INTERNATIONAL JOURNAL
OF
PHARMACEUTICAL SCIENCES
AND
RESEARCH

MUCOADHESIVE MICROPARTICLES OF CARBOXYMETHYL CHITOSAN FOR SITE SPECIFIC DELIVERY OF PANTOPRAZOLE: FORMULATION AND *IN VITRO* CHARACTERIZATION

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ABSTRACT

Carboxymethyl chitosan, a water soluble modified carboxymethyl substituted chitosan derivative have distinct and unique properties, rendering them effective to form selective permeable mucoadhesive film or membranes. In the formulation of chitosan microsphere an acidic environment is essentially required that may degrade acid sensitive moiety, peptide or protein drugs. Mucoadhesive microparticle of carboxymethyl chitosan was designed and developed for site specific sustained release of Pantoprazole sodium. Thus prolong the residence time at the absorption site by intimate contact with the mucus layer thereby increase bioavailability, reduce the frequency of dose administration and also prolong the drug release. The mucoadhesive microparticles were prepared by Orifice ionic gelation method using carboxymethyl chitosan in combination with Carbopol 934 and HPMC K15. Entrapment efficiency was in the range of 42.4 to 84.6 %. SEM studies revealed that microparticles were discrete, spherical and free flowing. Microparticle exhibited good mucoadhesive property in the *in vitro* wash off test and found that Carbopol 934 had greater mucoadhesive strength than that of HPMC K15. A sustained release of Pantoprazole sodium was obtained from mucoadhesive microparticle. Stability study of optimized batch was carried out and drug content found was retained with permissible limits and there was no significant difference in the drug content.

INTRODUCTION: The oral route of drug administration constitutes the most convenient and preferred means of drug delivery to systemic circulation of body. However, oral administration of most of the drugs in conventional dosage forms has short-term limitations due to their inability to restrain and localize the system at gastro-intestinal tract.

Microparticles constitute an important part of these particulate drug delivery systems by virtue of their small size and efficient carrier capacity.

Microparticles are the carrier linked drug delivery system in which particle size ranges from 1-1000 μm in diameter having a core of drug and entirely outer layers of polymers as coating material [1].

However, the success of these microspheres is limited due to their short residence time at site of absorption. It would, therefore be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membrane.

This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres. Bioadhesive microspheres have advantages like efficient absorption and enhanced bioavailability of the drugs due 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 [2].

Chitosan has both reactive amino and hydroxyl groups, can be used for modification of its physicochemical properties by cross linking carboxymethylation, galactosylation etc. for drug delivery and biomedical application [3]. Chemical modification of chitin and chitosan may have an advantage, because the modification with a hydrophilic reagent would be expected to result in hydrophilic chitin or chitosan while keeping the fundamental skeleton intact [4]. A modified carboxymethyl substituted chitosan can be prepared is water soluble and have distinct and unique properties, rendering them effective to form selective permeable film or membranes [5].

Additionally in the formulation of chitosan microsphere an acidic environment is essentially required to dissolve chitosan that may degrade acid sensitive moiety and peptide or protein drugs. Pantoprazole sodium, a drug of choice recommended in the treatment of peptic ulcer. Pharmacokinetic studies of pantoprazole revealed that it is well absorbed (at least 30%) and can be detected in the plasma within 1 h. It is also very susceptible to acidic environment [6].

The objective of present work was to design mucoadhesive multiparticulate system of water soluble chitosan derivative for delivery of pantoprazole at absorption specific site. The system is anticipated to protect drug loss by providing acid free environment during formulation and guard in upper GI tract results from inherent property of Eudragit L-100. Mucoadhesive properties of microparticles prepared by ionic gelation were evaluated to confirmed prolonged residence time of drug in GIT.

MATERIALS AND METHODS: Pantoprazole sodium was obtained as a gift sample from Dr. Reddy Ltd., Hyderabad. Carboxymethyl chitosan was purchased from Koyo chemical co. Ltd. Carbopol 934 and HPMC K15 were obtained as a gift sample from Corel Pharma Chem, Ahmedabad and Colorcon Asia Pvt. Ltd, Goa, India respectively. Calcium chloride was purchased from S.D fine Chemicals, Mumbai.

