PREPARATION AND CHARACTERIZATION OF FAMOTIDINE MICROCAPSULE EMPLOYING MUCOADHESIVE POLYMERS IN COMBINATION TO ENHANCE GASTRO RETENTION FOR ORAL DELIVERY

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ABSTRACT

A new sustained release microencapsulated drug delivery system employing sustained release polymers in combination has been proposed in this present study. The microcapsules were formulated by orifice ionic gelation technique using famotidine as the model drug and polymers combination forms like (carbopol-934 and hydroxy propyl methyl cellulose, carbopol-934 and sodium carboxy methyl cellulose, carbopol-934 and methyl cellulose, carbopol-934 and guar gum) against carbopol-934 only. Microcapsules were evaluated for particle size, percentage yield, flow properties, drug entrapment efficiency, surface morphology by scanning electron microscopy (SEM), sphericity measurement, percentage moisture loss, , wall thickness, swelling property, *in vitro* drug release profile, drug release kinetic study and mucoadhesion study by *in vitro* wash off test. The effect of drug and different polymer combination on *in vitro* drug release profile was examined. The famotidine microcapsules with good structure and satisfactory yield were produced. Microcapsules employing sustained release polymers used in combination, exhibited slow release of famotidine over 9 hours with zero order release kinetic fashion. It was concluded that the polymer possess substantial release controlling properties used in combination that could be used for sustained drug delivery. All data were verified statistically by employing one way ANOVA and found to be significant at 5 % level of significance.

Keyword : Famotidine, Microencapsulation, Mucoadhesion

INTRODUCTION

Sustained release (SR) drug delivery system significantly improve therapeutic efficacy of a drug. Drug release retarding polymers are the key performer in such systems. Much of the development in SR drug delivery systems is focusing in the preparation and use of polymers with specificity designed macroscopic and microscopic structural and chemical features. Number of natural, semi synthetic and synthetic polymer materials are used in the controlled delivery of drugs. Recent trend towards the use of vegetable and nontoxic products demands the replacement of synthetic additives with natural one[1]. The natural materials have been extensively used in the field of drug delivery for their easy availability, cost effectiveness, ecofriendliness, capable of multitude of chemical modifications, potentially degradable and compatible due to natural origin. Past research therefore studied and acknowledged various natural gum like agar, guar gum, chitosan, xanthium, sodium alginate and lotus bean gum etc. for potential pharmaceutical and biomedical application[2]. The development of efficient orally delivered mucoadhesive drug delivery system includes advantages like, enhanced bioavailability, targeted specific delivery to specific region of the GI tract, maximized absorption rate due to intimate contact with the absorbing membrane, improved drug protection by polymer encapsulation and longer gut transit time resulting in extended periods for absorption. Famotidine, a potent H_2-receeoter

antagonist was widely used in the treatment of peptic ulcer in a dose of 20 mg b.i.d associated with adverse effects like diarrhoea, dizziness, headache and anorexia etc. The plasma half life of drug was 2.5-3 hour as reported in literature, which may exhibits toxic effect in prolong use[3]. Hence an attempt was made in this current study to evaluate the efficacy of combine polymers in designing of sustained release mucoadhesive famotidine microcapsule for oral delivery.

MATERIALS AND METHODS

Materials

Famotidine was received as a gift sample from Nicholas Piramol India limited, Mumbai. Carbopol-934 was procured from Corel Pharma Ltd., Ahmadabad. Sodium carboxy methyl cellulose (SCMC), hydroxyl propyl methyl cellulose (HPMC), methyl cellulose and guar gum were obtained from S.D. fine chemicals, WB. All other chemical and reagents used in this study were of analytical grade and procured from authorized dealer.

