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Design and evaluation of microspheres loaded with cimetidine

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

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

ABSTRACT

Cimetidine loaded microspheres were prepared by Ionotropic gelation technique with different drug to carrier ratio. 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 C10, was selected as optimized formulation based on the physic chemical and release studies. In the* in vitro *release study of formulation C10 showed 95.35%, after 12h in a controlled manner, which is essential for anti ulcer therapy. The innovator Cimetidine conventional tablet shows the drug release of 96.15 within 1 h. The drug release of optimized formulation C10 followed zero order and Higuchi kinetics indicating diffusion controlled drug release.*

Key words: Cimetidine, chitosan, microspheres, scanning electron microscopy, release order kinetics.

INTRODUCTION

Oral drug administration is by far the most preferable route for taking medications. However, their short circulating half life and restricted absorption via a defined segment of intestine limits the therapeutic potential of many drugs. Such a pharmacokinetic limitation leads in many cases to frequent dosing of medication to achieve therapeutic effect. This results in pill burden and consequently, patient complains. Rational approach to enhance bioavailability and improve pharmacokinetic and pharmacodynamic profile is to release the drug in a controlled manner and site specific manner [1].

Microspheric drug delivery has advantage over various other dosage forms like we know for lungs disease now a days aerolised drugs are used for local delivery of drugs but it has disadvantage of shorter duration of action so for sustained release and reducing side effects and hence to achieve better patient compliance microspheres can be used. It also has advantage over liposomes as it is physicochemically more stable. Moreover the microspheres are of micron size so they can easily fit into various capillary beds which are also having micron size [2].

For the treatment of chronic diseases it is important to take medication several times, this may lead to fluctuating drug level in body. In order to avoid frequent drug administration and maintenance of therapeutic drug level in body it is essential to administer drug by a sustained release system. Drugs with short elimination half life are most suitable for sustained release formulations. Sustained delivery of drugs can be achieved by microspheres formulation [3].

The microsphere requires a polymeric substance as a carrier and a core material [4, 5]. Microspheres have been widely accepted as a mean to achieve oral and parenteral controlled release [6,7,8].

Peptic ulcer disease, also known as a peptic ulcer or stomach ulcer, is a break in the lining of the stomach, first part of the small intestine, or occasionally the lower esophagus. Common causes include the bacteria, *Helicobacter pylori* and non-steroidal anti-inflammatory drugs [9].

Cimetidine is histamine H_2-receptor antagonists, 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 2h. The aim of present work is to design and in vitro evaluation of Cimetidine microspheres to enhance its bioavailability and prolonged drug release [10].

MATERIALS AND METHODS

Materials:
Cimetidine pure drug was generous gift from Aurobindo Pharma Limited, Hyderabad, India. Sodium alginate was obtained from Pruthvi Chemicals, Mumbai. HPMC K 4 M & HPMC K 15 M was obtained from Rubicon labs, Mumbai. Xanthan gum, Guar gum, Kondagogu gum and sodium CMC were gifted from MSN Labs Ltd. Hyderabad. All other chemicals used were of analytical grade.

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

Table 1: Formulation trials for Cimetidine microspheres:

FORMULATION CODE	CIMETIDINE(G)	SODIUM ALGINATE	CALCIUM CHLORIDE
C1	2	1%	7%
C2	2	1.2 %	7%
C3	2	1.4%	7%
C4	2	1.6%	7%
C5	2	1.8%	7%
C6	2	2%	7%
C7	2	2.2%	7%
C8	2	1%	10%
C9	2	1.2%	10%
C10	2	1.4%	10%
C11	2	1.6%	10%
C12	2	1.8%	10%
C13	2	2%	10%
C14	2	2.2%	10%

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

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

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

Bulk density: Volume of the microspheres in the measuring cylinder was noted as bulk density.

$$\text{Bulk density} = \frac{\text{Wt of powder}}{\text{Bulk volume of powder}}$$

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

$$\text{Tapped density} = \frac{\text{Wt of microspheres}}{\text{Tapped volume of microspheres}}$$