Preparation of Microparticle: Microparticles were prepared by orifice ionic gelation method. Carboxymethyl chitosan and mucoadhesive polymer such as HPMC K15 and Carbopol 934 were dissolved in purified water to form a homogeneous polymer solution. The core material (drug) was added to this polymer solution and mixed to form a smooth viscous dispersion. This dispersion was added drop wise into 5% w/v $CaCl_2$ solution through a syringe 22 gauge. The added droplets were retained in the calcium chloride solution for 15 minutes to complete the curing reaction and to produce spherical rigid microparticles. The microparticles were collected and dried in an oven at 45°C for 4 hours.

Characterization of Mucoadhesive Microparticles:

1. **Micromeritic Properties [7, 8]:** The bulk density and tapped density of microparticles were measured in 10 ml of graduated cylinder. Microparticles were accurately weighed and introduced into a 10 ml of measuring cylinder. The initial volume was reported, and then the sample was tapped mechanically onto a hard surface from the height of 2.5 cm at intervals of 2 second per tapping. The tapping was done for 100 times. The initial bulk volume and tapped volume were reported from which, their respective densities were calculated. The bulk density and tapped density were calculated using following equation:

Bulk density   = Mass of microparticle/ volume of microparticle

Tapped density = Mass of microparticle / volume of microparticle after tapping

2. **Compressibility Index:** Compressibility index of all formulations was calculated by following equation:

Carr's index = $[1 - V/V_0] \times 100$

Where, V_0 = volume of microparticle before tapping

      V = volume of microparticle after tapping

3. **Hausner's Ratio:** Hausner's ratio was also calculated by using following equation:

Hausner's ratio   =   Tapped density / Bulk density

4. **Angle of Repose:** The angle of repose was determined by the fixed funnel method. Accurately weighed microparticles were placed in funnel. The height of funnel was adjusted in such a way that the tip of the funnel just touched to apex of the heap of the microparticles. It is the maximum angle possible between the surface of pile of microparticle and horizontal plane. The microparticle was allowed to flow through the funnel freely onto the surface. The diameter of microparticle cone was measured. The Angle of repose is calculated by using the following equation:

$$\tan(\theta) = h/r$$

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

Where,   h= Height of the microparticle heap and r= Radius of microparticle heap

5. **Particle Size and Shape:** The size and shape of Microparticle was determined using optical microscope. The diameters of 50 microparticles were measured randomly by optical microscope [8].

a) **Scanning Electron Microscopy:** The SEM photographs of microparticles of optimized formulation were obtained by scanning electron microscope using platinum sputter technique. A working distance of 500 μm and the particles were vacuum dried and 5-kV accelerating voltage was set as a processing parameters.

b) **FT-IR Spectroscopy:** The interaction between the pantoprazole and polymers were determined by using the FT-IR spectroscopy wherein infrared spectra of pantoprazole sodium, Carboxymethyl chitosan, Carbopol 934, HPMC K15 and Microparticles were carried out using the KBr disk method. The scanning range was 400 to 4000 cm^{-1} and the resolution was 1/cm.

c) **Production Yield:** The product yields of microparticles of various formulations were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microparticles and percent yields were calculated as per the formula mentioned below [9]:

Production Yield = [Practical mass (microparticles) / Theoretical mass (Polymer + Drug)] x 100

d) **Entrapment Efficiency:** The drug content of carboxymethyl chitosan microspheres was determined by crushing 50 mg microparticles in 100 ml NaOH solution followed by agitation with a magnetic stirrer for 12 hours to dissolve the polymer. The solution was then gently warmed for two hours to extract the drugs completely, filtered, and the resulting solution was analyzed by UV spectrophotometer for Pantoprazole at 297 nm.

