Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (4):371-380
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Design and evaluation of microspheres using anti-ulcer agent roxatidine

Arifa Begum S. K.[1,2]* and Basava Raju D.[3]

[1]*Vijaya Institute of Pharmaceutical Sciences for Women, Vijayawada, Andhra Pradesh, India*
[2]*Jawaharlal Nehru Technological University, Kukatpally, Hyderabad-500072, Telangana, India*
[3]*Shri Vishnu College of Pharmacy, Bhimavaram, Andhra Pradesh, India*

ABSTRACT

Present study aims to prepare and evaluate Roxatidine acetate HCl microspheres by ionotropic gelation method. Among all the formulations R9 was selected as optimized formulations for based on the physico chemical parameters and drug release studies. In the in vitro *release study of formulation R9 showed 95.12% after 12 h in a controlled manner, which is essential for disease like peptic ulcer. The in vitro release profiles from optimized formulations were applied on various kinetic models. The best fit with the highest correlation coefficient was observed in Higuchi model, indicating diffusion controlled principle. The innovator Rotane 150mg conventional tablet shows the drug release of 96.45 within 1 h. FT-IR and DSC analyses confirmed the absence of drug-polymer interaction. The results obtained from evaluation and performance study of different types of Roxatidine microspheres that system may be useful to achieve a controlled drug release profile suitable for peroral administration and may help to reduce the dose of drug, dosing frequency and improve patient compliance when compared with marketed product.*

Key words: Roxatidine, SEM, microspheres, peptic ulcer, Higuchi.

INTRODUCTION

Controlled drug delivery by encapsulating the drug inside polymeric carriers has made great progress in last two decades as it can enhance the drug release and decrease adverse effects [1, 2, 3, 4], by drug localization at the site of action and by controlling the drug release [5]. Moreover, entrapment inside the polymers can also shield the sensitive drugs (e.g., peptides/proteins) from chemical and enzymatic decomposition. Microspheres developed using biodegradable polymers are widely used to achieve controlled release of drugs [6, 7]. The chief advantage of using biodegradable polymers is that after performing their tasks they break down in a biologically friendly manner.

For the treatment of chronic diseases it is important to take medication several times, this may lead to fluctuating drug level in body. In order to avoid frequent drug administration and maintenance of therapeutic drug level in body it is essential to administer drug by a sustained release system. Drugs with short elimination half life are most suitable for sustained release formulations. Sustained delivery of drugs can be achieved by microspheres formulation [8].

Peptic ulcer disease, also known as a peptic ulcer or stomach ulcer, is a break in the lining of the stomach, first part of the small intestine, or occasionally the lower esophagus. An ulcer in the stomach is known as a gastric ulcer while that in the first part of the intestines is known as a duodenal ulcer. The most common symptoms are waking at night

with upper abdominal pain or upper abdominal pain that improves with eating. Common causes include the bacteria, *Helicobacter pylori* and non-steroidal anti-inflammatory drugs [9].

Roxatidine acetate is a specific and competitive histamine H_2 receptor antagonist, which is used to treat gastric ulcers, Zollinger–Ellison syndrome, erosive esophagitis, gastro-oesophageal reflux disease and gastritis. Roxatidine has less bioavailability (80%) and lesser half life of 5 hours [10]. The aim of present work is to design and in vitro evaluation of different types of microspheres of Roxatidine to enhance its bioavailability and prolonged residence time in stomach.

MATERIALS AND METHODS

Materials:
Roxatidine pure drug was generous gift from Aurobindo Pharma Limited, Hyderabad, India. Sodium alginate was obtained from Pruthvi Chemicals, Mumbai. Xanthan gum was gifted from MSN Labs Ltd. Hyderabad. All other chemicals used were of analytical grade.

