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Formulation and Evaluation of Mucoadhesive Microspheres Containing Cimetidine

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ABSTRACT

In the present research work mucoadhesive microspheres of Cimetidine were prepared using Ionotropic gelation technique. All the microspheres were characterized for particle size, scanning electron microscopy, FT-IR study, DSC, percentage yield, drug entrapment, stability studies and for *in vitro* release kinetics and found to be within the limits. Among all the formulations M12 was selected as optimized formulation based on the physicochemical and release studies. *In vitro* drug release study of optimized formulation M12 showed 99.12% after 12 h in a controlled manner, which is essential for anti ulcer therapy. The innovator Cimetine conventional tablet showed the drug release of 96.15% within 1 h. The drug release of Cimetidine optimized formulation M12 followed zero order and Higuchi kinetics indicating diffusion controlled drug release.

Keywords: Cimetidine, mucoadhesion, chitosan, gum kondagogu, xanthan gum.

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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 which show absorption window in the proximal gut and is the major obstacle to the development of controlled release formulation. A number of approaches have been developed to increase the residence time of dug formulation. One of the approaches is the formulation of Gastro retentive dosage forms in the form of Mucoadhesive microspheres. 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[1,2,3].

The success of these microspheres is limited 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 [4, 5, 6, 7].

Controlled release drug delivery systems that can be retained in stomach for long time are importants for drug that are degraded in intestine or for drugs like antacids or certain enzymes that should act locally in the stomach. If the drugs are poorly soluble in intestine due to alkaline pH, gastric retention may increase solubility before they are emptied, resulting in improved gastrointestinal absorption of drugs with narrow absorption window as well as for controlling release of drugs having site specific absorption limitation[8].

Peptic ulcer disease is a break in the lining of the stomach, first part of the small intestine, or occasionally the lower esophagus[9].

Cimetidine is histamine H_2-receptor antagonist, which is used to reduce the risk of stomach ulcers in patients treated with nonsteroidal anti-inflammatory drugs, which has less bioavailability (60%) and lesser half life of 2 hr [10]. The aim of present work is to design and *in vitro* evaluation of mucoadhesive microspheres of Cimetidine to enhance its bioavailability and prolonged residence time in stomach.

MATERIALS AND METHOD

Materials:

Cimetidine pure drug was generous gift from Aurobindo Pharma Limited, Hyderabad, India. Sodium alginate was obtained from Pruthvi Chemicals, Mumbai. Sodium alginate, Chitosan, Xanthan gum, Kondagogu gum and sodium CMC were gifted from MSN Labs Ltd., Hyderabad. All other chemicals used were of analytical grade.

Formulation of Cimetidine mucoadhesive microspheres

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

Table 1: Formulation trials for Cimetidine mucoadhesive microspheres

Formulation code	Cimetidine (g)	Sodium alginate	Sodium CMC(mg)	Calcium chloride	Xanthan gum	Gum kondagogu
M1	2	1 %	100	7%	1%	0.5%
M2	2	1.2 %	150	7%	1.2%	0.5%
M3	2	1.4%	200	7%	1.4%	0.5%
M4	2	1.6%	250	7%	1.6%	0.5%
M5	2	1.8%	300	7%	1.8%	0.5%
M6	2	2%	350	7%	2%	0.5%
M7	2	2.2%	400	7%	2.2%	0.5%
Formulatin code	**Cimetidine (g)**	**Sodium alginate**	**Chitosan (mg)**	**Calcium chloride**	**Xanthan gum**	**Gum kondagogu**
M8	2	1%	10	10%	1%	0.5%
M9	2	1.2%	15	10%	1.2%	0.5%
M10	2	1.4%	20	10%	1.4%	0.5%
M11	2	1.6%	25	10%	1.6%	0.5%
M12	2	1.8%	30	10%	1.8%	0.5%
M13	2	2%	35	10%	2%	0.5%
M14	2	2.2%	40	10%	2.2%	0.5%

Evaluation studies of Cimetidine mucoadhesive 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 (Ɵ) of microspheres measures the resistance to particles flow, and is calculated according to fixed funnel standing cone method. Where (Ɵ) 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.