Entrapment efficiency = [Practical drug content / Theoretical drug content] x 100

e) **Swelling Index:** The swelling indices of formulations were determined by immersing preweighed dried microparticles (20 mg) in 10 ml of NaOH at a temperature of 37º C and flask was shaken at 100 rpm by rotary shaker for 12 hrs. After 12 hours, the sample was removed, blotted with a piece of tissue paper to absorb excess water on surface and then reweighed. The difference in weight before and after soaking was found out. The swelling index was calculated from the following expression [9];

$$E_{sw} = [W_s - W_0 / W_0] \times 100$$

Where E_{sw} = Percent swelling of carboxymethyl chitosan microparticles at equilibrium.

W_s = Weight of swelled microparticles at equilibrium.

W_0 = Initial weight of at microparticles equilibrium.

f) **Mucoadhesive Study:** The mucoadhesive properties of microparticles were evaluated by *in vitro* adhesion testing method, known as wash off method [10]. The pieces of goat intestinal mucosa (2×2 cm) were tied on glass slides (3×1 inch) by rubber band. About 50 microparticles were counted and spread over the wet rinsed tissue specimen and wait for 10 min and immediately

thereafter the support were hung on the arm of a USP tablet disintegrating test machine. By operating the disintegration machine the tissue specimen was given slow regular up and down movement in the 1 L vessel containing NaOH at 37± 2°C. At the end of each hour, the machine was stopped and number of microparticles still adhering on the tissue was counted [11, 12].

Where, Nt = Number of microparticles adhered to tissue after each time interval. N_0 = Number of microparticles applied

g) *In vitro* **Drug Release Study:** The dissolution studies of mucoadhesive microparticles were carried out in a USP dissolution apparatus II (TDT 08L Electrolab) at a rotation speed of 100 rpm in a 900 ml medium at 37 ±0.5°C. The microparticles were placed in muslin cloth and tied to the paddle and transferred to dissolution medium NaOH and samples were taken at selected time intervals, filtered through Whatmann filter paper no. 41 and analyzed by UV spectrophotometer 2210 (Systronics, Ahmedabad) at 297 nm. The drug release data of the *in vitro* dissolution study was analyzed with various kinetics equations like zero order, first order, matrix, Peppas and Hixon crowell model. Coefficient of correlation (r) values were calculated for linear curves obtained by regression analysis of the plots [13].

h) **Stability studies** [14] **:** Stability studies were carried out at various temperatures. The samples were wrapped in a butter paper and placed in petri dishes. These were stored at room temperature (27±2°C) and at elevated temperature (45±2°C) for a period of 1 month. Then microparticles were analyzed for physical changes such as color, texture and entrapment efficiency.

RESULTS AND DISCUSSION:

a) **FT-IR Studies:** Drug polymer compatibility studies were carried out using FTIR spectroscopy to establish any possible interaction of Pantoprazole sodium with the polymer used in the formulation as shown in **Figure 1 to Figure 6**. Thus, results indicated that the characteristic absorption peak due to pure Pantoprazole sodium have appeared in the formulated microparticle, without any significant change in their position indicating no chemical interaction between Pantoprazole sodium and polymers.
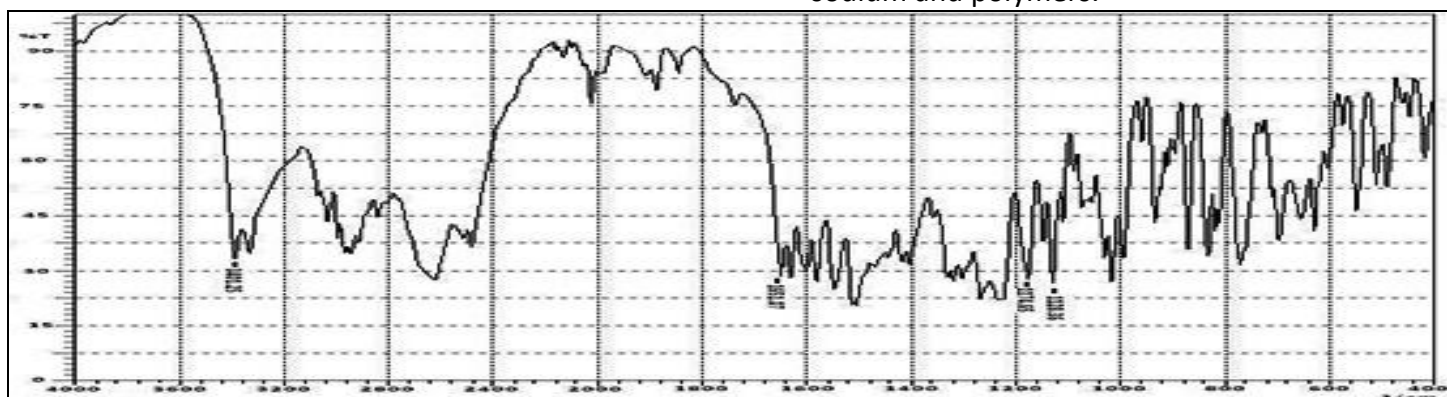