Preparation of Roxatidine microspheres:
Roxatidine microspheres were prepared with polymers like sodium alginate and calcium chloride by Ionotropic gelation method. Different formulation trials of Roxatidine were prepared using different concentration of polymer and cross linking agent. Total 14 formulations are developed using sodium alginate and calcium chloride in different concentrations. In this method weighed quantity of Roxatidine was added to 100ml sodium alginate solution and thoroughly mixed at 500 rpm. Resultant solution was extruded drop wise with the help of syringe and needle into 100ml aqueous calcium chloride solution and stirred at 100 rpm. After stirring for 10 minutes the obtained microspheres were washed with water and dried at 60 degrees-2hours in a hot air oven and stored in dessicater. [11]

Table 1: Formulation trials for Roxatidine microspheres

FORMULATION CODE	ROXATIDINE ACETATE HCL(mg)	SODIUM ALGINATE	CALCIUM CHLORIDE	XANTHAN GUM
R1	1500	1%	7%	1%
R 2	1500	1.2 %	7%	1.2 %
R 3	1500	1.4%	7%	1.4%
R 4	1500	1.6%	7%	1.6%
R5	1500	1.8%	7%	1.8%
R6	1500	2%	7%	2%
R7	1500	2.2%	7%	2.2%
R8	1500	1%	10%	1%
R9	1500	1.2%	10%	1.2%
R10	1500	1.4%	10%	1.4%
R11	1500	1.6%	10%	1.6%
R12	1500	1.8%	10%	1.8%
R13	1500	2%	10%	2%
R14	1500	2.2%	10%	2.2%

Micromeretic properties of Roxatidine microspheres:
Particle size:
The 100 microspheres were evaluated with respect to their size and shape using optical microscope fitted with an ocular micrometer and a stage micrometer. The particle diameters of more than 100 microspheres were measured randomly by optical microscope. [12]

Angle of repose:
Angle of repose (θ) of microspheres measures the resistance to particles flow, and is calculated according to fixed funnel standing cone method. Where (θ) is angle of repose, H/D is surface area of the free standing height of the microspheres heap that is formed on a graph paper after making the microspheres flow from glass funnel.

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

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.

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

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

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

Evaluation of Roxatidine microspheres:
Swelling index:
Swelling index was determined by measuring the extent of swelling of microspheres in the given medium. Exactly weighed amount of microspheres were allowed to swell in given medium. The excess surface adhered liquid drops were removed by blotting and the swollen microspheres were weighed by using microbalance. The hydro gel microspheres then dried in an oven at 60 degrees for 5h until there was no change in the dried mass of sample. The swelling index of the microsphere was calculated by using the formula. [14]

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

Drug entrapment efficiency and % yield:
In order to determine the entrapment efficiency, 10 mg of formulated microspheres were thoroughly crushed by triturating and suspended in required quantity of methanol followed by agitation to dissolve the polymer and extract the drug. After filtration, suitable dilutions were made and drug content assayed spectrophotometrically at 280nm using calibration curve. Each batch should be examined for drug content in a triplicate manner. [15]

% 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:
In vitro drug release studies for developed Roxatidine microspheres were carried out by using dissolution apparatus II paddle type (Electrolab TDL-08L). The drug release profile was studied in 900 ml of 0.1 N HCl at 37± 0.5^0C temperature at 100 rpm. The amount of drug release was determined at different time intervals of 0, 1, 2, 3, 4, 6, 8, 10& 12 hours by UV visible spectrophotometer (Shimadzu UV 1800) at 280nm. [16]

Kinetic modeling of drug release:
In order to understand the mechanism and kinetics of drug release, the result of the *in vitro* dissolution study of microspheres were fitted with various kinetic equations, like zero order [17] (percentage release Vs. time), first order [18] (log percentage of drug remaining to be released vs. time) and Higuchi's model [19] (Percentage drug release

vs. square root of time). Correlation coefficient (r^2) values were calculated for the linear curves obtained by regression analysis of the above plots.

