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Research Article

PREPARATION AND IN-VITRO EVALUATION OF OFLOXACIN MUCOADHESIVE MICROSPHERES

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ABSTRACT

Ofloxacin is a fluoroquinolone antibacterial agent. It is used in various urinary and respiratory tract infections,gonorrhea, and skin diseases.The purpose of this study was to prepare Ofloxacin mucoadhesive microspheres by ionotropic gelation technique using Sodium alginate and mucoadhesive substance Chitosan. Six different formulations (F1-F6) were prepared by using various formulation variables. The microspheres have been characterized invitro in the terms of their surface morphology, particle size,encapsulation efficiency, swelling ratio and mucoadhesivity. Drug entrapment efficiency of Ofloxacin was found at 294nm. Different formulation variables like polymer-polymer ratio, drug-polymer ratio and coating concentrations were considered. Almost spherical microspheres were obtained with sufficient swelling and Mucoadhesive property. Among all the 6 formulations the F6 has released drug in a sustained manner, for prolonged time. Dissolution study was followed at phosphate buffer (pH-7.4) for 8 hr.

Keywords: Ofloxacin, Sodium alginate, Ionotropic gelation, Chitosan and microspheres.

INTRODUCTION

Despite tremendous advancement in drug delivery,oral route remains the preferred route for the administration of therapeutic agents,low cost of therapy and ease of administration leads to higher levels of patient compliance.Conventional oral dosage forms such as tablets, capsules provide specific drug concentration in systemic circulation without offering any control over drug delivery and also cause great fluctuations in plasma drug levels.These have a disadvantage of a release all or nothing emptying process while the multiple unit particulate system pass through the GIT to avoid the vagaries of gastric emptying and thus release the drug more uniformly.Various approaches have been worked out to improve the retention of oral dosage form in the stomach, Eg.Bio-adhesive systems, Floating systems, Swelling or Expanding systems and High density systems.

Microsphere[1]carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery[2].However, the success of these microspheres is limited owing to their short residence time at the site of absorption.This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres.The term bio adhesion describes materials that bind to the biological substrates such as mucosal membranes.Adhesion of bioadhesive drug delivery devices to the mucosal tissue offers the possibility of creating an intimate and prolonged contact at the site of absorption. This prolonged residence time can result in the enhanced absorption and in combination with a controlled release[3,4]of drug, also improved patient compliance by reducing the frequency of administration. The epithelial adhesive properties of mucin have been applied in the development of gastro retentive drug delivery systems.

Ofloxacin[5,6]is a fluoroquinolone antibacterial agent. It is used in various urinary and respiratory tract infections,gonorrhea, and skin diseases. Normal dosage regimen varies from 200-600 mg administered twice orthrice a day, depending on severity of infection. In severe cases, long-term therapy may also berequired. Biological half-life of drug is from 5-6 hrs. As it requires frequent dosing to maintainthe therapeutic effect, it was chosen as a model drug for the present study. These particles consistof core material, which is the drug, and a coating material.

The coating materials can be of various types ranging from natural polymers, such as albumin, gelatin[7,8]chitosan and synthetics suchpoly(vinyl alcohol),poly(lactide-co-glycolide) and a combination of two polymers such aschitosan-sodium CMC,alginate-chitosan etc.Also literature survey revealed that not much workhas been done on sustained release drug release of ofloxacin,except for few workers.The natural mucoadhesive substance[9] (NMS) used in the present work was obtained from local market E.g.Chitosan[10].The NMS has considerable swelling behavior in water and particularly in buffer 7.4.This may be considered as significant for its use in mucoadhesive drug delivery, particularly for controlled release.

In this study ofloxacin, was chosen to be microencapsulated by the ionotropic gelation technique using sodium alginate and chitosan blend.The proposed system is expected to provide several advantages.Firstly,gelation of the aqueous solution of alginate/chitosan blend renders oral sustained drug delivery.Gastric retention time of microspheres enhances with the addition of mucoadhesive agent, resulting in the delivery of drug across the mucous membrane for an extended period of time in intestine.

So, the main aim of the present work was to prepare microspheres containing Ofloxacin by ionotropic gelation[11] technique and to characterize the controlled-release property.

