International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 7, 2016

Original Article

FORMULATION, OPTIMIZATION AND *IN VITRO* EVALUATION OF GASTRORETENTIVE MUCOADHESIVE MICROSPHERES OF FUROSEMIDE

SANDEEP KUMAR, ARUN NANDA*

*Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak, India 124001
Email: an_mdu@rediffmail.com

Received: 11 Mar 2016 Revised and Accepted: 20 May 2016

ABSTRACT

Objective: The objective of this study was to formulate and evaluate sustained release gastro retentive microspheres of furosemide using mucoadhesive polymers. It was expected that gastro retention plus mucoadhesion would contribute to extending the rate of drug release in the acidic medium *in vitro*, thereby projecting this formulation as a potential candidate for improvement of oral bioavailability of furosemide.

Methods: Mucoadhesive microspheres of furosemide were formulated by ionic gelation method by using two opposite charge mucoadhesive polymers (cationic chitosan and anionic sodium alginate). The formulations were optimized by employing 2^2 factorial design and characterized for *in vitro* evaluation i.e. drug entrapment efficiency, mucoadhesion study, drug release study, swelling study, etc.

Results: The microspheres formed were spherical in shape, and size ranged between 692-815 µm. Drug entrapment efficiency, % mucoadhesion and % drug release were ranged between 74.82-84.21 %, 22-43 % and 85.01-94.21 % respectively. DSC analysis revealed that there was no incompatibility between drug and excipients. The mechanism of drug release from microspheres followed Hixson-Crowell model. Comparison of drug release with marketed formulation (Lasix-40®) demonstrated the sustained release pattern of the gastro retentive mucoadhesive microspheres of Furosemide.

Conclusion: This work suggests that gastro retentive mucoadhesive microspheres, an effective drug delivery system for furosemide in improving the bioavailability of the drug.

Keywords: Furosemide, Mucoadhesive microsphere, Factorial design, Drug entrapment efficiency, Bioavailability

INTRODUCTION

Furosemide (FR) is a loop diuretic and chemically related to the sulphonamide. It has been chemically described as 4-chloro-2-[(furan-2-ylmethyl) amino]-5-sulfamoylbenzoic acid. It inhibits water reabsorption in the nephron by blocking the sodium-potassium-chloride cotransporter (NKCC-2) in the thick ascending limb of the loop of Henle [1]. Furosemide is widely used in the treatment of high blood pressure, edema associated with renal disease, nephritic syndrome, cirrhosis of the liver and congestive heart failure. The absolute bioavailability of Furosemide administered as a 100 mg tablet (equivalent 40 mg of Furosemide) is about 60-70 % [2]. FR is absorbed mostly in the stomach and upper small intestine, possibly due to its weak acidic properties (pKa 3.9) and it has a short half-life (1-2 h) [3]. The narrow absorption window of furosemide in the upper part of the GIT, together with its improved effect upon continuous drug input, provides a rationale for developing a gastro retentive dosage form for this drug [4].

Oral delivery of the drug is the most preferable route of drug delivery due to the ease of administration, patient compliance, and flexibility in the formulations. The major objective of oral controlled drug delivery system is to deliver drugs for a longer period of time to achieve better bioavailability, which should be predictable and reproducible [5]. Several approaches have been designed to retain the dosage forms in the stomach.

These methods include bio adhesive systems, swelling systems, expanding systems and floating systems. An oral sustained dosage form is particularly useful if the drug is absorbed throughout the GIT as the dosage form passes forward releasing the drug in GIT. One of the major limiting factor in oral sustained drug delivery is the short transit time which makes the drug remain at the absorption site for too short time to get absorbed completely from the desired site and there is no or little control over release of drug and thus effective concentration has to be achieved by multiple dosing. These problems can be overcome by the development of gastro retentive sustained release dosage forms [6]. Gastro retentive systems can remain in the gastric region for several hours and hence significantly prolong the gastric residence time of drugs. Prolonged gastric retention improves bioavailability, reduces drug waste, and improves solubility for drugs that are less soluble in a high pH environment. It has applications also for local drug delivery to the stomach and proximal small intestines. Gastro retention helps to provide better availability of new products with new therapeutic possibilities and substantial benefits for patients [7].