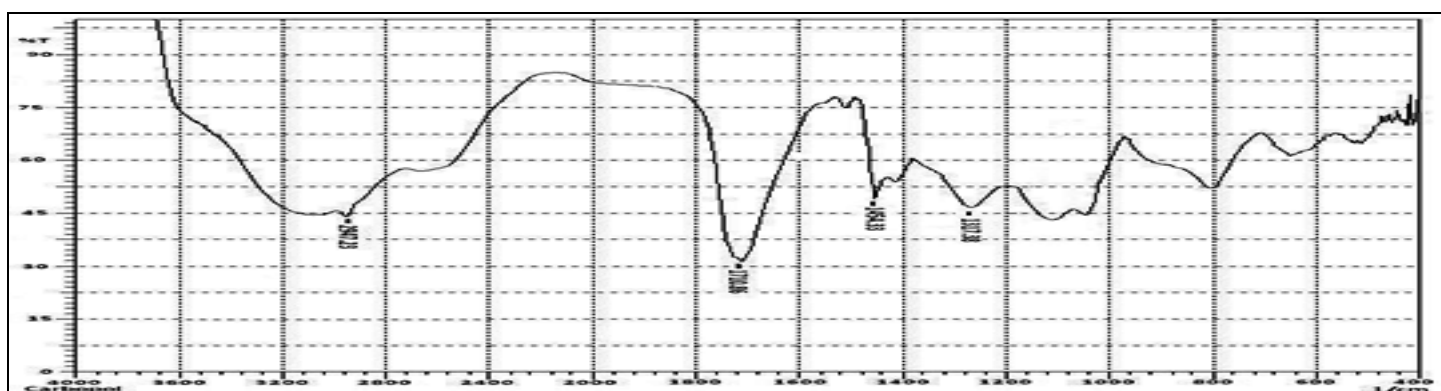FIGURE 1: IR SPECTRA OF PURE PANTAPRAZOLE SODIUM
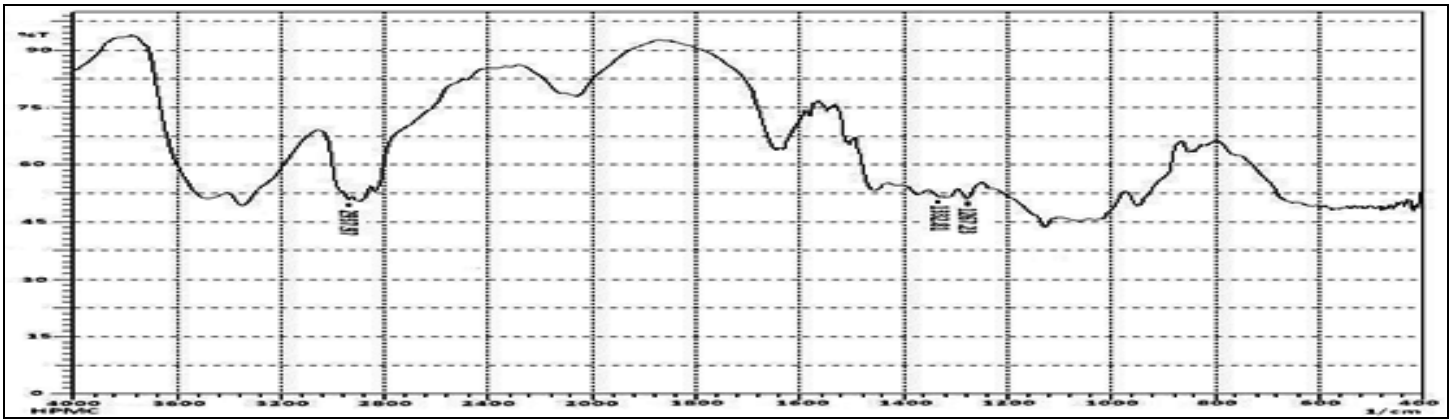

