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Formulation and evaluation of mucoadhesive microspheres of roxatidine acetate hydrochloride

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ABSTRACT

The intention of the present study is to formulate mucoadhesive microspheres containing roxatidine acetate hydrochloride by employing xanthan gum & gum olibanum as mucoadhesive agent and by adapting ionotropic gelation technique. Response Surface Composite design was employed to study the effect of independent variables, polymer concentration (X1), and sodium alginate concentration (X2) on dependent variables mucoadhesion time. The best batch exhibited a high drug entrapment efficiency of 95.01% and a swelling index of 96.23%; percentage mucoadhesion after 10 h was 97.01%. The drug release was also sustained for 12 h. The polymer-to-drug ratio had a more significant effect on the dependent variables. The prepared mucoadhesive microspheres were characterized for various properties like preformulation, flow properties, *in vitro* mucoadhesion, *in vitro* drug release, entrapment efficiency and surface properties. The external and internal surface morphological characteristics of mucoadhesive microspheres were investigated using Scanning Electron Microscope (SEM). The formulation which showed better flow properties, *in vitro* mucoadhesion, *in vitro* drug release and entrapment efficiency was selected as optimized formulation i.e., formulation MOG4. The *in vitro* release profiles from optimized formulations were applied on various release kinetic models of drug and suggested that the drug release from microspheres followed non-fickian diffusion. The optimized formulation MOG4 was subjected to stability studies for six months at $40^0 \pm 2^0$C & 75±5%RH as per ICH guidelines and result have not showed any changes in physical parameters, formulation parameters and *in vitro* release studies.

Keywords: Mucoadhesive microspheres, Roxatidine, Factorial design, *In vitro* study.

INTRODUCTION

Microspheres are discrete particles that make up a multiple unit system. Recently, much emphasis has been laid on the development of microspheres dosage forms in preference to single unit systems because of their potential benefits such as increased bioavailability, reduced risk of systemic toxicity, reduced risk of local irritation and predictable gastric emptying. Microspheres systems show better reproducible pharmacokinetic behavior than conventional (monolithic) formulations. The

incorporation of mucoadhesive polymers in the microspheres significantly increases the gastrointestinal transit time of microspheres [1, 2]. It has the desired characteristics suitable for developing mucoadhesive extended release formulations, which include its solubility in acidic pH and a shorter half-life of 3 - 5 h. Due to side effects of roxatidine a sustained release medication is required to get prolonged effect with reduced fluctuations in drug plasma concentration levels [3].

Roxatidine is used as an antiulcer drug; however, constipation remains one of its side effects [4]. Roxatidine is competitive inhibitor of histamine at the parietal cell H_2 receptor. It suppresses the normal secretion of acid by parietal cells and the meal-stimulated secretion of acid. It accomplish this by two mechanisms: histamine released by ECL cells in the stomach is blocked from binding on parietal cell H_2 receptors which stimulate acid secretion and other substances that promote acid secretion (such as gastrin and acetylcholine) have a reduced effect on parietal cells when the H_2 receptors are blocked, so heal the ulcers caused by *H. pylori* bacteria. Roxatidine acetate markedly reduces total pepsin output, but has no significant influence on serum pepsinogen I and gastrin levels in patients with peptic ulcer disease [5].

MATERIALS AND METHODS

Materials

Roxatidine was obtained as a gift sample from Aurobindo Pharma Limited, Hyderabad, India. Sodium alginate was obtained from Pruthvi Chemicals, Mumbai. Sodium Carboxy Methyl Cellulose, Xanthan gum and Gum olibanum were obtained from MSN Labs Ltd., Hyderabad. All other chemicals were of Pharmaceutical grade.

Method

Roxatidine mucoadhesive microspheres were prepared using polymers like sodium alginate, xanthan gum and gum olibanum employing ionotropic gelation method. Resposne Surface Composite design was employed to study the effect of independent variables, polymer concentration (X1), and sodium alginate concentration (X2) on dependent variables mucoadhesion time. Different formulations were prepared by using different concentrations of polymers and mucoadhesive agent as showed in **Table 1 & 2.**

Roxatidine mucoadhesive microspheres were prepared using polymers sodium alginate & xanthan gum and gum olibanum in different concentrations by ionotropic gelation method. In this method, weighed quantity of roxatidine was added to 100 ml sodium alginate solution containing, mucoadhesive polymer (xanthan gum and gum olibanum) and was mixed thoroughly at 500 rpm. Resultant solution was extruded drop wise with the help of syringe and needle into 100 ml aqueous calcium chloride solution kept stirring at 100 rpm. After stirring for 30 min, the obtained microspheres were washed with water and dried at 60^0C for 4 h in a hot air oven and stored in desiccators [6].

Table 1 (a): Optimization of Roxatidine acetate HCl Mucoadhesive Microspheres containing Xanthan Gum

Factor	Name	Minimum	Maximum	-1 Actual	+1 Actual	Mean	Std. Dev.
A	Sodium Alginate (%)	3.00	4.00	3.00	4.00	3.50	0.41
B	Xanthan Gum (%)	15.00	20.00	15.00	20.00	17.50	2.04

Table 1 (b): Composition of Roxatidine acetate HCl Mucoadhesive Microspheres containing Xanthan Gum

Formulation Code	Roxatidine Acetate HCl (mg)	Sodium Alginate (%)	Calcium Chloride (%)	Xanthan Gum (%)
MX1	1500	3.5	10	17.5
MX2	1500	4	10	20
MX3	1500	3.5	10	20

MX4	1500	3	10	17.5
MX5	1500	3	10	15
MX6	1500	4	10	15
MX7	1500	3	10	20
MX8	1500	3.5	10	15
MX9	1500	4	10	17.5

Table 2 (a): Optimization of Roxatidine acetate HCl Mucoadhesive Microspheres containing Xanthan Gum

Factor	Name	Minimum	Maximum	-1 Actual	+1 Actual	Mean	Std. Dev.
A	Sodium Alginate (%)	3.00	4.00	3.00	4.00	3.50	0.41
B	Gum Olibanum (%)	5.00	10.00	5.00	10.00	7.50	2.04

Table 2 (b): Composition of Roxatidine acetate HCl Mucoadhesive Microspheres containing Gum Olibanum

Formulation Code	Roxatidine Acetate HCl (mg)	Sodium Alginate (%)	Calcium Chloride (%)	Gum Olibanum (%)
MOG1	1500	3.5	10	7.5
MOG2	1500	3.5	10	10
MOG3	1500	4	10	10
MOG4	1500	3	10	5
MOG5	1500	4	10	5
MOG6	1500	3	10	10
MOG7	1500	3	10	7.5
MOG8	1500	3.5	10	5
MOG9	1500	4	10	7.5

Evaluation Studies of Roxatidine acetate HCl Mucoadhesive Microspheres

The Various evaluation test that to be conducted for prepared mucoadhesive microspheres follow as; [7]

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 37^0C and agitated horizontally. The sac were taken out of the medium after immersion for every one hour time interval, 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 [7].

$$Percentage\ mucoadhesion = \frac{no.\ of\ microspheres\ adhered}{no.\ of\ microspheres\ applied} * 100$$

In vitro Drug Release Studies [8]

Accurately weighed amount of microspheres from each batch were subjected to dissolution studies in triplicate manner. Release rate of drug from mucoadhesive microspheres was carried out using USP dissolution apparatus II;

Conditions for mucoadhesive microspheres:

- Performed using USP dissolution apparatus II.
- Dissolution medium – 0.1N HCl
- Temperature – 37 ± 0.5^0C

- Stirring speed – 100 rpm
- Bath volume – 900 ml
- Time intervals – 0, 2, 3, 4, 6, 8, 10 & 12 h.

The withdrawn volume was replaced with an equivalent volume of fresh dissolution medium to maintain the volume of dissolution medium constant. The sample solutions were analyzed for the concentration of drug by UV spectrophotometer. The amount of drug released was calculated from the calibration curve of the same dissolution medium.

Kinetic Modelling of Drug Release

In order to understand the kinetics and mechanism of drug release, the results 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 as cumulative percentage drug released Vs. square root of time. R^2 and n values were calculated for the linear curves obtained by regression analysis of the above plots [9, 10].