Mucoadhesive drug delivery systems (MDDS) possess additional advantages, such as close contact with the mucosal surface, when compared to other approaches. The MDDS provides a high surface to volume ratio and longer residence time, resulting in effective absorption and increased the bioavailability of the drug. The MDDS can also be tailored to adhere to any mucosal tissue in the GIT, including those found in the stomach. Thus, the MDDS offers an advantage for achieving localized, as well as systemic, controlled release of drugs [8]. Mucoadhesive microspheres include microparticles and microcapsules (having a core of the drug) of 1–1000 µm in diameter and consisting either entirely of a bioadhesive polymer or having an outer coating of it, respectively. Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery; but coupling of bioadhesive properties to microspheres has additional advantages, e. g., Efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucous layer, specific targeting of drugs to the absorption site achieved by anchoring plant lectins, bacterial adhesions and antibodies, etc. on the surface of the microspheres. Bioadhesive microspheres can be tailored to adhere to any mucosal tissue including those found in eye, nasal cavity, urinary and gastrointestinal tract, thus offering the possibilities of localized as well as systemically controlled release of drugs. Application of bioadhesive microspheres to the mucosal tissues of ocular cavity, gastric and colonic epithelium is used for administration of drugs for localized action. Prolonged release of

drugs and a reduction in the frequency of drug administration to the ocular cavity can highly improve the patient compliance [9].

Chitosan, a natural linear bio poly aminosaccharide is obtained by alkaline deacetylation of chitin, which is the second abundant polysaccharide next to cellulose. Chitin is a straight homopolymer composed of β-(1, 4)-linked N-acetyl-glucosamine units while chitosan comprises of copolymers of glucosamine and N-acetyl-glucosamine. Chitosan has one primary amino and two free hydroxyl groups for each C_6 building unit. Due to the easy availability of free amino groups in chitosan, it carries a positive charge and thus, in turn, reacts with many negatively charged surfaces/polymers and also undergoes chelation with metal ions [10].

Alginate is a copolymer, consisting of linear chains of α-L-glucuronic acid (G) and β-D-mannuronic acid (M) produced by marine brown algae. It is a useful biopolymer to prepare nanocapsules due to its good biocompatibility, biodegradability, non-toxicity and mucoadhesion properties. Glucuronic acids of alginate have the ability to exchange their Na^+ion and react with Ca^{2+}. In this reaction, the α-L-glucuronic acid groups will connect to each other by these divalent cations. Dimerization of alginate chains will also help them to join with many other chains which result in a gel network [11].

The present study aims to formulate and evaluate sustained-release gastro retentive microspheres of Furosemide using mucoadhesive polymers. Mucoadhesive microspheres had advantages such as efficient absorption and enhanced bioavailability of furosemide owing to a high surface to volume ratio, a much more intimate contact with the mucus layer and specific targeting to the absorption site. Sustained-release microspheres of furosemide would provide constant plasma concentration, with the less frequent administration and may also decrease the side effects to some extent. This could extend its safe administration and improve patient compliance.

MATERIALS AND METHODS

Materials

Furosemide was supplied as gift sample by Brawn Laboratories Ltd, Faridabad, India; the following materials were purchased from, Sodium alginate (Loba Chemie, Mumbai, India), Chitosan (Bio-Gen Extracts, New Delhi, India), Calcium chloride (Loba Chemie, Mumbai, India). All other reagents were of analytical grade.

Drug-excipient compatibility and stability study

Any interaction between drug and excipients was studied by DSC. Accurately weighed a sample of furosemide along with the excipients was hermetically sealed in an aluminum crucible. A blank hermetically sealed aluminum crucible was used as a reference pan. The system was purged with nitrogen gas at a flow rate of 60 ml/min and heating was done from 40-300 °C [1].

Factorial design and selection of optimized formulation

2^2 Factorial is the simplest factorial design in which two factors are studied at two levels, low and high. First of all amounts of sodium alginate and chitosan used were determined from the literature study and fixed minimum and maximum amount to be used. Then different formulations were designed according to 2^2 factorial designs. These formulations were prepared and tested for optimization parameters, i.e., entrapment efficiency, mucoadhesion study and release rate study. The amount of drug incorporated in each formulation was 1 g. The different formulations were prepared according to formula as shown in table 1.

Table 1: Different formulations according to formula

Formulation No.	Experiment	Drug (gram)	Sodium alginate (gram) A	Chitosan (w/v) B	Calcium chloride (w/v)
1	(1)	1	0.5 (Low)	0.5 % (Low)	3.0 %
2	A	1	2.0 (High)	0.5 % (Low)	3.0 %
3	B	1	0.5 (Low)	1.0 % (High)	3.0 %
4	Ab	1	2.0 (High)	1.0 % (High)	3.0 %

Inserting data in factorial design equations

Equation (1) for effect of factor A is

$$\text{Effect of factor A} = \frac{1}{2}[(ab+a)-(b+1)] \ldots\ldots\ldots (1)$$

Similarly, the effect of factor B is given by equation (2) as follows

$$\text{Effect of factor A} = \frac{1}{2}[(ab+b)-(a+1)] \ldots\ldots\ldots (2)$$

If the two factors have interaction between them, then the magnitude of interaction can be calculated by the following method.

