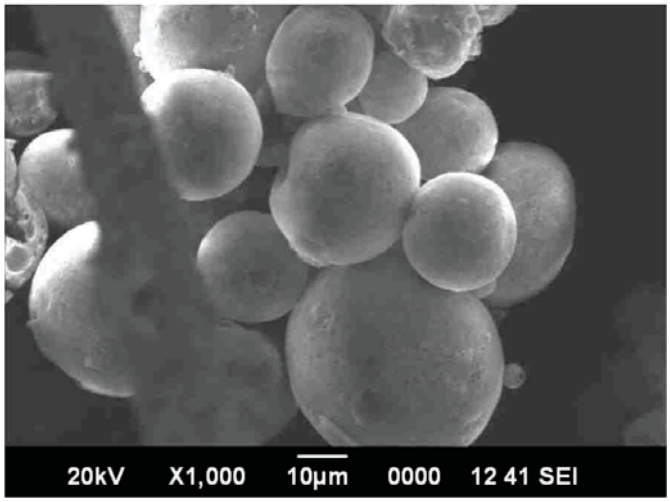

Figure 1. Scanning electron microphotograph of floating microparticles

This important difference in the size may be due to the existence of gas forming agent in the microparticles (Table 1). The permeable nature and sphere shaped microparticles are confirmed from their SEM photomicrographs as depicted in figure 1. As can be seen in the photomicrograph, there are many pores and cavities in the microparticles.

Table 1. Characterization of levofloxacin hydrochloride microparticles

Batch	Mean particle size (µm)	PDI	% In vitro Buoyancy	%Entrapment Efficiency (EE)
NFM	378 ± 19	0.44 ± 0.20	52.12 ± 1.22	89.13 ± 2.23
FM	490 ± 70	0.49 ± 0.12	72.15 ± 1.50	75.23 ± 4.25

The drug entrapment was found to be 89.13 ± 2.23. The extent of loading had affected the particle size distribution of microparticles. When the loading was high, the ratio of larger particles produced was also high. Chitosan in gelation medium improves the drug entrapment and drug loading in similar experiments performed by Ma et al., 2008.

All the gas-forming agent free microparticles in the SGF. In contrast, about 90% of the floating microparticles still floating after 24 h. The good buoyancy property of the microparticles may be due to the hollow nature of the microparticles which arised out because of the production of air bubbles during preparation. Existence of hollow cavities in microparticles enhances the drug release. Gas bubbles in the microparticles are supposed to accelerate the levofloxacin liberation. Current observation is in agreement with other studies stated using chitosan and alginate floating microparticles (Ma et al., 2008). This fact may be justified on basis of studies described by (Choi et al., 2002). They have found that $CaCO_3$ is present as an insoluble dispersion in neutral pH aqueous alginate solution; however, in acidic media, the $CaCO_3$ becomes water-soluble. $CaCO_3$ reacts with the acid to produce CO_2, simultaneously, the ionized Ca^{2+} ions promote internal gelation by cross-linking with the alginate carboxyl group. Thus, the addition of $CaCO_3$ did not considerably accelerated the drug release even though it enhanced bead porosity and pore size, as shown in Figure 2.

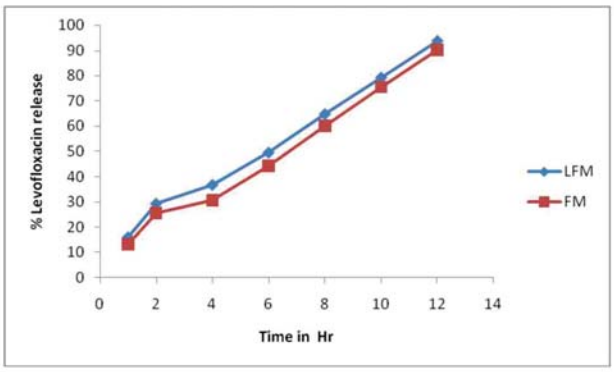

Figure 2. Cumulative release of levofloxacin from microparticles

Release pattern of levofloxacin in SGF (pH 2.0) from floating microparticles followed Higuchi matrix model and Peppas-Korsmeyer model. Regression analysis and Slope values proposed that the release of levofloxacin hydrochloride from floating microparticles followed non-Fickian diffusion mechanism (Table 2).

Conclusion

From the currently performed study it was determined that the floating microparticles could be employed for delivery of levofloxacin in stomach-jejunum transit which may improve bioavailability and ultimately lead to better patient

Table 2. The regression coefficients and rate constants for release of levofloxacin from floating microparticles in SGF (pH 2.0)

Formula -tion	Zero-order Model		First-order Model		H-M Model		P-K Model		H-C Model	
	R	k_1	r	k_1	r	k_1	r	k_1	r	k_1
NFM	0.7651	9.6844	0.782	-0.1590	0.9890	21.2500	0.9843	23.0652	0.9624	-0.0460
FM	0.7646	7.3463	0.9677	-0.1090	0.9891	17.6942	0.9935	21.1447	0.9182	-0.0546

compliance. Researchers are in progress to incorporate specific release modifiers such as eudragit which could be utilized to prolong the liberation of levofloxacin from Alginate/Chitosan microparticles.

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