International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.9, No.2, pp 124-133, 2016

Development & Evaluation of Mucoadhesive Microspheres of Roxatidine Acetate HCl

Arifa Begum. SK[1,2]*, 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 mucoadhesive microspheres of Roxatidine acetate HCl by ionotropic gelation method. Among all the formulations, M13 was selected as optimized formulation for mucoadhesive microspheres based on the evaluation parameters and drug release studies. *In vitro* release study of formulation M13 showed 99.4% 12 h in a controlled manner, which is essential for disease like peptic ulcer. The release order kinetics for M13 was best fit with the highest correlation coefficient was observed in Higuchi model, indicating diffusion controlled principle. The innovator Rotane 150 mg 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 studies of Roxatidine mucoadhesive microspheres that system may be useful to achieve a controlled drug release and targeting also achieved by mucoadhesion of the microspheres to the GIT may help to reduce the dose of drug, dosing frequency and improve patient compliance when compared with marketed product

Key words: Roxatidine, mucoadhesiveness, gum olibanum, chitosan, microspheres.

Introduction:

The most desirable and convenient method of drug administration is the oral route due to the ease of administration and patient compliance. One limitation for oral delivery is poor bioavailability and for the drug candidates who show absorption window in the proximal gut and is the major obstacle to the development of controlled release formulation. Microsphere carrier systems, made from natural polymers are attracting considerable attentions for several years, for sustained drug delivery. Today, those dosage forms which can control the release rates and which are target specific have a great impact in development of novel drug delivery systems. Microspheres are part of such novel delivery systems[1,2].

The term microsphere is defined as a spherical particle with size from 1µm to 1000µm. The microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature, and ideally having a particle size less than 200 micrometer[3]. Microspheres are one of the multiparticulate drug delivery systems and are prepared by Ionotropic gelation method by dropping drug loaded polymeric solution using syringe into the aqueous solution of polyvalent cations to obtain prolonged (or) controlled drug delivery to improve bioavailability or stability and to target drug to specific sites[4].

Mucoadhesive microspheres:

The success of normal microspheres is limited because due to short residence time at the site of absorption. Therefore, it would be advantageous to provide an intimate contact of the drug delivery systems with the absorbing membranes. This can be achieved by coupling bioadhesion characteristics to microspheres and formulating bioadhesive microspheres. These microspheres provide advantages such as efficient absorption and increased bioavailability of drugs owing to high surface-to-volume ratio, a much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site[5,6,7].

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*[8]**.**

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 h[9]. The aim of present work is to design and *in vitro* evaluation of microspheres of Roxatidine to enhance its bioavailability and prolonged residence time in stomach.

Materials and Methods

Mucoadhesive microspheres:

Formulation of Roxatidine mucoadhesive microspheres

Roxatidine mucoadhesive microspheres were prepared using different polymers like Sodium alginate, Calcium chloride, Chitosan, sodium CMC, Xanthan gum, Gum olibanum, Guar gum and Gum kondagogu by Ionotropic gelation method.

Table 1: Formulation trials for Roxatidine mucoadhesive microspheres

Formulation code	Roxatidine acetate HCl (mg)	Sodium alginate	Sodium CMC(mg)	Calcium chloride	Xanthan gum	Gum olibanum
M1	1500	1 %	100	7%	1%	0.5%
M2	1500	1.2 %	150	7%	1.2%	0.5%
M3	1500	1.4%	200	7%	1.4%	0.5%
M4	1500	1.6%	250	7%	1.6%	0.5%
M5	1500	1.8%	300	7%	1.8%	0.5%
M6	1500	2%	350	7%	2%	0.5%
M7	1500	2.2%	400	7%	2.2%	0.5%
Formulation Code	**Roxatidine Acetate HCl (mg)**	**Sodium Alginate**	**Chitosan (mg)**	**Calcium Chloride**	**Guar Gum**	**Gum Kondagogu**
M8	1500	1%	10	10%	1%	0.5%
M9	1500	1.2%	15	10%	1.2%	0.5%
M10	1500	1.4%	20	10%	1.4%	0.5%
M11	1500	1.6%	25	10%	1.6%	0.5%
M12	1500	1.8%	30	10%	1.8%	0.5%
M13	1500	2%	35	10%	2%	0.5%
M14	1500	2.2%	40	10%	2.2%	0.5%