The magnitude of the interaction term is then calculated in the same way as that of the main factors, i.e. the mean of the results of all experiments with a '+' in interaction column minus the mean of all those with a '-' in that column. Interaction of A and B can be calculated by the following equation (3).

$$\text{Magnitude of interaction} = \frac{1}{2}\times[(1+ab)-(a+b)] \ldots\ldots\ldots\ldots (3)$$

If the combined effect of the two factors had been to produce an effect greater than that produced by the factor individually, then the interaction is said to be synergistic. An interaction which produced a decrease is antagonistic.

Preparation of microspheres

Formulation of extended-release microspheres was done by using ionic gelation method [12]. Sodium alginate, an anionic mucoadhesive polymer was used as core material and cross-linked with chitosan which was cationic in nature and calcium chloride solution. Aqueous insoluble alginate-chitosan-calcium microspheres were formed by interaction between two oppositely charged polymers and cation exchange between Na^+ and Ca^{2+}. In this technique, sodium alginate was dissolved in purified water by simply agitating. Then drug was added into sodium alginate solution and ultra sonicated for 2 h to form a viscous dispersion. The resulting dispersion was added manually dropwise into different concentrations of chitosan and calcium chloride (3 %) solution through a syringe with a needle of size no. 26 gauge with gently stirring. The added droplets were retained in the calcium chloride and chitosan for 30 min to complete the curing reaction and to produce rigid spherical microspheres. The microspheres were collected by decantation, and the microspheres thus separated were repeatedly washed with purified water and dried at 45 °C for 12 h.

Evaluation of microspheres

The microspheres were evaluated for different parameters as described below:

1. Microencapsulation efficiency
2. Mucoadhesion study
3. *In vitro* drug release
4. Size distribution analysis
5. Swelling index study
6. Selection of mathematical models
7. Stability study

Microencapsulation efficiency

Furosemide content in microspheres was determined by UV-spectrophotometer (UV-Shimadzu, Japan). 100 mg microspheres

were crushed in a glass mortar-pestle, and the powdered microspheres were suspended in 100 ml pH 5.8 phosphate buffer. The solution was shaken occasionally and kept for 24 h. After 24 h, the solution was filtered to obtain a clear solution and the filtrate was analyzed for drug content spectrophotometrically at 277 nm [13, 14].

$$\text{Microencapsulation efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

Mucoadhesion study

For the mucoadhesion study, the use of goat intestinal mucosa had been approved by the Institutional Animal Ethics Committee, M. D. University, Rohtak with approval number Ph. Sci.-998, dated-08/08/2013. The freshly excised pieces of the intestinal mucosa (2×3 cm.) from goat were tied onto glass slides using thread. About 50 microspheres were spread onto each wet rinsed tissue specimen and immediately thereafter the slides were placed into the USP Type II dissolution apparatus containing pH 5.8 phosphate buffer operated at 50 rpm. At different time intervals up to 8 h the apparatus was stopped, and the numbers of microspheres still adhering to the tissue were counted. The percentage mucoadhesion was calculated [15-17].

$$\%\ \text{Mucoadhesion} = \frac{\text{No. of microspheres adhered}}{\text{No. of microspheres applied}} \times 100$$

***In vitro* drug release**

In an ideal situation, an extended release oral dosage form should be tested *in vitro* throughout the entire physiology pH (1-7.8) of the GI tract in order to simulate the *in vivo* conditions. The *in vitro* dissolution studies were performed using USP Type II dissolution apparatus at 50 rpm. The dissolution medium was kept in a thermostatically controlled water bath maintained at 37 °C±0.5 °C and volume of dissolution medium was 900 ml. Dissolution studies were carried out up to 8 h. An aliquot (5 ml) was withdrawn at specified time intervals, filtered through Whatman filter papers and drug content was determined by UV-Spectrophotometer at 277 nm. At each withdraw; 5 ml of fresh dissolution medium was replaced into the dissolution flask to maintain the sink condition. The phosphate buffer of pH 5.8 was used as dissolution media for the purpose of testing [18]. Microspheres equivalent to containing 40 mg of drug were used for *in vitro* release study. Dissolution data obtained was plotted as cumulative percentage drug release v/s time.

Size distribution analysis

Size distribution analysis of microspheres was performed by an optical microscope to determine the average size of microspheres. The microspheres were dispersed in liquid paraffin, and a drop off above dispersion was put on a glass slide and observed under a microscope. The diameter of 50 microspheres was determined using calibrated eyepiece micrometer and stage micrometer [14, 19]. The average diameter was calculated using the following formula:

$$\text{Average diameter} = \sum \frac{nd}{n}$$

Where,

n= number of microspheres, d= diameter of microspheres.

