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Formulation and evaluation of mucoadhesive microspheres of cimetidine

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ABSTRACT

The intention of the present study is to formulate mucoadhesive microspheres containing cimetidine by employing xanthan gum & gum kondagogu 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 97.12% and a swelling index of 96.98%; percentage mucoadhesion after 10 h was 98%. The drug release was also sustained for 12 h. 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* drug release and entrapment efficiency was selected as optimized formulation i.e., formulation MGK5. 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 formulations MGK5 was subjected to stability studies for six months at $40^0 \pm 2^0$C & 75±5%RH as per ICH guidelines and result showed that there were no changes in physical parameters, formulation parameters and *in vitro* release studies.

Keywords: Mucoadhesive Microspheres, Cimetidine, Factorial Design, *In vitro* study.

INTRODUCTION

Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel

drug delivery systems [1-3]. They have varied applications and are prepared using assorted polymers. However, the success of these microspheres is limited owing to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes [4]. This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres. Bioadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site. [5]

Cimetidine is a histamine H2 receptor antagonist, which is widely prescribed in gastric ulcers, duodenal ulcers and gastroesophageal reflux disease. It is poorly absorbed from the lower gastrointestinal tract and has a short elimination half-life (~ 2 h) [6]. The purpose of the work was to prepare cimetidine (CM) microspheres in order to achieve an extended retention in the upper GIT, which may result in enhanced absorption and thereby improved bioavailability.

MATERIALS AND METHODS

Materials

Cimetidine 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 Kondagogu were obtained from MSN Labs Ltd., Hyderabad. All other chemicals were of Pharmaceutical grade.

Method

Cimetidine mucoadhesive microspheres were prepared using polymers sodium alginate, chitosan, sodium CMC by ionotropic gelation method. A 3^2 full factorial design was employed to study the effect of independent variables, polymer-to-drug ratio (X1) and stirring speed (X2) on dependent variables percentage mucoadhesion, drug entrapment efficiency and swelling index. Different formulations were prepared by using different concentrations of polymers and mucoadhesive agent showed in **Table 1 & 2.** Cimetidine mucoadhesive microspheres were prepared using polymers sodium alginate & xanthan gum and gum kondagogu were used in different concentrations by ionotropic gelation method. In this method, weighed quantity of cimetidine was added to 100 ml sodium alginate, xanthan gum and gum kondagogu solution were 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 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 [7].

Table 1 (a): Optimization of Cimetidine 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 Cimetidine Mucoadhesive Microspheres containing Xanthan gum

Formulation Code	Cimetidine (g)	Sodium Alginate (%)	Calcium Chloride (%)	Xanthan Gum (%)
MX1	2	3.5	10	17.5
MX2	2	4.0	10	20.0
MX3	2	3.5	10	20.0
MX4	2	3.0	10	17.5
MX5	2	3.0	10	15.0
MX6	2	4.0	10	15.0

MX7	2	3.0	10	20.0
MX8	2	3.5	10	15.0
MX9	2	4.0	10	17.5

Table 2 (a): Optimization of Cimetidine 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 Kondagogu (%)	10.00	15.00	10.00	15.00	12.50	2.04

Table 2 (b): Composition of Cimetidine Mucoadhesive Microspheres containing Gum Kondagogu

Formulation Code	Cimetidine (g)	Sodium Alginate (%)	Calcium Chloride (%)	Gum Kondagogu (%)
MGK1	2	3.5	10	12.5
MGK2	2	3.5	10	15.0
MGK3	2	4.0	10	15.0
MGK4	2	4.0	10	10.0
MGK5	2	3.0	10	15.0
MGK6	2	4.0	10	12.5
MGK7	2	3.0	10	10.0
MGK8	2	3.5	10	10.0
MGK9	2	3.0	10	12.5

EVALUATION STUDIES

Cimetidine mucoadhesive microspheres were evaluated for determination of particle size, bulk density, tapped density, angle of repose, compressibility index, swelling index, drug entrapment efficiency and percentage yield [8-10].

Mucoadhesion 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 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 h, immediately repositioned as before in a similar tube containing 40 ml of fresh saline and unbound microspheres were counted [11]. The adhering percent was presented by the following equation.

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

In vitro Drug Release Studies

Release rate of drug from mucoadhesive microspheres was carried out using USP dissolution apparatus [12]. Accurately weighed amount of microspheres from each batch were subjected to dissolution studies in triplicate manner. At appropriate intervals up to 12 h, specific volume of aliquots were withdrawn and analyzed spectrophotometrically at 280 nm. 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.

Conditions for mucoadhesive microspheres:

- Performed using USP dissolution apparatus II.
- Dissolution medium – 0.1N HCl
- Temperature – 37 ± 0.5^{0}C
- Stirring speed – 100 rpm
- Bath volume – 900 ml
- Time intervals – 0, 2, 3, 4, 6, 8, 10 & 12 h.

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 [13].

Drug Excipient Compatability Studies:

The drug excipient compatibility studies were carried out by Fourier Transmission Infrared Spectroscopy (FTIR) method and Differential Scanning Colorimetry (DSC) method [14].

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 (DSC) 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 range under nitrogen atmosphere. Empty aluminum pan was used as a reference [15].

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

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 [16].

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, b0 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 [6].

RESULTS AND DISSCUSSION

Micromeritic Properties

Particle size of all the formulations containing xanthan gum MX1 to MX9 varied from 65.29 ± 0.13 µm to 87.12±0.13 and formulation containing gum kondagogu varied from MGK1 to MGK9 66.89±0.10 µm to 91.45±0.12 µm. The formulation MGK5 showed the particle size 70.04±0.11µm.

The bulk density of all the formulations containing xanthan gum MX1 to MX9 was measured and it was ranged from 0.63 g/cm³ to 0.75 g/cm³ and formulation containing gum kondagogu varied from MGK1 to MGK9 also measured and it was ranged from 0.63 g/cm³ to 0.89 g/cm³. The tapped density of all the formulations were measured and ranged between 0.62 g/cm³ - 0.91 g/cm³.

