CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1182428

Available online at: http://www.iajps.com Research Article

DESIGN AND EVALUATION OF MICROSPHERES LOADED WITH REBAMIPIDE

P. Kanteepan[1]and D.V.R.N.Bhikshapathi [2]*

[1]Research Scholar, Mewar University, Chittorgarh, Rajasthan, India.
[2]Research Supervisor, Mewar University, Chittorgarh, Rajasthan, India

Abstract:

In this present research study, we sought to develop novel Rebamipide microspheres and explore the influence of various concentrations of sodium alginate (polymer) and calcium chloride (cross-linking agent) on particle size, entrapment efficiency, and drug release of the same. These microspheres were prepared by ionotropic gelation technique for oral delivery in the treatment of gastro duodenal ulcers. The formulated microspheres were evaluated regarding size, shape, % EE, % yield, drug release and characterization was studied using FTIR, DSC, SEM analysis. Among the total S14 formulations, S7 formulation was optimized at 2.2% of sodium alginate, 7% of calcium chloride maintained 500rpm, for 2h at room temperature. The in vitro dissolution showed sustained release of Rebamipide upto 96.12% by diffusion mechanism over 12h, which followed the zero order and Korsmeyer-Peppas model (R^2 = 0.987, 0.993), respectively. The marketed product displayed the drug release of 95.15% within 1h. The optimized S7 formulation displayed the %EE 95.30, particle size 73.45±0.09 µm, % yield 96.30 and swelling index 95.26%. The optimized S7 formulation subjected to stability studies for 6months as per ICH guidelines, and concluded that significant difference was not observed for % yield, %EE, and drug release before and after stability studies, hence the formulation (S7) found stable. Finally, the prepared Rebamipide microspheres showed significant effect on gastritis in a controlled manner for prolonged period of time.

Key words: *Rebamipide, microspheres, ionotropic gelation method, DSC, sustained release.*

***Corresponding Author:**
D.V. R. N. Bhikshapathi,
Research Supervisor,
Mewar University, Chittorgarh,
Rajasthan, India
Mobile No: +91-9848514228
E-mail: dbpathi71@gmail.com

QR code

*Please cite this article in press as P. Kanteepan and D.V.R.N.Bhikshapathi., **Design and Evaluation of Microspheres Loaded With Rebamipide**, Indo Am. J. P. Sci, 2018; 05(02).*

INTRODUCTION:

According to the WHO (World Health Organization), 23% of gastro duodenal ulcers leads to malignancies are the main cause of illness and premature death in the world. Every year, an estimated 10 million people die of GI diseases, particularly chronic gastritis [1]. The advances in oral controlled drug delivery formulations had receive rising interest since these pharmaceutical systems present several advantages over conventional release such as: low cost, reduced dosing frequency, convenience, increased safety, easy therapeutic regime, intestinal absorption increased by the large surface area [2].

The lack of control on the drug release rate produces an immediate and short duration effect. One way to circumvent these drawbacks is the creation of a polymeric coverage around the Rebamipide through microspherification. A microsphere has a solid matrix wherein the API is finely dispersed. In the microspheres formation the premix between the API and the polymer solution before the spherification is essential [3, 4]. Furthermore, this polymeric coverage regulates the drug release rate, maintaining it at desired concentrations to show the effect at a specific place, and so allows for its absorption [5]. There are several encapsulation methods; among those ionic gelation is an interesting method, given its simplicity and versatility [6].

Rebamipide is an amino acid derivative used for the treatment of gastro duodenal ulcers and reduces the recurrence of gastric ulcers. Nowadays, Rebamipide is commercially delivered in solid dosage form (tablets, capsules). Rebamipide in oral administration will significantly metabolize into its inactive metabolite within liver and colonic environment so the efficacy would be reduced as well. One approach to this problem would be control the Rebamipide release and avoid the formation of its inactive metabolite hence increases the bioavailability at insitu level. Sustained release formulation of Rebamipide microspheres via oral route will reduce the frequency of administration, as well as dose and dose-dependent side-effects during the management of gastritis [7, 8].

Polymeric drug delivery system displays several advantages over the conventional dosage forms and it includes enhanced efficacy, patient compliance, reduced toxicity, and to control the encapsulated drug release. Sodium alginate is a anionic natural polysaccharide, prepared by mixture of D-mannuronic acid and L-glucuronic acid [9]. Sodium alginate is extensively used as carrier for drug delivery due to its biocompatibility and low toxicity. The widely used method for Rebamipide microspheres preparation is an ionotropic gelation method. This technique offers several advantages such as simple method of preparation no need to use of organic solvent, and, also easier to control. Sodium alginate could form gel in the presence of multivalent cations such as Ca2+, Zn2+, Ba2+ and Al3+ etc... by ionic cross-linking to form microspheres, it has been widely used in sustained drug release. Hence in this study calcium chloride is selected as cross-linking agent and because of its nontoxic and biocompatibility [10].