Drug excipient compatibility studies

The drug excipient compatibility studies were carried out by Fourier transmission infrared spectroscopy (FTIR) method, Differential Scanning Calorimetry (DSC) and SEM.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra for pure drug, physical mixture and optimized formulations were recorded using a Fourier transform Infrared spectrophotometer. The analysis was carried out in Shimadzu-IR Affinity 1 Spectrophotometer. The samples were dispersed in KBr and compressed into disc/pellet by application of pressure. The pellets were placed in the light path for recording the IR spectra. The scanning range was 400-4000 cm^{-1} and the resolution was 1 cm^{-1}.

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry studies were carried out using DSC 60, having TA60 software, Shimadzu, Japan. Samples were accurately weighed and heated in sealed aluminum pans at a rate of 10°C/min between 25 and 350°C temperature rang under nitrogen atmosphere, empty aluminum pan was used as a reference.

SEM studies

The surface and shape characteristics of pellets were determined by scanning electron microscopy (SEM) (HITACHI, S-3700N). Photographs were taken and recorded at suitable magnification.

Stability studies

The stability study of the optimized formulation was carried out under different conditions according to ICH guidelines. The optimized microspheres were stored in a stability chamber for stability studies (REMI make). Accelerated Stability studies were carried out at 40 ^{0}C / 75 % RH for the best formulations for 6 months. The microspheres were characterized for the percentage yield, entrapment efficiency & cumulative % drug released during the stability study period [20]

RESULTS AND DISCUSSION

Normal microspheres of Roxatidine acetate HCl

Figure 1: **Roxatidine acetate HCl normal microspheres**

Table 2: Micromeritic properties of Roxatidine acetate HCl microspheres

Formulation code	Particle size (µm)	Bulk density (g/cc³)	Tapped density (g/cc³)	Angle of repose	Carr's index
R1	61.12±0.08	0.66	0.69	28˚.74	15.34%
R2	65.29±0.13	0.74	0.72	29˚.67	13.34%
R3	67.43±0.04	0.76	0.73	30˚.54	12.12%
R4	69.67±0.09	0.79	0.73	31˚.15	14.23%
R5	73.45±0.04	0.89	0.75	27˚.93	14.56%
R6	92.45±0.09	0.92	0.76	26˚.21	13.95%
R7	82.45±0.09	0.94	0.76	25˚.54	14.32%
R8	67.45±0.04	0.66	0.59	27˚.93	14.56%
R9	68.45±0.09	0.67	0.62	23˚.24	08.95%
R10	81.23±0.14	0.69	0.64	28˚.91	14.32%
R11	85.12±0.08	0.71	0.66	25˚.74	12.34%
R12	87.29±0.13	0.74	0.68	27˚.67	13.34%
R13	91.43±0.04	0.76	0.73	29˚.54	12.12%
R14	94.13±0.09	0.87	0.78	28˚.15	11.23%

Roxatidine acetate HCl microspheres of 14 formulations were prepared by ionic gelation method evaluated for their various physic chemical parameters. All the formulations were evaluated for particle size, bulk density, tapped density, angle of repose and carr's index and found to be within the limits, the results were depicted in **Table 2.**

The results of % yield, entrapment efficiency and swelling index was found to be satisfactory which shown in **Table 3**. The formulation R9 showed the best percentage yield, entrapment efficiency and swelling index values of 96%, 97% and 94% respectively.

Table 3: Percentage drug yield, entrapment efficiency and swelling index of Roxatidine acetate HCl microspheres

Formulation code	Percentage yield	Entrapment efficiency	Swelling index
R1	70.00%	69.00%	64%
R2	71.00%	72.00%	69%
R3	81.00%	80.00%	70%
R4	83.87%	83.30%	71%
R5	86.30%	85.20%	79%
R6	91.30%	91.30%	87%
R7	96.30%	94.10%	91%
R8	76.00%	74.03%	69%
R9	96.00%	97.00%	94%
R10	84.00%	83.00%	75%
R11	86.09%	85.00%	84%
R12	87.50%	86.66%	90%
R13	93.30%	91.03%	91%
R14	85.30%	84.88%	89%

In vitro dissolution studies:

Dissolution studies were conducted for all 14 formulations and the % drug release was found to be in the range of 87.01% to 95.92%. The formulations R9 was developed using Roxatidine acetate HCl, sodium alginate in concentration of 2.2%, and calcium chloride 7%, shown highest % drug release of 95.92% within 12h and the results are depicted in **Table 4&5**.