Angle of repose of all the formulations was found satisfactory. The θ value of the formulation MGK5 was found to be 25˚.34 having good flow property.

The compressibility index values were found to be in the range of 9.34 to 14.34%. These findings indicated that the all batches of formulation exhibited good flow properties and depicted as shown in **Table 3 (a) and (b).**

Table 3 (a): Micromeritic Properties of Cimetidine 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 Cimetidine Mucoadhesive Microsphere containing Gum Kondagogu

Formulation Code	Particle Size (µm)	Bulk Density ($g/cm^{3)}$	Tapped Density ($g/cm^{3)}$	Angle of Repose (^{o})	Carr's Index
MGK1	66.89±0.10	0.72±0.02	0.68±0.01	30.24±0.2	12.12±0.12
MGK2	85.94±0.11	0.74±0.02	0.72±0.02	27.93±0.2	12.23±0.13
MGK3	88.94±0.11	0.79±0.03	0.75±0.01	25.34±0.13	09.34±0.14
MGK4	89.04±0.21	0.81±0.03	0.76±0.03	26.54±0.15	12.34±0.14
MGK5	**70.04±0.11**	**0.63**±0.11	**0.72**±0.04	**25.34**±0.1	**09.34**±0.04
MGK6	77.98±0.10	0.68±0.12	0.78±0.01	23.61±0.23	12.27±0.17
MGK7	89.54±0.21	0.73±0.11	0.89±0.01	27.61±0.14	10.92±0.12
MGK8	91.45±0.12	0.83±0.03	0.83±0.09	27.91±0.11	11.45±0.15
MGK9	81.45±0.21	0.89±0.01	0.77±0.08	32.61±0.12	13.83±0.13

Percentage Yield, Entrapment Efficiency & Swelling Index

The mucoadhesive microspheres of formulation showed the percentage yield values ranging from 75.45% to 99.30%. The entrapment efficiency values of all the 18 formulations ranged from 76.00% to 97.12%. All the formulations showed the swelling of microspheres. The swelling of the formulation MGK5 was found to be 96.98%. The formulation MGK5 showed better % yield, entrapment efficiency and swelling index of 99.30%, 97.12% and 96.98% respectively, when compared with other formulations and the results are showed in **Table 4 (a) and (b) .**

Mucoadhesion Study

The *in vitro* mucoadhesive test was carried out using chicken small intestine. All the 18 formulations of mucoadhesive microspheres were exposed to mucoadhesion test and setup for mucoahesion study was showed in **Figure 2 - 3** and results were depicted in **Table 4 (a) & 4 (b).**

The formulation MGK 5 was found to have the high percentage of mucoadhesive property and showed 98.00% of adhesion nature in 10 h.

Fig. 1(A) **Fig. 1 (B)**

Fig. 1: Pictorial Diagram Showing Mucoadhesive Property of Mucoadhesive Microspheres in Chic Intestine at 0 min (A) & after 8 h (B)

In vitro Drug Release Studies

The optimized formulation MGK 5 was found to provide the best drug release when compared with other formulations. The % drug release of formulation MGK5 was found to be 99.41 ± 0.16% in 12 h. The drug release of optimized formulation MGK5 was in controlled manner when compared with innovator product cimetine i.e. 96.15% within 2 h and results were showed in **Table 5 & 6** & **Figures 4 – 8.**

Table 4 (a): Evaluation Report of Cimetidine 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 Cimetidine Mucoadhesive Microspheres containing Gum Kondagogu

Formulation Code	Percentage Yield (%)	Entrapment Efficiency (%)	Swelling Index (%)	Mucoadhesion Time (h)
MGK1	84.00±	85.00±1.15	75.22±1.22	8.83
MGK2	89.00±	88.25±1.18	84.34±1.11	8.5
MGK3	98.90±	97.07±1.17	96.08±1.13	7.5
MGK4	90.72 ±	89.67±1.76	90.03±1.13	9
MGK5	**99.30**±0.16	**97.12**±1.11	**96.98**±1.54	**10**
MGK6	93.02±	96.95±1.13	96.58±1.22	9.75
MGK7	92.00±	91.03±1.03	94.08±1.32	9
MGK8	98.90±	97.74±1.04	96.79±1.65	7.83
MGK9	83.79±	95.4±1.54	98.54±1.45	8.83

Mathematical Modelling of Optimized Formula of Mucoadhesive Microspheres

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 zero order and Higuchi model, indicating diffusion controlled principle i.e., showed in **Table 7**. Further, the n value obtained from the Korsmeyer plots i.e., 1.075 suggested that the drug release from microspheres was anomalous non-fickian diffusion.