The objective of the present study is to develop calcium ion cross-linked sodium alginate blend MS by ionotropic gelation technique for oral targeting and assess the effect of various concentrations of sodium alginate and calcium chloride on entrapment efficiency, % yield, drug release, particle size. The developed blend microspheres were characterized by SEM, FTIR, and DSC analysis. Stability studies were carried out as per ICH guidelines. Furthermore, *in vitro* release properties of Rebamipide blend MS were studied in simulated gastrointestinal conditions.

MATERIALS AND METHODS:

Materials: Rebamipide was obtained from Daewoong Pharmaceutical Co. Ltd, Hyderabad., India as a gift sample. Sodium alginate was purchased from Pruthvi Chemicals, Mumbai, India, calcium chloride was obtained from SD Fine ltd, Mumbai., India. Remaining all chemicals used in this research study was of analytical grade.

Methods:

Preparation of Rebamipide microspheres:

The microspheres were formulated by ionotropic gelation method using the formulations as mentioned in Table 1. The alginate solution consisting various percentages of sodium alginate was ranges from 1% to 2.2% w/v. Initially, sodium alginate solution was prepared by solubilizing the polymer in deionized water using gentle heat, being stirred magnetically. On complete solution, a weighed quantity of Rebamipide was added to 100ml of each percentage solution to form homogeneous dispersions at 500rpm, maintained room temperature. The mixtures were sonicated for 30min to eliminate air bubbles that may have been formed during the stirring process. The alginate-API dispersions (100ml) were added drop wise via a 20-gauge needle fitted with a 10ml syringe into 100ml of 7% w/v and 10% w/v of calcium chloride solution, being stirred at 500rpm for 10min. The droplets from the dispersions instantly gelled into distinct Rebamipide- sodium alginate matrices upon contact with the calcium chloride solution (cross-linking agent). Later, the solution of calcium chloride was removed and the Rebamipide microspheres were washed with in deionized water. The Rebamipide microspheres were thereafter dried at 60°C for 2h in a hot-air oven [11].

Table -1: Formulation of Rebamipide microspheres

Formulation code	Rebamipide (mg)	Sodium alginate	Calcium chloride
S1	100	1%	7%
S2	100	1.2 %	7%
S3	100	1.4%	7%
S4	100	1.6%	7%
S5	100	1.8%	7%
S6	100	2%	7%
S7	100	2.2%	7%
S8	100	1%	10%
S9	100	1.2%	10%
S10	100	1.4%	10%
S11	100	1.6%	10%
S12	100	1.8%	10%
S13	100	2%	10%
S14	100	2.2%	10%

Evaluation of Rebamipide microspheres [12, 13]**:**

Size analysis:

Microsphere Size plays significant role in determining the drug release from it. Particle size analysis was made by optical microscopy technique, using calibrated eye piece and a stage micrometer, almost 100 particles were measured, and the results mentioned in the Table 2.

Angle of repose:

Angle of repose of the microspheres, is the maximum angle possible between the surface of the pile of microspheres and the horizontal plane, was obtained by fixed funnel method using the following formula.

$$\theta = \tan^{-1}(h/r)$$

θ = Angle of repose, h = height of the microsphere pile and d = diameter of the microsphere pile.

Bulk density:

Volume of the microspheres in the measuring cylinder was noted as bulk density.

$$\text{Bulk density} = \frac{\text{Wt of powder}}{\text{Bulk volume of powder}}$$

Tapped density: Change in the microspheres volume was observed in mechanical tapping apparatus.

$$\text{Tapped density} = \frac{\text{Wt of microspheres}}{\text{Tapped volume of microspheres}}$$

Compressibility index:

Also called as Carr's index and is computed according to the following equation.

$$\text{Carr's compressibility index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

Hausner's ratio:

Hausner's ratio of microspheres is determined by comparing the tapped density to the fluff density using the equation [13].

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Swelling index studies:

The swelling index of the microspheres is sign of the ability of the microspheres to absorb water and swell. For determining the swelling index, the accurately weighed quantity of microspheres was suspended in simulated gastro intestinal fluids. After 1h microspheres were transferred onto blotting paper to remove the excess moisture then weighed the swollen microspheres using a microbalance. After that swollen microspheres were dried in oven at 60°C for 5h until showed the constant weight. The increase and decrease in weight of microspheres used to calculate the swelling index [14].

Swelling index= (Mass of swollen microspheres - Mass of dry microspheres/mass of dried microspheres) 100.

Drug incorporation efficiency and %yield:

10mg of drug-loaded microspheres from each batch was crushed in a mortar then placed in 100ml conical flask containing 50ml of methanol. The microspheres were stirred to improve swelling and defragmentation of the cross-linked structure. This afforded leads to dissolution of Rebamipide. The solution was filtered through a membrane filter (0.45µm). Then the Rebamipide was quantified spectrophotometrically at 227nm [15]. The encapsulation efficiency and %yield was determined by using the following empirical formulas:

% Drug entrapment = Calculated drug concentration /Theoretical drug concentration x 100

% yield = [Total weight of microspheres / Total weight of drug and polymer] x 100

***In vitro* drug release studies:**

The drug release studies were performed in a USP dissolution testing apparatus II at 37 ± 0.5°C and maintained 100rpm in 0.1N HCl about 900ml. A quantity of microspheres equivalent to 100mg Rebamipide for each formulation was added to all drug release studies. The samples of 5ml were withdrawn at programmed time interval i.e. 0, 1, 2, 4, 6, 8, 10 & 12h and the same volume of fresh 0.1N HCl added immediately to maintain sink condition throughout the experiment. The aliquots of samples, following suitable dilution, were analyzed spectrophotometrically at 227nm to estimate the concentrations of Rebamipide in the test samples. The concentration of drug was calculated using a regression equation of the calibration curve developed in 0.1N HCl [16].

