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RESEARCH ARTICLE

FORMULATION AND CHARACTERIZATION OF MICROSPHERES OF SELECTED ANTI-INFECTIVE AGENT FOR URINARY TRACT INFECTION

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ABSTRACT

The main objective of the present study was to improve bioavailability of cephalexin and decrease the frequency of dosage form administration by increasing the encapsulation efficiency of the drug, residence time of the dosage form at the site of absorption and sustained release of the drug from the delivery system. Cephalexin is a first generation cephalosporin antibiotic and widely used in the treatment of respiratory tract infection. It has an extensive and highly variable hepatic first pass metabolism following oral administration having half life of 1.2 h. The usual dose of cephalexin is 250 mg/day with systemic bioavailability of 23-40% due to extensive "first-pass" metabolism and has a narrow absorption window. These characteristics make cephalexin a suitable drug candidate for mucoadhesive drug delivery system. Cephalexin-loaded mucoadhesive microspheres were prepared by ion gelation technique. The mucoadhesive microspheres were prepared by using sodium alginate alone and in combination with guar gum. The size distribution of microspheres had good mucoadhesive properties. The scanning electron microscopy and in vitro dissolution studies were performed to characterize the microspheres.

Keywords: Cephalexin, Sodium alginate, guar gum, ion gelation technique, Mucoadhesive microspheres.

INTRODUCTION

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then maintain the desired concentration. That is the drug delivery system should deliver the drug at a rate dictated by the needs of the body over a specified period of treatment. [1] The design of proper dosage form regimens is an important element in accomplishing this goal. An important issue in the development of these systems is:

- To avoid interknit and intersubject variations in GI residence time.
- To improve the absorption of poorly absorbed drug by using such systems. [2]

The term "sustained release" is used to describe a dosage form formulated to retard the release of a therapeutic agent such that its appearance into systemic circulation is delayed and/or prolonged and its plasma profile is sustained in duration. The onset of pharmacological action is often delayed and duration of its therapeutic effect is often sustained. Controlled release dosage forms are designed to release drug *in vivo* according to predictable rates that can be verified by *in vitro* measurements. Controlled release technology implies a quantitative understanding of the physicochemical mechanism of drug availability to the extent that the dosage

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form release rate can be specified. Various designations such as smart, targeted, intelligent, novel and therapeutic have been given to sustained release systems. [3, 4]

Cephalexin is a first generation cephalosporin antibiotic and widely used in the treatment of respiratory tract infection . It has an extensive and highly variable hepatic first pass metabolism following oral administration having half life of 1.2 h. In present work, a mucoadhesive gastro retentive microparticulate system (microspheres) for cephalexin was developed by ion gelation technique using aforementioned polymers. The microspheres were spherical in shape and the drug remained dispersed in the polymer matrix at amorphous state. Scanning electron microscopy, Differential scanning calorimetry and *in vitro* dissolution studies were performed to characterize the microspheres.

Therefore, the objective of the present study was the development and evaluation of anti-infective microspheres containing cephalexin using various mucoadhesive polymers for prolonged absorption. The method of microencapsulation is based on ion gelation technique involving alginate polymers alone and/or in combination with other mucoadhesive polymers.

MATERIALS AND METHODS

Cephalexin was obtained from ZIM Labs, Nagpur. Sodium alginate, ,calcium chloride , Potassium dihydrogen phosphate, Liquid Paraffin, Span 80, were purchased from purchased from Loba Chemical, Mumbai. .

Preparation of microspheres[5,6]:

Mucoadhesive microspheres containing cephalexin were prepared by ion gelation technique employing solutions of sodium alginate alone (0.5% w/v, 1% w/v, 1.5% w/v) Guar gum (1%) was dissolved in water by using magnetic stirrer. In another beaker sodium alginate (1%) dissolved in water. Cephalexin (250 mg) dissolved in distilled water then mixed the sodium alginate solution with guar gum the above solution was dropped using a hypodermic syringe into calcium chloride (3% w/v) solution.

