

Preparation and *In Vitro* Characterization of Microparticles Loaded with Cimetidine: Analysis of Dissolution Data using DDSolver

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SUMMARY. Preparation and *in vitro* characterization of floating microparticles loaded with cimetidine (FMC) for oral delivery was the objective of this study. Non-solvent addition coacervation technique using hydroxypropylmethylcellulose (HPMC) as the rate controlling polymer was employed to achieve FMC. Three formulations of FMC were prepared and optimized regarding encapsulation efficiency and dissolution kinetics. Among all formulations, FMC2 having 1:3 ratio of cimetidine:HPMC exhibited the better *in vitro* performance regarding encapsulation efficiency and dissolution kinetics than that of FMC1 and FMC3. In conclusion, the FMC can be designed via non-solvent addition technique.

INTRODUCTION

Cimetidine, histamine H₂ receptor antagonist (H₂ blocker), is widely prescribed for the treatment of gastro-esophageal reflux disease, ulcers and heartburn ^{1,2}. In general, it increases survival after gastric cancer and in particular the colorectal cancer ³.

Modified release formulations have been widely employed to get improved therapeutic objectives with numerous drugs ⁴. However, many physiological issues are faced during the preparation processes, *i.e.* failure to confine and control the dosage form within the desired physiological area of the gastrointestinal tract and the extremely uneven nature of the gastric emptying course, which may cause the unpredictable bioavailability and pharmacokinetics. However, the developments of gastro-retentive dosage forms having capability of stay for several hours in the gastric area has resulted in the considerable improvement in the bioavailability extending the gastric residence time of drugs and improving the solubility of drugs that are less soluble in high pH medium. Gastric retention of drug may also make easy the local drug delivery to the stomach and proximal small intestine ⁵.

The preparation of a system that remains buoyant in stomach environment due to its lower density compared to the gastric fluids has gained much importance. Alternatively, a floating system consisting of multiple units exhibit relative advantages compared to a single unit formulation ⁶ such as reduced dose dumping and controlled release of drug ^{7,8}. The idea of floating microparticles can also be used to reduce gastric irritation of weakly acidic drugs by evading direct exposure of the mucosa to drug and thus making available drug in low dosage for prolonged periods ⁶.

Microencapsulation is a method that is employed in the preparation of microparticle-based formulations. A number of microencapsulation methods such as non-solvent addition coacervation have been devised to prepare microparticles of different physico-chemical properties ⁹.

Therefore, this study involved the preparation of floating microparticles loaded with cimetidine (FMC) for the oral delivery and then *in vitro* characterization. The FMC were developed by using non-solvent addition coacervation technique and hydroxypropylmethylcellulose (HPMC) as the rate controlling polymer.

KEY WORDS: Cimetidine, Hydroxypropylmethylcellulose (HPMC), *In vitro* characterization, Microparticles.

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Materials and methods

Materials

Hydroxypropylmethylcellulose (HPMC, 40-60 cP) was supplied by Fluka (Switzerland). Cimetidine was gifted by Stand Pharma, Lahore, Pakistan. Dichloromethane and n-hexane were supplied by Merck (Germany). Liquid paraffin was provided by BDH (UK). All other chemicals were used without further purification and were purchased through commercial sources.

Preparation of microparticles

For the preparation of floating microparticles loaded with cimetidine (FMC), HPMC was dissolved in dichloromethane (1 g of polymer per 20 mL) at room temperature and cimetidine (1 g) was added to the polymer solution. For phase separation, liquid paraffin (two times the volume of dichloromethane) was incorporated slowly into the drug polymer solution with constant stirring for 2 h using magnetic stirrer operated at a speed of 700 rpm. The resulting drug polymer microparticles were filtered and three times washed with n-hexane and dried in air and oven at 40 °C to ensure complete removal of all solvents⁵. The microparticles (FMC1, FMC2 and FMC3) with different ratios (1:1, 1:2, and 1:3) of drug to polymer concentration were prepared and evaluated by *in vitro* testing.