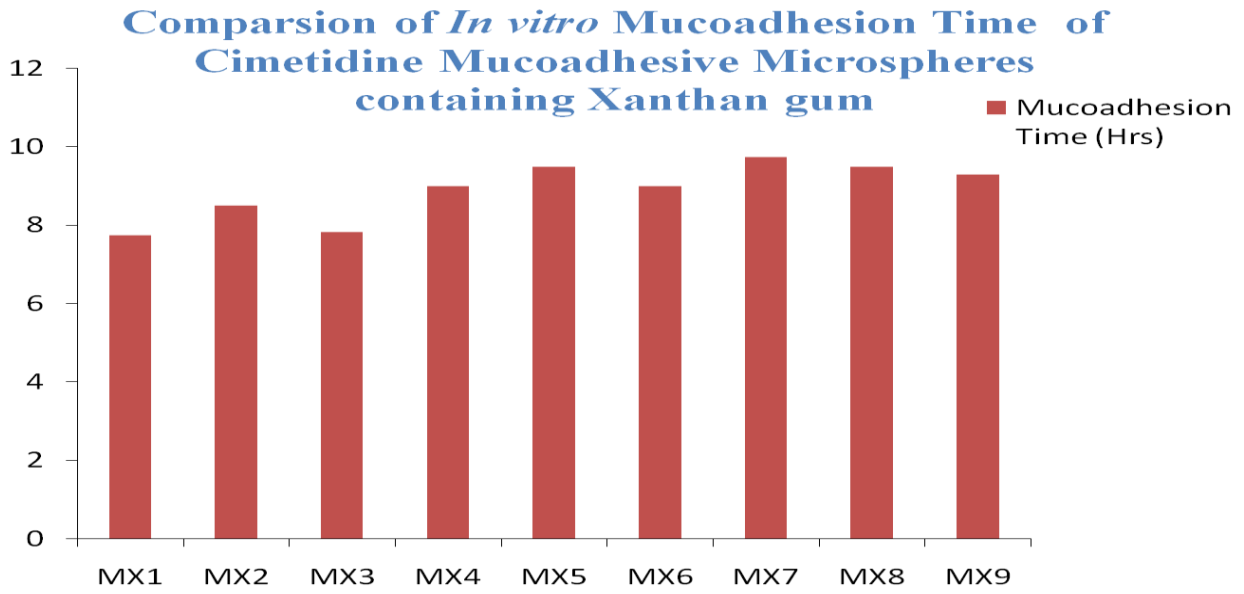

Fig. 2: Comparsion of *In vitro* Mucoadhesion Time of Cimetidine Mucoadhesive Microspheres containing Xanthan gum (MX1 to MX9)

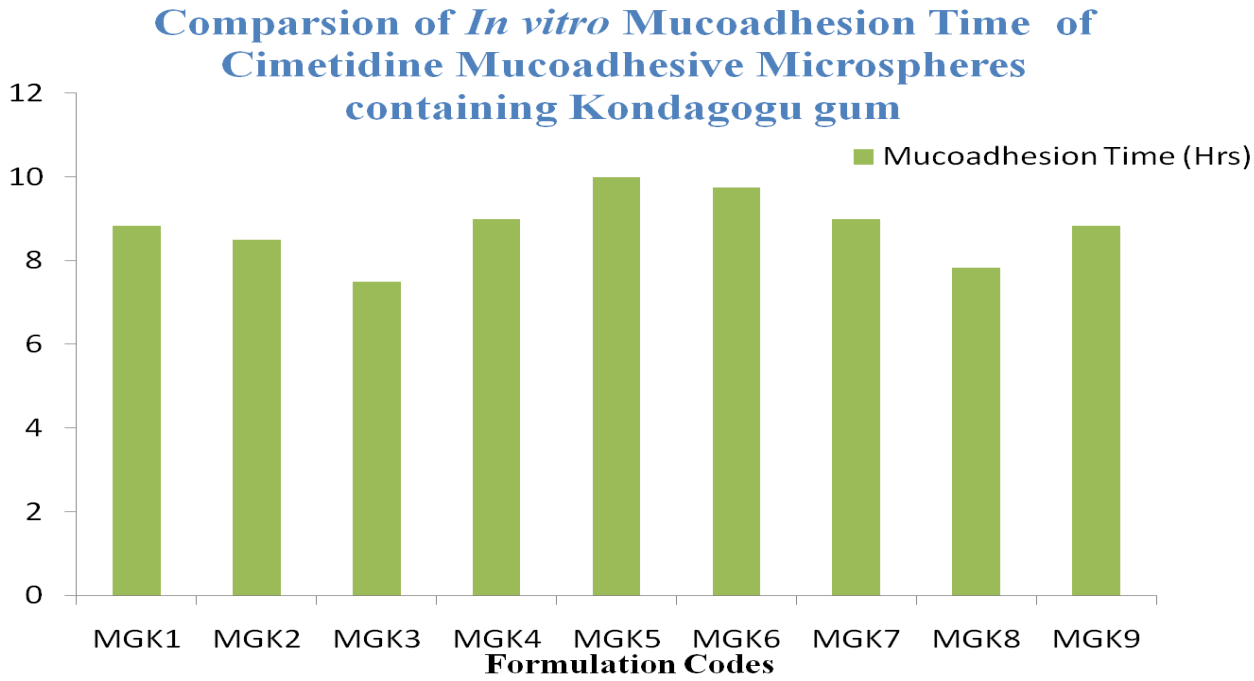

Fig. 3: Comparsion of *In vitro* Mucoadhesion Time of Cimetidine Mucoadhesive Microspheres containing Kondagogu gum (MGK1 to MGK9)

Table 5 (a): *In vitro* Release Study of Cimetidine 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	24.78±0.22	24.05±0.52	25.07±0.45	25.16±0.22	22.86±0.98
3	37.61±0.23	40.62±0.16	33.42±0.16	43.80±0.21	33.85±0.78
4	49.67±0.32	43.00±0.13	48.98±0.22	46.43±0.11	45.96±0.76
6	60.77±0.16	54.16±0.22	50.17±0.52	50.20±0.13	57.18±0.66
8	76.31±0.32	66.95±0.23	62.00±0.34	60.78±0.21	67.86±0.44
10	81.08±0.12	78.09±0.32	74.82±0.22	71.96±0.22	79.77±0.12
12	82.83±0.23	90.50±0.16	92.94±0.23	84.80±0.16	81.21±0.32

Table 5 (b): *In vitro* Cimetidine Mucoadhesive Microspheres containing Xanthan gum Formulations MX6 – MX9 and Innovator

Time (h)	MX6	MX7	MX8	MX9	Innovator (Cimetine 200 mg)
0	0±0	0±0	0±0	0±0	0±0
2	22.86±0.14	24.03±0.22	22.42±0.21	25.76±0.22	96.15±0.12
3	32.85±0.18	34.20±0.11	31.39±0.22	31.43±0.52	---
4	44.96±0.16	46.81±0.21	43.35±0.16	43.54±0.34	----
6	56.18±0.33	57.83±0.13	54.65±0.23	54.57±0.66	----
8	66.79±0.12	70.22±0.33	65.29±0.32	60.36±0.44	----
10	78.52±0.22	89.73±0.41	77.02±0.16	75.42±0.12	----
12	82.17±0.11	94.54±0.11	86.70±0.13	89.94±0.32	----

Table 6 (a): *In vitro* Release Study of Cimetidine Mucoadhesive Microspheres containing Gum Kondagogu Formulations MGK1 – MGK5