Microspheres formed immediately and were left into the original solution for 1 hr to ensure internal gelification. Then they were filtered, washed with alcohol and dried at room temperature

Particle size analysis [7]:

Particle size of microspheres was determined by using an optical microscope under regular polarized light, and the mean particle size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer.

Swelling index [8]:

The swelling index of the microspheres is an indication of the capacity of the microspheres to absorbed water and swell. For estimating swelling index, the microspheres (50) were weighed initially then suspended in pH 1.2 and 7.4 phosphate buffer. After every 1 h microspheres were removed, surface water trapped with tissue paper and weighed. The increase in weight of microspheres is used for calculation of swelling index.

$$\text{Swelling index} = \frac{\text{Weight of wet microspheres} \times 100}{\text{Weight of dry microspheres}}$$

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Determination of encapsulation efficiency [9]:

The amount of cephalexin present in the microspheres was determined by extracting the drug into phosphate buffer of (pH 7.4). Accurately weighed 0.1g powdered microspheres were extracted in to 50 ml of phosphate buffer (pH 7.4) by magnetic stirring for a period of 2h. The solution was filtered through Whatman filter paper no.5, suitably diluted and estimated for drug content spectrophotometrically at 263 nm using UV–Visible Spectrophotometer.

Encapsulation efficiency was calculated by the following formula:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100$$

Measurement of mucoadhesive strength [10. 11]:

The mucoadhesive properties of the microcapsules were evaluated by *in vitro* wash off test as reported by Lehr *et al*. The test was performed at both simulated gastric fluid (pH 1.2) and simulated intestinal fluid (phosphate buffer, pH 7.4). The freshly excised pieces of intestinal mucosa (3×2 cm) from goat was tied onto glass slides (3×1 inch) using thread. About 50 microspheres were spread onto each wet rinsed tissue specimen and immediately thereafter the slides with suitable support were hung onto one the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing 1.2 pH and pH 7.4 buffer solution. At the end of every hour the number of microspheres still adhering onto tissue was counted.

Morphology[12]:

Surface morphology of microspheres was investigated by Scanning Electron Microscopy (SEM) using JSM 6380A (JOEL, Japan). The microspheres, coated with Platinum by ion sputtering using Auto fine coater JFC-1600 (JOEL, Japan), for 20 s at 1.1V under argon atmosphere were mounted onto metal stubs using double-sided carbon adhesive tapze and the scanning electron micrographs were take.

***In vitro* drug release studies[13]:**

The *in vitro* drug release studies were performed using Dissolution Apparatus USP using simulated gastric fluid pH 1.2 for twelve hours. An accurately weighed amount of drug loaded mucoadhesive microspheres equivalent to 50 mg of cephalexin, was added to 900 ml of dissolution medium and the release of cephalexin,from mucoadhesive microspheres was investigated at about 100 rpm at temp 37 $^{\circ}$C ± 0.5 $^{\circ}$C. During dissolution 5 ml aliquot was withdrawn at different time intervals of 1 to 12 h and same was replaced with equal volume of fresh medium. The withdrawn samples were filtered through Whatmann filter paper no.42 and absorbance was measured at

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263 nm using UV-Visible Spectrophotometer. Cumulative percent drug released was found out at each time interval and graph was plotted between cumulative % drug released and time in h.

Treatment of drug release data with different kinetic equations[14]:

Analysis of drug release from microspheres was performed with a flexible model that can identify the contribution to overall kinetics, mechanism of drug release and the dissolution data obtained for optimized formulation was treated with the different release kinetic equations.