MATERIALS AND METHODS

Materials

Ofloxacin was obtained from Micro Labs, Bangalore. The polymers Sodium alginate and Chitosan were obtained from Karnataka Fine Chem Pvt.Ltd (Bangalore).

Preparation of microspheres

Accurately weighed about 1.4gm of sodium alginate and kept aside, then it was dispersed in 100ml of distilled water by using magnetic stirrer at 40^0C.Then after complete dispersion, added accurately 1gm of Ofloxacin then the stirring was continued until complete and uniform dispersion was obtained.Then the chitosan[12]solution was prepared by dispersing the 5mg of chitosan powder in 10 ml of distilled water by heating at 40^0 C.Then the above prepared chitosan solution was added to the homogenous dispersion of sodium

alginate containing 1g of drug (Ofloxacin) which was homogenized thoroughly with the help of magnetic stirrer.

The resulting bubble free dispersion was added manually drop wise with a 5 ml syringe (22 gauze needle) into 100ml of (10%w/v) calcium chloride solution ($cacl_2$) and stirred in a 250ml beaker.The gelation time of 15min was allowed to complete the curing reaction and produce spherical and rigid microspheres.The beads so prepared were collected by decantation, washed with water and dried in hot air oven at 60^{0}C for 2 hours.The process was applied to 6 different formulations by using varying proportions of chitosan and sodium alginate[12] (i.e., F1-F6).

Table 1: Formulations with different formulation variables

Formulation Code	Ofloxacin(mg)	Sodium Alginate (mg)	NMS (Chitosan) (mg)	Cross linking Agent $cacl_2$ (%w/v)
F1	1000	1400	5	10
F2	1000	1600	10	10
F3	1000	1800	15	10
F4	1000	2000	20	10
F5	1000	2200	25	10
F6	1000	2400	30	10

Characterization of Microspheres[13]

Particle size analysis

The sample of prepared microspheres was randomly selected and their size was determined using an optical microscope (Olympus, India) with the help of eye piece and stage micro meter.

Entrapment efficiency

The drug entrapment efficiency of beads was estimated by dispersing the beads in 100 ml of phosphate buffer at 7.4 by vigorous shaking on mechanical shaker for 12 hr. Then, the solution was filtered, and the lansoprazole content was assayed by a UV spectro photometer at 294 nm.

The entrapment efficiency of micro beads was calculated using the following formula.

$$\text{Entrapment efficiency} = \frac{\text{Estimated percentage drug loading}}{\text{Theoretical percentage drug loading}} \times 100$$

Swelling study

The swelling studies of beads were performed in aqueous swelling media with pH 7.4 buffer at 37.5 ± 0.5^{0}C.The swelling ratio,S_{wt},was calculated from the following expression.

$$S_{wt} = [(W_t - W_0) / W_0] \times 100$$

Where, Wt and W_0 are weight of sample Swollen at time‘t’ and weight of the original sample respectively.

Evaluation of mucoadhesive property

Apparatus used

Chicken intestine (2x2cm), glass slides,USP tablet disintegration apparatus, phosphate buffer pH 7.4.

Method

The mucoadhesive property ofmicrobeads was evaluated by an in vitro adhesion testing method known as wash-off method.Freshly excised pieces of chicken intestinal mucous were mounted on to glass slides with cotton thread.About 20 microbeads were spread on to each prepared glass slide and immediately thereafter the slides were hung to USP II tablet disintegration test,when the test apparatus was operated, the sample is subjected to slow up and down movement in the test fluid at 37^{0}C contained in a 1-litre vessel of the apparatus.At an interval of 30min up to 8 hours the machine is stopped and number of beads still adhering to mucosal surface was counted. The test was performed at intestinal (phosphate buffer pH 7.4) condition.

In vitro drug release study

The release of Ofloxacin from the microbeads was studied in phosphate buffer pH 7.4 as medium using dissolution test apparatus paddle type at 37± 0.2^0 C with a rotating speed of 50 rpm.A sample of microbeads equivalent to 40 mg of ofloxacinwas used in each test.At present time intervals 5ml aliquots were withdrawn and replaced by an equal volume of fresh dissolution medium.The samples were withdrawn through a membrane filter and were analyzed for Ofloxacincontent spectro photo metrically at 294 nm using the UV-Visible Spectrophotometer.