Preparation of microcapsules[4]

Mucoadhesive microcapsule were prepared by orifice ionic gelation method with polymers combinations such as carbopol and HPMC, carbopol and SCMC, carbopol and methyl cellulose, carbopol and guar gum, against carbopol only, in the drug-polymer ratio of 1:3 w/w using famotidine as model drug. Famotidine loaded microcapsule were prepared by ionic gelation method. Briefly 200 mg of sodium alginate, 100 mg of polymer (First carbopol-934 alone and then carbopol-934 with other polymers in ratio of 1:1 w/w) and 100 mg of drug were dispersed in 10 ml water with a constant stirring at 300 rpm for 30 min. The resultant dispersion was added drop wise through a syringe (17 gages) into the $CaCl_2$ solution (10 % w/v). The so formed microcapsules (1:3 w/w ratio) were kept for 30 min for complete reaction and afterwards, microcapsule were recovered by filtration through a sintered glass filter, under vacuum, dried in hot air oven at 60°C for 1 hour.

Percentage Yield[5]

The microcapsules were evaluated for percentage yield and percent drug entrapment. The yield was calculated as per the equation,

$$\text{Percentage yield} = \frac{\text{Weight of microcapsule recovered}}{\text{Weight (drug + polymer)}} \times 100$$

Particle size measurement[5]

The size of the prepared microcapsules was measured by the optical microscopy method using a calibrated stage micrometer. Particle size was calculated by using equation,

$X_g = 10 \times [(n_i \times \log X_i) / N]$, Where, X_g is geometric mean diameter, n_i is number of particle in range, X_i is the mid point of range and N is the total number of particles. All the experimental units were analyzed in triplicate (n=3).

Flow properties of microcapsules[6,7]

Flowability of microcapsules was investigated by determining Angle of repose, bulk density, Carr's index and Hausner ratio. The angle of repose was determined by fixed funnel method. The microcapsules were tapped using bulk density apparatus (Excel Enterprises, Kolkata) for 1000 taps in a cylinder and the change in volume were measured. Carr index and Hausner ratio were calculated by the formula,

Carr index (%) = $(D_f - D_0) \times 100 / D_f$ and Hausner ratio = D_f / D_0, where, D_f is poured density; D_0 is tapped density.

Drug entrapment efficiency (DEE)[5]

Drug loaded microcapsules (100 mg) were powdered and suspended in 100 ml water solvent system. The resultant dispersion was kept for 30 min for complete mixing with continuous agitation and filtered through a 0.45 μm membrane filter. The drug content was determined spectrophotometrically (UV-Visible-1700, Shimadzu, Japan spectrophotometer) at 265 nm using a regression equation derived from the standard graph ($r^2 = 0.9978$). The drug entrapment efficiency (DEE) was calculated by the equation, DEE = (Pc / Tc) X 100, Where, Pc is practical content, Tc is the theoretical content. All the formulations were analyzed in triplicate (n=3).

Scanning Electron Microscopy (SEM)[8]

The SEM analysis was carried out using a scanning electron microscope (LEO, 435 VP, U.K.). Prior to examination, samples were mounted on an aluminium stub using a double sided adhesive tape and making it electrically conductive by coating with a thin layer of gold (approximately 20 nm) in vacuum. The scanning electron microscope was operated at an acceleration voltage of 05 kV and resolution of 4000.

Percentage moisture loss[9]

The famotidine loaded microcapsules was evaluated for percentage moisture loss which sharing an idea about its hydrophilic nature. The microcapsules weighed (W_1) initially kept in desiccator containing calcium chloride at 37°C for 24 hours. The final weight (W_2) was noted when no further change in weight of sample was observed.

Moisture loss = $[(W_1 - W2)/ W2] X 100$. All the experimental units were studied in triplicate (n=3).

Determination of sphericity[10]

The particle shape was measure by computing circulatory factor (S). The tracing obtained from optical microscopy were used to calculate Area (A) and perimeter (P).

This will indicate the approximate shape of the prepared microcapsule calculated by the equation, $S = P^2/ 12.56 \times A$. All the experimental units were studied in triplicate (n=3).