Percentage yield

The dried microparticles were weighed to calculate the percentage yield of formulation obtained using Eq. [1]:

$$\text{Percentage yield} = \frac{\text{Total amount of microparticles}}{\text{Total weight of drug and polymers}} \times 100 \quad [1]$$

Buoyancy percentage

A weighed quantity (0.3 g) of dried microparticles was spread over the surface of a medium (900 mL of 0.1 M HCl containing 0.02% Tween 80) in USP type II dissolution apparatus. The medium was agitated for 12 h using a paddle at a speed of 100 rpm. The settled and the floating portions of FMC were separate, dried and weighed to calculate percentage buoyancy using Eq. [2]¹⁰.

$$\text{Percentage buoyancy} = \frac{\text{Microparticles remained floating}}{\text{Total mass of microparticles}} \times 100 \quad [2]$$

Percentage drug loading

Cimetidine content in FMC was evaluated by thoroughly triturating the weighed sample of microparticles and suspended in a minimal vol-

ume of methanol (10 mL). To separate HPMC precipitates, the suspension was adequately diluted with water and filtered. To calculate drug content, the filtrate was analyzed spectrophotometrically at 218 nm using Eq. [3]¹¹.

$$\text{Encapsulation efficiency} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100 \quad [3]$$

Size measurements

Size distribution of FMC was determined by optical microscopy using stage micrometer, slide and calibrated eyepiece by counting at least 100 microparticles.

In vitro release of drug from microparticles

In vitro drug release from FMC having 100 mg cimetidine and control formulation (immediate release tablets) was assessed using USP apparatus-I (Pharma Test, Germany) stirred at 100 rpm. The release studies were conducted in phosphate buffer pH 6.8 as dissolution media (900 mL) kept at 37 ± 0.5 °C. The 5 mL of dissolution samples were collected at predetermined time points using an automated collector after filtering through Whatmann filters (0.45 µm). To maintain the sink condition, the volume was replenished each time with the same amount of fresh dissolution fluid already maintained at 37 ± 0.5 °C. All drawn samples were analyzed at 218 nm for cimetidine assay using a double beam UV/VIS-spectrophotometer¹¹. All experiments were performed three times.

Application of kinetic models

The dissolution data of all FMC and control formulation was analyzed using various kinetics models such as zero order, first order, Higuchi, and Korsmeyer-Peppas¹² to elaborate the mode of drug release from formulations developed. Dissolution data was assessed employing DDSolver, new software designed for the kinetic analysis of dissolution profiles. DDSolver is a menu-driven add-in program for Microsoft Excel written in Visual Basic for applications¹³.

X-ray diffractometry (XRD)

X-ray diffractometry of FMC, cimetidine and HPMC was conducted to assess the influence of microencapsulation technique on the crystallinity of cimetidine using D8 Discover (Bruker, Germany). The samples were scanned in the 8 to 70° diffraction angle range under the following conditions: Cu-K_α radiation 1.5406 Å° (source), 4°/min scan speed, scintillation detector, primary slit 1 mm and secondary slit 0.6 mm¹⁴.

Thermal analysis

Differential scanning calorimetry (DSC) (TA Instruments, USA) of 10 mg powdered samples of FMC, cimetidine and HPMC were conducted to assess polymer-drug interaction. The samples were placed in an aluminum pan under a nitrogen flow of 40 mL/min at a heating rate of 10 °C/min¹⁴.

Micromeritics studies

By using the usual tapping process, tap density was assessed using a measuring cylinder (10 mL). Taped density and Compressibility index (Ci) were calculated by Eqs. [4] and [5], respectively⁹.

$$\text{Tapped density} = \frac{\text{Weight of microparticles}}{\text{Volume of microparticles after 100 tapings}} \quad [4]$$

$$Ci = \frac{\text{Initial volume} - \text{Final volume}}{\text{Initial volume}} \times 100 \quad [5]$$

Hausner's ratio, another index of flowability of microparticles is determined by Eq. [6]⁹:

$$\text{Hausner's ratio} = \frac{\text{Volume before taping}}{\text{Volume after taping}} \quad [6]$$

Angle of repose was measured by funnel method. The radius (r) of cone base and the height (b) of the heap formed were measured for calculation of angle of repose (θ) by Eq. [7]⁹:

$$\theta = \tan^{-1}b/r \quad [7]$$

where r = radius of heap and b = height of heap.