MORPHOLOGY

Surface morphology of microspheres was investigated by Scanning Electron Microscopy (SEM) using JSM 6380A (JOEL, Japan). The microspheres, coated with Platinum by ion sputtering using Auto fine coater JFC-1600 (JOEL, Japan), for 20 s at 1.1V under argon atmosphere were mounted onto metal stubs using double-sided carbon adhesive tape and the scanning electron micrographs were taken

TABLE 1: FORMULATIONS OF MUCOADHESIVE MICROSPHERES OF CEPHALEXIN

Formulation code	Drug (mg)	Sodium Alginate (%)(w/v)	Guar gum (%)(w/v)	Crosslinker (calcium chloride %w/v)
AF1	250	0.5	0.5	3%
AF2	250	0.5	1	3%
AF3	250	0.5	1.5	3%
AF4	250	1	0.5	3%
AF5	250	1	1	3%
AF6	250	1	1.5	3%
AF7	250	1.5	0.5	3%
AF8	250	1.5	1	3%
AF9	250	1.5	1.5	3%

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TABLE 2: VALUES OF PARTICLE SIZE, SWELLING INDEX AND % ENCAPSULATION EFFICIENCY FOR MUCOADHESIVE MICROSPHERES OF CEPHALEXIN

Formulation code	Mean Particle size (µm)	% swelling index		% Encap -sulation Efficiency
		pH 1.2	pH 7.4	
AF1	1130.25 ± 4.58	282.52 ± 2.84	630.40 ± 5.12	68.34
AF2	1250.30 ± 3.35	268.37 ± 1.14	621.88 ± 4.85	76.29
AF3	1168.21 ± 5.12	159.67 ± 1.68	614.69 ± 3.23	81.16
AF4	978.60± 7.46	192.42 ± 2.86	564.26 ± 4.68	80.22
AF5	1156.52 ± 4.38	279.13 ± 1.28	556.56 ± 3.76	83.28
AF6	980.2 ± 6.54	166.49 ± 1.63	544.42 ± 4.13	85.05
AF7	1210.30± 8.66	281.44 ± 2.34	453.12 ± 5.51	74.28
AF8	1189.6± 9.36	174.99 ± 1.92	447.79 ± 3.62	79.04
AF9	1270.80± 8.15	258.29 ± 1.82	440.32 ± 4.24	86.13

Mean ± SD, *n = 3*

TABLE 3: RESULTS OF *IN VITRO* WASH OFF TEST AT pH 1.2 CEPHALEXIN LOADED MICROSPHERES FORMULATIONS

Formu-lation code	Percentage of microspheres adhered to goat intestine mucosa					
	Time in h					
	In 0.1M HCl (pH 1.2)					
	0	1	2	4	6	8
AF1	50	50 ± 0.0	48 ± 1.15	48 ± 1.0	44 ± 0.57	36 ± 0.57
AF2	50	48 ± 1	44 ± 0.0	40 ± 1.1	38 ± 1.0	34 ± 1.0
AF3	50	50 ± 0.0	48 ± 0.0	46 ± 1.15	44 ± 1.0	38 ± 1.15
AF4	50	48 ± 0.0	46 ± 1	46 ± 1	42 ± 0.57	38 ± 0.57
AF5	50	46 ± 0.0	44 ± 1.0	44 ± 1.0	42 ± 1.15	34 ± 0.0
AF6	50	46 ± 0.57	46 ± 0.57	44 ± 0.57	42 ± 0.0	36 ± 1.0
AF7	50	48 ± 0.0	48 ± 0.0	48 ± 0.57	46 ± 1	40 ± 0.57
AF8	50	50 ± 0.0	50 ± 0.0	48 ± 0.0	46 ± 1	40 ± 0.0
AF9	50	48 ± 0.0	48 ± 0.57	46 ± 1.0	44 ± 0.0	38 ± 0.57

SD ± (n=5)

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TABLE 4: RESULTS OF *IN VITRO* WASH OF TEST AT pH 7.4 OF DILTIAZEM HYDROCHLORIDE LOADED MICROSPHERES FORMULATIONS