RESULTS AND DISCUSSION

Determination of Particles Size

The effect of different parameters on particle size of micro beads has been summarized in the table No.2. Increase in gel concentration increases the mean particle size of the beads.

This is due to the increase in viscosity, which in turn increase the droplet size during addition of the polymer solution to the cross-linking agent solution. Particle size also increases by increasing the drug load.

Entrapment efficiency

The drug entrapment efficiency of different formulations has been summarized in the Table No.3.The Ofloxacin being highly soluble in water is having tendency to diffuse out to the aqueous medium even though the sufficiently higher drug entrapment to the gel beads prepared with the chitosan could be achieved that might be resulted due to hindered diffusion of the medicament through the gel barrier formed by the chitosan.It was observed that, as the concentration of chitosan increases, viscosity of resulting gel increases and thereby increases in entrapment efficiency.

Table 2: Particles Size Analysis

Particle size(µm)	No. of Particles						Mean particle size (in µm)						Average Particle Size (micrometers)					
	F1	F2	F3	F4	F5	F6	F1	F2	F3	F4	F5	F6	F1	F2	F3	F4	F5	F6
10-20	0	0	0	0	0	0	0	0	0	0	0	0						
21-30	4	3	0	2	1		267	298	302	321	335	598	416.	496.	521.	537.	562.	623.
31-40	18	22	34	31	29	43	321	347	382	397	429	457	25	125	625	735	50	375
41-50	11	12	24	38	41	12	386	391	408	436	451	497						
51-60	12	15	21	42	19	28	413	453	439	480	513	542						

61-70	16	13	12	52	49	36	492	529	571	553	572	596
71-80	23	23	10	24	38	48	521	576	597	574	631	659
81-90	19	20	7	48	17	29	598	651	693	692	697	738
91-100	13	15	5	28	31	41	621	724	781	849	872	900

Table 3: Entrapment Efficiency

Formulation code	Weight taken (mg)	Media Qty (mL)	Entrapment efficiency
F_1	50	100	67.50%
F_2	50	100	75.02%
F_3	50	100	82.52%
F4	50	100	86.78%
F5	50	100	88.76%
F6	50	100	91.87%

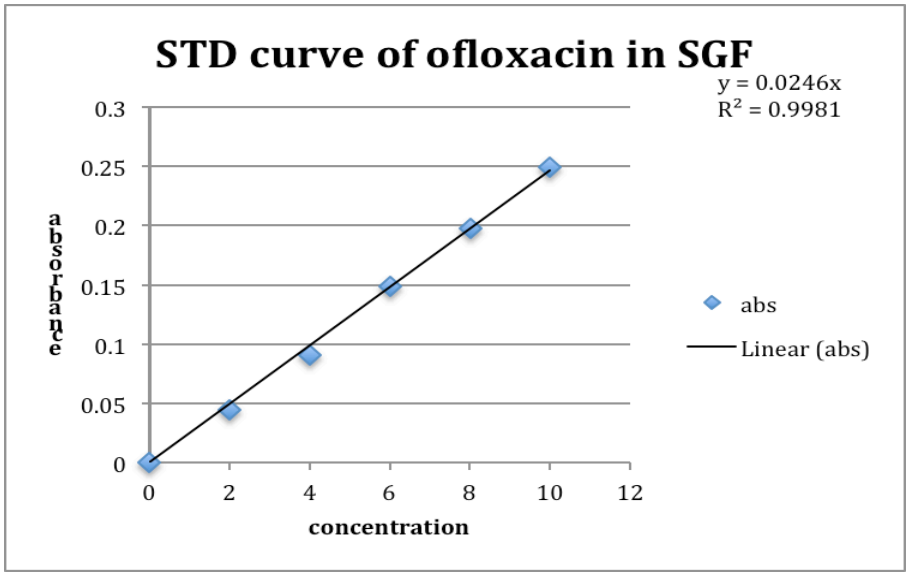

Fig. 1: Standard plot for entrapment efficiency of Ofloxacin

Swelling factor

The swelling behavior of microbeads was determined gravimetrically. The results indicated that as the amount of polymer (formulations F1, F2, F3, F4, F5 &F6) in microbeads was increased the swelling ratio also proportionately increased. Results given in Table No.4.The higher percentage of chitosan in micro beads renders high swelling and gel formation. So the inclusion of chitosan in alginate gel opens an option for the manufacture of cross linked matrix devices for gastrointestinal delivery.