Stability studies

The FMC (1 g) were sealed in an air-tight amber color glass bottles and stored at 20 °C/60% RH, 30°C/65% RH, and 40 °C/75% RH. The microparticles were assessed for drug content and dissolution behavior (as described above) weekly for eight weeks. One bottle was used in each assessment⁹.

Statistical analysis

One-way ANOVA for significance at $P < 0.05$ was used for analysis of release profiles using SPSS version 12.0.

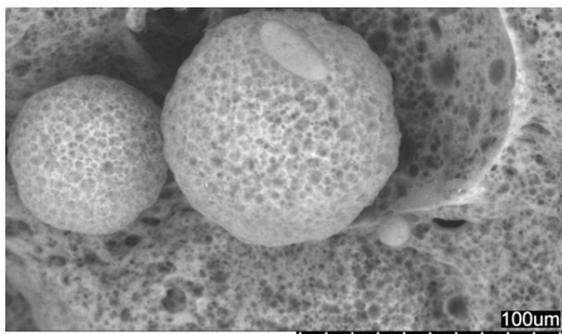


Figure 1. Scanning electron microscopic photograph of FMC2.

RESULTS AND DISCUSSION

Physico-chemical features of microparticles

The SEM photograph (Fig. 1) of FMC2 exhibits round and smooth surface of microparticles.

Mean size, encapsulation efficiency and percentage yield of FMC are given in Table 1. There is a significantly ($p < 0.05$) increasing trend in size and encapsulation yield with the increase in polymer concentration. Same pattern of the effect of HPMC concentration has already been presented previously⁵. The increase in size of microparticles from FMC1 (having lowest polymer concentration) to FMC3 (having highest polymer concentration) could be due to increase in thickness of polymer covering around the core drug. Non-significant ($p > 0.05$) change was observed increase in percentage yield. The microparticles floated for a prolonged time on the surface of the dissolution medium with a buoyancy of 63.4 to 67.7 % for all three formulations.

In vitro dissolution study of microparticles and release kinetics

In vitro dissolution study of microparticles was carried out by making them sink in the dissolution medium (phosphate buffer pH 6.8) using stainless steel sieves. The release of cimetidine from microparticles was calculated as cumulative percent drug release. After dissolution

Formulations	FMC1	FMC2	FMC3
Drug:Polymer	1:1	1:2	1:3
Encapsulation efficiency (Mean \pm S.D.) (%)	88.28 \pm 3.59	94.92 \pm 4.14	96.48 \pm 3.23
Percentage yield (Mean \pm S.D.)%	96.24 \pm 1.19	97.92 \pm 1.27	97.15 \pm 1.52
Size (Mean diameter) (Mean \pm S.D.), μ m	133.62 \pm 15.11	147.94 \pm 10.84	154.19 \pm 14.52

Table 1. Evaluation of physical characteristics of cimetidine microparticles.

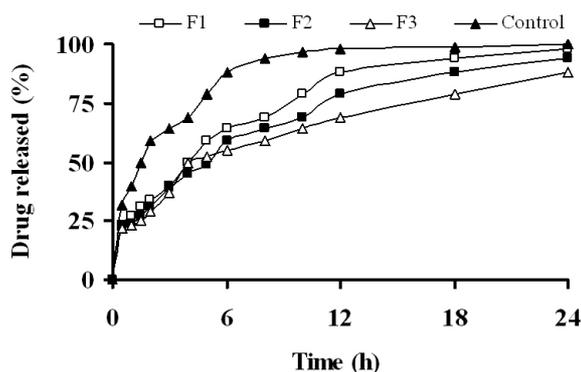


Figure 2. Release pattern of cetrizine from microparticles M1, M2 and M3.

of 24 h, the cumulative percentage drug release from the prepared formulation was greater than 80%.