Drug Excipient Compatibility Studies

The drug excipient compatibility studies were carried out by Fourier Transmission Infrared Spectroscopy (FTIR) method and Differential Scanning Colorimetry (DSC) [11, 12].

SEM Studies

The surface morphology of microspheres was determined by scanning electron microscopy (SEM) (HITACHI, S-3700N) [13, 14].

Stability Studies

Accelerated stability studies were carried out at 40°C/75% RH for the best formulations for 6 months according to ICH guidelines [15]. The microspheres were characterized for the percentage yield, entrapment efficiency & cumulative % drug released during the stability study period.

Factorial Design

A statistical model incorporating interactive and polynomial terms was used to evaluate the responses:

$Y = b_0 + b_1X_1 + b_2X2 + b_{12}X_1X_2 + b_{11}X^2{}_1 + b_{22}X^2{}_2$;

Where, Y is the dependent variable, b_0 is the arithmetic mean response of the 9 runs, and bi is the estimated coefficient for the factor X_i. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when 2 factors are simultaneously changed. The polynomial terms (X^1 2 and X^2 2) are included to investigate nonlinearity.

RESULTS AND DISCUSSION

Preparation of Roxatidine acetate HCl Mucoadhesive Microspheres:

Mucoadhesive microspheres of roxatidine acetate HCl were formulated by ionic gelation method, using different polymers like sodium alginate, xanthan gum and gum olibanum in different concentrations in according to assigned quantities in **Table 1 (b) and Table 2 (b).**

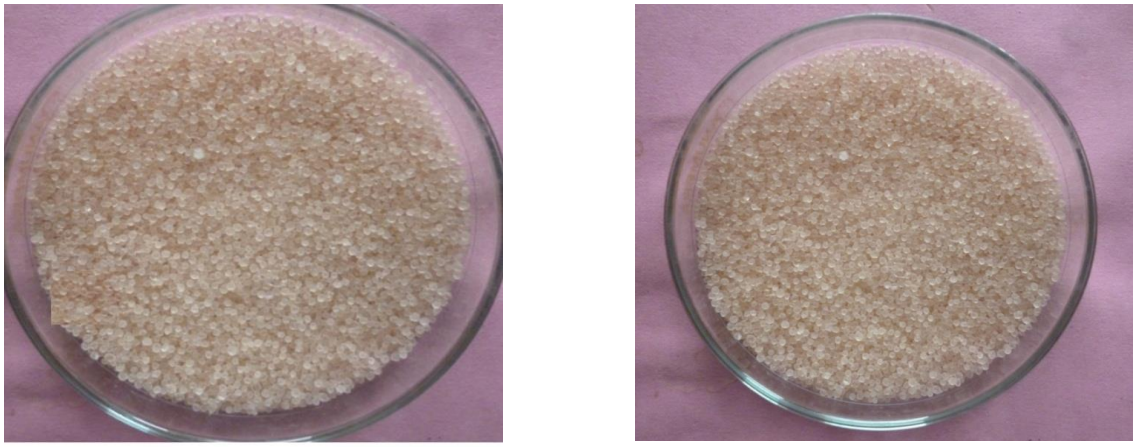


Fig. 1: Roxatidine acetate HCl Mucoadhesive Microspheres

Micromeritic Properties

The particle size of all the prepared formulations was found to be in the range of 65.25 ± 0.21 µm to 90.04 ± 0.11 µm. The formulation **MOG4** showed the particle size 65.25 ± 0.21 µm. The bulk density of all the prepared formulations was measured and it was ranged from 0.63 g/cm³ to 0.89 g/cm³. The tapped density of all the prepared formulations was measured and ranged between 0.65 g/cm³ - 0.91 g/cm³. Angle of repose of all the formulations was found to be satisfactory. The θ value of the formulation **MOG4** was found to be 22˚.91 having good flow property. The compressibility index values were found to be in the range of 11.00 to 14.34. The compressibility of **MOG4** was found to be 12.00%.These findings indicated that the all batches of formulation exhibited good flow properties.

Table 3 (a): Micromeritic Properties of Roxatidine acetate HCl Mucoadhesive Microspheres containing Xanthan gum

Formulation Code	Particle Size (µm)	Bulk Density ($g/cm^{3)}$	Tapped Density ($g/cm^{3)}$	Angle of Repose (^{o})	Carr's Index (%)
MX1	68.29±0.13	0.63±0.01	0.62±0.02	26.67±0.3	13.34±0.01
MX2	73.43±0.04	0.65±0.02	0.69±0.03	25.54±0.6	12.12±0.02
MX3	78.67±0.09	0.67±0.15	0.73±0.05	25.15±0.5	12.23±0.01
MX4	79.45±0.21	0.69±0.01	0.75±0.12	28.91±0.1	11.00±0.04
MX5	83.42±0.12	0.72±0.04	0.79±0.06	27.93±0.9	12.20±0.08
MX6	85.34±0.09	0.75±0.08	0.82±0.05	28.54±0.7	13.00±0.02
MX7	87.12±0.13	0.76±0.01	0.91±0.02	27.91±0.6	11.20±0.04
MX8	69.43±0.09	0.66±0.07	0.61±0.01	26.91±0.5	14.34±0.03
MX9	72.46±0.09	0.68±0.12	0.63±0.01	27.91±0.4	12.11±0.02

Table 3 (b): Micromeritic Properties of Roxatidine acetate HCl Mucoadhesive Microspheres containing Gum Olibanum

Formulation Code	Particle Size (µm)	Bulk Density ($g/cm^{3)}$	Tapped Density ($g/cm^{3)}$	Angle of Repose (^{o})	Carr's Index (%)
MOG1	75.29±0.13	0.63±0.01	0.62±0.01	29.67±0.2	11.34±0.05
MOG2	73.43±0.04	0.65±0.01	0.69±0.02	27.54±0.2	13.12±0.01
MOG3	78.67±0.09	0.67±0.02	0.73±0.02	26.15±0.3	14.23±0.01
MOG4	**65.25±0.21**	**0.63**±0.03	**0.65**±0.04	**22.91**±0.1	**12.00**±0.01
MOG5	83.42±0.12	0.72±0.05	0.79±0.05	27.93±0.2	13.00±0.02
MOG6	85.34±0.09	0.75±0.06	0.82±0.05	28.54±0.3	13.00±0.03
MOG7	77.12±0.13	0.83±0.07	0.83±0.06	22.81±0.2	13.45±0.01
MOG8	90.04±0.11	0.63±0.06	0.72±0.04	28.61±0.4	12.74±0.05
MOG9	81.45±0.21	0.89±0.05	0.77±0.03	25.61±0.4	13.83±0.06

Percentage Yield, Entrapment Efficiency and Swelling Index

The prepared mucoadhesive microspheres formulations showed the percentage yield values ranging from 75.45% to 98.6%. The entrapment efficiency values of all the 18 formulations ranged from 76.00% to 98.00%. It was found that the formulation **MOG4** showed the best percentage yield and entrapment efficiency values of 98.6% and 98.00% respectively when compared with other formulations. All the formulations containing xanthan gum and gum olibanum showed the swelling of microspheres. The swelling of the formulation MOG4 was found to be highest i.e., 97.07%.

Mucoadhesion Study

The *in vitro* mucoadhesive test was carried out using chicken small intestine (Jain SK *et al*., 2007). The mucoadhesive microspheres of all formulations prepared using xanthan gum (**MX1** to **MX9)** and gum olibanum (**MOG1** to **MOG9)** showed mucoadhesive time ranged from 6.5 h to 10.17 h with more than 90% mucoadhesion. The high percentage of mucoadhesive property i.e. 98% was observed for formulation **MOG4** in 10.17 h.