Bulk density = Wt of powder / Bulk volume of powder

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

Tapped density =Wt of powder/ Tapped volume of powder

Compressibility index:

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

Carr's compressibility index =Tapped density - Bulk density X 100/ Tapped density

Hausner's ratio:

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

Hausner's ratio =Tapped density / Bulk density

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 particular wavelength 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

Mucoadhesiveness:

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 2cm 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 was incubated at 37^0C and agitated horizontally. The sac was taken out of the medium after immersion for 1, 2, 3, 4, 5, 6, 7 and 8h, immediately repositioned as before in a similar tube containing 40ml of fresh saline and unbound microspheres were counted. The adhering percent was presented by the following equation[16].

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

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

In vitro drug release studies for developed Cimetidine 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 218nm.[17]

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[21] (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 range 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

Mucoadhesive microspheres

Figure 1: Cimetidine mucoadhesive microspheres

Table 2: Formulated Cimetidine mucoadhesive microspheres

Formulation code	Particle size (µm)	Bulk density (g/cc)	Tapped density (g/cc)	Angle of repose	Carr's index
M1	65.29±0.13	0.63	0.62	29°.67	13.34%
M2	73.43±0.04	0.65	0.69	30°.54	12.12%
M3	78.67±0.09	0.67	0.73	31°.15	12.23%
M4	79.45±0.21	0.69	0.75	28°.91	11.00%
M5	83.42±0.12	0.72	0.79	27°.93	12.20%
M6	85.34±0.09	0.75	0.82	28°.54	13.00%
M7	87.12±0.13	0.76	0.91	27°.91	11.20%
M8	69.43±0.09	0.66	0.61	30°.91	14.34%
M9	72.46±0.09	0.68	0.63	27°.91	12.11%
M10	76.89±0.10	0.72	0.68	30°.24	12.12%
M11	85.94±0.11	0.74	0.72	27°.93	12.23%
M12	88.94±0.11	0.79	0.75	25°.34	09.34%
M13	89.04±0.21	0.81	0.76	26°.54	12.34%
M14	91.45±0.21	0.83	0.83	27°.91	11.45%

All fourteen formulations were evaluated for various micromeretic and physic chemical parameters and the results are tabulated in **Table 2.** Among all the formulations M12 shown best results of particle size, bulk density, tapped density, angle of repose and carr's index of 88.94±0.11, 0.79, 0.75, 25°.34 and 9.32% respectively.

Table 3: Percentage yield and entrapment efficiency of Cimetidine Mucoadhesive microspheres Formulations:

Formulation code	Percentage yield	Entrapment efficiency	Swelling index	Mucoadhesiveness
M1	75.45%	76.00%	72.11%	71.00%
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	96.03%	95.03%	91.12%	92.00%
M7	92.01%	90.01%	84.23%	85.00%
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	98.90%	97.07%	96.08%	96.00%
M13	92.00%	91.03%	94.08%	91.50%
M14	90.72%	89.67%	90.03%	88.00%

The percentage yield and entrapment efficiency of all the formulations were measured by assay method and found to be within the limits. The formulation M12 showed good percentage yield and entrapment efficiency, swelling index and mucoadhesiveness of 98.90%, 97.07%, 96.08% and

96.00% respectively and the results were depicted in Table 3 and pictorial diagram of mucoadhesive study was shown in Figure 2.

(A) **(B)**

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

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

Cimetidine microspheres were evaluated for in vitro drug release studies in 0.1N HCL and the results are depicted in Table 4. The formulation M12 shows best drug release of 99.12% within 12h. The drug release of optimized formulation M12 was in controlled manner when compared with innovator product Cimetine i.e 96.12% within 1h.