FIGURE 2. IR SPECTRA OF CARBOPOL 934

FIGURE 3: IR SPECTRA OF HPMC K15

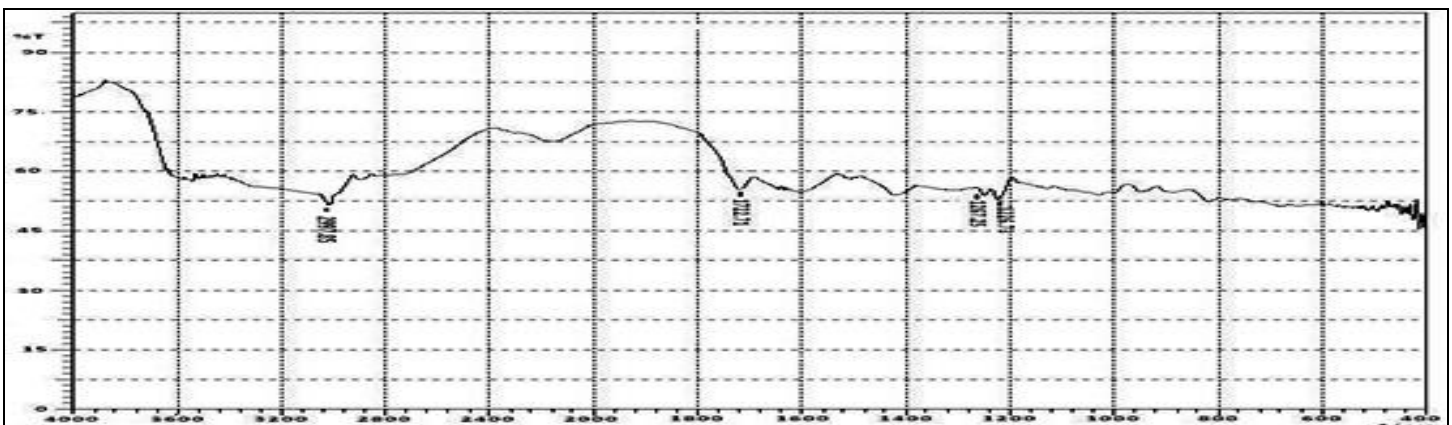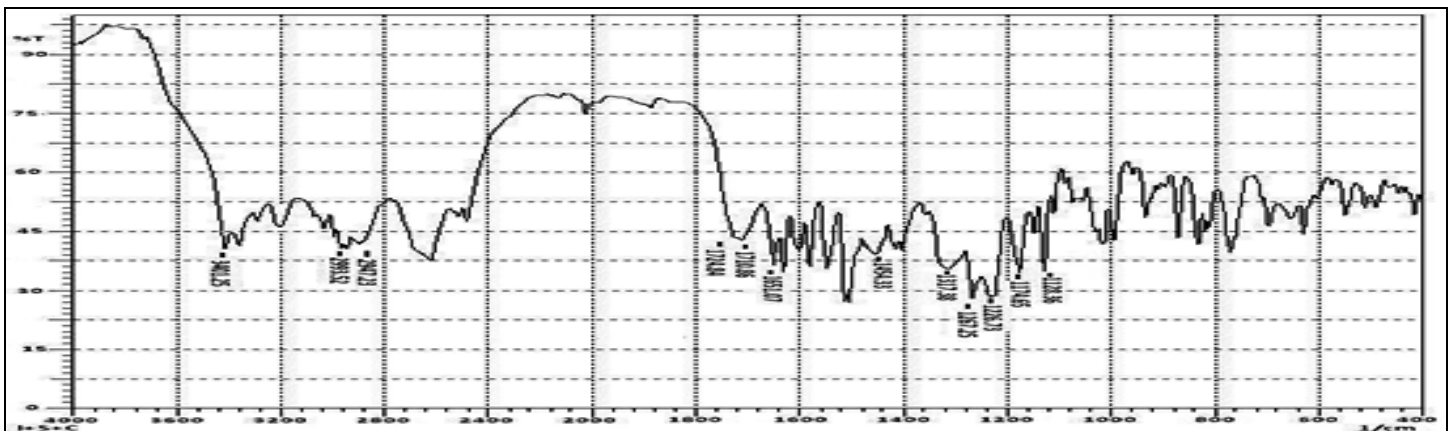**FIGURE 4: IR SPECTRA OF CARBOXYMETHYL CHITOSAN**