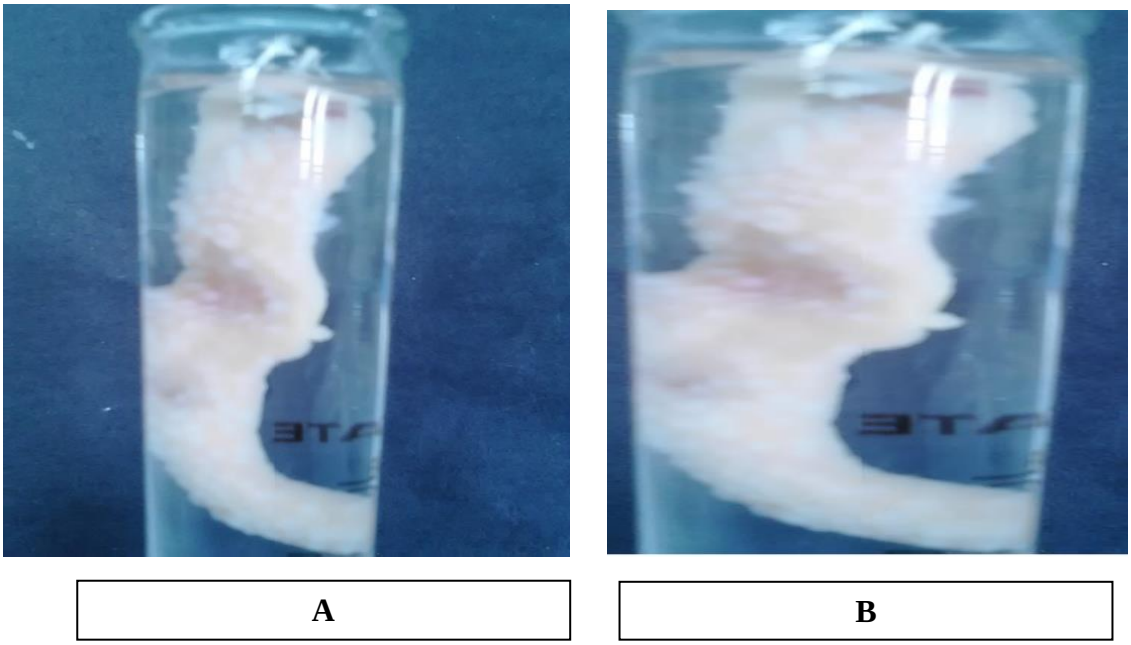


Fig. 2: Pictorial Diagram Showing Mucoadhesive Property of Mucoadhesive Microspheres in Chic Intestine at 0 min (A) & after 8 h (B)

Table 4 (a): Evaluation Report of Roxatidine acetate HCl Mucoadhesive Microspheres containing Xanthan gum

Formulation Code	Percentage Yield (%)	Entrapment Efficiency (%)	Swelling Index (%)	Mucoadhesion Time (h)
MX1	75.45±1.43	76.00±1.86	72.11±1.14	7.75
MX2	81.38±2.43	82.03±1.32	78.34±1.07	8.5
MX3	82.97±2.56	84.04±1.72	82.89±1.28	7.83
MX4	85.00±2.31	86.00±1.87	84.56±1.46	9
MX5	87.02±2.12	88.72±1.98	85.23±1.21	9.5
MX6	96.03±1.54	95.03±1.22	91.12±1.42	9
MX7	**96.10**±0.43	**97.01**±1.73	**91.23**±1.53	**9.75**
MX8	81.08±1.87	80.02±1.39	69.12±1.08	9.5
MX9	83.00±2.41	82.05±1.57	70.12±1.22	9.30

Table 4 (b): Evaluation Report of Roxatidine acetate HCl Mucoadhesive Microspheres containing Gum Olibanum

Formulation Code	Percentage Yield (%)	Entrapment Efficiency (%)	Swelling Index (%)	Mucoadhesion Time (h)
MOG1	95.59±1.09	90.00±1.58	80.02±1.04	6.5
MOG2	93.7±1.12	92.4±1.27	82.02±1.54	7.75
MOG3	96.8±1.26	84.04±1.45	97.40±1.34	7.25
MOG4	**98.6**±1.78	**98.00**±1.58	**97.07**±1.04	**10**
MOG5	96.7±1.32	91.03±1.39	88.25±1.18	8.33
MOG6	96.8±1.39	90.65±1.58	91.00±1.05	9.25
MOG7	96.8±1.02	91.57±1.22	87.70±1.07	9.17
MOG8	95.1±1.39	89.34±1.27	89.67±1.06	8.5
MOG9	95.2±1.39	90.24±1.35	84.61±1.22	9.75

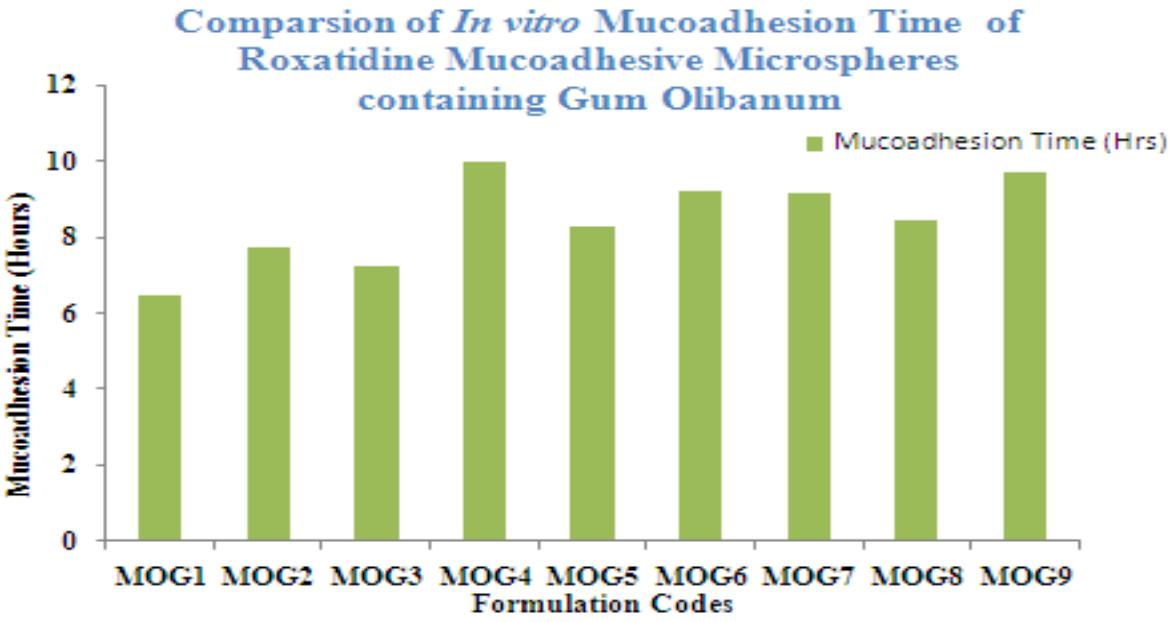


Fig. 3: Comparsion of *In vitro* Mucoadhesion Time of Roxatidine acetate HCl Mucoadhesive Microspheres containing Gum olibanum