Kinetic modeling of drug release:

The release mechanism of drug is investigated by fitting the data into several kinetic models like Zero order, First order, Higuchi's model and Korsmeyer-Peppas. Fitness of the data into several kinetic equations was determined by calculating the (r^2) correlation coefficient [17].

Drug excipient compatibility studies

Fourier transmission infrared spectroscopy (FTIR), Differential Scanning Calorimetry (DSC) and Scanning electron microscopy (SEM) were used to signify the drug-excipient compatibility [18,19].

Fourier transform infrared spectroscopy (FTIR)

The FTIR technique can be used to recognize the functional groups in the sample and drug-excipient compatibility. FTIR spectra of pure Rebamipide, physical mixtures and optimized formulation were recorded by using FTIR (SHIMADZU). Weighed quantity of KBr and excipients were taken in the ratio 100: 1 and mixed by mortar. The samples were made into pellet/disk by the application of pressure. Then the FTIR spectra were recorded between 4000 and 400 cm^{-1}

Differential Scanning Calorimetry (DSC)

The Differential Scanning Calorimetry measurements were performed on DSC-60 associated with TA-60 software. Accurately weighed Samples were placed in aluminum pan and sealed before heating under nitrogen flow (300 ml/min) at a scanning rate of 10°C min^{-1} from 25°C to 350°C. An empty aluminum pan was used as reference.

SEM studies

Surface nature of microspheres includes size and shape was examined with the help of Scanning Electron Microscope (HITACHI, S-3700N). The microspheres were dried completely prior to analysis and SEM was carried out at different magnifications of 15.0 kv × 7mm, 15 kv x 7.3mm, 15Kv x 6.7mm.

Stability studies

Stability studies were conducted at 40°C ± 2°C/75% RH ± 5% RH for 6months using stability chamber (Thermo Lab, Mumbai). Samples were withdrawn at predetermined intervals of 0, 30, 60, 120, and 180 days period according to ICH guidelines. Various *in vitro* parameters like % yield, entrapment efficiency and *in vitro* release studies were evaluated [20].

RESULTS AND DISCUSSIONS:

Micromeretic properties of Rebamipide microspheres

Fig. 1: **Rebamipide microspheres**

Micromeretic parameters:

The results of micromeretic parameters and swelling index of Rebamipide microspheres were summarized in Table 2. Comparatively formulation S7 shown the better results such as particle size, bulk density, tapped density, angle of repose, Carr's index and swelling index of 73.45±0.09µm, 0.58 g/cc^3, 0.50 g/cc^3, 21˚.54, 9.12% and 95% respectively.

Table 2: Micromeritic properties of Rebamipide sodium alginate microspheres

Formulation code	Particle size (µm)	Bulk density (g/cc^3)	Tapped density (g/cc^3)	Angle of repose	Carr's index	Swelling index (%)
S1	71.12±0.08	0.68±0.12	0.63±0.24	25˚.74±0.12	11.14±0.02	74.23±0.18
S2	75.29±0.13	0.73 ±0.15	0.70±0.17	28˚.67±0.35	12.34±0.14	79.14±0.32
S3	83.45±0.04	0.75±0.21	0.72±0.31	30˚.54±0.25	10.42±0.22	71.23±0.33
S4	79.67±0.09	0.81 ±0.33	0.65 ±0.17	30˚.15±0.16	11.23±0.31	74.29±0.17
S5	77.43±0.04	0.78 ±0.17	0.71 ±0.13	28˚.93±0.22	14.56±0.17	79.46±0.22
S6	92.45±0.09	0.90 ±0.22	0.75 ±0.20	25˚.24±0.36	13.95±0.26	91.14±0.37
S7	73.45±0.09	0.58 ±0.16	0.50 ±0.33	21˚.54±0.28	9.12±0.39	95.26±0.26
S8	84.12±0.08	0.67±0.31	0.73 ±0.29	27˚.93±0.17	14.56±0.12	79.48±0.11
S9	77.45±0.09	0.75±0.23	0.62± 0.24	25˚.54±0.22	13.95±0.09	71.19±0.10
S10	80.23±0.14	0.68 ±0.24	0.73 ±0.35	22˚.91±0.35	10.32±0.22	74.23±0.25
S11	66.45±0.04	0.65 ±0.27	0.59 ±0.19	23˚.74±0.19	12.34±0.37	85.56±0.14
S12	86.29±0.13	0.73 ±0.15	0.62 ±0.21	25˚.67±0.27	11.34±0.21	94.42±0.23
S13	90.43±0.04	0.66 ±0.19	0.56 ±0.38	25˚.54±0.13	10.12±0.15	93.29±0.35
S14	93.13±0.09	0.86 ±0.30	0.78 ±0.21	29˚.15±0.08	12.23±0.33	89.21±0.21

All the formulations were evaluated for percentage yield and entrapment efficiency and the results are depicted in Table 3. The formulation (S7) was shows better results of Percentage yield and Entrapment efficiency, 96.30% and 95.30% respectively. The entrapment efficiency increased by increasing the sodium alginate concentration but reduces inturn by increasing calcium chloride. This might be due to saturation of calcium binding sites of the polymer with drug.