In vitro drug release study

The drug-polymer ratio was found to affect the drug entrapment, particle size and ultimately the drug release characteristics of the prepared micro beads.At higher drug-polymer ratio the drug release from the micro beads was faster as compared to lower drug/polymer ratio.

This may be due to the increase in the drug/polymer ratio with an increase in the amount of drug loaded in the polymer,suggesting that higher amount of drug is released per unit area of exposed surface of the polymer matrix.A significant decrease in rate and extent of drug release was observed with the increase in polymer concentration in micro beads and is attributed to an increase in the density of polymer matrix and in the diffusion path length that the drug molecules have to traverse.

The prolongation of the release rate from the hydro gel beads with increase of chitosan concentration reflects the concomitant increases in gel strength which is a determining factor in this case since the release of drugs in polymer matrices are mainly through the diffusion of the drug through the pores of the polymer network which can be significantly reduced in size by increasing the polymer concentration.The initial higher release from the beads reflects the lower diffusional resistance of these core beads compared with that of the coated beads caused by the absence of a barrier against drug diffusion.

Mucoadhesion test

The adhesion of microspheres to the intestinal mucosa of chicken was evaluated as the mean percent of microspheres remain adhered after a defined period of washing.Results indicating that the polymer to drug ratio had a significant effect on mucoadhesive property.The greater the concentration of the polymer associated with chitosan-alginate matrix, greater will be the adhesion.An increase in drug load has no such effect on mucoadhesive property.

Table 4: Swelling Factor

Formulation code	Wt. of spheres taken(mg)	Initial wt.	Final wt.	Swelling (%)

		(mg)	(mg)	
F1	10	10	12.6	26%
F2	10	10	14.2	42%
F3	10	10	16.5	65%
F4	10	10	17.3	73%
F5	10	10	18.7	87%
F6	10	10	19.3	93%

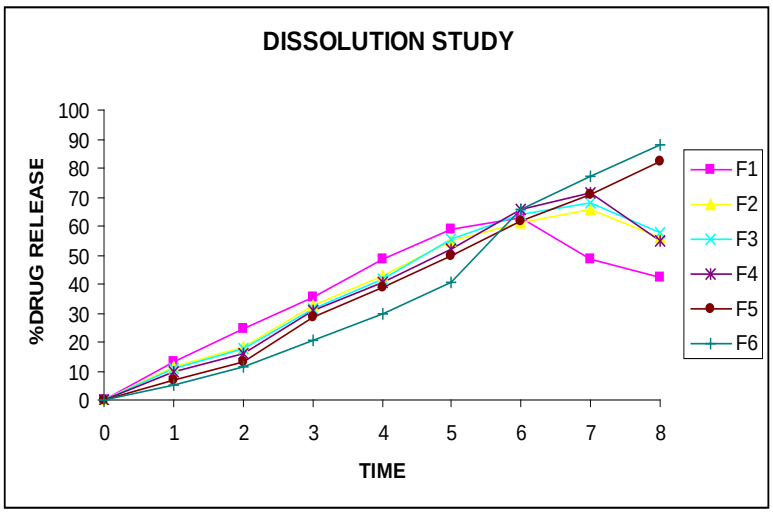

Fig. 2: Comparison study of In vitro drug

Table 5: Mucoadhesion Test

Formulation code	**No. of microspheres**		**Percentage of adhesion** (%)
	Initial	**Final**	
F1	20	12	60
F2	20	14	70
F3	20	15	75
F4	20	17	85
F5	20	18	90
F6	20	19	95

CONCLUSION

Ofloxacin release from these mucoadhesive micro-spheres[14,15]were slow and extended over longer period of time and dependent over ratio of polymers.These studies demonstrated that Ofloxacin can be encapsulated into microspheres having chitosan and sodium alginate backbone by micro orifice syringe gelation technique. In conclusion the performed studies suggested that chitosan may be a promising candidate for oral controlled drug delivery system because of its gel forming ability and sustaining the release of drug.Among F1-F6 formulations, F6 formulation has better controlled drug release profile because F6 formulation contains more mucoadhesive polymer concentration compared to other formulations.

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