Procedure for the preparation of Roxatidine mucoadhesive microspheres:

The Roxatidine mucoadhesive microspheres were prepared by using ionotropic gelation technique. In this method weighed quantity of Roxatidine acetate HCl was added to 100 ml sodium alginate, Sodium CMC solution and other polymers, thoroughly mixed at 500 rpm. Resultant solution was extruded drop wise with the help of syringe and needle into 100 ml aqueous calcium chloride solution and stirred at 100 rpm. After stirring for 30 minutes the obtained microspheres were washed with water and dried at 60 degrees-4 hours in a hot air oven and stored in desiccators.

Evaluation studies of Roxatidine acetate HCl mucoadhesive microspheres:

Micromeretic properties like particle size, angle of repose, bulk density, Tapped density, Compressibility index, Hausner's ratio and evaluation parameters like Swelling index, Drug entrapment efficiency and % yield, mucoadhesive study and *In vitro* dissolution studies were performed.

Mucoadhesive study

The *In vitro* Mucoadhesive test was carried out using small intestine from chicken. The small intestinal tissue was excised and flushed with saline. Five centimeter segment of jejunum were averted using a glass rod. Ligature was placed at both ends of the segment. 100 microspheres were scattered uniformly on the averted sac from the position of 2 cm above. Then the sac was suspended in a 50 ml tube containing 40 ml of saline by the wire, to immerse in the saline completely. The sac were incubated at 37 ^{0}C and agitated horizontally. The sac were taken out of the medium after immersion for 1, 2, 3, 4, 5, 6, 7 and 8 h, immediately repositioned as before in a similar tube containing 40 ml of fresh saline and unbound microspheres were counted. The adhering percent was presented by the following equation[9].

Mucoadhesion= (No. of microspheres adhered/ No. of microspheres applied) X 100

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

In vitro drug release studies for developed Roxatidine acetate HCl 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 ^{0}C 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 h by UV visible spectrophotometer (Shimadzu UV 1800) at 280 nm[10].

Kinetic modeling of drug release:

In order to understand the kinetics and mechanism of drug release, the result of the in vitro dissolution study of microspheres were fitted with various kinetic equations like Zero order as cumulative percentage drug released Vs time, First order as log percentage of drug remaining to be released Vs time, Higuchi's model cumulative percentage drug released Vs square root of time. r^2 and K values were calculated for the linear curves obtained by regression analysis of the above plots.

Drug excipient compatibility studies

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

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 and cumulative % drug released during the stability study period.

Results and Discussion:

Mucoadhesive microspheres

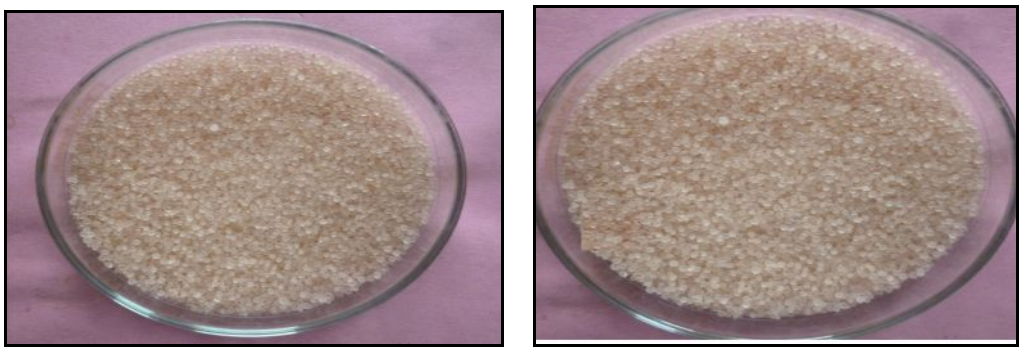


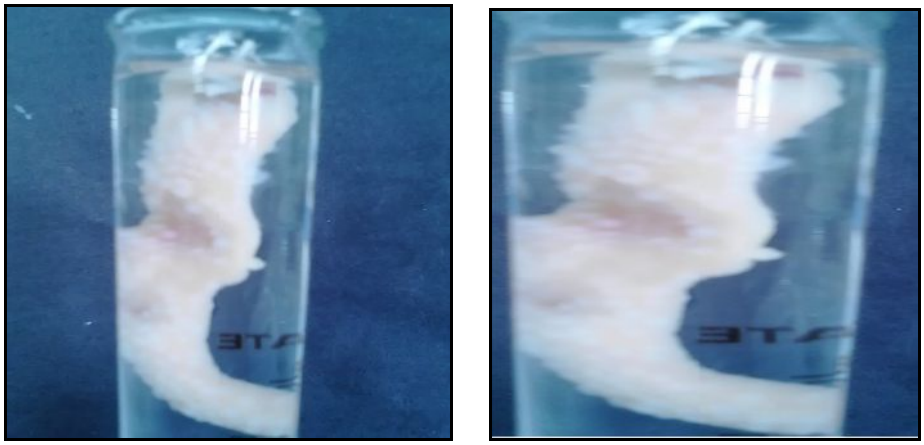
Figure 1: Roxatidine acetate HCl Mucoadhesive microspheres

Table 2: Micromeretic properties of Roxatidine acetate HCl microspheres

Formulation code	Particle size (µm)	Bulk density ($g/cm^{3)}$	Tapped density ($g/cm^{3)}$	Angle of repose	Carr's index
M1	65.29±0.13	0.63	0.62	29°.67	11.34%
M2	73.43±0.04	0.65	0.69	30°.54	13.12%
M3	78.67±0.09	0.67	0.73	31°.15	14.23%
M4	79.45±0.21	0.69	0.75	26°.91	12.00%
M5	83.42±0.12	0.72	0.79	27°.93	13.00%
M6	85.34±0.09	0.75	0.82	28°.54	13.00%
M7	77.12±0.13	0.74	0.67	25°.81	12.20%
M8	69.43±0.09	0.66	0.64	30°.91	13.34%
M9	72.46±0.09	0.68	0.63	27°.91	14.11%
M10	76.89±0.10	0.72	0.68	30°.24	13.12%
M11	85.94±0.11	0.74	0.72	27°.93	12.23%
M12	88.94±0.11	0.79	0.75	26°.34	11.34%
M13	67.12±0.13	0.76	0.61	22°.81	8.20%
M14	91.45±0.21	0.83	0.83	26°.91	13.45%

The particle size was measured by using optical microscopy. All the formulations M1 to M14 varied from 65.29±0.13 µm to 89.04±0.21 µm. The formulation M13 shows the particle size 67.12±0.13 µm. The bulk density and tapped density of all the formulations M1 to M14 were measured and they are ranged from 0.63 g/cm^3 to 0.83 g/cm^3 and 0.61 g/cm^3 to 0.91 g/cm^3. Angle of repose of all the formulations was found satisfactory results. And the formulation M13 was found to be 22°.81 having good flow property. The compressibility index values were found to be in the range of 6 to 13.00%. These findings indicated that the all batches of formulation exhibited good flow properties. The compressibility index of M13 was found to be 8.20%.