The rate of drug release was highest (as evident from highest release rate constants) from formulation FMC1 as compared to FMC2 and FMC3, which could be due to the use of low polymer concentration in FMC1 compared to other two formulations (Fig. 2). However, the values of release rate constants are higher for control compared to FMC1 which indicates the sustained release of drug from microparticles compared to control (immediate release). The sustained release of drug from FMC is also confirmed from the values of $t_{50\%}$ (time for the release of 50% of total amount of drug). Most sustained release of drug was observed from FMC3 that contain highest amount of polymer contents. Cimetidine release was decreased with increase in polymer concentration, due to an increase in hydrophobic encapsulating wall thickness around the drug core. The drug release started with the swelling of HPMC, creation of channels acting as the route for drug and then

in due course cimetidine leached out into dissolution medium. These results are in accordance to the previous presentations^{5,15}.

For kinetic analysis of dissolution data, several mathematical equations have been reported in literature. The zero order model elaborates that drug release is independent of its concentration. The first order model shows that release rate is concentration dependent. Higuchi model describes that drug release from formulation matrix is directly proportional to square root of time. Korsmeyer-Peppas power law describes the drug release mechanism from polymeric systems¹⁶. Thus, dissolution data were fitted to these models to explain drug release mode from the FMC prepared. On the basis of best goodness of fit, the most appropriate model was selected. The drug release constant (k) and coefficient of determination (R^2) obtained from zero order, first order, Higuchi and Korsmeyer-Peppas models are presented in Table 2.

Among all kinetic models, Higuchi's model was best fit to the dissolution data as evident from the highest values of R^2 . It elaborates that the release of drug from formulations is diffusion controlled and is directly proportional to the square root of time. Diffusion based release of drug from FMC is also confirmed from the values of " n " ($n < 0.45$) obtained from the Korsmeyer-Peppas model.

X-ray diffractometry (XRD)

X ray diffraction patterns of FMC, cimetidine and HPMC in Fig. 3 were obtained and confirmed the presence of crystalline structure of cimetidine in the FMC. Cimetidine being entrapped into the HPMC offers different preferred orientation resulting in a different pattern, yet confirming its presence as a crystalline sub-

Models	Formulations	M1	M2	M3	Control
Zero Order	K_0	5.834	5.397	4.998	6.700
	$t_{50\%}$ (h)	8.571	9.265	10.005	7.463
	R^2	0.3082	0.3918	0.2667	-0.7118
First Order	K_1	0.178	0.144	0.127	0.390
	R^2	0.9585	0.9407	0.8786	0.9591
Higuchi	KH	23.317	21.423	19.998	28.104
	R2	0.9522	0.9717	0.9494	0.6663
Korsmeyer-Peppas	KKP	28.958	25.545	25.560	48.272
	R2	0.9781	0.9878	0.9846	0.9458
	N	0.408	0.426	0.396	0.267

Table 2. Data showing release behavior of microparticles prepared.

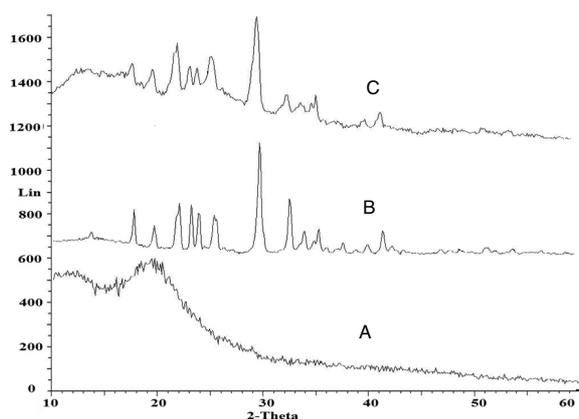


Figure 3. X-ray diffractograms of HPMC (A), cimetidine (B) and FMC (C).

stance. The grinding of the drug loaded microparticles prior to the analysis during sample preparation may also have affected its XRD pattern.