Loose surface crystals study[10]

The Famotidine encapsulated microcapsules prepared by different combination of polymers were evaluated by loose surface crystal study to observe the excess drug present on the surface of microcapsules. From each batch, 100 mg of microcapsule was shaken in 20 ml of 0.1N HCl for 5 min and then filtered through whatman filter paper 41. The amount of drug present in filtrate was determined spectroscopically and calculated as a percentage of total drug content. All the experimental units were studied in triplicate (n=3).

Determination of swelling properties[8]

The dynamic swelling property of microcapsules in the dissolution medium was determined. Microcapsules of known weight were placed in dissolution solution for 6 hr and the swollen microcapsules were collected by a centrifuge and the wet weight of the swollen microcapsules was determined by first blotting the particles with filter paper to remove absorbed water on surface and then weighing immediately on an electronic balance. The percentage of swelling of microcapsules in the dissolution media was

then calculated by using equation, $S_w = [(W_t-W_o)/W_o] \times 100$, where, S_w= percentage of swelling of microcapsules, W_t = weight of the microcapsules at time t, W_o = initial weight of the microcapsules. All the experimental units were studied in triplicate (n=3).

Determination of wall thickness[11,12]

Wall thickness of microcapsules was determined by method of Luu et al using equation, $h = [r\,(1\text{-}P)\,d_1/3\{Pd_2 + (1\text{-}P)\,d_1\}] \times 100$, where, h= wall thickness, r = arithmetic mean radius of microcapsules, d_1 and d_2 are densities of core and coat material respectively, P is the proportion of medicament in microcapsules. All the experimental units were studied in triplicate (n=3).

In vitro drug release[13]

In vitro drug release study was carried out in USP type-II dissolution test apparatus. Microcapsules were placed in basket of dissolution vessel containing 900 ml of 0.1N HCl maintained at (37±1) °C and stirred at 50 rpm. Aliquots of samples (5 ml) at an interval of 1 hour were withdrawn and filtered through a whatman filter paper. The samples were analyzed for famotidine content by UV-Visible spectrophotometer at 265 nm .All the experimental units were analyzed in triplicate (n=3).

In vitro drug release kinetic studies

Kinetic model had described drug dissolution from solid dosage form where the dissolved amount of drug is a function of test time. In order to study the exact mechanism of drug release from the microsphere, drug release data was analyzed according to zero order[14], first order[15], Higuchi square root[16], Korsmeyer-Peppas model[17]. The criteria for selecting the most appropriate model were chosen on the basis of goodness of fit test.

Mucoadhesion test by *In vitro* wash off method[18]

A piece of stomach mucosa (5 × 2 cm) was taken from local slaughter house. It was mounted on to glass slides with adhesive. About 100 microcapsules were spread on to each wet rinsed tissue specimen and immediately thereafter the support was hung on the arm of a USP tablet disintegrating test machine. By operating the disintegrating test machine the tissue specimen was given a slow regular up and down movement in the test fluid at 37°C taken in the vessel of the machine. At the end of every one hour up to 10 hours, the machine was stopped and number of microcapsules still adhering onto the tissue was counted.

Statistical Analysis[19]

Statistical data analyses were performed using the one way ANOVA at 5 % level of significance ($p < 0.05$) and standard error mean (SEM).

RESULTS AND DISCUSSIONS

The composition of famotidine loaded mucoadhesive microcapsules using combined polymers in ratio of 1:3 w/w of drug polymer was designed and prepared by orifice ionic gelation method. The obtained microcapsules were found to be non aggregated. The generalized microparticulation protocol depends on, choice of ingredient, successful preparation of microcapsules and optimization at every preparative steps. The percentage yield was found to be in the ranges of 67.7 to 87.5 % and the yield was found satisfactory in all the formulations as reported in Table 1. The formulation F3 is showing maximum yield. The optical microscopy revealed that all microcapsules thus obtained, were opaque, discrete and spherical particles with smooth