Formu-lation code	Percentage of microspheres adhered to goat intestine mucosa					
	Time in h					
	In 7.4 phosphate buffer					
	0	1	2	4	6	8
AF1	50	42 ± 1.0	38 ± 1.0	32 ± 1.0	28 ± 1.5	26 ± 1.0
AF2	50	48± 0.0	44 ± 0.0	42 ± 1.5	36 ± 0.0	28 ± 0.57
AF3	50	50 ± 0.0	46 ± 0.0	40 ± 0.0	38 ± 0.0	32 ± 0.0
AF4	50	46 ± 0.57	44 ± 1.0	40 ± 1.5	36 ± 0.57	31 ± 1.0
AF5	50	48 ± 1.0	44 ± 1.0	42 ± 0.57	40 ± 1.0	34± 0.57
AF6	50	50 ± 0.0	50 ± 0.0	46 ± 0.0	42 ± 0.0	36 ± 1.1
AF7	50	48 ± 0.5	46 ± 0.5	40 ± 1.0	38 ± 0.57	32± 0.57
AF8	50	50 ± 0.0	44 ± 1.0	38 ± 0.5	32 ± 1.0	37± 1.15
AF9	50	46 ± 1.0	44 ± 1.0	36 ± 0.57	30 ± 1.0	24 ± 0.57

SD ± (n=5)

TABLE 5: KINETIC TREATMENT OF DRUG RELEASE DATA OF VARIOUS BATCHES

Formulat-ion Code	Zero order equation	First order equation	Higuchi's equation	Korsmeyer Peppas equation	n
	R^2				
AF1	0.975	0.978	0.937	0.985	0.724
AF2	0.984	0.960	0.895	0.990	0.765
AF3	0.956	0.852	0.835	0.978	0.789
HF1	0.980	0.914	0.916	0.991	0.709
HF2	0.969	0.881	0.918	0.988	0.737
HF3	0.982	0.934	0.915	0.992	0.821
CF1	0.968	0.945	0.911	0.990	0.793
CF2	0.970	0.897	0.886	0.978	0.819
CF3	0.964	0.913	0.864	0.977	0.836

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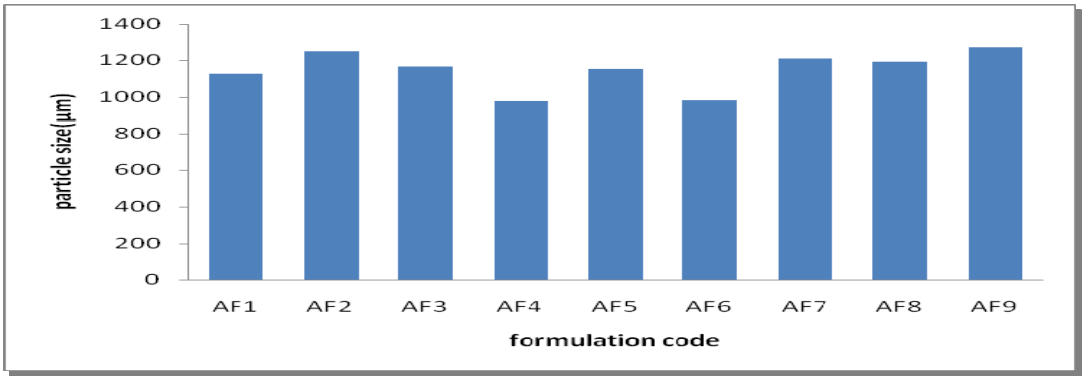

Figure 1: Particle size of cephalexin mucoadhesive microspheres formulations

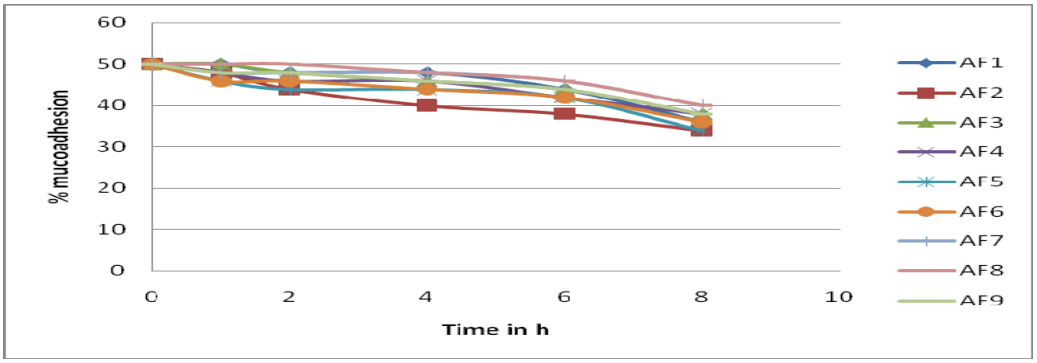

Figure 2: Mucoadhesion behaviour of cephalexin mucoadhesive microspheres formulations in pH 1.2