Table 4: *In vitro* cumulative % drug release of Cimetidine Mucoadhesive microspheres Formulations:

Time in (h)	M1	M2	M3	M4	M5	M6	M7	Innovator (Cimetine 200mg)
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	16.81±0.22	18.62±0.52	16.44±0.45	12.06±0.22	10.08±0.98	10.27±0.14	10.6±0.22	96.15±0.12
2	35.59±0.23	32.97±0.16	29.61±0.16	26.69±0.21	21.35±0.78	18.5±0.18	24.36±0.11	---
4	57.97±0.32	50.16±0.13	46.38±0.22	43.48±0.11	36.73±0.76	27.75±0.16	35.92±0.21	----
6	78.61±0.16	71.06±0.22	59.34±0.52	58.95±0.13	48.64±0.66	45.31±0.33	60.81±0.13	----
8	94.04±0.32	83.2±0.23	72.61±0.34	70.53±0.21	57.08±0.44	68.06±0.12	72.36±0.33	----
10	93.24±0.12	96.78±0.32	81.65±0.22	84.71±0.22	68.34±0.12	75.93±0.22	86.89±0.41	----
12	91.69±0.23	93.56±0.16	93.18±0.23	90.65±0.16	80.19±0.32	88.72±0.11	92.13±0.11	----

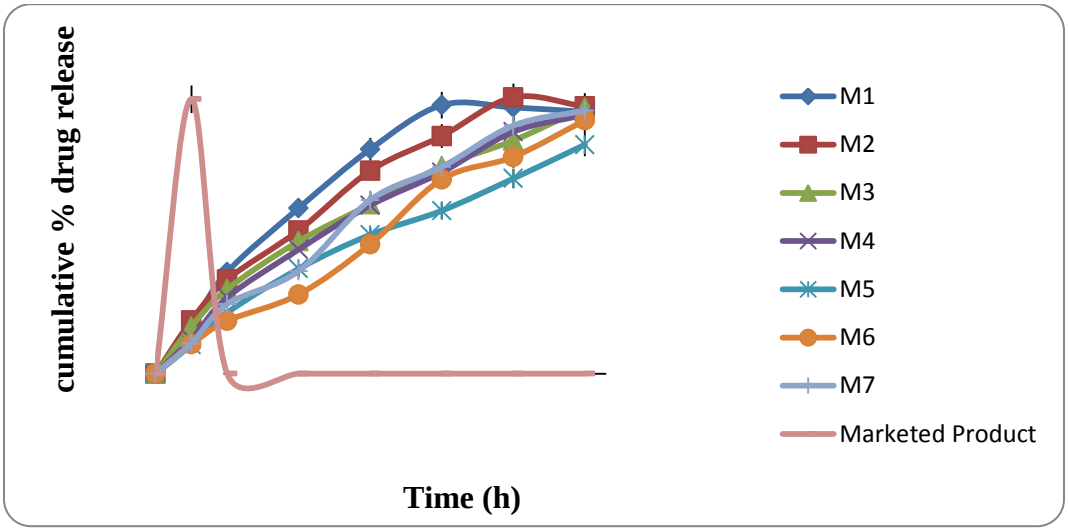

Figure 3: *In-vitro* cumulative % drug release of Cimetidine Mucoadhesive microspheres formulations

Table 5: *In vitro* cumulative % drug release of Cimetidine mucoadhesive microspheres formulation

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	9.31±0.33	8.4±0.12	13.96±0.32	11.17±0.16	12.41±0.22	9.67±0.12	8.22±0.12
2	16.48±0.52	17.79±0.22	24.73±0.16	19.78±0.15	20.76±0.23	17.41±0.32	14.08±0.22
4	26.76±0.33	30.61±0.43	37.62±0.11	32.12±0.11	35.82±0.32	24.36±0.16	20.7±0.22
6	37.72±0.56	40.53±0.44	53.29±0.21	45.27±0.16	50.62±0.34	31.76±0.17	37.02±0.32
8	50.24±0.52	47.56±0.52	62.4±0.12	60.29±0.32	67.73±0.16	45.63±0.22	58.79±0.87
10	63.21±0.51	61.95±0.33	72.59±0.33	75.85±0.16	81.09±0.22	51.53±0.32	71.84±0.32
12	78.05±0.55	76.82±0.22	81.23±0.32	83.69±0.52	99.12±0.13	80.64±0.16	85.39±0.22