Table 4: In vitro cumulative % drug release of Roxatidine acetate HCl microspheres

Time (h)	R1	R2	R3	R4	R5	R6	R7
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	16.4±0.22	15.42±0.11	14.91±0.61	14.08±0.14	14.74±0.11	12.02±0.11	11.42±0.12
2	25.4±0.32	29.37±0.16	22.98±0.21	22.11±0.21	20.25±0.21	27.18±0.16	24.81±0.21
4	36.8±0.11	40.59±0.21	36.56±0.32	30.98±0.18	37.91±0.14	36.93±0.34	38.56±0.22
6	66.8±0.22	56.34±0.17	50.72±0.16	46.19±0.32	55.26±0.26	52.54±0.32	50.19±0.32
8	84.9±0.35	72.67±0.32	74.41±0.52	62.86±0.11	74.73±0.22	78.78±0.16	65.43±0.12
10	93.16±0.35	90.59±0.16	96.67±0.22	80.59±0.32	93.34±0.36	92.96±0.32	83.03±0.16
12	92.04±0.33	91.46±0.11	93.45±0.43	92.06±0.17	91.67±0.15	89.65±0.22	93.64±0.22

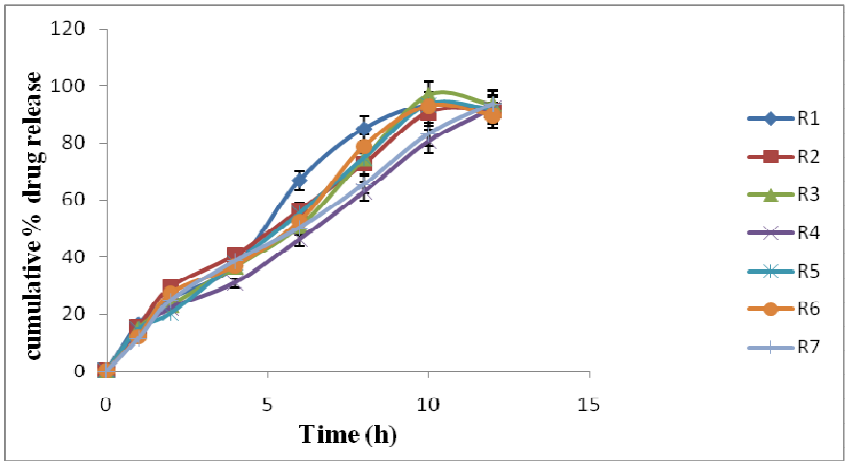

Figure 2: In vitro cumulative % drug release of Roxatidine acetate HCl sodium alginate microspheres formulation

Table 5: In vitro cumulative % drug Roxatidine acetate HCl sodium alginate release of microspheres formulation

Time (h)	R8	R9	R10	R11	R12	R13	R14	Innovator (Rotane 150mg)
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	12.68±0.32	12.06±0.16	11.13±0.35	10.52±0.24	11.82±0.16	12.85±0.32	12.12±0.21	96.45±0.12
2	27.19±0.15	20.57±0.115	19.68±0.33	22.61±0.15	24.16±0.22	19.17±0.23	27.65±0,22	--
4	45.05±0.11	35.26±0.15	27.73±0.32	35.42±0.16	38.94±0.15	25.81±0.15	35.02±0.11	---
6	54.84±0.54	43.18±0.16	41.01±0.16	48.94±0.16	47.08±0.55	43.37±0.16	49.74±0.32	---
8	72.13±0.45	62.73±0.67	56.36±0.15	63.71±0.32	59.62±0.16	61.85±0.44	61.59±0.16	---
10	95.64±0.16	82.32±0.32	73.53±0.44	76.13±0.56	70.19±0.32	70.68±0.15	78.25±0.11	---
12	92.87±0.32	95.92±0.11	89.04±0.35	88.25±0.43	87.67±0.15	87.19±0.33	87.01±0.32	---