Mucoadhesion study:



(A) **(B)**

Figure 2: Pictorial diagram showing mucoadhesive property of mucoadhesive microspheres in Chic Intestine at 0 min (A) & after 8 hr (B)

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

Formulation code	Percentage yield	Entrapment efficiency	Swelling index	Mucoadhesiveness
M1	75.45%	76.00%	72.11%	69.00%
M2	81.38%	82.03%	78.34%	78.00%
M3	82.97%	84.04%	82.89%	71.00%
M4	85.00%	86.00%	84.56%	78.00%
M5	87.02%	88.72%	85.23%	80.00%
M6	93.03%	93.03%	94.12%	91.00%
M7	86.05%	85.01%	88.23%	90.00%
M8	81.08%	80.02%	69.12%	83.00%
M9	83.00%	82.05%	70.12%	82.00%
M10	84.00%	85.00%	75.22%	85.00%
M11	89.00%	88.25%	84.34%	87.00%
M12	92.00%	91.00%	91.09%	92.50%
M13	96.05%	95.01%	96.23%	95.00%
M14	90.72%	89.67%	90.03%	88.00%

The percentage release and entrapment efficiency of all the formulations were measured by assay method. The mucoadhesive microspheres of formulation M1 to M14 shows the percentage yield values ranges from 75.45% to 96.05% and entrapment efficiency of 76% to 95%. All the formulations M1 to M14 showed the swelling of microspheres. The swelling index of the formulation M13 was found to be 96.23%. The formulation M13 shows the best percentage yield and entrapment efficiency values of 96.05% and 95.01% respectively when compared with other formulations.

Table 4: *In-vitro* cumulative % drug release of Roxatidine acetate HCl Mucoadhesive microspheres Formulations:

Time in hours	M1	M2	M3	M4	M5	M6	M7	Innovator (Rotane 150 mg)
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	18.21±0.32	16.51±0.11	16.51±0.22	15.26±0.23	15.19±0.11	14.09±0.16	14.09±0.22	96.45±0,12
2	39.32±0.15	33.62±0.21	35.32±0.11	33.67±0.15	29.02±0.16	26.33±0.43	26.33±0.24	---
4	50.21±0.11	50.02±0.31	51.73±0.65	48.07±0.11	45.31±0.13	35.75±0.88	35.75±0.15	----
6	64.46±0.16	67.63±0.22	66.72±0.43	60.96±0.16	55.43±0.12	55.06±0.76	55.06±0.17	----
8	81.08±0.32	83.47±0.32	75.23±0.16	79.28±0.21	71.98±0.21	73.53±0.54	73.53±0.54	----
10	88.39±0.16	90.36±0.17	85.31±0.32	93.27±0.33	88.53±0.11	80.42±0.34	80.42±0.55	----
12	91.27±0.99	93.44±0.77	91.82±0.22	90.74±0.17	93.22±0.16	91.14±0.21	87.14±0.76	----

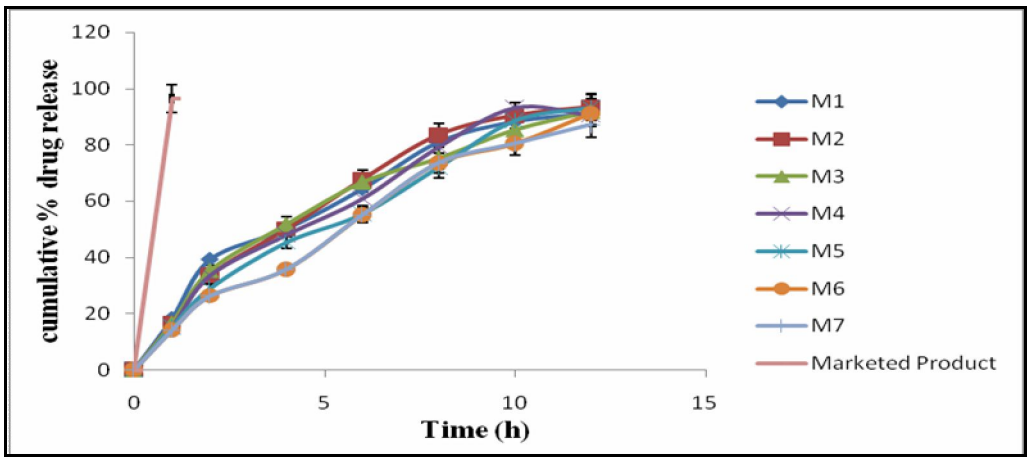


Figure 3: *In-vitro* cumulative % drug release of Roxatidine acetate HCl Mucoadhesive microspheres formulations