Time (h)	MGK 1	MGK 2	MGK 3	MGK 4	MGK 5
0	0±0	0±0	0±0	0±0	**0±0**
2	25.15±0.22	27.59±0.52	28.67±0.11	27.59±0.16	**29.00±0.41**
3	31.28±0.23	32.83±0.13	34.26±0.23	32.09±0.22	**44.96±0.11**
4	46.15±0.16	48.79±0.32	40.60±0.11	48.80±0.32	**52.26±0.22**
6	51.98±0.11	53.55±0.22	45.55±0.32	53.73±0.11	**67.46±0.16**
8	67.11±0.13	59.30±0.13	60.35±0.23	68.96±0.13	**72.56±0.52**
10	71.56±0.32	64.16±0.19	86.26±0.32	73.62±0.13	**87.14±0.22**
12	86.42±0.52	94.39±0.16	93.81±0.11	80.36±0.11	**99.41±0.16**

Table 6 (b): *In vitro* Release Study of Cimetidine Mucoadhesive Microspheres containing Gum Kondagogu Formulations MGK6 – MGK9 and Innovator

Time (h)	MGK 6	MGK 7	MGK 8	MGK 9	Innovator (Cimetine 200 mg)
0	0±0	0±0	0±0	0±0	0±0
2	19.01±0.16	28.24±0.22	19.02±0.52	29.42±0.16	96.15±0.12
3	24.38±0.13	34.59±0.13	24.67±0.32	34.68±0.44	---
4	31.13±0.13	41.72±0.13	31.82±0.52	41.83±0.24	----
6	46.87±0.16	56.68±0.13	47.48±0.62	57.57±0.66	----
8	52.05±0.13	61.81±0.13	52.82±0.11	62.36±0.44	----
10	78.26±0.23	76.32±0.32	67.83±0.52	78.98±0.23	----
12	87.00±0.11	83.56±0.52	85.13±0.52	89.41±0.22	----

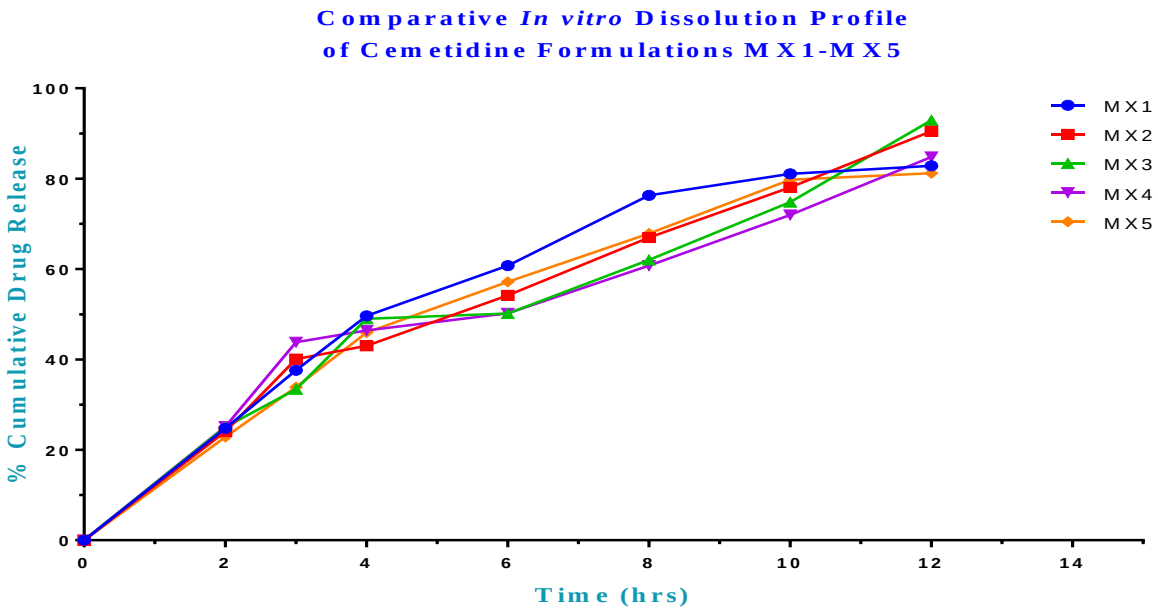

Fig. 4: *In vitro* Release Profiles of Cimetidine Mucoadhesive Microsphere containing xanthan gum formulations MX1 – MX5