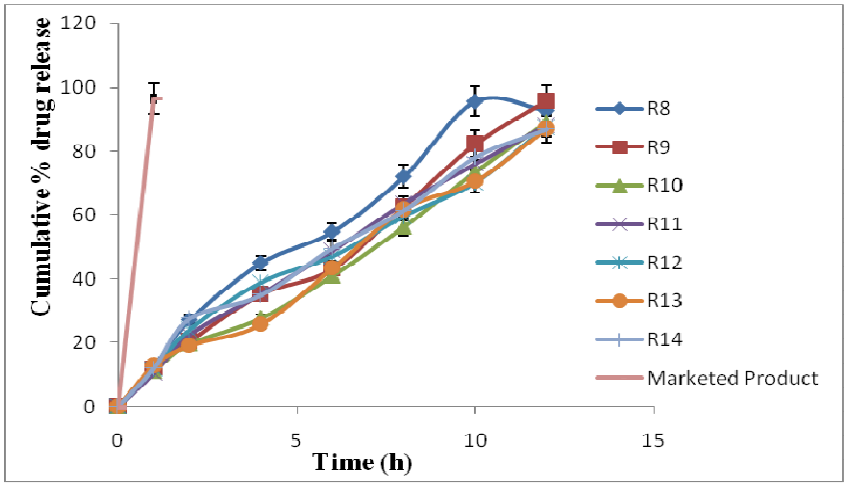

Figure 3: In vitro cumulative % drug Roxatidine acetate HCl normal microspheres

Mathematical modeling of cimetidine optimized microspheres (R9):

Table 6: Release order kinetics of optimized normal microspheres (R9)

Formula Code	Zero Order		First Order		Higuchi		Korsmeyer-Peppas	
	R^2	K	R^2	K	R^2	K	R^2	N
R9	0.992	7.711	0.851	0.094	0.924	27.62	0.989	1.062

From the above results it is apparent that the regression coefficient value closer to unity in case of zero order plot i.e.0.992 indicates that the drug release follows a zero order mechanism. This data indicates a lesser amount of

linearity when plotted by the first order equation. Hence it can be concluded that the major mechanism of drug release follows zero order kinetics. Further, the translation of the data from the dissolution studies suggested possibility of understanding the mechanism of drug release by configuring the data in to various mathematical modeling such as Higuchi and Korsmeyer plots. The mass transfer with respect to square root of the time has been plotted, revealed a linear graph with regression value close to one i.e. 0.924 starting that the release from the matrix was through diffusion. Further the n value obtained from the Korsmeyer plots i.e. 1.062 suggest that the drug release from microspheres was anomalous Non fickian diffusion.

DRUG EXCIPIENT COMPATABILITY STUDIES:

Fourier Transform Infrared Spectroscopy (FTIR)