Table 3: Percentage drug yield & entrapment efficiency of Rebamipide microspheres

Formulation code	Percentage yield (%)	Entrapment efficiency (%)
S1	81.00±0.22	81.56±0.17
S2	83.00±0.19	85.30±0.21
S3	81.00±0.32	79.98±0.33
S4	82.87±0.26	82.00±0.18
S5	89.30±0.30	89.20±0.27
S6	92.30±0.13	79.60±0.09
S7	96.30±0.27	95.30±0.11
S8	82.42±0.30	83.50±0.37
S9	77.00±0.15	79.60±0.25
S10	82.56±0.29	78.75±0.19
S11	81.09±0.07	79.62±0.23
S12	94.30±0.36	78.23±0.38
S13	87.50±0.22	78.16±0.16
S14	85.30±0.14	78.88±0.05

***In vitro* drug release studies**

In vitro dissolution studies were conducted and the results are shown in Table 4 and 5 and in Figure 2 and 3. Among all the formulations the highest drug release was found from S7 i.e 96.12±0.16% upto 12h and the drug release from marketed product was 95.15±0.23% within 1 h.

Table 4: *In vitro* Cumulative % drug release of Rebamipide sodium alginate microspheres S1 to S7 and marketed product

Time (h)	S1	S2	S3	S4	S5	S6	S7	Marketed product (%)
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	13.09±0.12	12.65±0.2	12.25±0.15	11.83±0.19	11.33±0.16	11.21±0.11	11.05±0.32	95.15±0.23
2	26.08±0.15	24.76±0.21	22.34±0.18	21.34±0.18	20.34±0.36	20.15±0.23	20.04±0.21	---
4	36.77±0.18	37.20±0.16	37.90±0.16	38.40±0.25	39.20±0.39	41.23±0.29	41.40±0.25	---
6	49.09±0.19	49.30±0.19	50.90±0.36	51.30±0.25	51.70±0.34	52.73±0.28	49.80±0.36	---
8	59.23±0.06	60.30±0.21	61.20±0.26	62.30±0.33	64.30±0.36	65.46±0.16	66.60±0.31	---
10	70.25±0.18	70.90±0.23	71.20±0.18	71.30±0.36	73.30±0.22	80.45±0.12	82.90±0.21	---
12	81.34±0.35	82.30±0.52	83.20±0.39	85.50±0.31	87.30±0.25	92.17±0.16	96.12±0.16	---

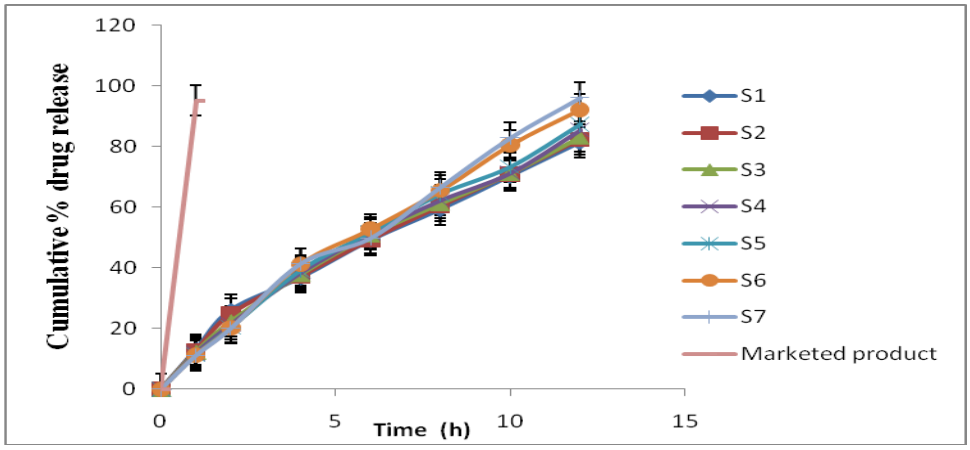

Fig. 2: *In vitro* Cumulative % drug release of Rebamipide sodium alginate microspheres formulations S1 to S7 with marketed product

Table 5: *In vitro* Cumulative % drug Rebamipide sodium alginate release of microspheres formulations S8 to S14