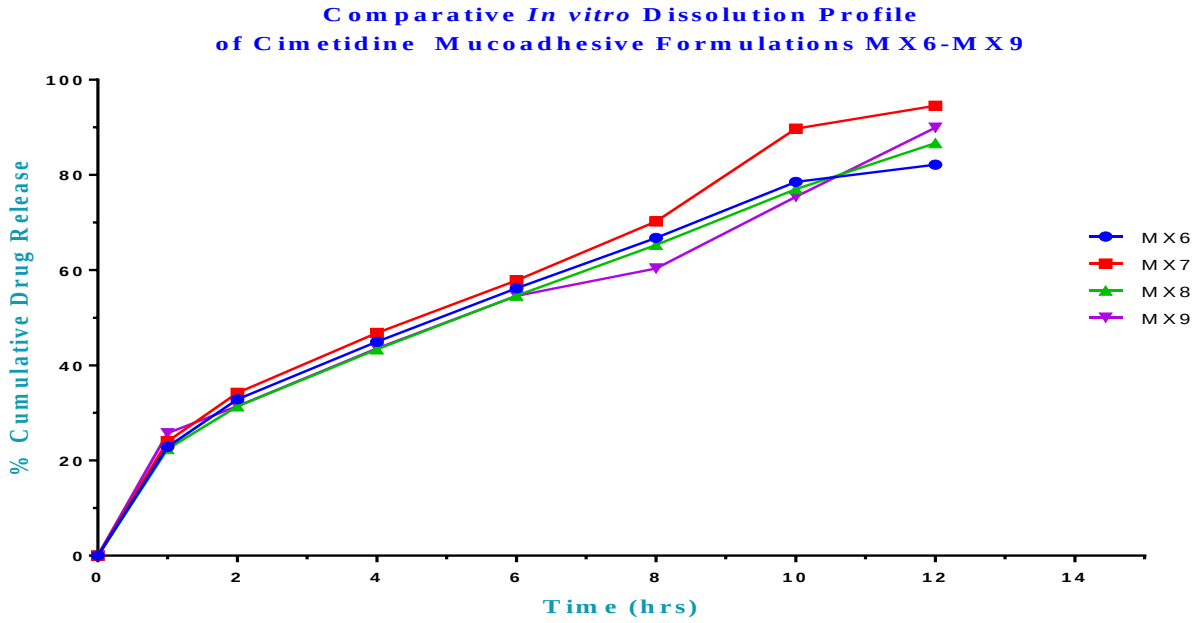

Fig. 5: *In vitro* Release Profiles of Cimetidine Mucoadhesive Microspheres containing Xanthan gum Formulations MX6 – MX9

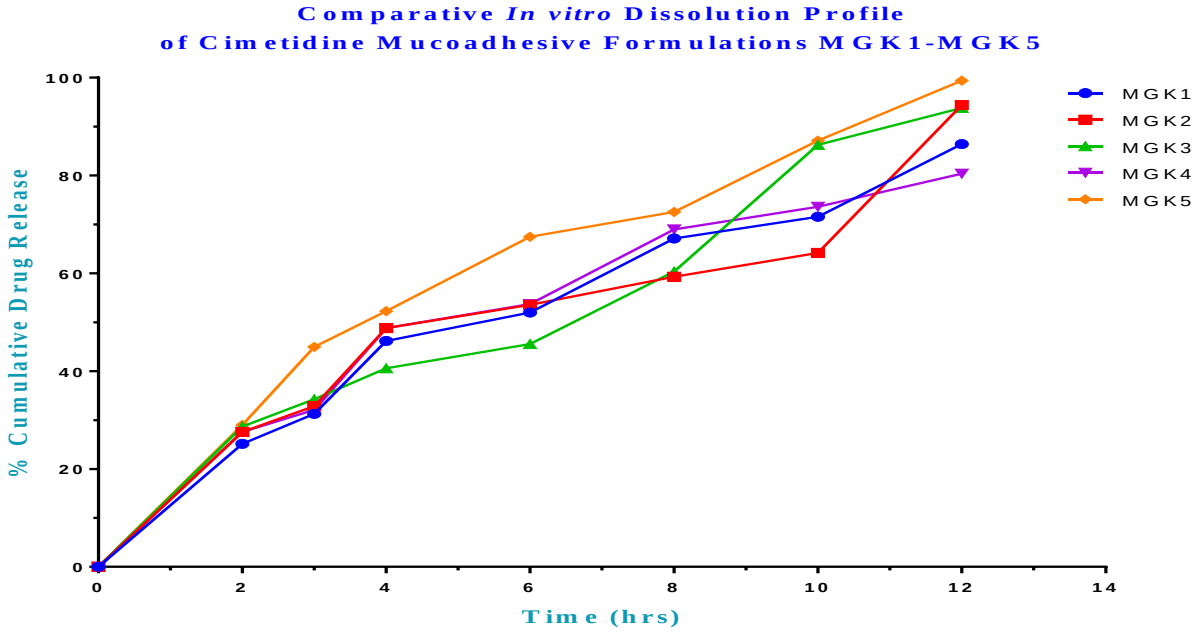

Fig. 6: *In vitro* Release Profiles of Cimetidine Mucoadhesive Microsphere containing Gum Kondagogu formulations MGK1 – MGK5

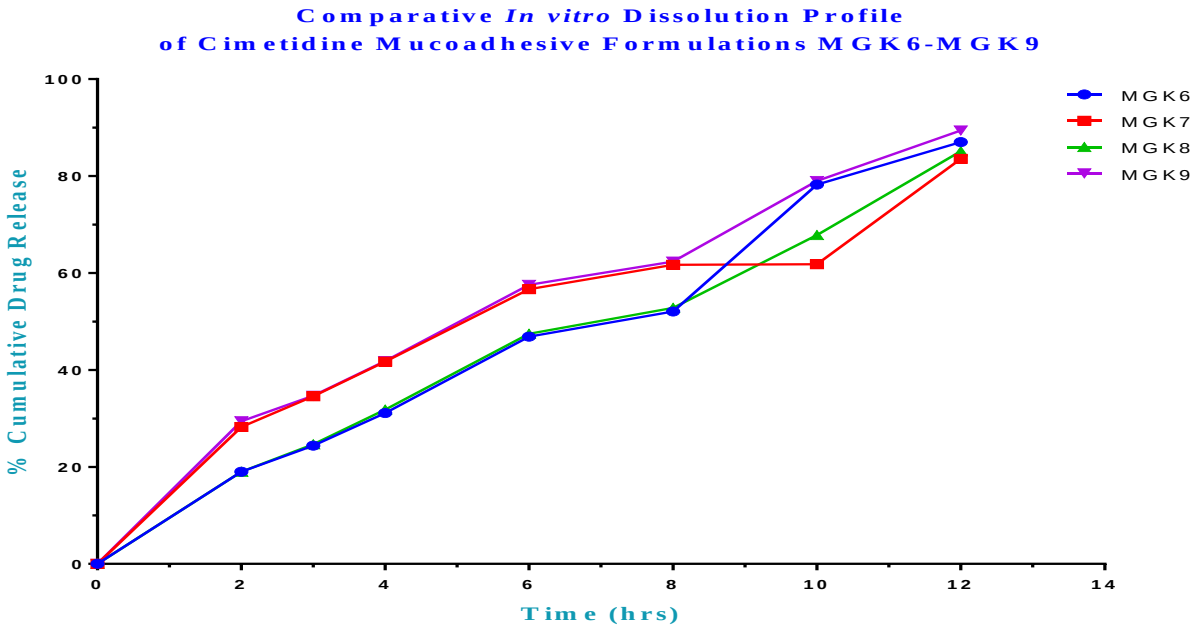

Fig. 7: *In vitro* Release Profiles of Cimetidine Mucoadhesive Microspheres containing Gum Kondagogu Formulations MGK6 – MGK9