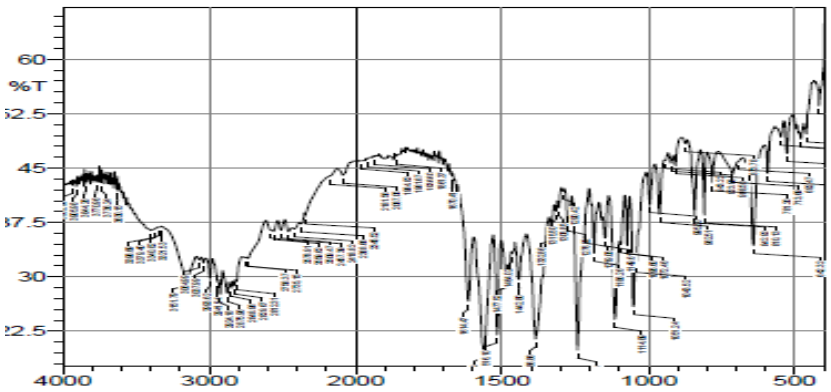

Figure 4: FT-IR spectrum of pure drug Roxatidine acetate HCl

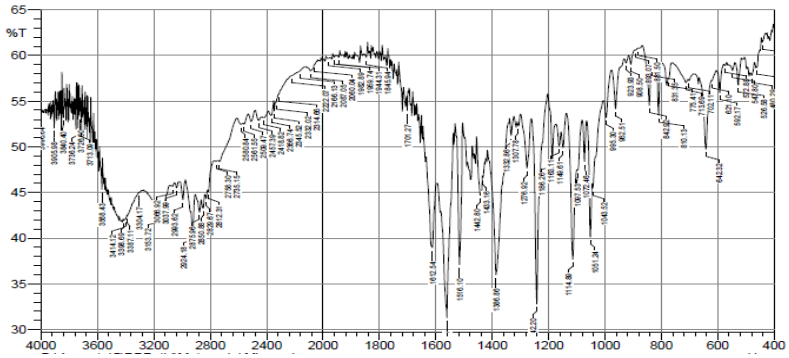

Figure 5: FT-IR spectrum of physical mixture (Roxatidine+Sodium alginate+$CaCl_2$)

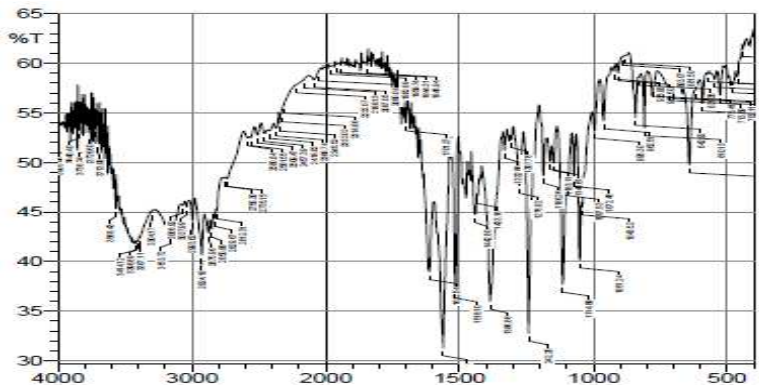

Figure 6: FT-IR spectrum of Roxatidine optimized formulation R9

FTIR was carried out to check the drug excipient interaction. The FTIR peak of Roxatidine acetate HCl is almost similar to that of the peak obtained with excipient and all the peaks of the functional group is in proper range. Hence, it can be concluded that the drug Roxatidine acetate HCl was found to be compatible with the excipient used in the designed formulation.

DSC Studies:

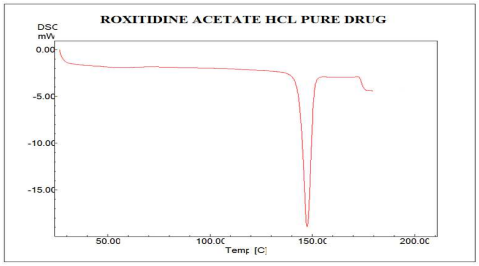

Figure 7: DSC thermogram of Roxatidine acetate HCl pure drug

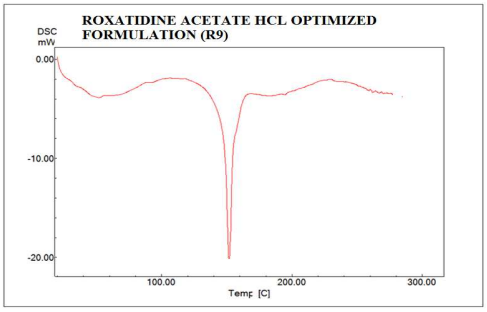

Figure 8: DSC thermogram of Roxatidine acetate HCl optimized microspheres (R9)