Swelling index study

The swelling study was carried for the prepared microspheres. The pre-weighed microspheres were immersed in 100 ml of medium (pH 5.8 phosphate buffer) and maintained at temperature 37 °C±0.5 °C for 6 h of the study period. At predetermined time intervals (1, 2, 3, 4, 5 and 6 h); the swollen microspheres were removed from the solution, immediately wiped with a paper towel to remove droplets of surface and weighed. The swelling index (S. I.) was calculated according to the following formula: [13, 17]

$$\text{Swelling Index} = \frac{Wt - Wo}{Wo}$$

Where, W_{0} = initial weight of dry microspheres, and W_t = weight of swollen microspheres at time t.

Selection of mathematical models

Several theories/kinetics models have been published, to elucidate the resulting drug release kinetics from extended release dosage forms. There are several models to represent the drug dissolution profiles where drug release is a function of time (t) related to the amount of drug dissolved from pharmaceutical dosage system.

The drug release data was modeled into various rate equations including zero order, first order, Higuchi equation, Hixson-Crowell plot and Peppas-Korsemeyer equation. The best-fit model was selected on the basis of greatest R^2 value [20, 21].

Stability study

Stability studies from an integral part of the formulation development process as the stability of the active components is a major criterion in determining its acceptance. In order to assess the stability of drug product, accelerated stability studies were conducted for the optimized batch of model drug microspheres. Microspheres were stored in glass vials for one month at storage condition 40 °C±2 °C/75 % RH±5 %. The products charged for stability are to be monitored for the physical appearance, assay, and dissolution [22].

***In vitro* release study of optimized formulation vs marketed formulation**

Dissolution studies were performed for a marketed immediate release tablet formulation (Lasix, 40® mg) using the same dissolution procedure as described above. A graph was plotted between cumulative percent drug release from optimized formulation and marketed formulation v/s time. The percent drug release per hour was studied in the same way and compared with optimized formulation (microspheres).

RESULTS AND DISCUSSION

Preparation of microspheres

Formulation of furosemide extended-release microspheres was done by using ionic gelation method. With the help of optical microscopy, microspheres were found to be discrete, large and spherical, free flowing, monolithic matrix and had rigid surfaces.

Determination of microencapsulation efficiency

The test for encapsulation efficiency was carried out to ascertain the amount of drug encapsulated in microspheres. The results obtained are reported in table 2. From the results obtained, it can be inferred that there is proper distribution of Furosemide in the microspheres. The encapsulation efficiency was found to be between 76.6 to 84.21%.

Mucoadhesion study

The mucoadhesive study showed that even at the end of 8 h, 22-43 % of microspheres were still adhering to the mucosa which shows that the prepared microspheres are having good mucoadhesion. The results are tabulated in table 3.

Table 2: Microencapsulation efficiency

Formulation	Experiment	Microencapsulation efficiency* (%)
1	(1)	74.82±0.47
2	A	78.95±1.64
3	B	76.6±2.57
4	Ab	84.21±0.78

*values are mean of triplicate±SD

Table 3: Percentage of microspheres remained adhered after each time interval

Formulations	% mucoadhesion* of microspheres after each interval							
	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h
1	95±0.82	88±0.51	72±0.46	52±2.13	47±0.58	32±1.85	27±0.68	22±0.56
2	88±1.23	77±0.98	70±0.73	65±0.48	62±0.35	53±1.29	42±0.95	37±0.48
3	90±0.67	80±2.43	75±0.39	68±0.81	53±1.58	42±0.92	38±0.46	32±0.35
4	95±0.95	78±1.66	72±0.65	67±0.53	60±0.88	55±0.48	47±0.62	43±0.80

*Results were expressed in average±SD (n=3)

***In vitro* drug release rate studies**

The *in vitro r*elease rate studies were performed in phosphate buffer media. Microspheres equivalent to containing 40 mg drug were put into dissolution medium. Percentage of drug released after each time interval is depicted in table 4.

Optimization of formula

Results of microencapsulation efficiency, mucoadhesion study and release rate study with the levels of factors A and B are shown in table 5.

From the above results in table 6, the factor A, i.e. sodium alginate was found more important than factor B, *i.e.* chitosan. So, now the amount of chitosan was fixed to its maximum level and the amount of sodium alginate was varied between minimum and maximum to obtained optimized formula.

Formulation A and D were prepared earlier. Now formulation B and C were prepared and evaluated for optimization parameters i.e. drug entrapment efficiency, mucoadhesion and release rate study. Results of all these studies are shown in table 7.