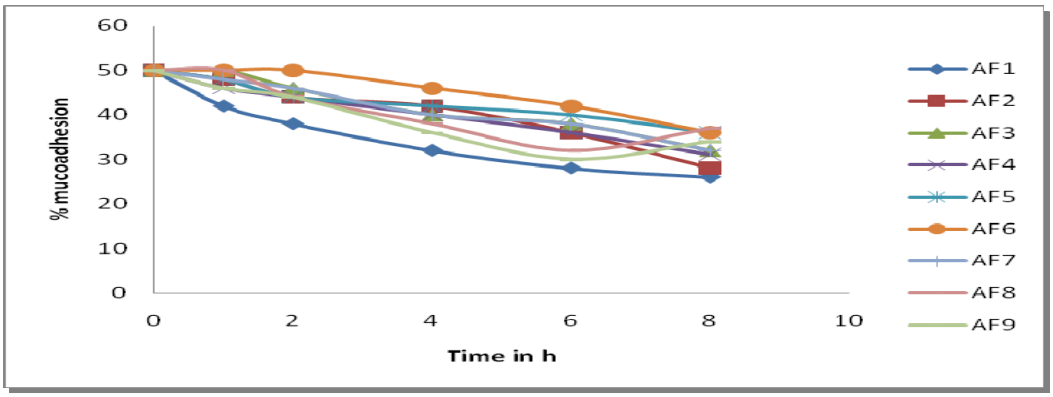

Figure 3: Mucoadhesion behaviour of Diltiazem hydrochloride mucoadhesive microspheres formulations in pH 7.4

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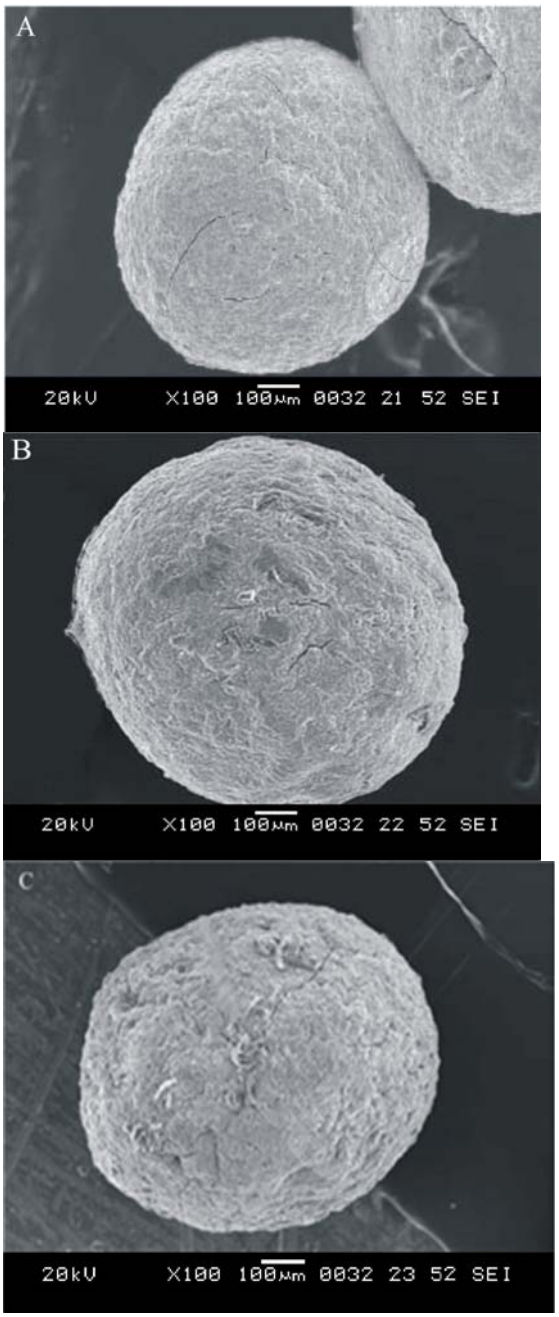