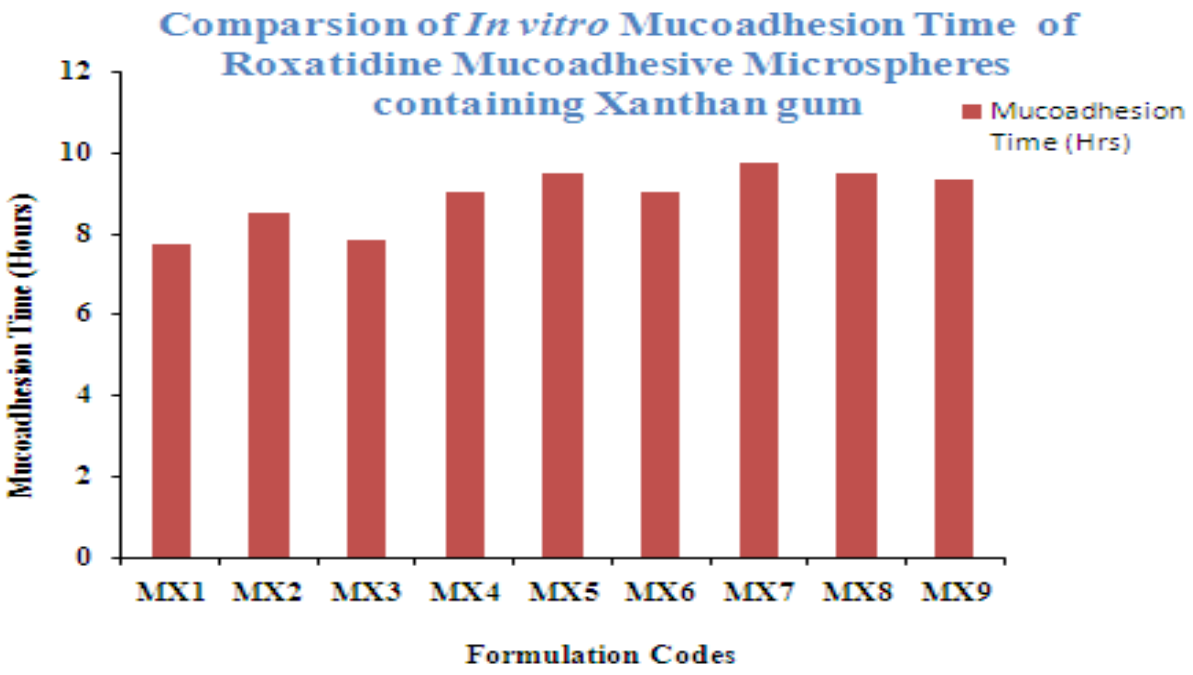


Fig. 4: Comparsion of *In vitro* Mucoadhesion Time of Roxatidine acetate HCl Mucoadhesive Microspheres containing Xanthan gum

In vitro Drug Release Studies

The optimized formulation **MOG4** was found to provide the best drug release when compared with other formulations. The percentage drug release of formulation **MOG4** was observed to be 99.4 ± 0.11 % in 12 h. The drug release of optimized formulation **MOG4** was in controlled manner when compared with innovator product rotane i.e. 96.15% within 2 h.

Mathematical Modelling of Optimized Formula of Mucoadhesive Microspheres

In the view of establishment of release mechanism and quantitatively interpreting and translate mathematically the dissolution date was being plotted. From the results of drug release kinetic studies, it was apparent that the regression coefficient value closer to unity in case of zero order plot i.e., 0.991 indicated that the drug release followed a zero order mechanism. This data indicated a lesser amount of linearity when plotted by the first order equation. Hence, it can be concluded that the major mechanism of drug release followed 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 modelling 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 stating that the release from the matrix was through diffusion. Further the n value obtained from the Korsmeyer plots i.e., 0.327 suggested that the drug release from the mucoadhesive microspheres of roxatidine was anomalous non-fickian diffusion.

Table 5 (a): *In vitro* Release Profiles of Roxatidine acetate HCl Mucoadhesive Microspheres containing Xanthan gum Formulations MX1 – MX5

Time (h)	MX1	MX2	MX3	MX4	MX5
0	0±0	0±0	0±0	0±0	0±0
2	18.21±0.32	16.51±0.11	16.51±0.22	15.26±0.23	15.19±0.11

3	39.32±0.15	33.62±0.21	35.32±0.11	33.67±0.15	29.02±0.16
4	50.21±0.11	50.02±0.31	51.73±0.65	48.07±0.11	45.31±0.13
6	64.46±0.16	67.63±0.22	66.72±0.43	60.96±0.16	55.43±0.12
8	81.08±0.32	83.47±0.32	75.23±0.16	79.28±0.21	71.98±0.21
10	88.39±0.16	90.36±0.17	85.31±0.32	93.27±0.33	88.53±0.11
12	91.27±0.99	93.44±0.77	91.82±0.22	90.74±0.17	93.22±0.16

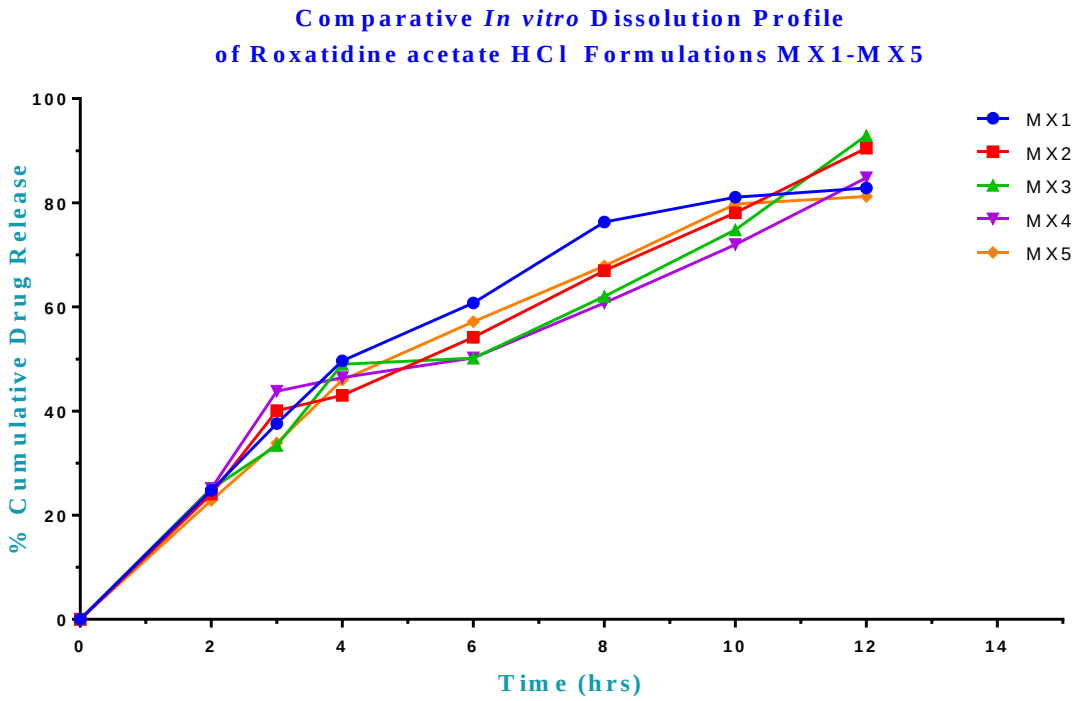


Fig. 5: *In vitro* Release Profiles of Roxatidine acetate HCl Mucoadhesive Microspheres containing Xanthan gum Formulations MX1 – MX5

Table 5 (b): *In vitro* Release Profiles of Roxatidine acetate HCl Mucoadhesive Microspheres containing Xanthan gum Formulations MX6 – MX9 and Innovator

Time (h)	MX6	MX7	MX8	MX9	Innovator (Rotane 150 mg)
0	0±0	0±0	0±0	0±0	0±0
2	22.86±0.14	24.03±0.22	14.09±0.16	14.09±0.22	96.15±0.12
3	32.85±0.18	34.20±0.11	26.33±0.43	26.33±0.24	---
4	44.96±0.16	46.81±0.21	35.75±0.88	35.75±0.15	----
6	56.18±0.33	57.83±0.13	55.06±0.76	55.06±0.17	----
8	66.79±0.12	70.22±0.33	73.53±0.54	73.53±0.54	----
10	78.52±0.22	89.73±0.41	80.42±0.34	80.42±0.55	----
12	82.17±0.11	94.54±0.11	91.14±0.21	87.14±0.76	----