Table 4: Percent *in vitro* drug release after specified interval of time

Formulation	Cumulative percent drug release* after following time interval							
	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h
1	28±0.38	44±1.42	48±0.45	57±0.75	67±1.23	76±0.78	81±0.48	89±0.13
2	40±0.44	49±0.86	60±0.73	69±0.91	79±0.84	83±0.45	87±0.59	93±0.67
3	26±0.72	40±1.23	45±0.51	54±0.88	64±1.06	71±0.69	77±1.18	85±0.79
4	38±0.41	45±0.65	57±0.81	67±0.7	72±0.52	79±0.57	84±0.93	89±1.08

*n=3, average of three determinations±SD

Table 5: Results of entrapment efficiency, percent mucoadhesion, and percent drug release after 8 h

Experiment	Factor A Sodium alginate (gram)	Factor B Chitosan (w/v)	Interaction of A and B	Entrapment efficiency*	% mucoadhesion* after 8 h	Cumulative % drug release* after 8 h
(1)	0.5 (-)	0.5 % (-)	+	74.82±0.47	22±0.56	89±0.13
A	2.0 (+)	0.5 % (-)	-	78.95±1.64	37±0.48	93±0.67
B	0.5 (-)	1.0 % (+)	-	76.6±2.57	32±0.35	85±0.79
Ab	2.0 (+)	1.0 % (+)	+	84.21±0.78	43±0.80	89±1.08

*n=3, average of three determinations±SD

Table 6: The magnitude of the interaction between different parameters

Effect of factor based on microencapsulation efficiency		Effect of factor based on mucoadhesion		Effect of factor based on drug release study	
Effect of factor A	Effect of factor B	Effect of factor A	Effect of factor B	Effect of factor A	Effect of factor B
5.87	3.52`	13	8	4.5	-3.67
Magnitude of interaction					
1.74		-2		–0.175	

Table 7: Results of microencapsulation efficiency, mucoadhesion and release rate study

Formulation	Drug (gram)	Sodium alginate	Chitosan	Microencapsulation efficiency*	% mucoadhesion* after 8 h	% drug release* after 8 h
A	1	0.5	1.0 %	76.6±0.86	32±3.0	85.01±0.76
B	1	1.0	1.0 %	75.6±2.34	38±2.5	91.37±1.25
C	1	1.5	1.0 %	79.14±1.65	36±1.5	94.21±2.47
D	1	2.0	1.0 %	84.21±0.58	43±2.0	89.35±1.63

*n=3, average of three determinations±SD

Percent release of formulation B was found better than other formulations after each interval of time. Microencapsulation efficiency of formulation B was less, but mucoadhesion was good as compared to other formulations.

Microspheres prepared according to formulation B were regular in shape and almost spherical. So, formulation B was found to be optimized formulation. It was further evaluated for remaining parameters.

Compatibility study

Compatibility study of drug and excipients was determined by using Differential Scanning Calorimetry apparatus Q 10. As demonstrated in the graphs, furosemide exhibited a characteristic, sharp exothermic peak at 222.28 °C which was associated with an initial and small melting point followed by a sharp decomposition peak of the drug. The alginate polymer showed wide endothermic pick at 116 °C that indicates glass Transition Temperature (Tg) point of polymer and decomposition peak at about 301 ºC. DSC analysis of chitosan showed a broad endothermic peak at about 86.76 °C. In DSC graph of optimized formulation B the characteristics peak of furosemide was almost unchanged indicating the absence of strong interactions between the components and suggesting drug-excipients compatibility in all the formulations examined. DSC graphs obtained for furosemide, sodium alginate, chitosan and optimized formulation for compatibility study are shown in fig. 1 (A), (B), (C) and (D) respectively.