surfaces further confirmed by SEM study. The geometric diameter of the microcapsules lies in the ranges of 380.1 to 723.4 μm as shown in Table 1. All the formulations having excellent flow properties as given in Table 1. The drug entrapment efficiency (DEE) of all the formulations was reported as high profile ranges from 30.3 to 96.7 % may be due to use of polymers in combination, as abridged in Table 1. The maximum DEE was shown by formulation F3. The shape of famotidine microcapsule as evidenced from the scanning electron photomicrograph was spherical and uniformly distributed shown in Fig 1. The percentage of moisture loss was found in a range 2.24 to13.26 % tabularized in Table 2. The minimum moisture content was observed with formulation F3. The results ensure the presence of diminutive water content which can be due to the involvement of water in process method and hydrophilic property of mucoadhesive polymers. But the lessen proportion of water obtained indicates its proper drying and instant hardening of microcapsule due to quick gelation occurred between calcium chloride and sodium alginate facilitate the storage behavior of the formulations. The famotidine loaded mucoadhesive microcapsules obtained having circularity factor very close to 1.00, which confirm their sphericity, as represented in Table 2. These loose surface crystal studies lend a hand to estimate the excess amount of drug attached on the surface of microcapsules after a successful drug entrapment. The study was executed with various prepared formulations and the results were tabularized in Table 2. The swelling indexes of microcapsules were found satisfactory and shown in Table 2, indicates the hydrophlilicity property of the polymers with

establishing the fundamentals that the increase in swelling index may depends on the use of polymers in combination in formulations. The wall thickness was found to be highest 3.888 μm for F2 in comparison to other formulations as shown in Table 2. The wall thickness of the microcapsules mainly built up with polymer content in formulations. The i*n vitro* famotidine releases from microcapsules prepared by combination of polymers were studied. All the formulations were found to be release famotidine in a controlled manner for a prolonged period over 9 hours which is represented graphically in Fig 2. Among all the formulations, F3 was found to release famotidine in a controlled manner with constant fashion over extended period of time. To illustrate the kinetic of drug release from microcapsules, release data was analyzed according to different kinetic equations depicted in Table 3. Release data of all formulations except F1 and F5 following zero order kinetic showing a constant release profile, independent of formulation variations. The drug release of the formulations F1 and F5, have a diffusion controlled release pattern which is dependent on concentration of release retarding polymer as they are best fit in Higuchi square root kinetic model. The Table 3 showed that all the formulations release the drug by diffusion following Fickian ($n<0.5$) transport mechanism except the formulation F5, which follow non-Fickian ($n>0.5$) transport mechanism. Statistical verification with one way ANOVA method attested the fact that the drug release data were found significant at 5 % level of significance ($p < 0.05$). The order of mucoadhesion property among all the formulations was found as F1>F2>F3>F4>F5, as shown in Fig 3. The formulations F1, F2 and F3, are showing good bioadhesion.

Table 1 : Yield, particle size, flow properties and DEE of various famotidine microcapsule formulations.

Formu- lations Code	Yield (%) (X±S.D.)	Particle size D_g (µm) (X ± S.D)	Carr's index	Hausner's ratio	Angle of repose	DEE (%) (X±S.D.)
F1	56.2±0.1	512.2 ± 0.031	10.11	1.09	24.8°	49.2±0.04
F2	85.9±0.2	558.4 ± 0.017	8.94	1.02	22.2°	44.8±0.21
F3	87.5±0.5	427.2 ± 0.028	9.18	1.04	21.7°	96.7±0.12
F4	67.7±0.3	739.3 ± 0.018	8.16	1.01	21.7°	30.3±0.16
F5	82.9±0.4	518.4 ± 0.011	9.34	1.03	20.1°	32.4±0.18

F1 – Drug: Carbopol-934 (1:3), F2 - carbopol and HPMC (1:3), F3 - carbopol and SCMC (1:3), F4 - carbopol and methyl cellulose (1:3), F5 - carbopol and guar gum (1:3).