Table 5: *In-vitro* cumulative % drug release of Roxatidine acetate HCl mucoadhesive microspheres formulations

Time (h)	M8	M9	M10	M11	M12	M13	M14
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	10.21±0.66	8.96±0.11	10.83±0.56	6.51±0.22	7.63±0.22	11.23±0.22	8.21±0.11
2	17.7±0.32	16.05±0.15	19.22±0.66	14.33±0.15	17.44±0.21	24.91±0.18	18.82±0.21
4	28.52±0.55	26.56±0.16	27.83±0.98	21.57±0.22	24.89±0.15	33.51±0.87	29.64±0.22
6	40.71±0.32	38.45±0.17	36.54±0.43	30.08±0.32	37.97±0.16	43.52±0.98	45.75±0.32
8	56.54±0.22	52.36±0.26	49.86±0.32	42.72±0.11	49.86±0.12	60.94±0.87	54.96±0.16
10	70.66±0.34	72.04±0.12	61.37±0.11	59.23±0.43	60.64±0.32	69.48±0.16	66.18±0.17
12	88.43±0.45	88.55±0.32	83.45±0.32	78.74±0.22	72.17±0.21	99.4±0.22	79.03±0.42

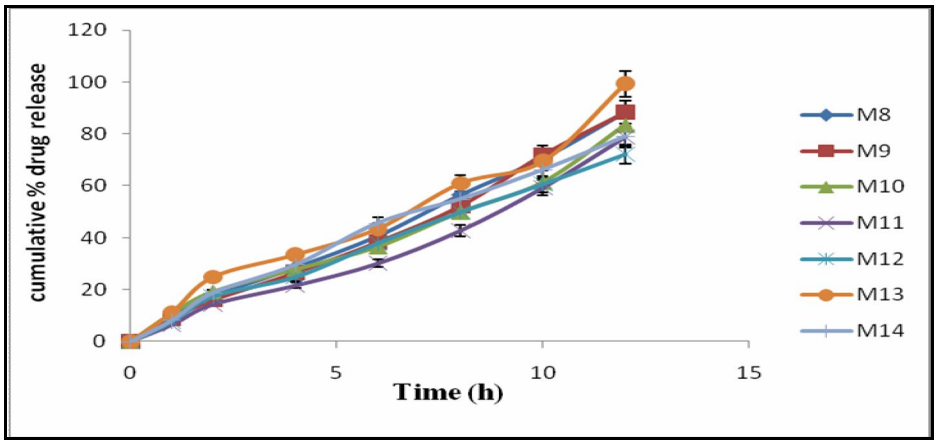


Figure 4: *In-vitro* cumulative % drug release of Roxatidine acetate HCl mucoadhesive microspheres formulations

Mathematical modeling of optimized formula of mucoadhesive microspheres:

Table 6: Release order kinetics of optimized formulation (M13) of mucoadhesive microspheres:

Formulation Code	Zero Order		First Order		Higuchi		Korsmeyer-Peppas	
	R^2	K	R^2	K	R^2	K	R^2	N
M13	0.991	7.562	0.830	0.093	0.937	27.29	0.984	1.077

From the above results it is apparent that the regression coefficient value closer to unity in case of zero order plot i.e.0.991 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, t-he 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. 0.937 starting that the release from the matrix was through diffusion. Further the n value obtained from the Korsmeyer plots i.e. 1.077 suggest that the drug release from floating tablet was anomalous Non fickian diffusion.