Time (h)	S8	S9	S10	S11	S12	S13	S14
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	13.54±0.16	12.23±0.18	12.21±0.22	18.54±0.18	15.21±0.33	16.62±0.16	21.63±0.23
2	26.40±0.15	25.23±0.25	25.80±0.16	24.40±0.15	24.23±0.21	22.01±0.31	32.01±0.26
4	38.20±0.23	39.90±0.18	40.40±0.21	41.20±0.16	44.10±0.18	39.24±0.25	40.83±0.23
6	50.30±0.26	51.92±0.22	53.60±0.22	54.30±0.32	54.20±0.21	53.83±0.85	53.79±0.21
8	62.35±0.28	63.27±0.24	63.30±0.21	64.30±0.22	68.20±0.33	63.03±0.11	64.06±0.24
10	69.90±0.34	71.10±0.32	72.60±0.21	79.90±0.32	83.32±0.25	73.22±0.25	75.56±0.25
12	71.30±0.32	74.20±0.16	78.50±0.36	83.42±0.21	84.36±0.52	84.41±0.21	85.70±0.21

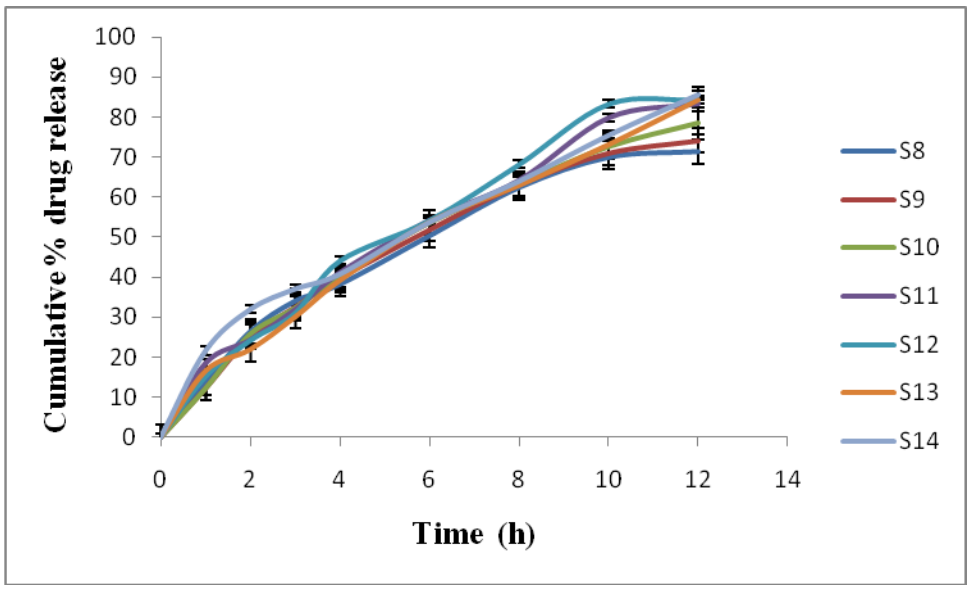

Fig. 3: *In vitro* Cumulative % drug release of Rebamipide microspheres

Mathematical modeling of Rebamipide optimized microspheres (S7)

Table 6: Release order kinetics of optimized normal microspheres (S7)

Formula Code	Zero Order	First Order	Higuchi	Korsmeyer-Peppas	
	R^2	R^2	R^2	R^2	n
S7	0.987±0.017	0.866±0.012	0.890±0.019	0.993±0.014	0.822±0.011
Marketed product		0.926			

To find out the drug release mechanism, the controlled release Rebamipide microspheres were treated in several mathematical models like Zero order, First order, Higuchi model and Korsmeyer-Peppas model. The drug release data plotted and correlation coefficients (R^2) was calculated from the linear curve slope portions. It is observed that drug released from sustained release microsphere followed zero order and Korsmeyer-Peppas model. The optimized formulation n value was 0.822 followed non fickian diffusion signifying, principles of both diffusion and erosion mechanism involved. The marketed product followed the first order kinetics. The results were mentioned in Table 6.

Drug excipient compatibility studies

Fourier Transform Infrared Spectroscopy (FTIR)