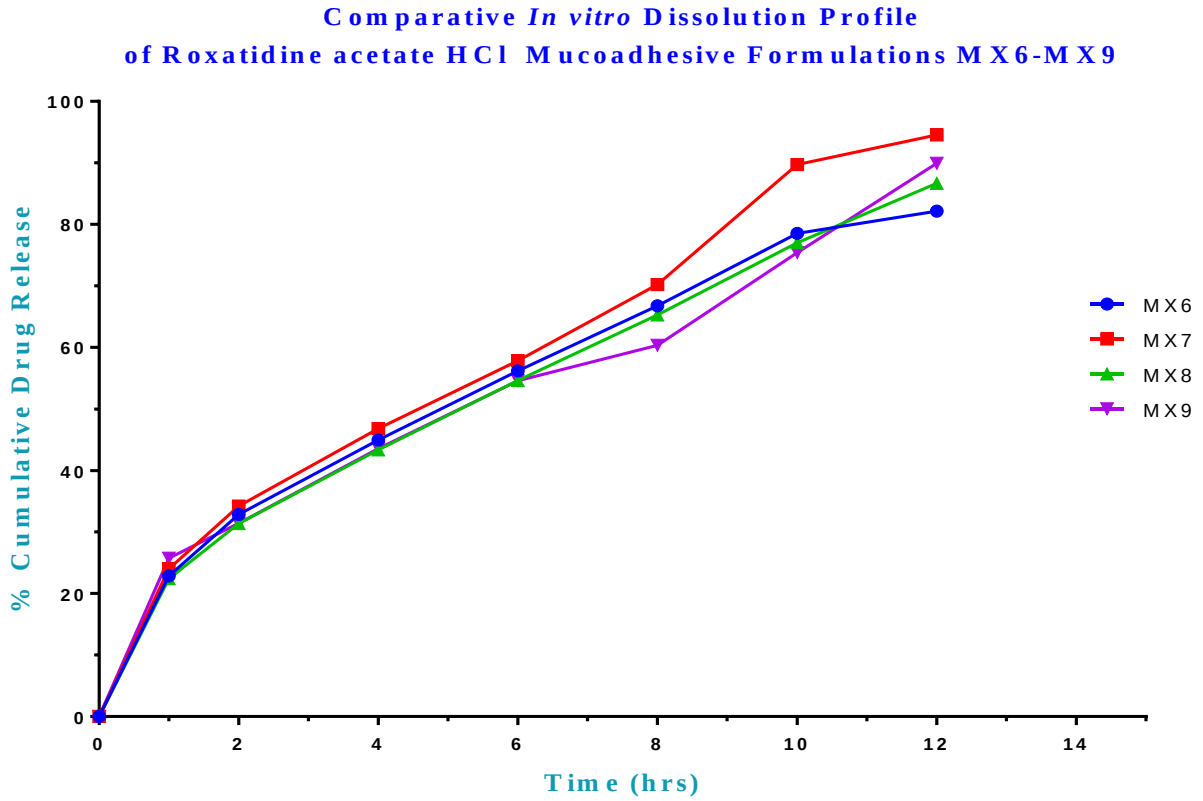


Fig. 6: *In vitro* Release Profiles of Roxatidine acetate HCl Mucoadhesive Microsphere containing xanthan gum formulations MX6 – MX9

Table 6 (a): *In vitro* Release Profiles of Roxatidine acetate HCl Mucoadhesive Microspheres containing Gum Olibanum Formulations MOG1 – MOG5

Time (h)	MOG1	MOG2	MOG3	MOG4	MOG5
0	0±0	0±0	0±0	**0±0**	0±0
2	28.96±0.22	10.21±0.66	18.78±0.11	**11.23±0.22**	8.96±0.11
3	36.05±0.23	17.7±0.32	24.03±0.23	**24.91±0.21**	16.05±0.15
4	47.65±0.16	28.52±0.55	32.05±0.11	**33.51±0.14**	26.56±0.16
6	58.45±0.11	40.71±0.32	46.85±0.32	**43.52±0.12**	38.45±0.17
8	62.36±0.13	56.54±0.22	51.38±0.23	**60.94±0.32**	52.36±0.26
10	82.04±0.32	70.66±0.34	66.14±0.32	**79.48±0.38**	72.04±0.12
12	93.55±0.52	88.43±0.45	77.01±0.11	**99.63±0.11**	88.55±0.32

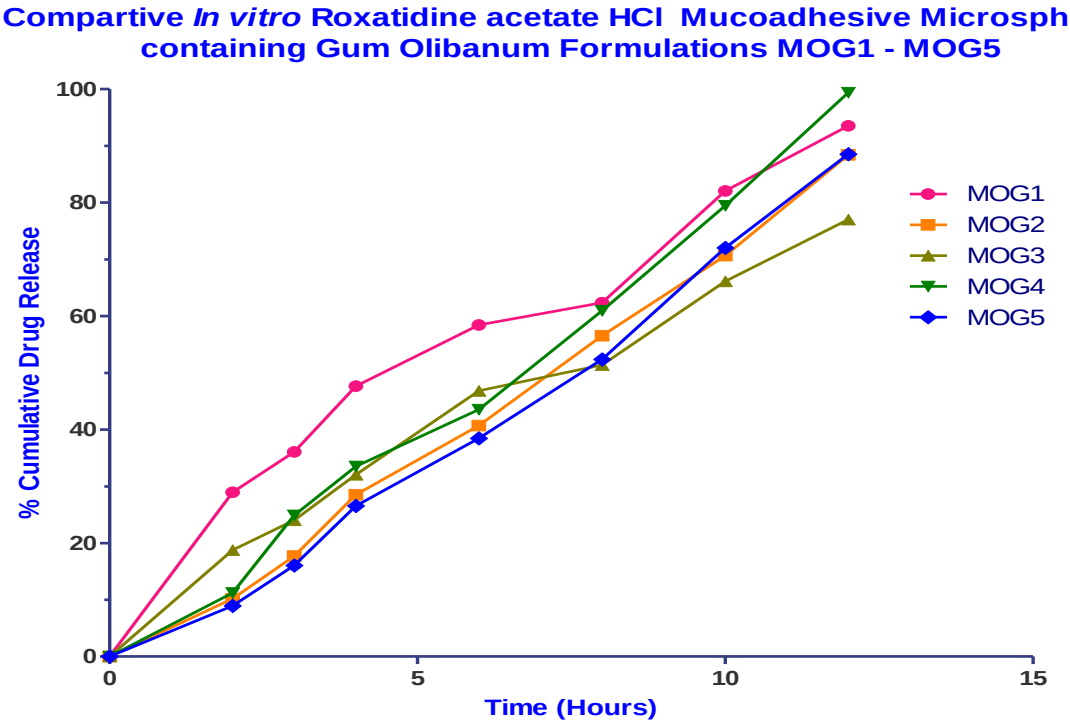


Fig. 7: *In vitro* Release Profiles of Roxatidine acetate HCl Mucoadhesive Microspheres containing Gum Olibanum Formulations MOG1 – MOG5

Table 6 (b): *In vitro* Release Profiles of Roxatidine acetate HCl Mucoadhesive Microspheres containing Gum Olibanum Formulations MOG6 – MOG9 & Innovator

Time (h)	MOG6	MOG7	MOG8	MOG9	Innovator (Rotane 150 mg)
0	0±0	0±0	0±0	0±0	0±0
2	10.83±0.56	10.21±0.22	19.86±0.41	21.15±0.16	96.15±0.12
3	19.22±0.66	17.7±0.13	25.52±0.11	31.94±0.44	---
4	27.83±0.98	30.71±0.13	33.06±0.22	42.82±0.24	----
6	36.54±0.43	40.78±0.13	48.33±0.16	55.82±0.66	----
8	49.86±0.32	56.54±0.13	53.64±0.52	63.53±0.44	----
10	61.37±0.11	70.66±0.32	67.05±0.22	77.72±0.23	----
12	83.45±0.32	90.43±0.52	76.23±0.16	78.48±0.22	----