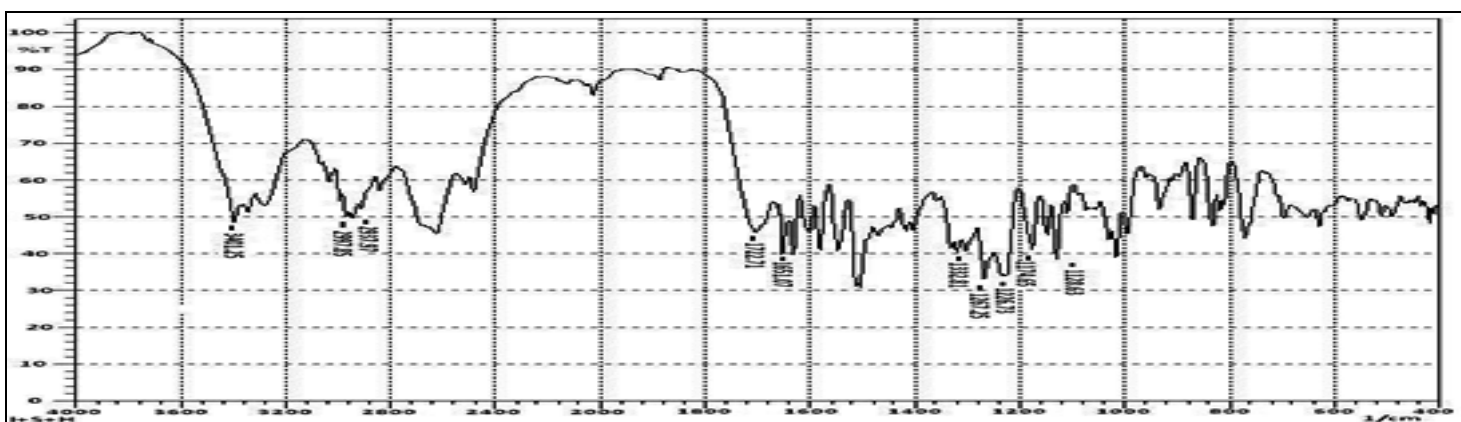**FIGURE 5: IR SPECTRA OF FORMULATION CMC-C4**

FIGURE 6. IR SPECTRA OF FORMULATION CMC- H3

b) **Micromeritic Properties:** The prepared formulations were evaluated for bulk density, tapped density, carr's index and hausner's ratio, angle of repose. The results are shown in **Table 1.** The mean particle size increased with increasing polymer proportion which is due to a significant increase in viscosity, thus leading to an increased droplet size and finally a higher microparticle size. Microparticles of Pantoprazole sodium using carboxymethyl chitosan in combination with Carbopol 934 exhibited a size range of 780.18 µm to 963.5 µm and microparticles of Pantoprazole sodium using carboxymethyl chitosan in combination with HPMC K15 exhibit a size range of 765.7 µm to 939.4µm. Increase in the ratio of polymer tends to form the particles more spherical and obtained uniform size spheres. The difference in the shape of microcapsules is observed, representing that microparticle containing higher amount of carboxymethyl chitosan are more spherical and regular as compared to that of microparticle having lower percent of carboxymethyl chitosan. Such results may be due to as the polymer proportion increases the spherical nature of microparticle also increases.