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

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

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

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

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

***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 [16].

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

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

Drug excipient compatibility studies

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

Fourier transform infrared spectroscopy (FTIR)

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

Differential Scanning Calorimetry (DSC)

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

SEM studies

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

Stability studies

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

RESULTS AND DISCUSSION

Micromeretic properties of cimetidine microspheres

Figure 1: **Cimetidine microspheres**

Table 2: Micromeretic properties of Cimetidine microspheres

Formulation code	Particle size (µm)	Bulk density (g/cc³)	Tapped density (g/cc³)	Angle of repose	Carr's index	Swelling index
C1	61.12±0.08	0.66	0.69	26˚.74	12.34%	64%
C2	65.29±0.13	0.74	0.72	29˚.67	13.34%	69%
C3	67.43±0.04	0.76	0.73	30˚.54	11.12%	70%
C4	69.67±0.09	0.79	0.73	31˚.15	13.23%	71%
C5	73.45±0.04	0.89	0.75	27˚.93	14.56%	79%
C6	92.45±0.09	0.92	0.76	26˚.21	13.95%	87%
C7	81.45±0.09	0.94	0.76	28˚.54	12.32%	75%
C8	67.45±0.04	0.66	0.59	27˚.93	14.56%	69%
C9	78.45±0.09	0.67	0.62	27˚.54	13.95%	70%
C10	82.45±0.09	0.69	0.64	22˚.91	9.32%	95%
C11	85.12±0.08	0.71	0.66	25˚.74	12.34%	84%
C12	87.29±0.13	0.74	0.68	27˚.67	14.34%	93%
C13	91.43±0.04	0.76	0.73	26˚.54	11.12%	92%
C14	94.13±0.09	0.87	0.78	29˚.15	14.23%	89%

All fourteen formulations were evaluated for various micromeretic and physic chemical parameters and the results are tabulated in **Table.** Among all the formulations C10 shown best results of particle size, bulk density, tapped density, angle of repose, carr's index and swelling index of 82.45±0.09, 0.69, 0.64, 22˚.91, 9.32% and 95% respectively.

Table 3: Percentage drug yield & entrapment efficiency of Cimetidine microspheres

Formulation code	Percentage yield	Entrapment efficiency
C1	70.00%	69.00%
C2	71.00%	72.00%
C3	81.00%	80.00%
C4	83.87%	83.30%
C5	86.30%	85.20%
C6	91.30%	91.30%
C7	86.30%	90.10%
C8	76.00%	74.03%
C9	81.00%	82.00%
C10	96.30%	97.70%
C11	86.09%	85.00%
C12	87.50%	86.66%
C13	93.30%	91.03%
C14	85.30%	84.88%

The percentage yield and entrapment efficiency of all the formulations were measured by assay method and found to be within the limits. The formulation C10 shows good percentage yield and entrapment efficiency of 96.30% and 97.00% respectively and the results were depicted in **Table 3**.

***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 and 5**. The formulation C10 shows best drug release of 95.35% within 12h. The drug release 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 microspheres formulations

Time (h)	C1	C2	C3	C4	C5	C6	C7	Innovator (Cimetine 200mg)
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	24.57±0.11	20.09±0.12	24.06±0.12	12.29±0.11	10.11±0.22	14.95±0.15	12.32±0.22	96.15±0.12
2	46.18±0.23	34.45±0.22	39.51±0.32	23.49±0.32	18.42±0.21	22.42±0.23	21.44±0.26	--
4	70.45±0.16	51.28±0.16	54.19±0.32	32.52±0.21	33.08±0.16	39.44±0.16	34.23±0.12	---
6	86.56±0.32	68.31±0.16	69.41±0.32	41.64±±0.41	46.15±0.11	56.63±0.43	47.29±0.45	---
8	94.48±0.24	79.67±0.32	81.55±0.33	59.14±0.42	59.62±0.21	63.43±0.16	60.46±0.43	---
10	93.29±0.14	93.32±0.29	93.51±0.16	71.02±0.99	70.06±0.16	75.09±0.22	78.34±0.44	---
12	90.65±0.22	91.84±0.22	90.16±0.32	81.77±0.22	84.36±0.21	86.92±0.12	85.69±0.16	---