Table 2 : Physical parameters of various famotidine microcapsule formulations.

Formu- lations Code	Moisture loss (%) (X±S.D.)	Circularity factor (s) (X±S.D.)	Surface drug content (%) (X±S.D.)	Swelling index (%) (X±S.D.)	Wall thickness (µm) (X±S.D.)
F1	4.11±0.014	1.01± 0.001	15.91±0.23	80±0.13	2.051±0.21
F2	3.19±0.032	1.01±0.002	16.74±0.31	80±0.33	3.117±0.18
F3	2.24±0.017	1.05±0.005	11.03±0.12	87±0.23	1.808±0.25
F4	13.26±0.025	1.06±0.001	24.25±0.32	73±0.13	1.248±0.14
F5	2.47±0.023	1.01±0.004	20.41±0.13	69±0.43	0.774±0.27

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	21293.043	4	5323.26	27.8949	0.03221	2.86608
Within Groups	383.11321	20	19.1557			
Total	21676.156	24				

Table 3 : Drug release kinetic studies of microcapsule formulations.

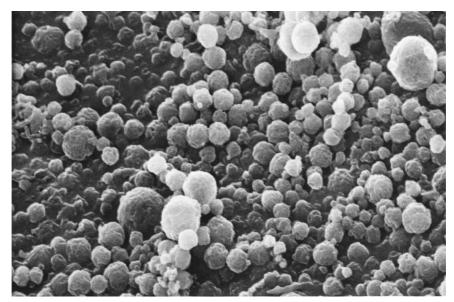| Formulations	Zero order kinetics	First order knetics	Higuchi square root equation	Korsmeyer-Peppas model	
	Regression co-efficient (r)				n
F1	0.9818	0.9131	0.9997	0.9872	0.4050
F2	0.9997	0.8968	0.9832	0.9543	0.2034
F3	0.9988	0.9200	0.9936	0.9234	0.4870
F4	0.9957	0.9144	0.9875	0.9675	0.2857
F5	0.9830	0.9106	0.9990	0.9788	0.5150

Fig. 1 : Scanning electron photomicrograph of prepared microcapsules (f3) under lower resolution 5 Kv x 200.
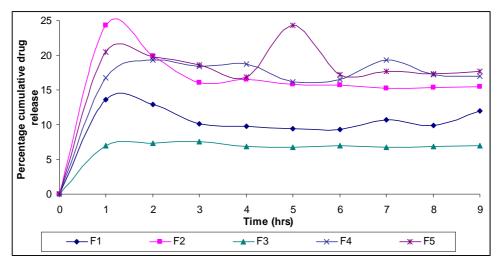

Fig. 2 : *In vitro* **drug release profile of different famotidine microcapsule formulations.**
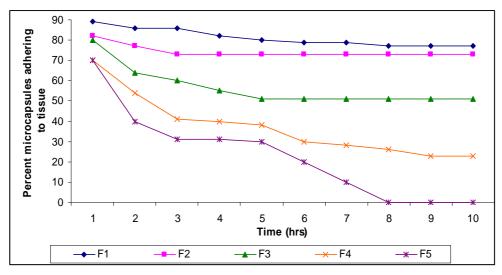

Fig. 3 : Mucoadhesion measurement of various famotidine microcapsules by *in vitro* **wash-off test.**

CONCLUSION

The formulation F3 containing drug: polymer ratio 1:3 with polymer combination of carbopol-934 and SCMC was found to be the best microcapsule formulation, regarding all the properties evaluated in order to achieve objective of this study. The novel formulation design facilitated the optimization and successful development of famotidine microcapsule formulations. Our data concluded that choice of combination of polymers instead of single polymer may be an effective strategy for the designing and development of famotidine mucoadhesive microcapsule for easy, reproducible and cost effective method to prove its potential for safe and effective controlled for oral drug delivery therapy.

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