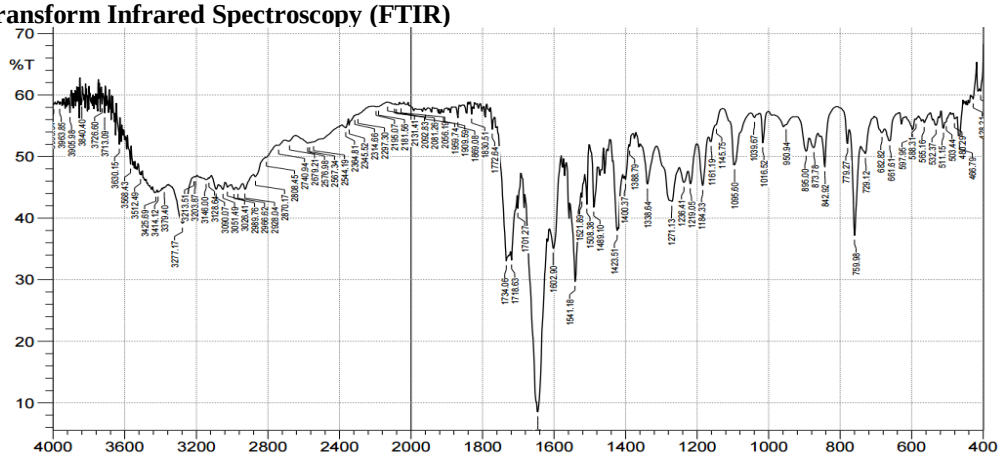

Fig. 4: FTIR spectrum of pure drug Rebamipide

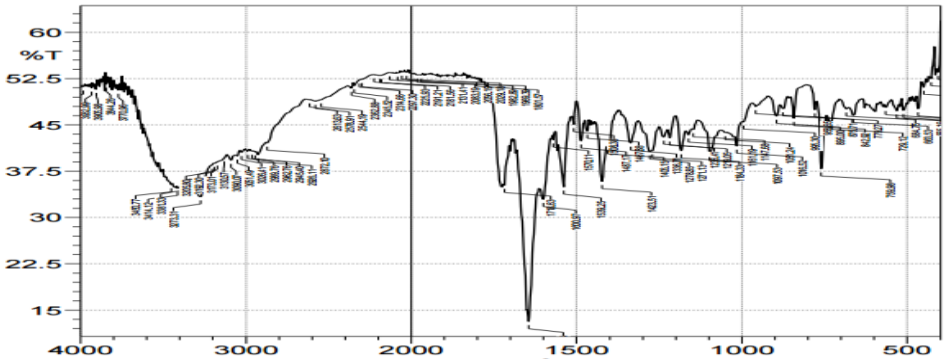

Fig. 5: FTIR spectrum of physical mixture

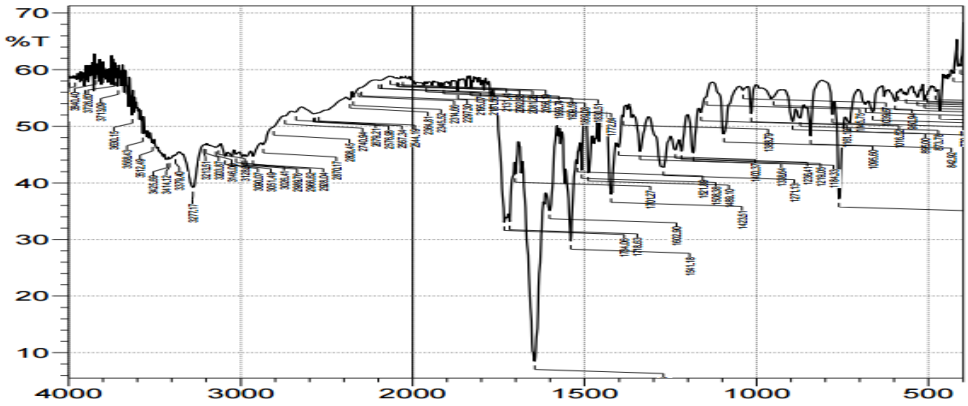

Fig. 6: FTIR spectrum of Rebamipide optimized formulation (S7) of microspheres

The FTIR spectrum of pure drug showed characteristic sharp peaks of amines stretching (═N–H and CH_2) vibration at 3420.32–3379.48 cm^{-1} and alkane stretching (–CH_3, –CH_2 and –CH) vibration at 2938.73 cm^{-1}. Also exhibited C═O stretch at 1740.2 cm^{-1} due to aldehydes and C═O–NH stretching at 1650.90 cm^{-1}. A selective stretching vibration at 1580.57 cm^{-1} and 1525.80 cm^{-1} for primary and secondary amine was also observed. For functional groups like –C–H bend alkanes and –C–H rock alkanes stretch showed vibrations at 1450.78 cm^{-1} and 749.57 cm^{-1} respectively (Figure 4). There were no new significant bands observed in the physical mixture (Figure 5) and optimized formulation (Figure 6), which confirms that no interaction takes place between the drug polymers.

DSC Studies:

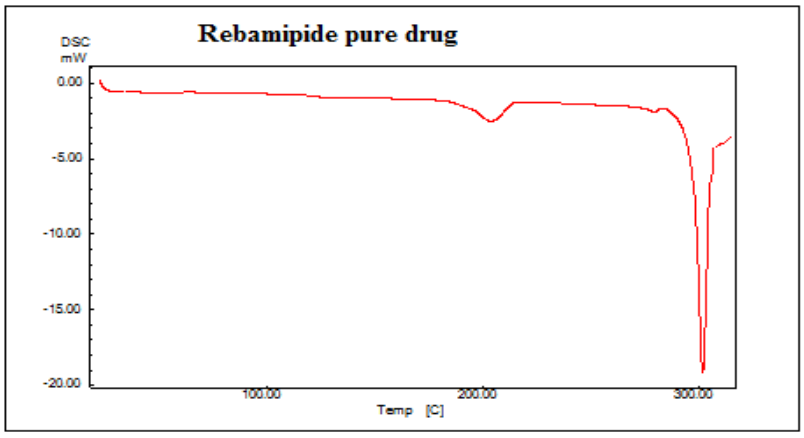

Fig. 7: DSC thermogram of pure drug Rebamipide

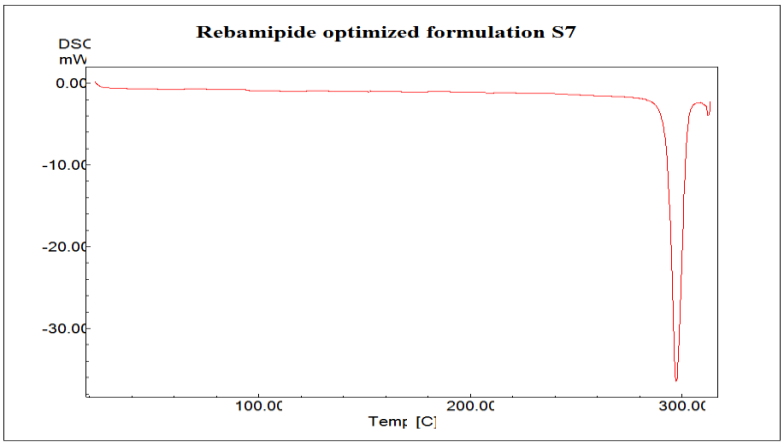

Fig. 8: DSC thermogram of optimized Rebamipide microspheres (S7)

The DSC thermograms of pure drug (Figure 7) and optimized formulation S7 was shown in Figure 8. Rebamipide exhibited a sharp endothermic peak at 290˚C corresponding to its melting point. The thermogram of microsphere loaded with Rebamipide (S7) exhibited a sharp endotherm melting point at 288˚C hence it indicated that no significant interaction between drug and excipients.

SEM of Rebamipide microspheres

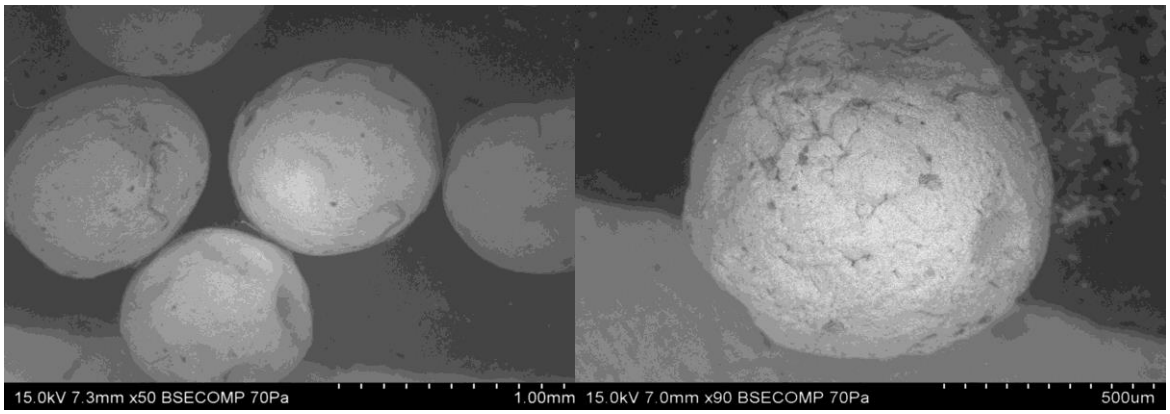

Fig. 9: Scanning electron micrographs of Rebamipide microspheres

The SEM photomicrographs of the dried sodium alginate microspheres were shown in Figure 9. It was observed that shape of microspheres seems to be spherical with fairly rough outer surface. The surface was rough due to polymer matrix density which justifies the controlled release of the drug.

Stability studies:

From the stability studies it was observed that there was no significant change in results before and after stability studies hence the optimized formulation S7 found to be stable (Table 7).

Table 7: Staility studies of optimized normal microspheres

Retest time for optimized formulation (S7)	Percentage yield	Entrapment efficiency (%)	*In-vitro* drug release profile (%)
0 days	96.30±0.17	95.30±0.24	96.12±0.13
30 days	95.66±0.23	94.68±0.19	95.46±0.22
60 days	94.53±0.35	94.43±0.32	94.73±0.15
120 days	95.12±0.08	93.17±0.21	95.42±0.27
180 days	94.23±0.16	94.45±0.15	94.78±0.31

CONCLUSION:

Thus, spherical microspheres with a coat consisting of Sodium alginate for the controlled release of Rebamipide showed the higher encapsulation efficiency was successfully prepared by ionotropic gelation technique. Rebamipide release from the microspheres was found to be slow, controlled release over a period of 12h. The drug release followed the zero order and Korsmeyer-Peppas model indicated the release was controlled by diffusion and swelling and relaxation of polymer chain. The results of the present study indicated promising potential of microspheres in the delivery of drugs with lower half lives and less bioavailability with controlled release of Rebamipide in the management of gastric ulcers.

REFERENCES:

1.Hailin Zhang, Weifeng Li , Xiumei Wang. Anti-ulcerogenic effect of cavidine against ethanol-induced acute gastric ulcer in mice and possible underlying mechanism. International immunopharmacology, 2016;38:450-459.