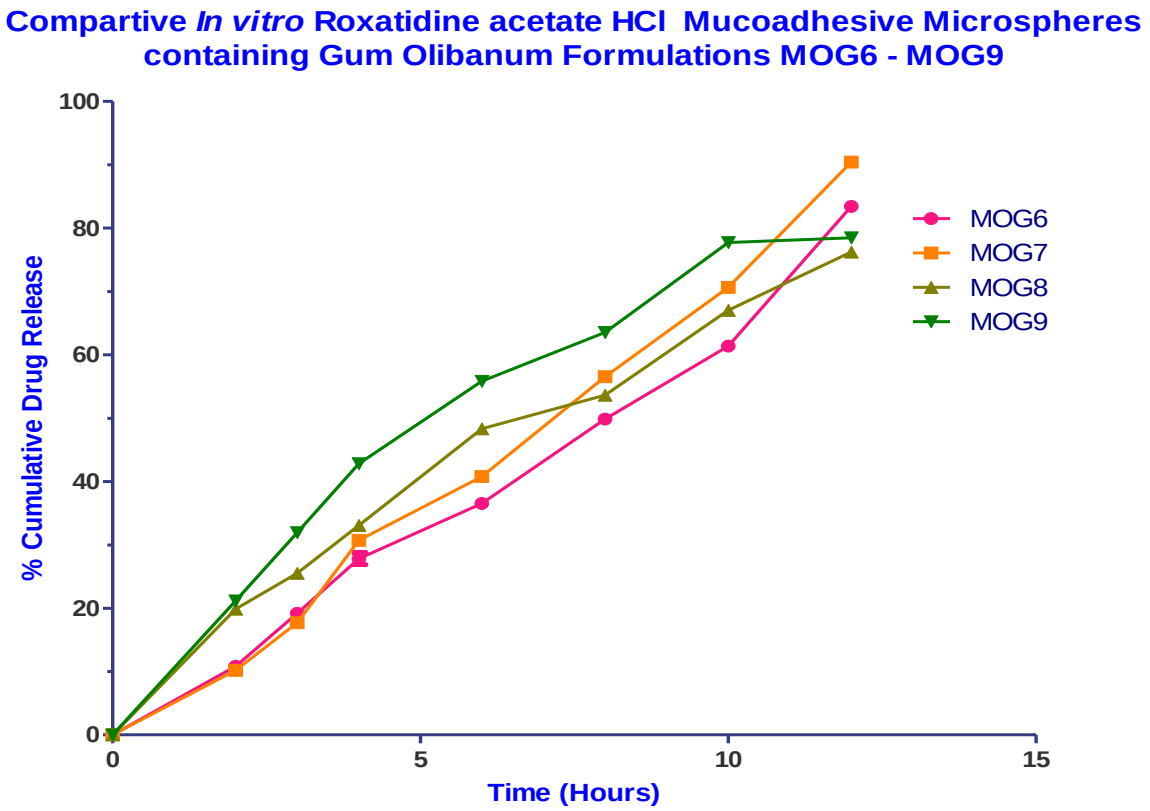


Fig. 8: *In vitro* Release Profiles of Roxatidine acetate HCl Mucoadhesive Microspheres containing Gum Olibanum Formulations MOG6 – MOG9

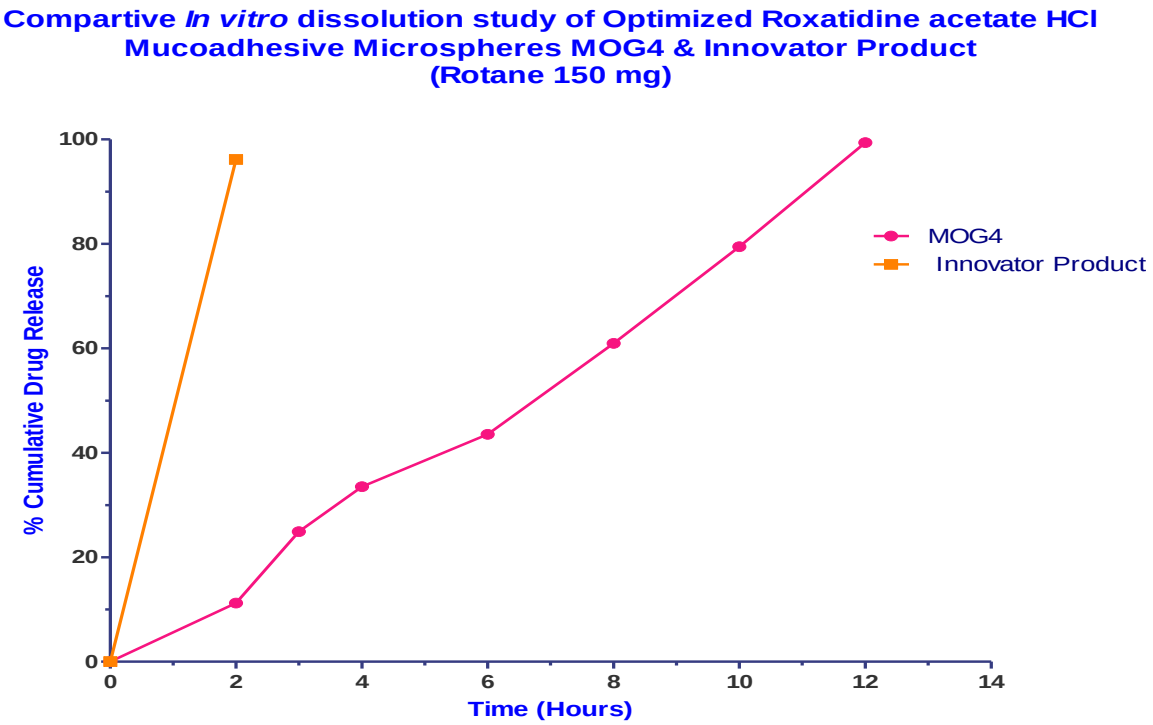


Fig. 9: Comparative *In vitro* Dissolution Profile of optimized Roxatidine acetate HCl Mucoadhesive Formulations MOG 4 & Innovator

Table 8: Release Kinetics of Optimized Formulation of Mucoadhesive Microspheres

S. No.	Formulation Code	Zero order (R^2)	First order (R^2)	Higuchi (R^2)	Korsmeyer-peppas (R^2)	Korsmeyer-peppas (n)
1.	**MOG4**	0.991	0.987	0.987	0.974	0.327

Drug Excipient Compatibility Studies

Fourier Transform Infrared Spectroscopy (FTIR)