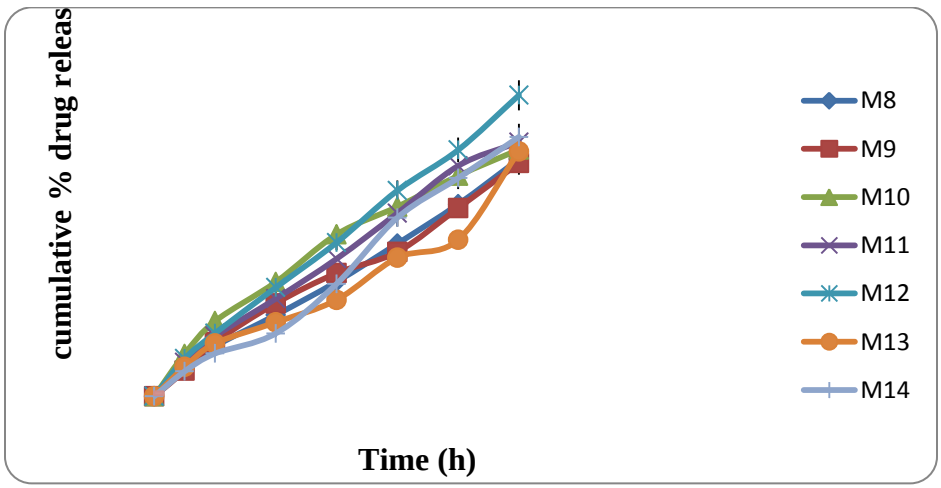

Figure 4: *In vitro* cumulative % drug release of Cimetidine mucoadhesive microspheres formulations

Mathematical modeling of optimized mucoadhesive microspheres M12:

Table 6: Release kinetics of optimized formulation of mucoadhesive microspheres:

Formulation Code	Zero Order		First Order		Higuchi		Korsmeyer-Peppas	
	R^2	K	R^2	K	R^2	K	R^2	N
M12	0.997	7.851	0.837	0.102	0.944	28.36	0.997	1.075

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. Further the n value obtained from the Korsmeyer plots i.e. 1.075 suggest that the drug release from microspheres was anomalous Non fickian diffusion.

CHARECTERIZATION:

FTIR:

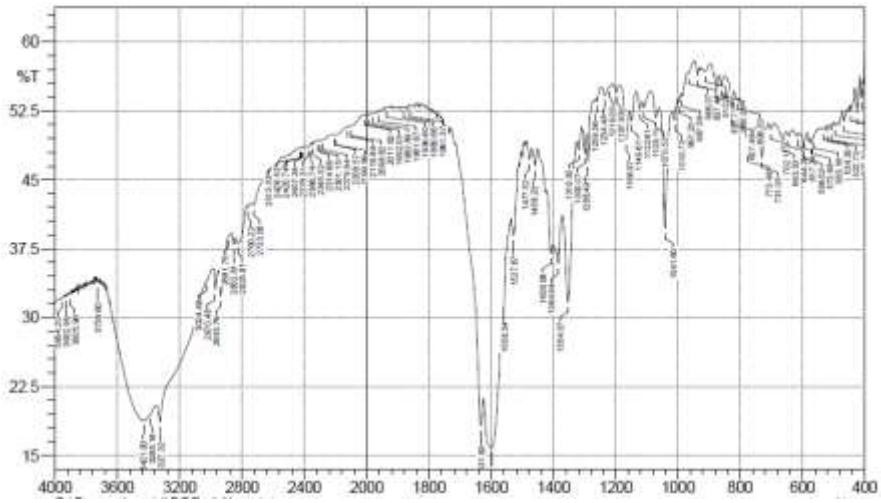

Figure 5: FT-IR spectrum of pure drug Cimetidine

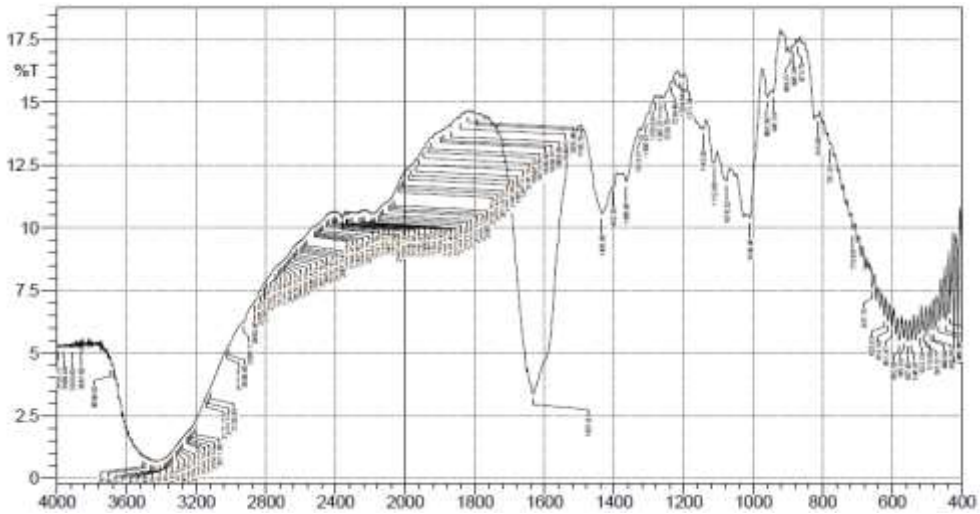

Figure 6: FT-IR spectrum of Cimetidine optimized formulation M12

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 5) and optimized formulation (M12) (Figure 6) and results found that there were no possible interaction between drug and polymer (Figure).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 =N-H stretching (amides), 3237.06 = symmetric vibration, 3103.86= C-H stretching vibration. From the FTIR graphs of drug polymer mixture, it was found that the same peaks of the drug are available. Since it proves that there is no incompatibility with the polymers.

DSC Studies:

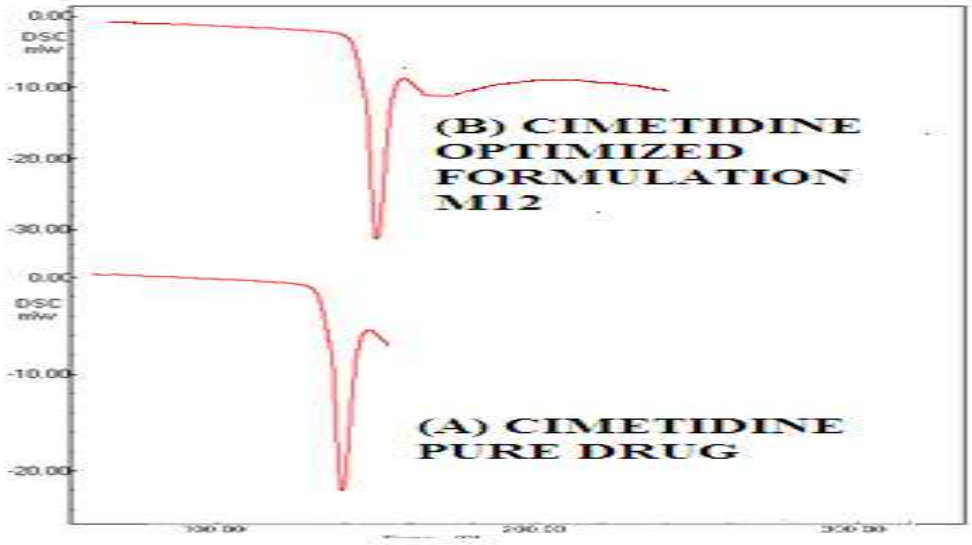

Figure 7: DSC thermogram of Cimetidine pure drug (A) and optimized formulatin M12 (B)

DSC was used to detect interaction between Cimetidine and excipients. The thermogram of Cimetidine (Figure 7) exhibited a sharp endotherm melting point at 141^{o}C. The thermogram of microsphere loaded with Cimetidine exhibited a sharp endotherm melting point at142^{o}C. There is no considerable change observed in melting endotherm of drug in optimized formulation (M12). It indicates that there is no interaction between drug & excipients used in the formulation.