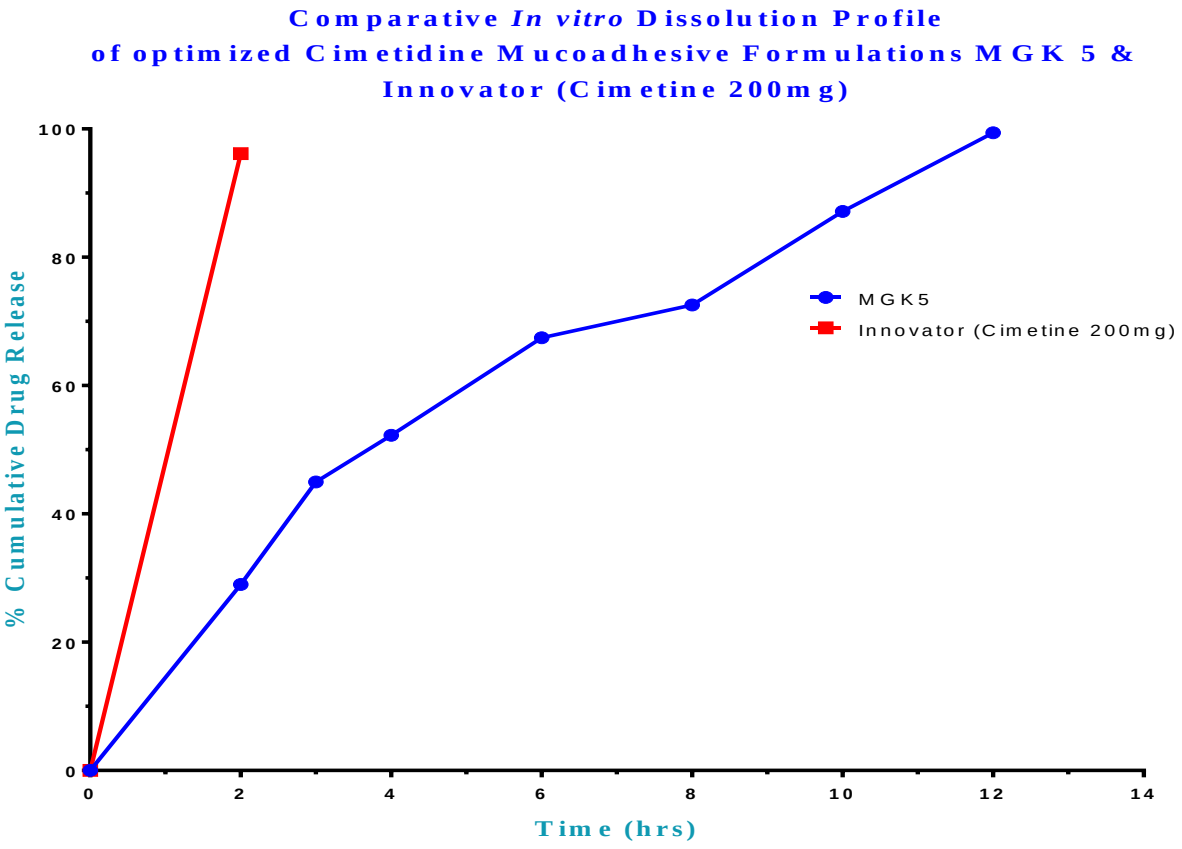

Fig. 8: Comparative *In vitro* Dissolution Profile of optimized Cimetidine Mucoadhesive Formulations MGK 5 & Innovator (Cimetine 200 mg)

Table 7: 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.	**MGK5**	0.999	0.979	0.948	0.712	1.075

Characterization of Cimetidine Mucoadhesive Microspheres

Fourier Transform Infrared Spectroscopy (FTIR)

Drug polymer interaction was checked by comparing the IR spectra of the physical mixture of drug with the excipients used with the IR spectrum of pure drug **Figure 9 (a)** and optimized formulation (MGK5) **Figure 9 (b),** and results found that there were no possible interaction between drug and polymer. The FTIR spectrum of cimetidine showed peaks corresponding to (C-H) bending at 1346.36 cm^{-1} and aromatic group (C=C) at 1501.63 cm^{-1}, alkane group (C-C) at 1202.66 cm^{-1}, Amine group (C-N) at 1281.74 cm^{-1}, Imines (C=N) at 1630.90 cm^{-1}, and (N-H) stretching at 3141.18 cm^{-1}. The peaks of the pure drug were found to be 3505.69 cm^{-1} = N-H stretching (amides), 3237.06 cm^{-1} = symmetric vibration, 3103.86 cm^{-1} = C-H stretching vibration. From the FTIR graphs of drug polymer mixture **Figure 9 (b)**, it was found that the same peaks of the drug are available. Therefore, it was evident that there was no incompatibility with the polymers.