Figure 4: SEM photomicrograph of drug-loaded (A) sodium alginate microsphere, (B) sodium alginate- guar gum microsphere, (C) cephalexin sodium alginate guar gum microsphere

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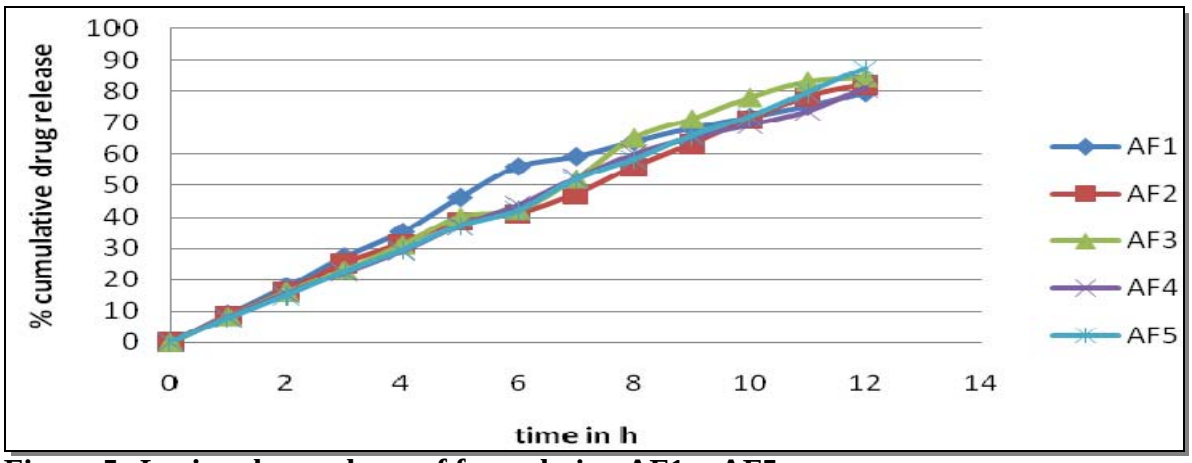