Drug excipient compatibility studies:

Fourier Transform Infrared Spectroscopy (FTIR)

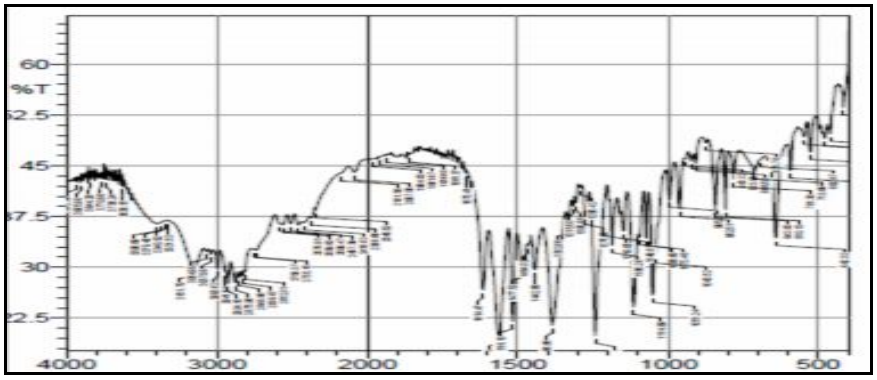


Figure 5: FT-IR spectrum of pure drug Roxatidine acetate HCl

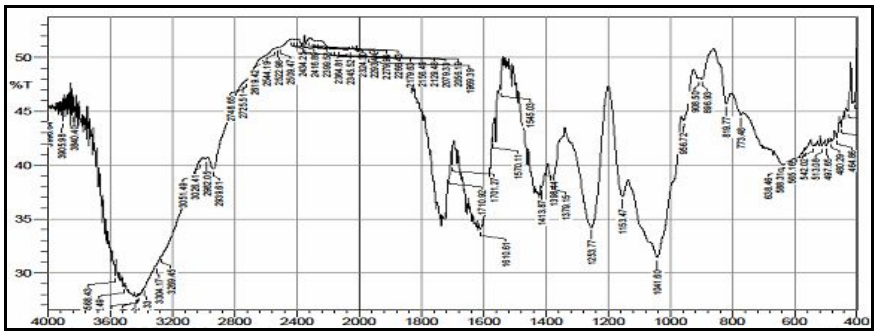


Figure 6: FT-IR spectrum of Gum Kondagogu

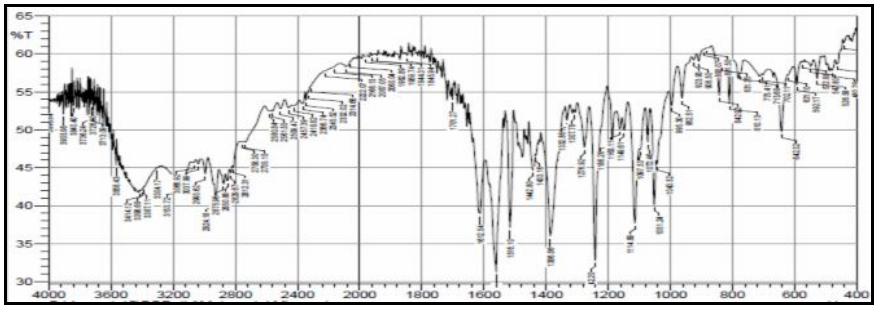


Figure 7: FT-IR spectrum of physical mixture

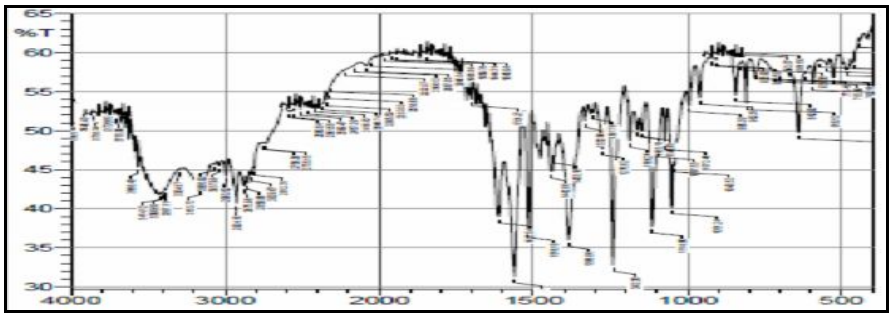


Figure 8: FT-IR spectrum of Roxatidine optimized formulation M13

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:

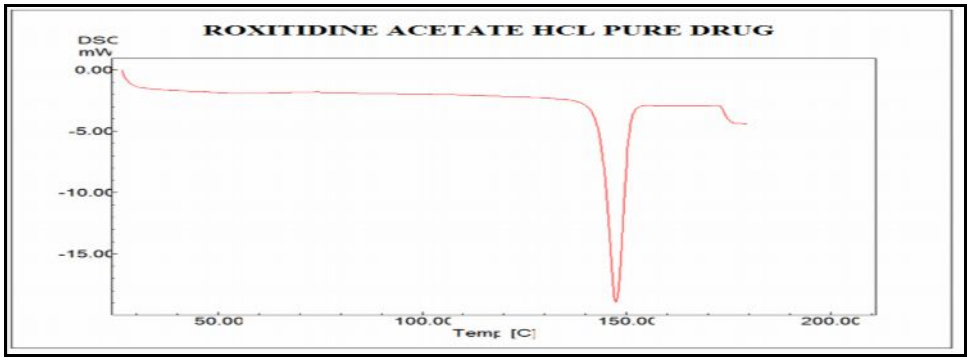


Figure 9: DSC thermogram of Roxatidine acetate HCl pure drug

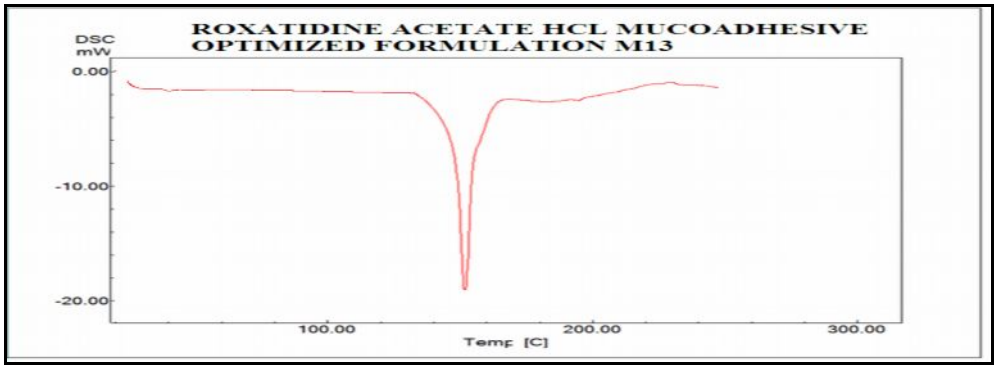


Figure 10: DSC thermogram of Roxatidine acetate HCl mucoadhesive optimized microspheres (M13)

DSC was used to detect interaction between Roxatidine acetate HCl and excipients. The thermogram of pure Roxatidine acetate HCl **(Figure 9)** exhibited a sharp endotherm melting point at 147 ^{0}C. The thermogram of optimized microspheres loaded with Roxatidine acetate HCl (M13) exhibited a sharp endotherm melting point at149 ^{0}C **(Figure 10)**. The DSC thermogram of sodium alginate was also shown in Figure. 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:

SEM of Roxatidine acetate HCl mucoadhesive microspheres

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

Mucoadhesive microspheres:

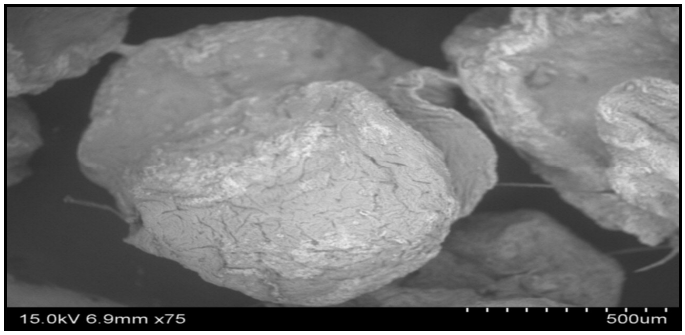


Figure 11: Scanning electron micrographs of Roxatidine acetate HCl mucoadhesive microspheres (M13)