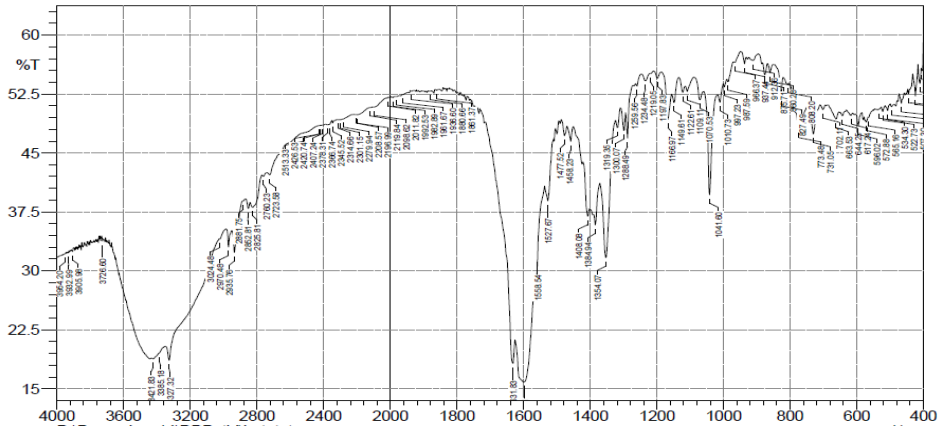

Fig. 9 (a): **FT-IR Spectrum of Pure Drug Cimetidine**

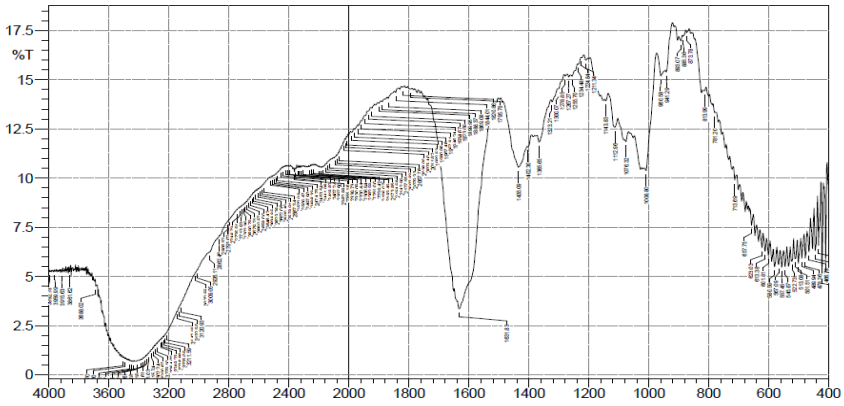

Fig. 9 (b): **FTIR Spectrum of Cimetidine Optimized Formulation MGK 5**

Differential Scanning Calorimetry (DSC)

DSC was used to detect interaction between cimetidine and excipients. The thermogram of cimetidine **Figure 10 (a)**, exhibited a sharp endotherm melting point at 141°C. The thermogram of microsphere loaded with cimetidine exhibited a sharp endotherm melting point at 142°C. There was no considerable change observed in melting endotherm of drug in optimized formulation (MGK5) **Figure 10 (b)**. It indicated that there was no interaction between drug & excipients used in the formulation and results were revealed in **Table 9**.

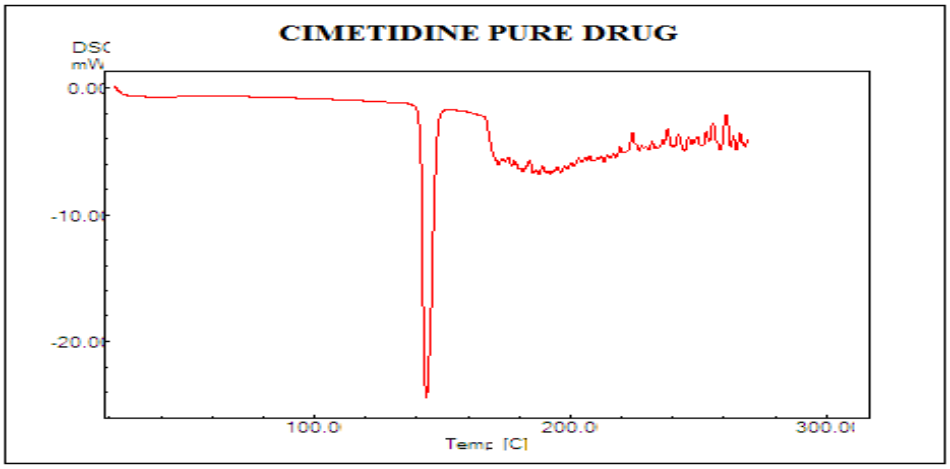

Fig. 10 (a): DSC Thermogram of Cimetidine Pure Drug

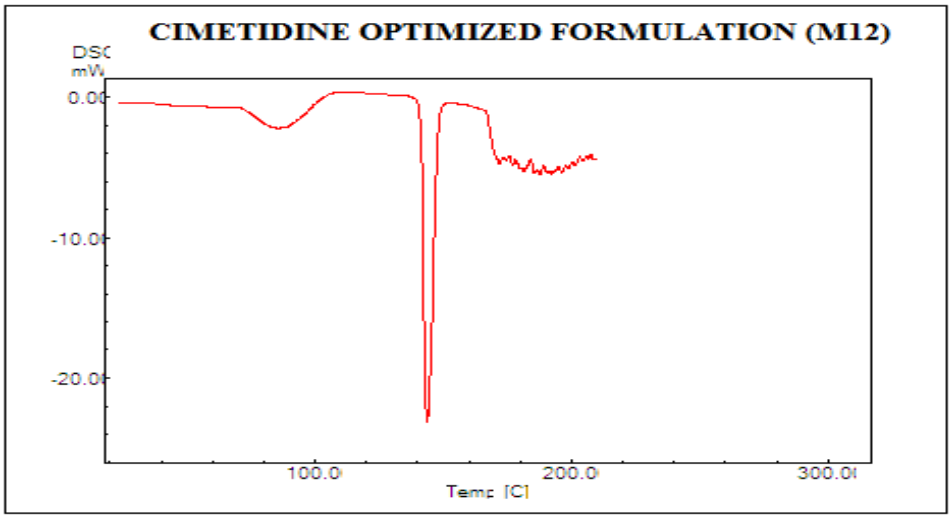

Fig. 10 (b): DSC Thermogram of Cimetidine Optimized Microspheres (MGK5)

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

Name of the Ingredient	Melting Point (^{0}C)
Cimetidine Pure Drug	141
Sodium Alginate	490
Cimetidine Optimized Formulation (MGK5)	142

Scanning Electron Microscopy Studies of Cimetidine Mucoadhesive Microspheres

The external and internal morphology of mucoadhesive microspheres were studied by Scanning Electron Microscopy. SEM photographs **Figure 11**, revealed that microspheres were discrete and spherical in shape with outer surface association of drug with polymer. The pores on microspheres surface help in drug release by diffusion mechanism.