Figure 5: *In vitro* drug release of formulation AF1 toAF5

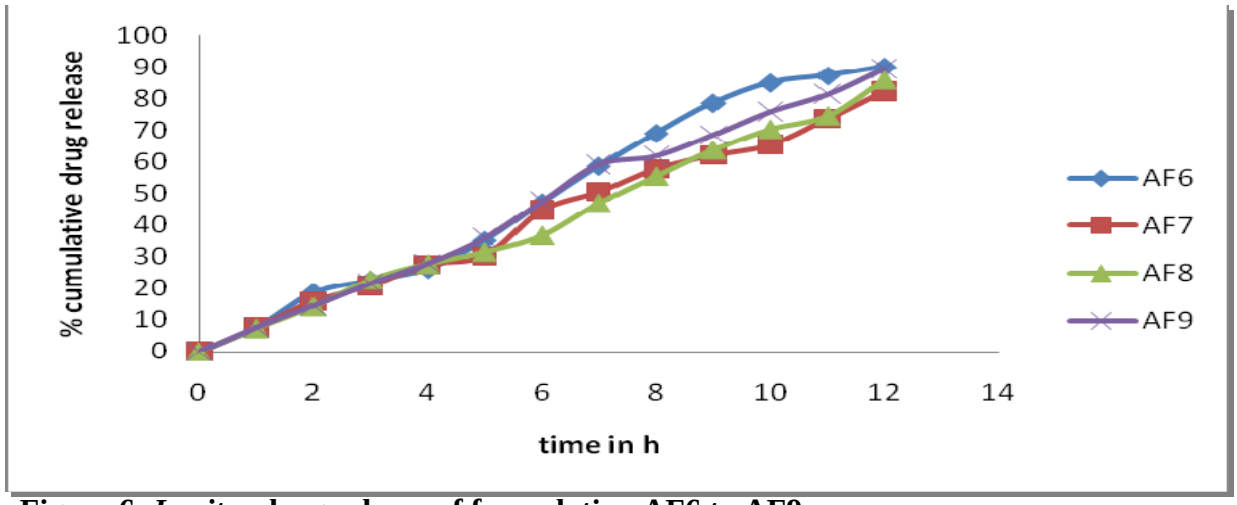

Figure 6: *In vitro* drug release of formulation AF6 to AF9

RESULTS AND DISCUSSION

Mucoadhesive microspheres were prepared using ion gelation technique. The concentration of the polymers and the concentration of the cross-linker agent were optimized by initial trial and error method. Three concentrations of the sodium alginate 0.5% w/v, 1% w/v and 1.5% w/v were tried which gave the formation of well formed microspheres. At concentrations above 1.5% w/v the viscosity was found to be very high as a result the drop wise extrusion of the polymer solution from the syringe became difficult to control. Thus a 0.5% w/v, 1% w/v and 1.5% w/v solution was chosen for the further studies. Similarly, a number of concentrations of calcium chloride were tried as a cross-linker agent. It was observed that at concentrations below 2% the microspheres formed were not strong enough and lost their shape while drying. It was also observed that at concentrations above 4%, the microspheres formed showed excessive shrinkage as a result, the shape was once again not retained. Thus, the optimum concentration of the cross-linker agent was chosen as 3%. Thus different batches of mucoadhesive microspheres

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were prepared using different concentration of guar gum (Table 1).

The particle size of mucoadhesive microspheres varied from 980.2 ± 6.54 µm to 1270.80 ± 8.15 µm (Table 8 and Figure 12). It was observed that the size of the microspheres was in increasing trend with increasing the alginate concentration. This is due to the increase in viscosity, which in turn increases the droplet size during addition of the polymer dispersion to the harvesting medium(Table 2)

The swelling behavior of the microspheres was studied by measuring the water uptake at certain time intervals in both (pH 1.2) and phosphate buffer (pH 7.4) . It was observed that the increase in polymer as well as cross-linker concentration decrease the swelling behavior of the microspheres. Sodium alginate is a polyelectrolyte, can exhibit swelling properties that are pH dependant. the ratio of water uptake by the microspheres was low and independent of time as compared to that obtained at phosphate buffer, pH 7.4.(table 2)

Three different concentrations of sodium alginate (0.5%, 1% and 1.5%) were used. The higher encapsulation efficiency was observed as the concentration of alginate increased. This is due to the greater availability of active barium binding sites in the polymeric chains and consequently the greater degree of cross linking. The highest incorporation efficiency (90.57) was achieved with 1% w/v sodium alginate in combination with 1.5% guar gum

The microspheres consisting of sodium alginate alone and in combination with guar gum exhibited good mucoadhesive properties as observed in *in vitro* wash-off test. The rapid wash-off observed at simulated intestinal pH is due to the ionization of carboxyl acid group and other functional groups in the polymers, which increase their solubility and reduce adhesive strength. The wash-off test was faster at simulated intestinal pH (7.4) than that at simulated gastric pH 1.2.

The microspheres were found to be discrete, spherical, free flowing and of the monolithic matrix type. The SEM photomicrographs of drug-loaded microspheres showed that the microspheres were almost spherical in shape with rough and nonporous surface and it also indicated that the drug was dispersed at amorphous state in the polymer matrices (Figure 4)

The cumulative percent drug release curve of the drug loaded sodium alginate microspheres showed the drug release from the microspheres decreased as the concentration of sodium alginate increased suggesting that drug release could be controlled by varying the polymers (Figure 5, 6).