TABLE 1. MICROMERITICS PROPERTIES OF MUCOADHESIVE MICROPARTICLE

Batch ratio	Bulk density (g/ml)	Tapped density (g/ml)	Carr`s index (%)	Hausner ratio	Angle of repose (θ)
CMC-C1	0.975	1.170	16.66	1.20	33.69
CMC-C2	0.871	1.016	14.33	1.16	29.74
CMC-C3	1.012	1.157	12.53	1.14	29.05
CMC-C4	0.969	1.145	15.41	1.18	27.14
CMC-C5	1.039	1.228	15.39	1.18	26.00
CMC- H1	1.13	1.36	16.91	1.20	31.21
CMC- H2	0.885	1.03	14.32	1.16	29.05
CMC- H3	0.928	1.08	14.07	1.16	27.75
CMC- H4	1.344	1.512	11.14	1.12	27.14
CMC- H5	0.950	1.055	9.95	1.11	24.94

c) **Scanning Electron Microscopy:** The photographs of the optimized batch taken by Scanning electron microscopy are shown in (**Figure 7 and 8**). The SEM Photograph indicated that the microparticles were spherical and completely covered the coat polymer. The results revealed that the microparticles containing higher amount of carboxymethyl chitosan are more spherical and regular as compared to microparticle containing lower amount of carboxymethyl chitosan, indicates that the spherical nature of microparticle depends on the concentration of polymer.
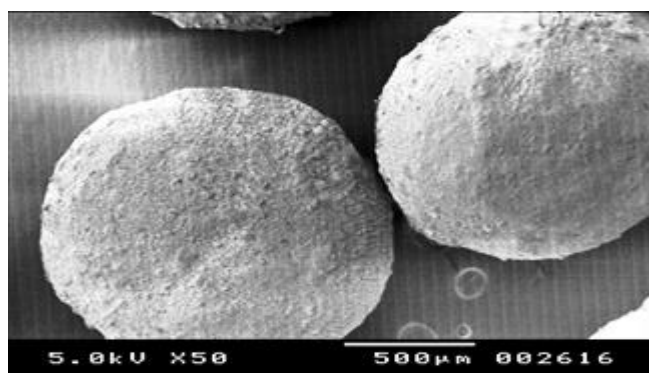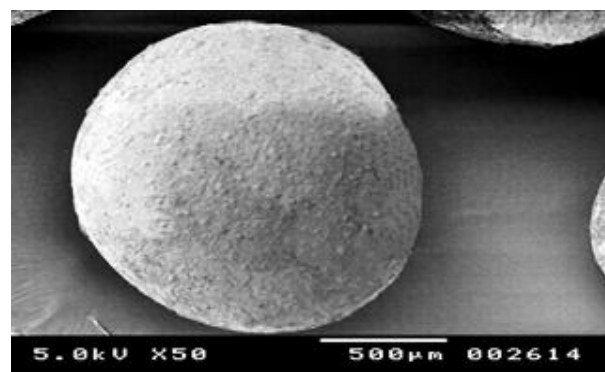

FIGURE 8: SEM OF OPTIMIZED BATCH CMC- H3

FIGURE 7: SEM OF OPTIMIZED BATCH CMC-C4

d) **Production yield, Entrapment efficiency and Swelling index:** The formulated microparticles were evaluated for production yield, drug entrapment efficiency and swelling index. The results are shown in **Table 2**. The result revealed that the production yield of batch CMC-C1 to CMC-C5 was found in the range 74 to 92 % and that of CMC- H1 to CMC- H5 was found in the range of 70 to 89 %. Percentage Drug Entrapment efficiencies (%EE) of batch CMC-C1- CMC-C5 was found in the range of 54.12 ± 0.48 to 81.68 ± 0.15 % and of batch CMC- H1- CMC- H5 was found in the range of 41.32 ± 0.33 to 78.4 ± 0.33 %.