DSC was used to detect interaction between Roxatidine acetate HCl and excipients. The thermogram of pure Roxatidine acetate HCl (Figure) exhibited a sharp endotherm melting point at 147 ^{0}C. The thermogram of optimized microspheres loaded with Roxatidine acetate HCl (R9) exhibited a sharp endotherm melting point at149 ^{0}C **(Figure 7).** The DSC thermogram of sodium alginate was also shown in **Figure 8.** The DSC thermogram of microsphere loaded with Roxatidine acetate HCl retained properties of pure Roxatidine acetate HCl. There is no considerable change observed in melting endotherm of drug in optimized formulation. It indicates that there is no interaction between drug & excipients used in the formulation.

Scanning Electron Microscopy studies:

SEM of Roxatidine acetate HCl normal microspheres

The external and internal morphology of controlled release microspheres were studied by Scanning Electron Microscopy.

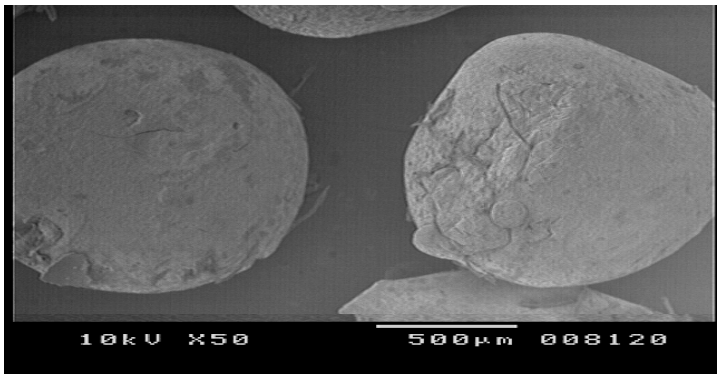

Figure 9: Scanning electron micrographs of Roxatidine acetate HCl microspheres

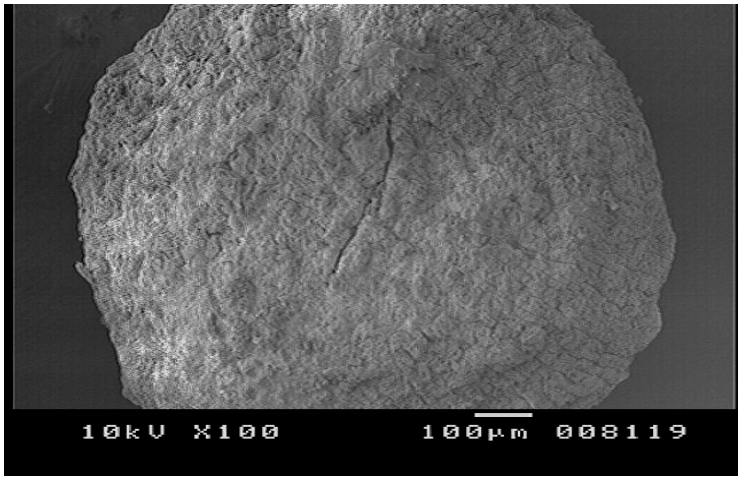

Figure 10: Scanning electron micrographs of Roxatidine acetate HCl microspheres

Morphology of the various formulations of Roxatidine acetate HCl microspheres prepared was found to be discrete and spherical in shape **(Figure 9&10).** The surface of the Roxatidine acetate HCl microspheres was rough due to higher concentration of drug uniformly dispersed at the molecular level in the sodium alginate matrices. There are no crystals on surface which states that is drug is uniformly distributed.