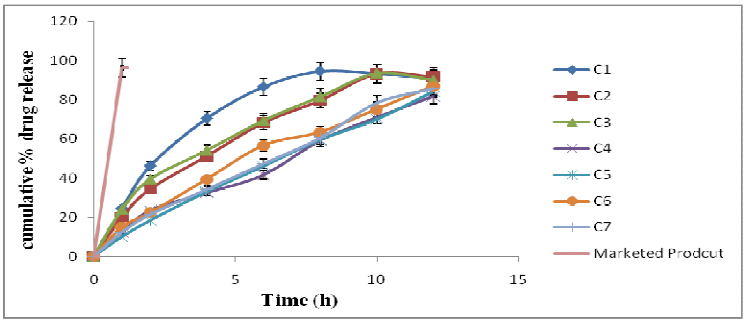

Figure 2: *In vitro* cumulative % drug release of Cimetidine microspheres

Table 5: *In vitro* cumulative % drug Cimetidine microspheres

Time (h)	C8	C9	C10	C11	C12	C13	C14
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	17.19±0.23	15.18±0.23	11.98±0.11	10.13±0.22	9.56±0.22	11.12±0.16	10.96±0.32
2	29.89±0.12	25.77±0.16	20.67±0.21	21.92±0.52	16.43±0.16	18.4±0.23	18.65±0.33
4	43.58±0.15	36.37±0.15	34.35±0.16	28.67±0.32	28.71±0.15	25.87±0.16	26.41±0.13
6	56.98±0.22	47.31±0.34	51.63±0.22	35.98±0.16	38.78±0.32	35.14±0.15	37.08±0.22
8	70.7±0.43	63.63±0.21	66.45±0.32	52.93±0.23	50.92±0.22	41.88±0.15	50.73±0.18
10	87.32±0.16	77.02±0.13	80.43±0.34	65.01±0.33	66.38±0.13	56.87±0.12	64.84±0.32
12	92.69±0.13	87.23±0.12	95.35±0.16	76.31±0.15	79.14±0.15	68.24±0.15	72.11±0.12

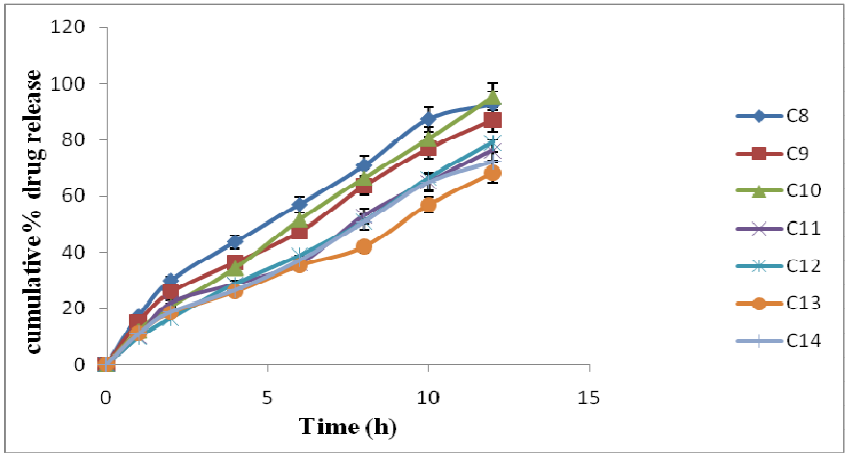

Figure 3: *In vitro* cumulative % drug Cimetidine sodium alginate microspheres

Mathematical modeling of cimetidine optimized microspheres (C10):

Table 6:Release order kinetics of optimized microspheres (C10)

Formula Code	Zero Order		First Order		Higuchi		Korsmeyer-Peppas	
	R^2	K	R^2	K	R^2	K	R^2	N
C10	0.997	7.815	0.845	0.099	0.943	28.21	0.997	1.063

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. 1.063 suggest that the drug release from microspheres was anomalous Non fickian diffusion.