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

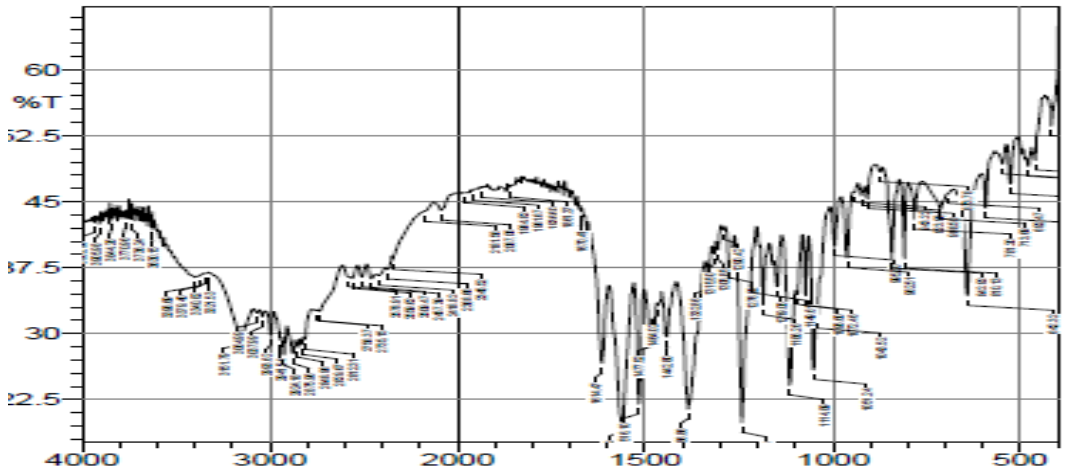


Fig. 10 (a): FT-IR Spectrum of Pure Drug Roxatidine acetate HCl

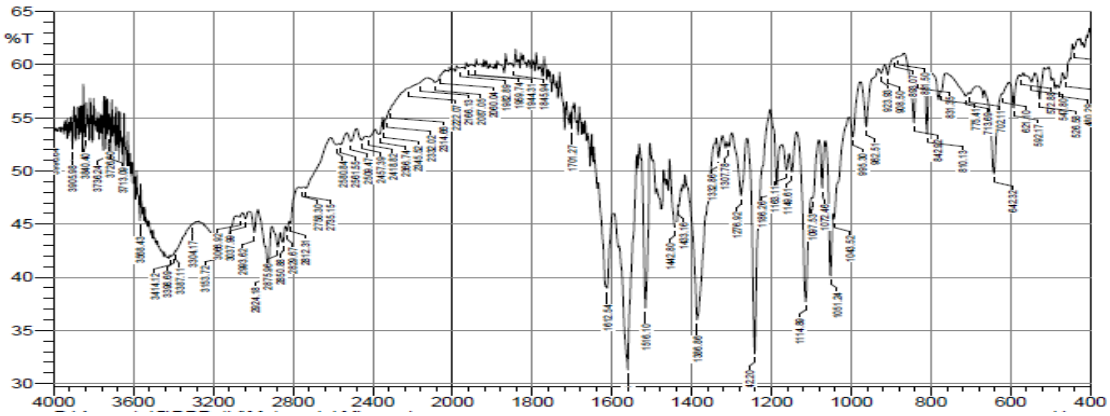


Fig. 10 (b): FT-IR Spectrum of Physical Mixture

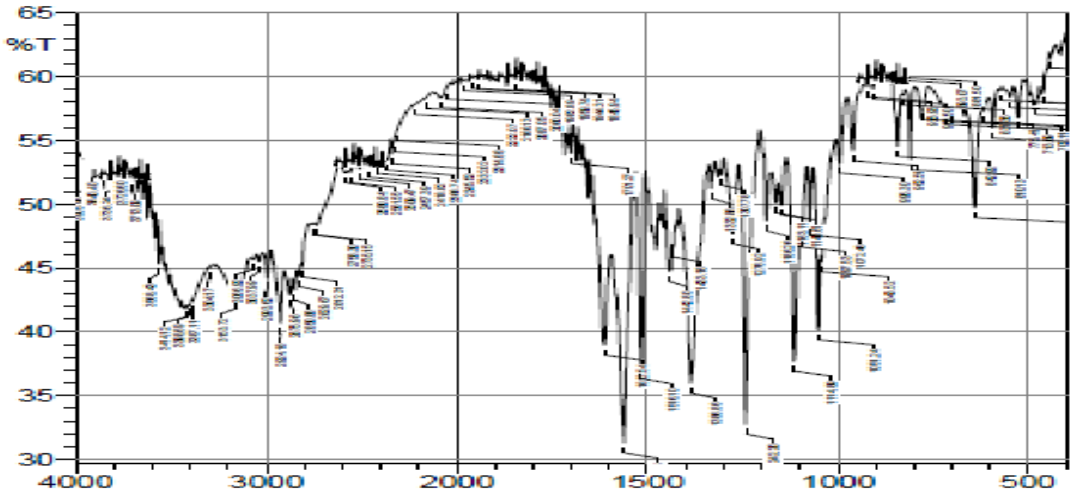


Fig. 10 (c): FT-IR Spectrum of Roxatidine Optimized Formulation MOG4

DSC Studies

DSC was used to detect interaction between roxatidine acetate HCl and excipients. The thermogram of pure roxatidine acetate HCl exhibited a sharp endotherm melting point at 147^0C. The thermogram of optimized microspheres loaded with roxatidine acetate HCl (MOG4) exhibited a sharp endotherm melting point at 151^0C. The DSC thermograms of sodium alginate, gum olibanum were also studied. The DSC thermogram of optimized microsphere formulation **(MOG4 or M13)** retained properties of pure roxatidine acetate HCl. There was no considerable change observed in melting endotherm of drug in optimized formulation. It indicated that there was no interaction between drug & excipients used in the formulation.

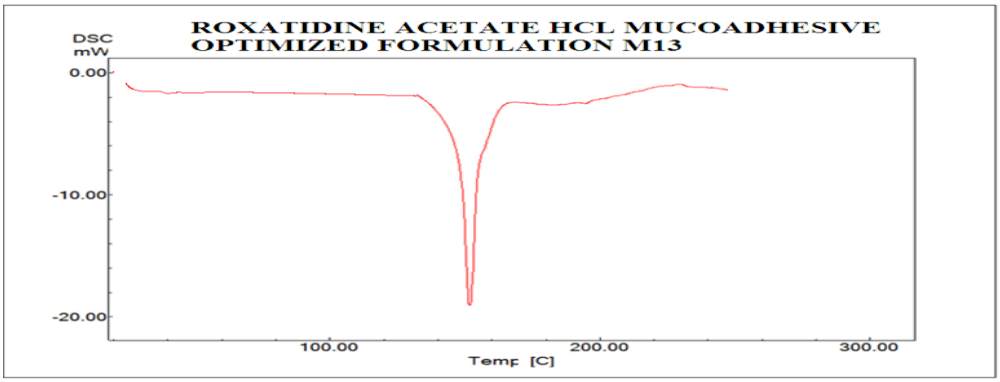


Fig. 11 (a): DSC Thermogram of Roxatidine acetate HCl Pure Drug

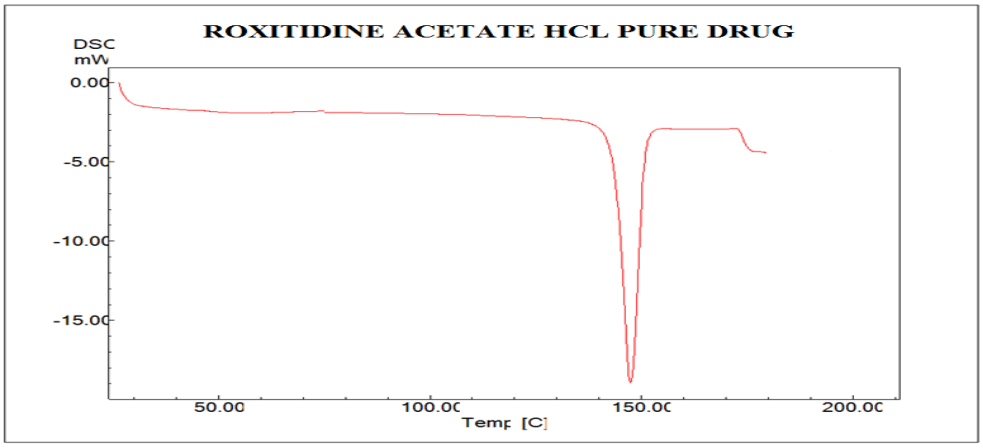


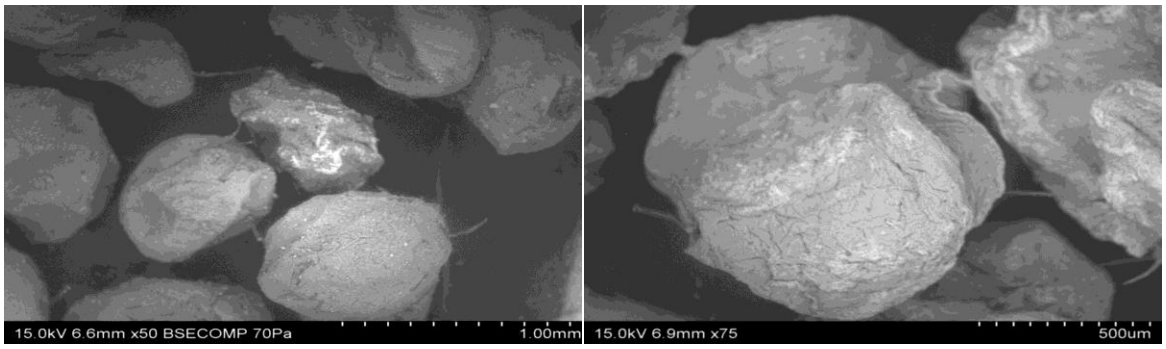
Fig. 11 (b): DSC Thermogram of Roxatidine acetate HCl Mucoadhesive Optimized Microspheres (MOG4 or M13)

Table 9: Melting Points of Drug, Polymers & Optimized Formulation

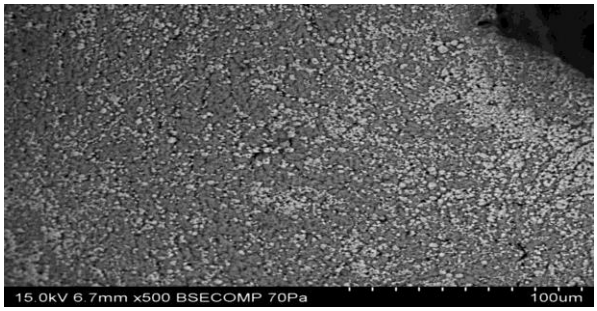
Name of the Ingredient	Melting Point ^{0}C
Roxatidine acetate HCl Pure Drug	147^{0}C
Sodium Alginate	490^{0}C
Roxatidine acetate HCl Optimized Formulation **(MOG4 or M13)**	151^{0}C

Scanning Electron Microscopy Studies of Roxatidine acetate HCl Mucoadhesive Microspheres

The external and internal morphology of controlled release microspheres were studied by Scanning Electron Microscopy. Morphology of the various formulations of roxatidine acetate HCl microspheres prepared was found to be discrete and spherical in shape. 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 were no crystals observed on surface which proved that the drug was uniformly distributed.