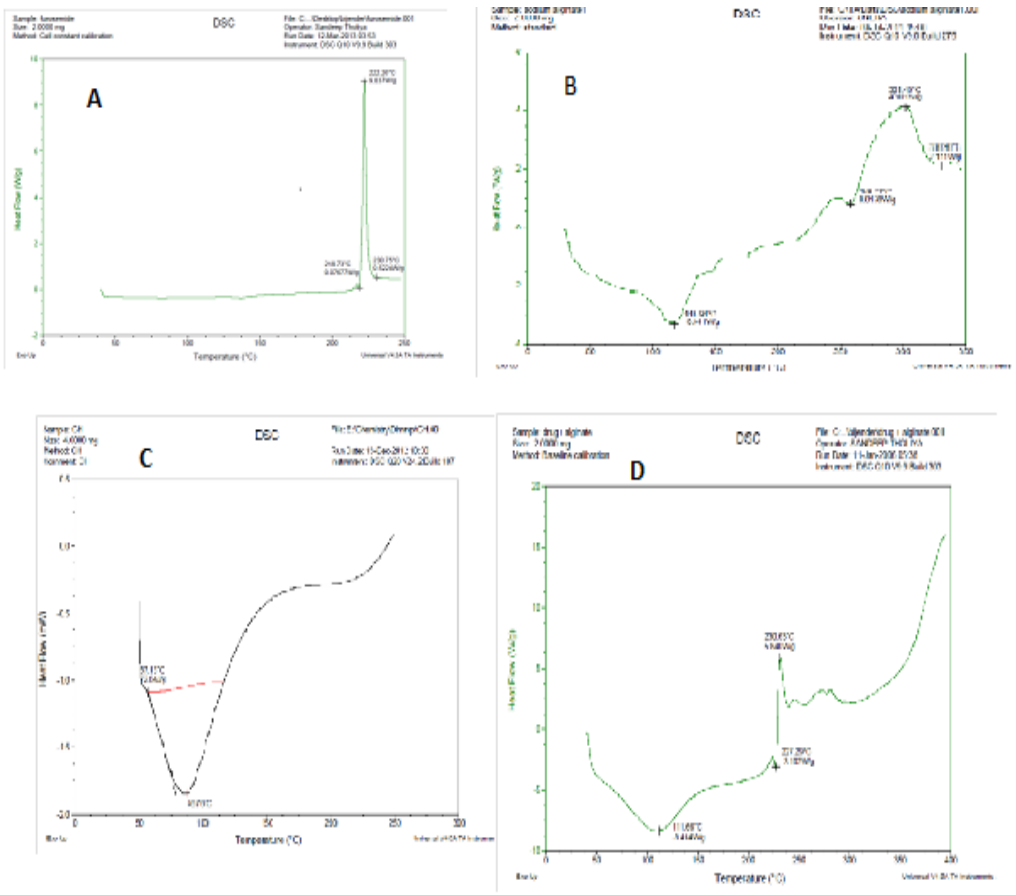

Fig. 1: DSC graphs obtained for furosemide (A), sodium alginate (B), chitosan (C) and optimized formulation (D)

Stability study

Stability studies for the furosemide encapsulated beads and microspheres did not show any significant changes in their properties after the end of one month. No change in drug appearance, drug content and dissolution results were observed after storage of optimized formulation at 40 °C/75 % R. H. for one month.

Shape and size analysis

Optimized formulation (B) was evaluated for shape and size analysis with the help of optical microscopy. Drug-loaded microspheres were found to be discrete, large, spherical, free flowing, monolithic matrix and had smooth surfaces. The size of microspheres was found to be in the range of 692 to 815 µm. The average size of microspheres was found 766 µm.

Swelling index study

Swelling increases with time because polymer gradually absorbs water due to its hydrophilicity. The outer layer of the polymer hydrates, swells, and a gel barrier is formed at the outer surface. The adhesive and cohesive properties of mucoadhesive polymers are generally affected by their swelling behavior. The percent swelling of the Optimized formulation (B) was found to be 308 %.

Plotting of release data in various models

Based on R^2 values Hixson-Crowell plot has greater R^2 value, i.e. 0.992. So the release follows Hixson-Crowell model. The significance of this model is that the dissolution occurs in planes which is parallel to drug surface if the dimension of dosage form diminish proportionality, in such a manner that the initial geometry form keeps constant all the time. R^2 values of different plots are shown in table 8.

Comparison of release rate studies with marketed formulation

Dissolution studies were performed for a marketed immediate release tablet formulation (Lasix, 40® mg) using the same dissolution procedure as described above. The comparative dissolution profile of marketed immediate release (Lasix) and best formulation (B) is tabulated in table 9.

A comparison of optimized mucoadhesive microspheres formulation of furosemide with conventional release marketed formulation (Lasix, 40® mg) as shown in fig. 2 confirmed the sustained-release behavior of the optimized formulation.

Table 8: R^2 value of different plots

Formulation	R^2 value of different plots				
	Zero order	First order	Higuchi plot	Hixson-Crowell	Korsmeyer-Peppas
B	0.957	0.982	0.991	0.992	0.986

Table 9: Comparative dissolution profile Lasix immediate release tablet and best formulation (B)

Time (h)	1	2	3	4	5	6	7	8
% Cumulative Drug Release of Marketed Product*	87.48± 0.56	96.9± 1.12	97.2± 0.81	97.8± 0.72	98.3± 1.48	98.7± 1.87	99.4± 0.95	99.8± 0.42
% Cumulative Drug Release of Optimized Formulation B*	20.11 ±0.95	37.36 ±1.65	53.5 ±0.87	61.72 ±1.12	72.57 ±0.46	83.11 ±2.14	89.62 ±0.74	91.37 ±1.25

*n=3, average of three determinations±SD

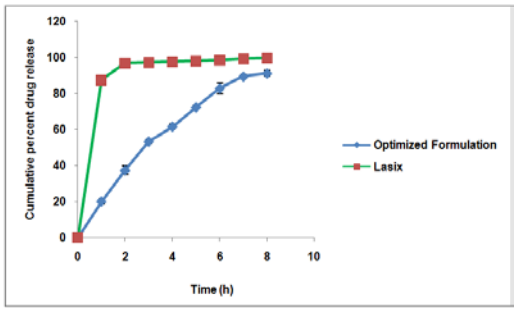

Fig. 2: Comparison of release rate study between optimized formulation and marketed formulation (n=3, average of three determinations±SD)

DISCUSSION

Mucoadhesive microspheres of furosemide were prepared by the ionic gelation method from polymers sodium alginate and chitosan using 2^2 full factorial design. The particle size of microspheres was determined by optical microscopy ranged from 692 to 815 µm. The particle size of the formulations prepared were in consonance with that reported by other workers, such as 718-1092 µm (Fentie *et al.*, 2015) [4], 827-1026 µm (Arora & Budhiraja, 2012) [7], 1025-1358 µm (Prasad *et al.,* 2011) [12], 726-1200 µm (Das & Senapati, 2008) [23] and 998-1143 µm (Singhavi *et al.*, 2012) [24].