Drug excipient compatibility studies
Fourier Transform Infrared Spectroscopy (FTIR)
FTIR:

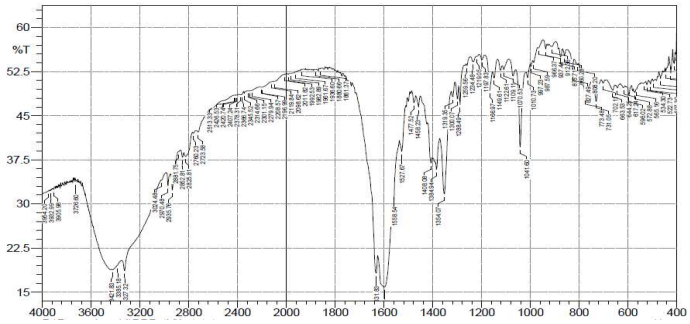

Figure 4: FT-IR spectrum of pure drug Cimetidine

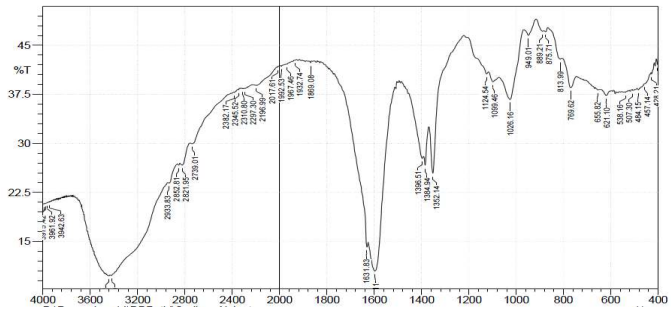

Figure 5: FT-IR spectrum of Cimetidine + Sodium alginate+$CaCl_2$

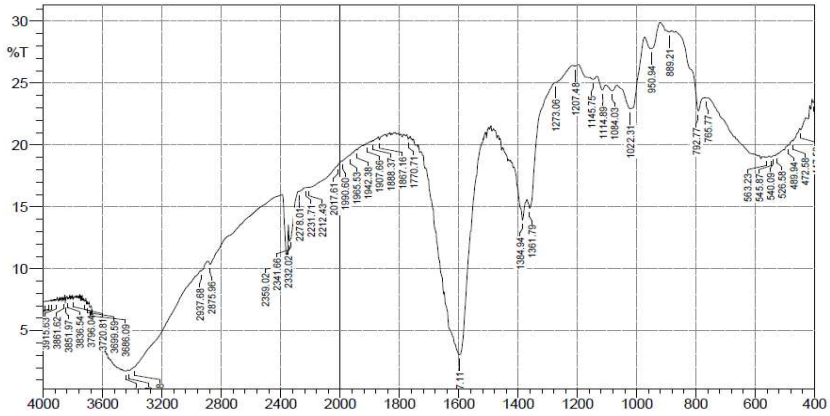

Figure 6: FT-IR spectrum of Cimetidine optimized microspheres (C10)

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 4)** and optimized formulation (C10) **(Figure 6)** and results found that there were no possible interaction between drug and polymer **(Figure 5).**The FTIR spectrum of Cimetidine (Figure) 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:

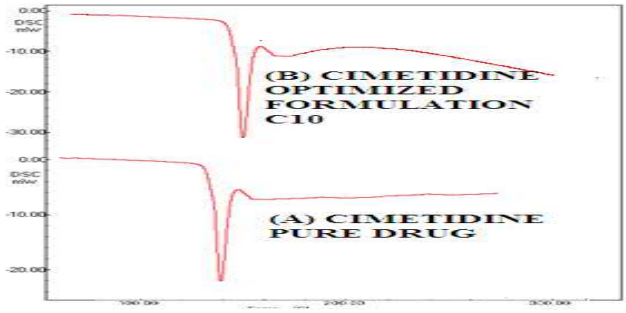

Figure 7: DSC thermogram of Cimetidine pure drug (A) and optimized formulatin C10 (B)