Scanning electron microscopy studies:

Cimetidine mucoadhesive microspheres:

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

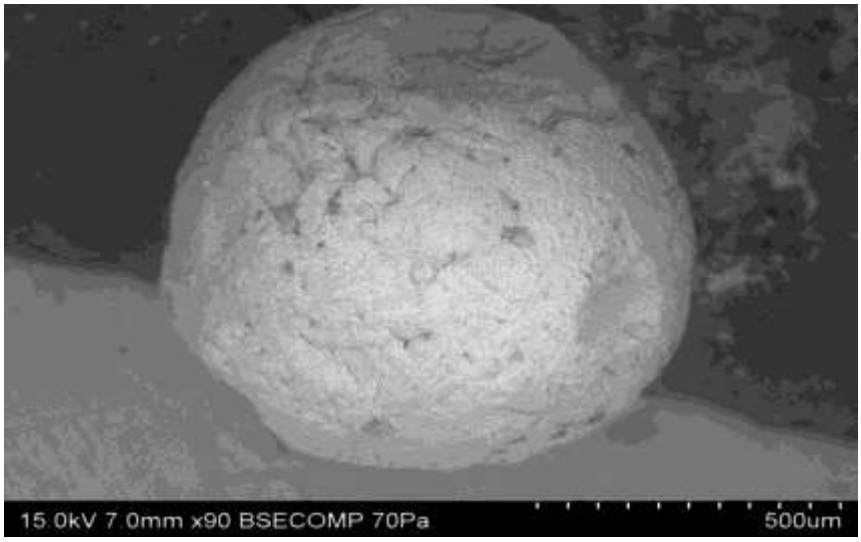

Figure 8: Scanning electron micrographs of Cimetidine optimized mucoadhesive microspheres (M12)

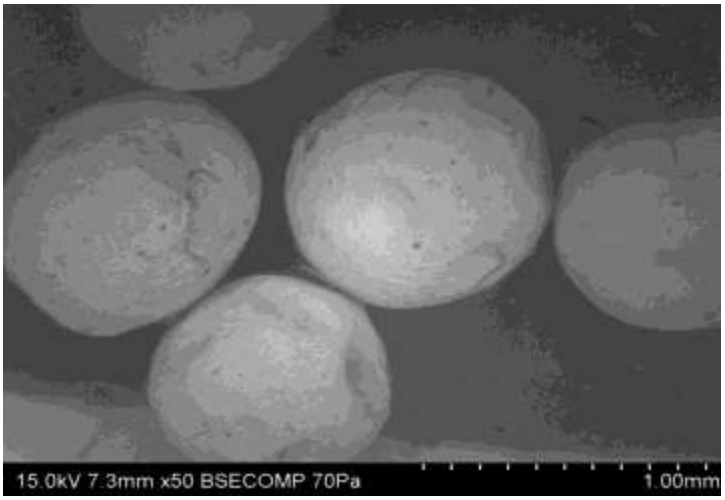

Figure 9: Scanning electron micrographs of Cimetidine optimized mucoadhesive microspheres (M12)

SEM photograph 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.

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 with minor differences.

CONCLUSION

In vitro data obtained for mucoadhesive microspheres of Cimetidine showed good drug entrapment and % yield. Microspheres of different size and drug content could be obtained by varying the formulation variables. Diffusion was found to be the main release mechanism. Mucoadhesive and floating microspheres exhibited prolonged and controlled release effect compared to Innovator product. Among all the formulations M12 was selected as optimized formulation based on the physico chemical and release studies. *In vitro* drug release study of formulation M12 showed 99.12% after 12 h in a controlled manner, which is essential for disease like peptic ulcer. The innovator Cimetine conventional tablet showed the drug release of 96.15% within 1 h.

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