Thermal analysis

A sharp endothermic peak between 145 to 150 °C is present in the DSC thermogram of pure cimetidine (Fig. 4). The FMC thermograms also showed a similar endothermic peak in the same temperature range. This further confirms the absence of any chemical interaction between drug and polymer.

Micromeritics studies

Micromeritics of microparticles was expressed in terms of tapped density, Hausner’s ratio, compressibility index and angle of repose

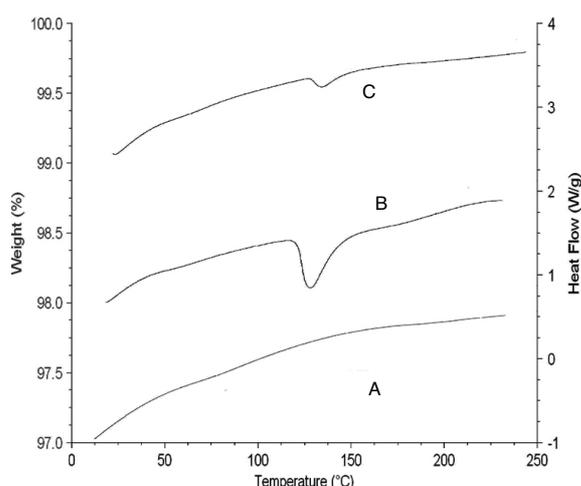


Figure 4. Thermal analysis (DSC) of HPMC (A), cimetidine (B) and FMC (C).

for its application in the preparation of microparticles. It can be concluded from results (Table 3) that FMC studied exhibit excellent flowability and compressibility.

Stability studies

The stability studies showed that microparticles remained stable at 40 ± 1 °C (HT) and 25 ± 1 °C (RT), so as to prepare stable microparticles. In Table 4, percentage residual drug contents of the M1, M2 and M3 microparticles are shown at 40 ± 1 °C (HT) and 25 ± 1 °C (RT) after storage of 1, 2, and 3 months. In percent residual drug content, non-significant (p > 0.05) changes were observed in the prepared formulations at ambi-

Formulations	M1	M2	M3
Taped density (g/mL)	0.186 ± 0.03	0.223 ± 0.07	0.239 ± 0.04
Hausner’s ratio	1.19 ± 0.16	1.25 ± 0.11	1.28 ± 0.12
Compressibility index (%)	16 ± 1.19	20 ± 1.45	22 ± 1.23
Angle of repose (degree)	26.32 ± 2.15	27.56 ± 3.79	27.98 ± 3.25

Table 3. Micromeritics studies of microparticles prepared.

Storage (Months)	Study at 25 ± 1 °C (RT) Percentage of cimetidine			Study at 40 ± 1 °C (HT) Percentage of cimetidine		
	M1	M2	M3	M1	M2	M3
0	100	100	100	100	100	100
1	98.64	98.92	99.4	98.31	98.41	98.29
2	98.11	98.43	98.84	98.01	97.89	97.76
3	97.92	98.06	97.74	97.08	97.39	97.15

Table 4. Percentage of cimetidine after stability studies of microparticles.

ent and accelerated conditions of storage indicating all formulations remained stable at room temperature and accelerated storage conditions.

CONCLUSIONS

The floating microparticles loaded with cimetidine can be prepared using hydroxypropylmethylcellulose as the rate controlling polymer through non-solvent addition coacervation method. The drug and polymer are compatible as depicted by x-ray diffractograms and differential scanning calorimetry. This study elaborates that the developed optimized floating microparticles loaded with cimetidine can maintain a therapeutic level of cimetidine for about 24 h, however in this context *in vivo* studies are in progress in our laboratory.

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