2.Jan Schulze, Stephan Hendrikx, Michaela Schulz-Siegmund. Microparticulate poly (vinyl alcohol) hydrogel formulations for embedding and controlled release of polyethyleneimine (PEI)-based nanoparticles. Acta Biomaterialia, 2016;45:210-222.

3.Haogang Duan, Shaoyu Lü, Hongyan Qin. Co-delivery of zinc and 5-aminosalicylic acid from alginate/N-succinyl-chitosan blend microspheres for synergistic therapy of colitis. International Journal of Pharmaceutics, 2016;1-38.

4.Girod Fullana.S, Ternet.H, Freche M. Controlled release properties and final macro porosity of a pectin microspheres–calcium phosphate composite bone cement. Acta Biomaterialia, 2010;6: 2294–2300.

5.Junzi Wu, Gareth R. Williams, Christopher Branford-White. Liraglutide-loaded poly (lactic-co-glycolic acid) microspheres: Preparation and in vivo evaluation. European Journal of Pharmaceutical Sciences, 2016; 92: 28–38.

6.Chickering III D.E, Jacob J.S, Desai T.A. Bioadhesive microspheres: III. An in vivo transit and bioavailability study of drug-loaded alginate and poly (fumaric-co-sebacic anhydride) microspheres. Journal of Controlled Release, 1997; 48: 35–46.

7.Akira Terano, Tetsuo Arakawa, Toshiro Sugiyama. Rebamipide, a gastro-protective and anti-inflammatory drug, promotes gastric ulcer healing following eradication therapy for Helicobacter pylori in a Japanese population: a randomized, double-blind, placebo-controlled trial. J Gastroenterol, 2007; 42:690–693.

8.Fujioka.T, Arakawa. T, shimoyama.T. Effects of Rebamipide, a gastro-protective drug on the Helicobacter pylori status and inflammation in the gastric mucosa of patients with gastric ulcer: a randomized double-blind placebo-controlled multicenter trial. Aliment Pharmacol Ther, 2003; 18 (1): 146–152.

9.Remedios Guadalupe Gomez-Mauricio, Argia Acarregui, Francisco Miguel Sánchez-Margallo. A preliminary approach to the repair of myocardial infarction using adipose tissue-derived stem cells encapsulated in magnetic resonance-labelled alginate microspheres in a porcine model. European Journal of Pharmaceutics and Biopharmaceutics, 2013; 84: 29–39.

10.Sougata Jana, Arindam Das, Amit Kumar Nayak. Aceclofenac-loaded unsaturated esterified alginate/gellan gum microspheres: In vitro and in vivo assessment. International Journal of Biological Macromolecules, 2013;57: 129– 137.

11.Sergio Benavides, Pablo Cortés, Javier Parada. Development of alginate microspheres containing thyme essential oil using ionic gelation. Food Chemistry 2016; 204: 77–83.

12.Loreana Lacerda, Alexandre Luis Parize, Valfredo Fávere. Development and evaluation of pH-sensitive sodium alginate/chitosan microparticles containing the antituberculosis drug rifampicin. Materials Science and Engineering C, 2014;39: 161–167.

13.Sanjay K. Motwani, Shruti Chopra, Sushma Talegaonkar. Chitosan–sodium alginate nanoparticles as submicroscopic reservoirs for ocular delivery: Formulation, optimization and in vitro characterization. European Journal of Pharmaceutics and Biopharmaceutics, 2008;68: 513–525.

14.Shanmuganathan.S, Shanumugasundaram.N, Adhirajan.N. Preparation and characterization of chitosan microspheres for doxycycline delivery. Carbohydrate Polymers, 2008;73: 201–211.

15.Antonio J. Ribeiro Ronald J. Neufeld, Philippe Arnaud. Microencapsulation of lipophilic drugs in chitosan-coated alginate microspheres. International Journal of Pharmaceutics 1999;187: 115–123.

16.Savita Sonawane, Mangesh Bhalekar, Shamkant shimpi. Preparation and evaluation of microspheres of xyloglucan and its thiolated xyloglucan derivative. International Journal of Biological Macromolecules, 2014; 1-7.

17.Silvia Vicini, Maila Castellano, Marco Mauri. Gelling process for sodium alginate: New technical approach by using calcium rich micro-spheres. Carbohydrate Polymers, 2015;134: 767–774.

18.Bo Li, Zhongning Liu, Jingwen Yang. Preparation of bioactive β-tricalcium phosphate microspheres as bone graft substitute materials. Material science and engineering C, 2016; 70(2): 1200-1205.

19.Guijin Liu, Shaomin Li, Yinxia Huang. Incorporation of 10-hydroxycamptothecin nanocrystals into zein microspheres. Chemical engineering science, 2016; 155:405-414.

20.Yan Li, Haibing He, Qiao Wang, Xing Tang. Preparation, stability and pharmacokinetics evaluation of lipid microspheres loading a promising antitumor candidate, Timataxel. Asian Journal of Pharmaceutical Sciences, 2016;11: 771–779.