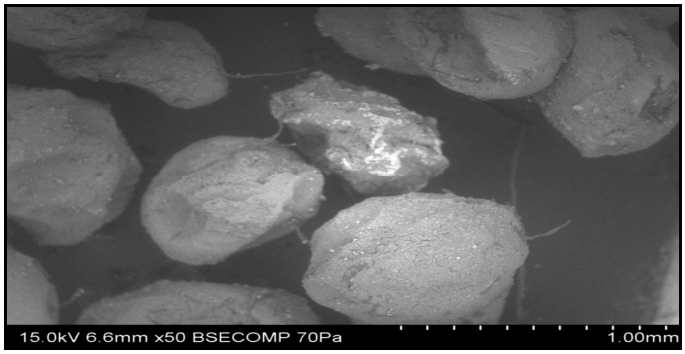


Figure 12: Scanning electron micrographs of Roxatidine acetate HCl mucoadhesive microspheres (M13)

Morphology of the various formulations of Roxatidine acetate HCl microspheres prepared was found to be discrete and spherical in shape **(Figure 11 & 12)**. The surface of the mucoadhesive 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 (M13) was selected for stability studies on the basis of high cumulative % drug release. Stability studies were conducted for Percentage yield, Entrapment efficiency & *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 with minor differences.

Conclusion:

In vitro data obtained for mucoadhesive microspheres of Roxatidine showed good drug entrapment and % yield. In the present study, an attempt was made to prepare mucoadhesive and 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 M13 was selected as optimized formulation based on the physicochemical studies and drug release studies. In the *in vitro* release study of formulation M13 showed 99.4% after 12 h in a controlled manner, which is required for disease like peptic ulcer. The *in vitro* release profiles from optimized formulation M13 was 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 150 mg conventional tablet showed 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. Gogel MC & Amin AF., Formulation optimization of controlled release diclofenac sodium microspheres using factorial design, J Control Release, 1998,51(2-3),115-22, 1998.
2. Chaudhari A, Jadhav KR & Kadam VJ., An over view: Microspheres as a nasal drug delivery system, International Journal of Pharmaceutical Sciences Review and Research, 2010, 5, 8-17.
3. Sudhamani T, Naveen Kumar Reddy K, Ravikumar VR, Revathi R, Ganesan V., preparation and evaluation of ethyl cellulose microspheres of ibuprofen for sustained drug delivery. Int. J. pharma Res and dev. 2010, 1,119-125.
4. Ikeda K, Murata K, Kobayashi M, Noda K., Enhancement of bioavailability of dopamine via nasal route in beagle dogs. Chem Pharm Bull, 1992,40(8),2155–2158;
5. Nagai T, Nishimoto Y, Nambu N, Suzuki Y, Sekine K., Powder dosage forms of insulin for nasal administration, J Control Release, 1984, 1, 15–22.
6. Schaefer MJ & Singh J., Effect of additives on stability of etoposide in PLGA microspheres, Drug Dev Ind Pharm, 2001, 27(4), 345-50.
7. Najm, WI., "Peptic ulcer disease." Primary care, 1991, 38 (3), 383–94.
8. Murdoch D, McTavish D.,"Roxatidine acetate. A review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic potential in peptic ulcer disease and related disorders". *Drugs* 1991, 42 (2), 240–260.
9. SK Jain, Nitin K Jain, Y Gupta, A Jain, D Jain, M Chaurasia., Mucoadhesive chitosan microspheres for non-invasive and improved nasal delivery of insulin, 2007, (4) (69) 498-504.
10. Shanmugarathinam D. Vidhyeswari and A. Puratchikody., Formulation, characterization and in-vitro evaluation of acrylic polymer loaded aceclofenac microspheres, International Journal of Pharma and Bio Sciences, 2011, (2), 253-57.

*****