TABLE 2: PRODUCTION YIELD, ENTRAPMENT EFFICIENCY AND SWELLING INDEX OF MUCOADHESIVE MICROPARTICLE

Batch Code	Product yield (%)	EE (%)[*]	Swelling index
CMC-C1	74	54.12 ± 0.48	0.40
CMC-C2	92	64.10 ± 0.21	0.55
CMC-C3	90	67.28 ± 0.36	0.85
CMC-C4	90.28	78.71 ± 0.39	0.95
CMC- C5	85	81.68 ± 0.15	1.05
CMC- H1	70	41.32 ± 0.33	0.25
CMC- H2	83.2	53.1 ± 0.62	0.45
CMC- H3	86.6	68.4 ± 0.49	0.70
CMC- H4	78.8	72.1 ± 0.86	0.90
CMC- H5	89	78.4 ± 0.33	0.95

It was observed that the drug entrapment efficiency of the prepared microparticles increases progressively with an increase in the proportion of respective polymers. Carboxymethyl chitosan concentration along with mucoadhesive polymer increases may also reduce loss of drug in curing medium due to formation of dense matrix structure. Increase in polymer proportion increases the viscosity of the dispersed phase. The higher viscosity of the polymer solution at the highest polymer proportion would be expected to decrease the diffusion of the drug into the external phase which would result in higher entrapment efficiency. The higher incorporation efficiency was observed as the proportion of carboxymethyl chitosan increased. This may be attributed to the greater availability of active calcium binding sites in the polymeric chains and consequently the greater degree of cross linking as the quantity of carboxymethyl chitosan increased, resulting in the formation of nonporous microspheres.

The degree of swelling is expressed as the percentage of water in hydrogel at any instant during swelling. The swelling property of microparticles was studied in NaOH. As the polymer to the drug ratio increases, the degree of swelling increased from 40 to 105 % for microparticles of batch CMC-C1- CMC-C5 and 25 to 95 % for microparticles of batch CMC- H1- CMC- H5. It can be concluded from the data shown in Table 2 that with increase in the polymer ratio, the degree of swelling also enhanced. So it can be stated that amount of polymer directly affects the degree of swelling.

e) **Mucoadhesive Study:** Optimized batch CMC-C4 showed 50 % of microparticles were still adhering and CMC- H4 showed 46 % of microparticle remains adhere at the end of 8 hour. Mucoadhesive microparticles are anticipated to take up water from the underlying mucosal tissue by absorbing, swelling and capillary effects leading to considerable stronger adhesion. From the technique which was used for mucoadhesive strength determination it was found that Carbopol 934 had greater mucoadhesive strength than that of HPMC K15. Carbopol possess various carboxyl group, when mobile at the wet mucosal surface, they orientate these mucoadhesive site towards mucosa and make interaction through hydrogen bonding. Also greater swelling rate of Carbopol 934 results in large surface of polymer that is expand to the mucosal layer resulting in increase in no. of hydrogen bonding between the polymer and mucosal layer. Thus, increase the mucoadhesive strength of polymer.

f) ***In vitro* Drug Release Study:** The formulation coded as CMC-C4 and CMC-C5 containing carboxymethyl chitosan along with Carbopol 934, sustained release of drug up to 12 hr found to be 84.1 ±0.43 and 74.2 ±0.65 respectively. The formulation CMC- H4 and CMC- H5 containing carboxymethyl chitosan along with HPMC K15 also showed sustained release of 82.6 ±0.43 and 71.4 ±0.46 was observed with increase in ratio of polymer at the end of 12 hr. It is attributed to fact that carboxymethyl chitosan hydrated faster under alkaline condition and built up the diffusion barrier more rapidly resulting in slower release in the basic phase. As the polymer to the drug ratio was increased the extent of drug release decreases. A significant decrease in the rate and extent of the drug release is due to the higher density of polymer matrix that results in increased diffusion path length through which the drug molecule have to traverse. The release would depend on diffusion of Pantoprazole through the insoluble matrix of carboxymethyl chitosan polymer in NaOH and a sustained drug release behavior was observed.

g) **Stability Studies:** Two formulation coded CMC-C4 and CMC- H4 were chosen for stability studies. The stability of preparation is an important factor to estimate the quality of dosage form. The stability data did not show any significant change in color, texture and entrapment efficiency. Thus we may conclude that, the drug does not undergo degradation on storage.

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