In this study the prepared cephalexin-loaded mucoadhesive microspheres showed a maximum encapsulation efficiency of 90.57% as well as sustained the drug release over 12 h. The microspheres were spherical in shape and the drug remained dispersed in the polymer matrix at amorphous state. Microspheres of the drug were having no apparent effect on particle size, swelling index and other characteristics. The prepared microspheres exhibited good mucoadhesive properties as observed in *in vitro* wash-off test. The mucoadhesion study revealed that, the prepared formulations are having good mucoadhesion ability to prolong the residence time, which may directly correlated with the bioavailability of cephalexin.

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CONCLUSION

Mucoadhesive polymers Guar gum in combination with sodium alginate provides extended gastric retention and has ability to coat gastric mucosa uniformly. The use of such mucoadhesive polymers offers attractive approach in the development of targeted formulations for the gastrointestinal tract.

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REFERENCES

1. Garg S, Vasir JK, Tambwekar K. Bioadhesive microspheres as a controlled drug delivery system. Int J Pharm 2003;255:13-32.
2. Chowdary KR, and Srinivasa RY. Mucoadhesive drug delivery systems: review of current status. Indian Drug 2000;37(9):400-406.
3. Chong-Kook K, Eun-Jin L. The controlled release of blue dextran from alginate beads. Int J Pharm 1992;79:11–19.
4. Mirghani A, Idkaidek NM, Salem MS, Najib NM. Formulation and release behavior of diclofenac sodium in compritol 888 matrix beads encapsulated in alginate. Drug Dev Ind Pharm 2000;26(7):791–795.
5. Liu XD, Yu WY, Zhang Y, Xue WM, Yu WT, Xiong Y, Ma XJ, Chen Y, Yuan Q. Characterization of structure and diffusion behavior of Ca-alginate beads prepared with external or internal calcium sources. J Microencapsul 2002;19:775-782.
6. Sambathkumar R, Venkateswaramurthy N, Vijayabaskaran M, Perumal P. Formulation of clarithromycin loaded mucoadhesive microspheres by emulsification-internal gelation technique for anti *helicobacter pylori* therapy. Int J Pharm and Pharmaceutical Sci 2011;3(2):173-179.
7. Trivedi P, Verma AM, Garud N. Preparation and characterization of aceclofenac microspheres. Asian J Pharm 2008;4:110-15.
8. Rasala TM, Lohiya GK, Kale VV, Avari JG. Comparative study of ionotropic gelation technique to entrap diltiazem HCl in mucoadhesive microparticulate system. J Pharm Res 2010;3(7):1531-1534.
9. Lehr CM, Bowstra JA, Tukker JJ, Junginer HF. Intestinal transit time of bioadhesive microspheres in an in situ loop in the rat. J Control Release 1990;13:51-61.
10. Ramesh V, Medicott N, Razzak M, Tucker IG. Microencapsulation of FITC -BSA into poly (ε - caprolactone) by a water-in-oil-in-oil solvent evaporation technique. Trends Biomater Artif Organs 2002;15(2):32-36.
11. Gibaud S, Bonneville D, Astier A. Preparation of 3,4-diaminopyridine microparticles by solvent-evaporation methods. Int J Pharm 2002;242:197-201.
12. Albertinia B, Passerinia N, Sabatinoa MD, Vitali B, Brigidi P, Rodrigueza L. Polymer–lipid based mucoadhesive microspheres prepared by spray-congealing for the vaginal delivery of econazole nitrate. Eur J Pharm Sci 2009;60:53-62.
13. Barhate SD, Rupnar YS, Sonvane RM, Pawar KR, Rahane RD. Formulation and evaluation of floating microspheres of ketorolac trometamol. Ind J Pharm Res Dev 2009;1(9):1-8.
14. Das MK, Rao KR. Evaluation of zidovudine encapsulated ethyl cellulose microspheres prepared by water-in-oil-in-oil (w/o/o) double emulsion solvent diffusion technique. Acta Pol Pharm Drug Res 2006;63(2):141-48.