A **B**



C

Fig. 12: Scanning Electron Micrographs of Roxatidine acetate HCl Mucoadhesive Microspheres (MOG4 or M13)

Stability Studies

Optimized formulation **(MOG4 or M13)** was selected for stability studies on the basis of high cumulative % drug release. Stability studies were conducted for 6 months according to ICH guidelines. From the results shown in **Table 10**, it was concluded that, optimized formulation is stable and retained their original properties with minor differences.

Table 10: Stability Studies of Optimized Mucoadhesive Microspheres

Retest Time For Optimized Formulation (MOG4)	Percentage Yield (%)	Entrapment Efficiency (%)	*In-vitro* Drug Release Profile (%)
0 days	98.6	98.00	99.4±0.11
30 days	98.58	97.95	99.4±0.32
60 days	98.57	97.55	98.4±0.25
120 days	98.57	97.53	98.4±0.14
180 days	98.57	97.53	98.4±0.19

Response Surface Central Composite Design Graphs of Roxatidine acetate hydrochloride Mucoadhesive Microspheres

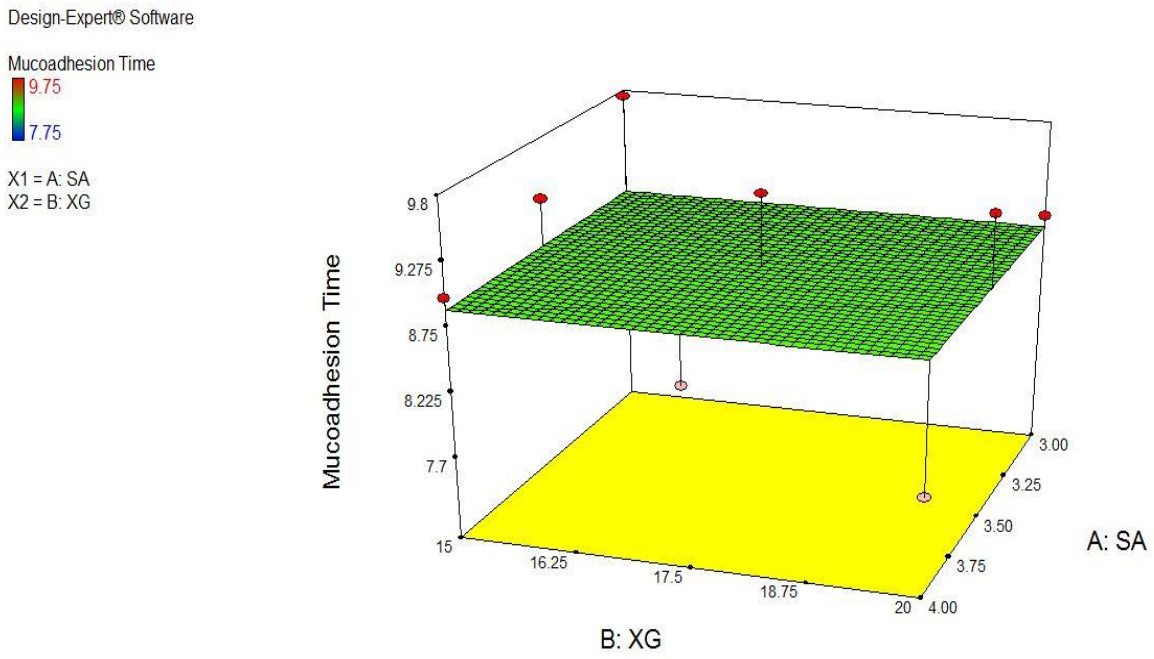


Fig. 12: Response Surface Central composite Design Graphs of Roxatidine acetate HCl Mucoadhesive Microspheres containing Xanthan gum

- Final equation in terms of coded factors

Mucoadhesion Time = 2.85+0.17*A-0.4*B+0.12*A*B+0.35*A ^{2} +0.45*B ^{2}

- Final equation in terms of actual factor

Mucoadhesion time = 49.925-11.21667*SA-3.03667*XG+0.1*SA*XG+1.400*SA ^{2} +0.072*G^2

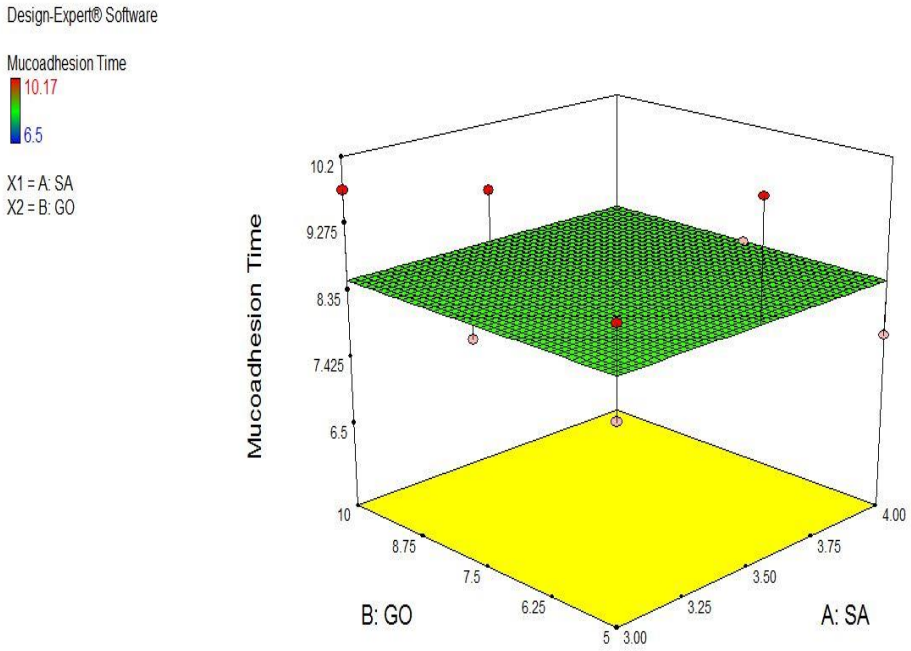


Fig. 13: Response Surface Central composite Design Graphs of Roxatidine acetate HCl Mucoadhesive Microsphere containing Gum Olibanum .

The model F-Value of 7.49 implies the model is significant

- Final equation in terms of coded factors:

mucoadhesion time = 5.33+0.042*A-0.083*B-0.69*A*B

- Final equation in terms of actual factor:

Mucoadhesion time = -9.145+10.20*SA+1.891*GO-0.55*SA*GO

CONCLUSION

Resposne Surface Composite design was employed to study the effect of independent variables, polymer concentration (X1) and sodium alginate concentration (X2) on dependent variables mucoadhesion time. The microspheres of the formulation containing gum olibanum i.e., **MOG4** exhibited a high percentage mucoadhesion of 98 % after 10 h, 98.00% drug entrapment efficiency and swelling index of 97.07%. The optimized formulations **MOG4** showed 99.63% cumulative drug release. The Response Surface Central composite Design Graphs indicated that there was influence of mucoadhesive polymers on mucoadhesion time. It also indicated that the mucoadhesive microspheres of roxatidine could sustain the release of the drug for 12 h.

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