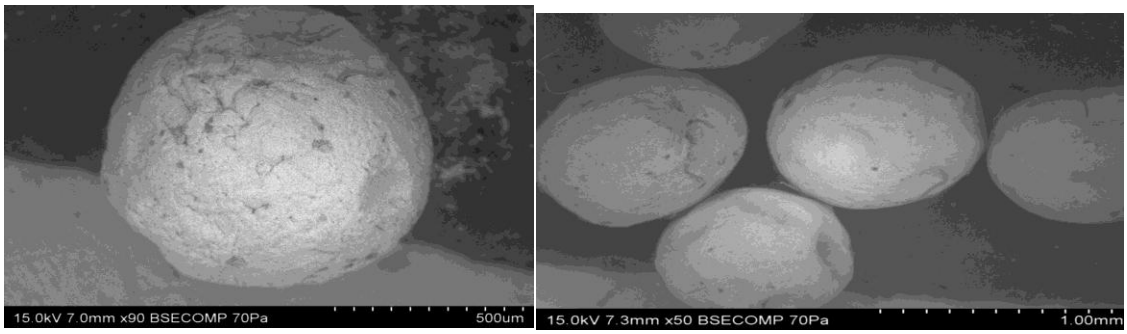

Fig. 11: Scanning Electron Micrographs of Cimetidine Optimized Mucoadhesive Microspheres (MGK5)

Stability Studies

Stability studies were conducted for the optimized formulation for 6 months according to ICH guidelines. From these results, it was concluded that the optimized formulation was stable and retained their original properties with minor differences which depicted in **Table 10**.

Table 10: Stability Studies of Optimized Mucoadhesive Microspheres (MGK 5)

Retest Time for Optimized Formulation (MGK5)	Percentage Yield (%)	Entrapment Efficiency (%)	*In-vitro* Drug Release Profile (%)
0 days	99.30	97.12	99.41±0.16
30 days	98.78	97.04	99.32±0.22
60 days	98.74	96.83	99.11±0.26
120 days	98.72	96.75	98.78±0.36
180 days	98.72	96.74	98.78±0.22

Response Surface Central composite Design Graphs of Cimetidine Mucoadhesive Microspheres

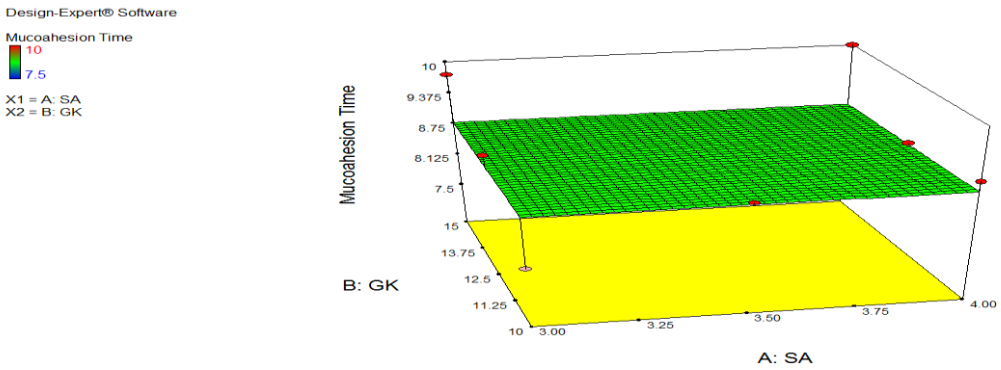

Fig. 10: Response Surface Central composite Design Graphs of Cimetidine Mucoadhesive Microspheres containing Xanthan Gum

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

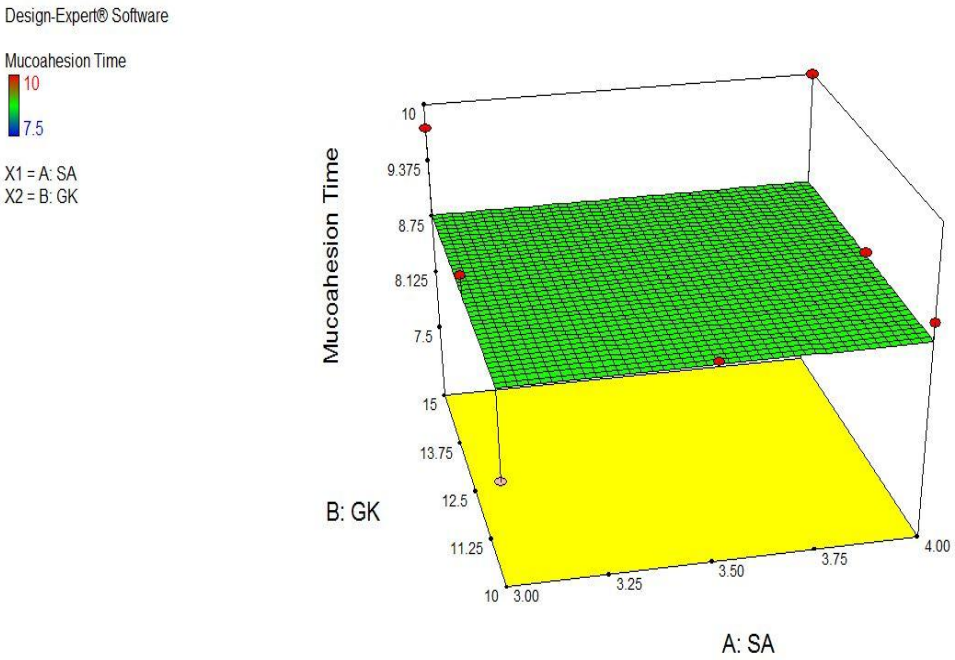

Fig. 11: Response Surface Central composite Design Graphs of Cimetidine Mucoadhesive Microspheres containing Gum Kondagogu.

Final equation in terms of coded factors

Mucoadhesion Time = 10.11

Final equation in terms of actual factor

Mucoadhesion Time = 10.11

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 best batch exhibited a high percentage mucoadhesion of 98% after 10 h, 97.12 % drug entrapment efficiency and swelling index of 96.98%. The optimized formulations MGK5 showed 99.41 % 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 cimetidine could sustain the release of the drug for 12 h.

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