Microencapsulation efficiency of microspheres was found to be in the range of 74.82 to 84.21%. The incorporation efficiency increased progressively with increasing sodium alginate concentration. The low incorporation efficiency of alginate beads cross-linked with Ca^{2+}could be attributed to the formation of porous beads ensuring the diffusion of the drug out of the beads at the time of curing. The result was similar to that reported by Das & Senapati, (2008) [23] and Singhavi *et al.,* (2012) [24].

The swelling index of the formulation was found 308%. Diffusion of the drug significantly depends on the water content of microspheres. As polymer chain becomes more hydrated and gel becomes more diluted, the disentanglement concentration may be reached (i.e. the critical polymer concentration below which the polymer chain disentangles and detaches from the gelled matrix) which result in swelling. Consequently, faster and greater swelling of microspheres might lead to increased dimension of microspheres resulting to an increasing diffusion pathway and thus, a reduction in diffusion rate. So, the drug release was found to be high initially and then gradually decreased.

In vitro mucoadhesion of the microspheres was tested (table 3). Microspheres with a high concentration of chitosan and sodium alginate showed more adherences (43% after 8 h) to gastric mucosa, indicating that chitosan and sodium alginate provided gastric adherence, due to the strong electrostatic attraction between the polymers and the mucus glycoproteins. The result was similar to that reported by Singhavi *et al.,* (2012) [24].

It was found that there was a decrease in drug release with an increase in mucoadhesive polymer content. This could be attributed to the greater degree of swelling upon hydration with greater mucoadhesive polymer content in the microspheres which leads to increase in the diffusional path length that slows down drug release. The principle of gelation or crosslinking of sodium alginate with $CaCl_2$ is based on the formation of a tight junction between the glucuronic acid residues. The number of apparent cross-linking points formed with calcium alginate gel beads increased with increasing alginate concentration in the formulation. The increase in the apparent crosslinking density delayed the alginate gel disintegration in phosphate buffer due to the retardation of Ca^{2+}exchange with $Na^+$and eventually caused an increase in microencapsulation efficiency and sustained percent drug release. Marketed preparation (Lasix) released 87.48 % drug in 1 h whereas optimized formulation released 91.37% of the drug in 8 h. The dissolution profile of marketed and optimized formulations showed the immediate and sustained release of drug respectively. Our results are in agreement with the reports of Fentie *et al.*, (2015) [4]; Arora & Budhiraja, (2012) [7]; Marina *et al.*, (2012) [14] and Das & Senapati, (2008) [23].

The *in vitro* dissolution data were analyzed by different kinetic models in order to find out the R^2 value, which describes the drug release mechanism. The values of coefficient of correlation (R^2) obtained for the respective model are listed in table 8. In this case, data fitted best to the Hixson–Crowell model (R^2= 0.992), that describes the drug releases by dissolution mechanism that occurs upon a change in surface area and diameter of particles (Lima *et al.*, 2015) [25].

CONCLUSION

Gastroretentive mucoadhesive microspheres of furosemide with good mucoadhesive strength could be successfully prepared by ionic gelatin method using sodium alginate and chitosan as polymer. This method was simple, reproducible and produced microspheres of regular shape and size. *In vitro* release studies indicated that there was a slow and sustained release of drug for all the formulations. Among all the formulations, formulation B microspheres showed the good microencapsulation efficiency, higher mucoadhesive strength and *in vitro* drug release. The size of microspheres was found to be in the range of 692 µm to 815 µm. The percent swelling of microspheres was found to be 308 %. The kinetic studies suggested that the drug was released by Hixson-Crowell model. The selected formulation B was found to be stable during the accelerated stability studies. Thus, the mucoadhesive gastro retentive microspheres of a furosemide present potential drug delivery system for improvement of oral bioavailability of this drug.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. ElMeshad AN, El-Ashmoony MM. Floating furosemide gel beads: *in vitro* and *in vivo* evaluation. J Drug Delivery Sci Technol 2012;22:317-25.