Stability studies:
Optimized formulation was selected for stability studies on the basis of high cumulative % drug release. Stability studies were conducted by performing Percentage yield, %Entrapment efficiency and *In-vitro* drug release profile for 6 months according to ICH guidelines. From these results it was concluded that, optimized formulation is stable and retained their original properties.

CONCLUSION

In the present study, an attempt was made to prepare Roxatidine floating microspheres, which were characterized for particle size, scanning electron microscopy, FT-IR study, DSC, percentage yield, %drug entrapment, stability studies and found to be within the limits. Among all the formulations R9 was selected as optimized formulations based on the physico chemical studies and drug release studies. In the *in vitro* release study of formulation R9 showed 95.12% of drug release after 12 h in a controlled manner, which is essential for disease like peptic ulcer. The *in vitro* release profiles from optimized formulations were applied on various kinetic models. The best fit with the highest correlation coefficient was observed in Higuchi model, indicating diffusion controlled principle. The innovator Rotane 150mg conventional tablet shows the drug release of 96.45 within 1 h. FT-IR and DSC analyses confirmed the absence of drug-polymer interaction. It may be concluded from the result obtained from evaluation and performance study of Roxatidine microspheres that system may be useful to achieve a controlled drug release profile suitable for peroral administration and may help to reduce the dose of drug, dosing frequency and improve patient compliance.

REFERENCES

[1] T. Kristmundsdóttir; K. Ingvarsdóttir; *J Microencapsul*, **1994**, 11, 633–639.
[2] K Bolourtchian; R Karimi; *J Microencapsul*, **2005**, 22, 529–538;
[3] K.N.S. Rani; A.G. Goundalkar; K. Prakasam; *Indian J Pharm Sci*, **1994**, 56, 45–50.
[4] S Gaur; Y Purohit; *Artif Cells Nanomed Biotechnol*, **2014**.
[5] *P. Goyal; S. Gill; U.D. Gupta; Artif Cells Blood Substit Immobil Biotechnol*, **2011**, 39, 330–334.
[6] S.K. Jain; G. Rai; D.K. Saraf; *Pharm Tech*, **2004**, 28, 66–71;
[7] D. Singh; M.R. Singh; *Artif Cells Blood Substit Immobil Biotechnol*, **2012**, 40, 345–353.
[8] K Nazia; Md. Irshad; K S Anupam; S G Sudhir; *Der Pharmacia Lettre*, **2012**, 4, 815-820.
[9] WI Najm; "Peptic ulcer disease." Primary care **2011**, 38, 383–94.

[10] D Murdoch; D McTavish; *Drugs*, **1991**, 42, 240–260.
[11] M.K Das; *Indian Journal of Pharmaceutical Sciences*, **2008**, 70, 77-84.
[12] P Trivedi; A. M. L Verma; N Garud; *Asian J. Pharm*. **2008**, 110-115.
[13] T Ashok; C Harish; M Patel; A Dangi; D. P. Chatterjee; *Panacea Journal of Pharmacy and Pharmaceutical Sciences* **2015**, 4, 654-679.
[14] G Rajput; F Majmudar; J Patel; R Thakor; NB Rajgor; *Sys Rev Pharm*, **2010**, 1, 36-44.
[15] R Deveswaran; R Manavalan; V Madhavan; S Bharath; *Int. J. PharmTech. Res*, **2010**, 2, 2319-2326.
[16] A. Shanmugarathinam; D. Vidhyeswari; A. Puratchikody; *International Journal of Pharma and Bio Sciences*, **2011**, 2, 253-57.
[17] B Saparia; RSR Murthy; A Solanki; *Indian J Pharm Sci*, **2002**, 64, 48–52.
[18] I El-Gibaly; *Int J Pharm*. **2002**, 249, 7–21.
[19] T Higuchi; *J Pharm Sci*, **1963**, 52, 1145–9.
[20] PM Dandagi; VS Mastiholimath; AP Gadad; SR Iliger; *IJPS*, **2007**, 69, 402-407.