DSC was used to detect interaction between Cimetidine and excipients. The thermogram of pure drug Cimetidine **(Figure 7)** exhibited a sharp endotherm melting point at 141 ^{0}C (Table no). The thermogram of microsphere loaded with Cimetidine (C10) exhibited a sharp endotherm melting point at143 ^{0}C **(Figure 7)**. It indicates that there is no interaction between drug & excipients used in the formulation.

SEM of Cimetidine microspheres

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

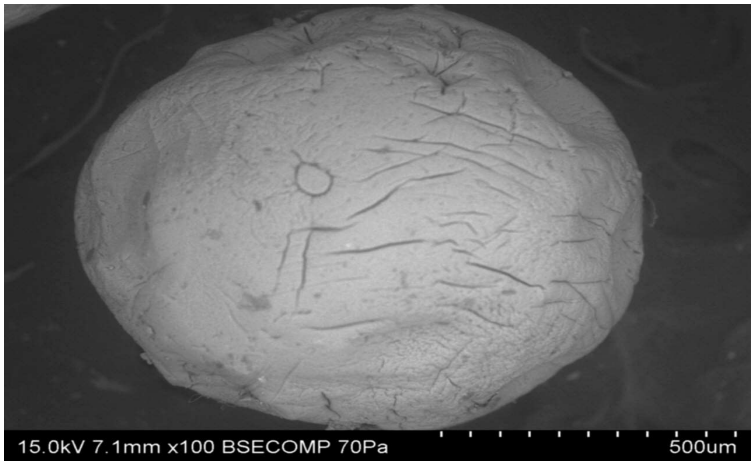

Figure 8: Scanning electron micrographs of Cimetidine microspheres

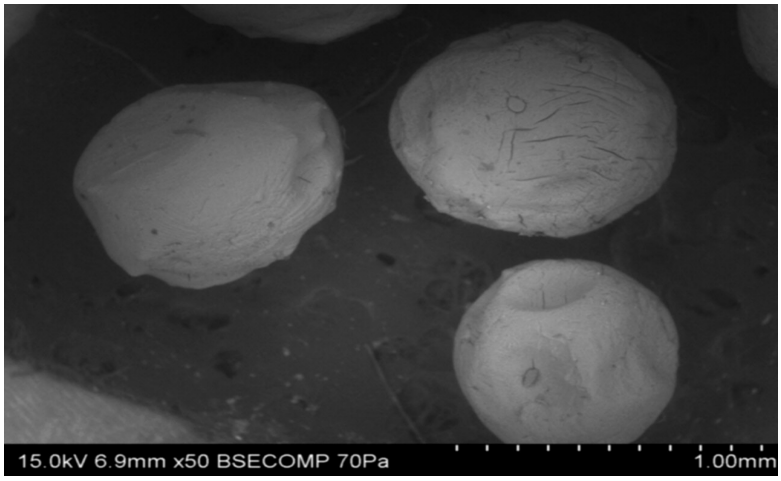

Figure 9: Scanning electron micrographs of Cimetidine microspheres

Morphology of the various formulations of Cimetidine microspheres prepared was found to be discrete and spherical in shape **(Figure 9)**. The surface of Cimetidine 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 (C10) 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

From the above data, it could be concluded that Cimetidine microspheres exhibited prolonged and controlled release effect compared to Innovator product. Prepared Cimetidine microspheres 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 C10 was selected as optimized cimetidine formulations based on the physic chemical and release studies. In the *in vitro* release study of formulation C10 showed 95.35%, after 12 h in a controlled manner, which is essential for disease like peptic ulcer. The innovator Cimetine conventional tablet shows the drug release of 96.15 within 1 h.

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