2. Longhia M, Garneroa C, Chattahb AK. Improving furosemide polymorphs properties through supramolecular complexes of β-cyclodextrin. J Pharm Biomed Anal 2014;95:139-45.
3. Ritschel WA. Targeting in the gastrointestinal tract: new approaches-methods find. Clin Exp Pharmacol Physiol 1991;13:313-36.
4. Fentie M, Belete A, Gebre-Mariam T. Formulation of sustained release floating microspheres of furosemide from ethylcellulose and hydroxypropyl methylcellulose polymer blends. J Nanomed Nanotechnol 2015;6:1-5.
5. Makwana A, Sameja K, Parekh H, Pandya Y. Advancements in controlled release gastro retentive drug delivery system: a review. J Drug Delivery Ther 2012;2:12-21.
6. Soni RP, Patel AV, Patel RB, Patel MR, Patel KR, Patel NM. Gastroretentive drug delivery systems: a review. Int J Pharm World Res 2011;2:1-24.
7. Arora S, Budhiraja RD. Chitosan-alginate microcapsules of amoxicillin for gastric stability and mucoadhesion. J Adv Pharm Technol Res 2012;3:68-74.
8. Patil H, Tiwari RV, Repka MA. Recent advancements in mucoadhesive floating drug delivery systems: a mini review. J Drug Delivery Sci Technol 2015;31:65-71.
9. Vasir JK, Tambwekar K, Garg S. Bioadhesive microspheres as a controlled drug delivery system. Int J Pharm 2003;255:13-32.
10. Sinha VR, Singla AK, Wadhawan S, Kaushik R, Kumria R, Bansal K, *et al.* Chitosan microspheres as a potential carrier for drugs. Int J Pharm 2004;274:1-33.
11. Rafiee A, Alimohammadian MH, TaranehGazon, Riazi-rad F, Fatemi SMR, Parizadeh A, *et al.* Comparison of chitosan, alginate and chitosan/alginate nanoparticles with respect to their size, stability, toxicity and transfection. Asian Pac J Trop Dis 2014;4:372-7.
12. Prasad BSG, Setty CM, Gupta VRM, Devanna D, Devi MR. Aceclofenac-loaded alginate microspheres prepared by ionotropic gelation method: effect of formulation variables on encapsulation efficiency, morphology and release characteristics. Pharm Lett 2011;3:10-24.
13. Chowdary KPR, Srinivasa RY. Preparation and evaluation of mucoadhesive microcapsules of indomethacin. Indian J Pharm Sci 2003;65:49-52.
14. Marina K, Ansu J, Prabhakara P, Girish SN. Mucoadhesive microspheres of famotidine for gastro retentive drug delivery. Int J Drug Dev Res 2012;4:59-64.
15. Hari PR, Chandy T, Sharma CP. Chitosan/calcium alginate microcapsules for intestinal delivery of nitrofurantoin. J Microencapsulation 1996;13:319-29.
16. Ahmed MG, Satish KBP, Kiran KGB. Formulation and evaluation of gastric-mucoadhesive drug delivery systems of captopril. Int J Curr Pharm Res 2010;2:26-32.
17. Hemant KSY, Singh MN, Shivakumar HG. Chitosan/Sodium tripolyphosphate cross-linked microspheres for the treatment of gastric ulcer. Pharm Lett 2010;2:106-13.
18. Burra S, Galipelly SK. Enhancement of solubility and dissolution rate of furosemide through liquid solid technique. Pharm Lett 2010;2:321-8.
19. Patel JK, Bodar MS, Amin, Patel MM. Formulation and optimization of mucoadhesive microspheres of metoclopramide. Indian J Pharm Sci 2004;66:300-5.
20. Siepmann J, Peppas NA. Modelling of drug release from delivery systems based on hydroxypropyl methylcellulose. Adv Drug Delivery Rev 2001;48:139-57.
21. Costa P, Lobo JM. Modeling and comparison of dissolution profiles. Eur J Pharm Sci 2001;13:123-33.
22. Setia A, Kumar M, Goyal N, Mahadevan N. A formulation and characterization of gastro retentive mucoadhesive quetiapine fumarate tablets. J Global Pharm Technol 2012;6:12-7.
23. Das MK, Senapati PC. Evaluation of furosemide loaded alginate microspheres prepared by ionotropic external gelation technique. Acta Pol Pharm 2007;64:253-62.
24. Singhavi DJ, Takarkhede K, Khan S, Yeole P. Floating and mucoadhesive system of furosemide: development and *in vitro* evaluation. Thai J Pharm Sci 2012;36:12-23.
25. Lima SAC, Ferreira M, Chaves LL, Reis S. Optimization of nanostructured lipid carriers loaded with methotrexate: A tool for inflammatory and cancer therapy. Int J Pharm 2015;492:65-72.

How to site this article

- Sandeep Kumar, Arun nanda. Formulation, optimization and *in vitro* evaluation of gastro retentive mucoadhesive microspheres of furosemide. Int J Pharm Pharm Sci 2016;8(7):392-398