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Original article

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FORMULATION AND IN VITRO EVALUATION OF GASTRORETENTIVE FLOATING DRUG DELIVERY SYSTEM OF RITONAVIR

Moumita BISWAS[1], Roop Narayan GUPTA[1], Rabinarayan PARHI[2]*, Kalyan Kumar SETHI[2], Suvendu Kumar SAHOO[2].

[1]Birla Institute of Technology, Department of Pharmaceutical Sciences, Meshra, Ranchi, Jharkhand, INDIA
[2]GITAM University, GITAM Institute of Pharmacy, Gandhi Nagar Campus, Rushikunda, Visakhapatnam-530045, Andhra Pradesh, INDIA

Abstract

The purpose of the research work was to develop a floating drug delivery system of ritonavir (RN) in order to prolong the gastric residence time and increase its bioavailability. The floating tablets of RN were prepared by direct compression technique, using polymers such as different grades of hydroxypropyl methylcellulose (HPMC, Methocel E15LV, E50LV, K100LV and K4M) and polyvinyl pyrrolidone (PVP K30). Sodium bicarbonate was used as gas releasing agent. The formulations were optimized on the basis of matrix integrity, duration of floating, swelling behavior and in vitro drug release. Except series FA, where floating time was 10 hr, other series such as FB, FC and FD were showing more than 12 hr of floating time. The highest average swelling index of 170.68 ± 0.45 was found in FD3 batch. The in vitro drug release indicated extended release of RN and more than 62 percent of drug was released at the end of the 12 hr for all the batches. The mechanism of RN release from the floating tablets for FA, FB and FC series is anomalous diffusion transport and follows zero order kinetics, but FD series indicated Higuchi kinetics with release rate exponent (n) of 0.44. Further, the scanning electron microscopy showed porous structured formed on the tablet surface at different times (0, 3, 6, 9 and 12 hr) of dissolution for the selected batch FC3. Finally, FC3 batch showed no significant change in above parameters after storage at room temperature (28-32°C), 40°C and 50°C for one month.

Key words: *Ritonavir, Floating tablet, Extended release, Higuchi kinetics, Gas formation.*

Ritonavir'in Gastroretentif Yüzen İlaç Taşıyıcı Sisteminin Formülasyonu ve İn Vitro Değerlendirilmesi

Bu çalışmanın amacı Ritonavir (RN)'in gastrik kalış zamanını uzatmak ve biyoyararlanımını artırmak için yüzen ilaç taşıyıcı sistemini hazırlamaktır. RN yüzen tabletleri, farklı hidroksipropil metil selüloz (HPMC, Methocel E15LV, E50LV, K100LV ve K4M) and polivinil pirolidon (PVP K30) türevleri kullanılarak direk basım tekniği ile hazırlanmıştır. Sodyum bikarbonat gaz salım ajanı olarak kullanılmıştır. Formülasyonlar matriks bütünlüğü, yüzme süresi, şişme davranışı ve in vitro ilaç salımı temel alınarak optimize edilmiştir. Yüzme süresi 10 saat olan FA serisi hariç FB, FC ve FD gibi diğer seriler 12 saatten fazla yüzme zamanı göstermiştir. En yüksek ortalama şişme indeksi 170.68 ± 0.45 olarak FD3 serisinde tespit edilmiştir. İn vitro salım çalışmalarında bütün serilerde 12 saat sonunda % 62'den daha fazla RN uzatılmış salımı elde edildiği bildirilmiştir. FA, FB ve FC serilerinden RN salım mekanizmaları anormal difüzyon transportu ve takiben sıfırıncı derece kinetiği göstermiştir ancak FD serileri salım hızı katsayısı (n) 0.44 olan Higuchi kinetiğini göstermiştir. Buna ilaveten, taramalı elektron mikroskopisi seçilmiş seri olan FC3 için değişik zaman aralıklarında (0,3,6,9 ve 12 saat)tablet yüzeyinde poröz yapı oluştuğunu göstermiştir. Sonuç olarak FC3 serisinde oda sıcaklığı (28-32°C), 40°C ve 50°C'de 1 ay saklama sonrasında önemli bir değişiklik bulunmamıştır.

Anahtar kelimeler: *Ritonavir, Yüzen tablet, Uzatılmış salım, Higuchi kinetiği, Gaz oluşumu.*

***Correspondence:** E-mail: bhu_rabi@rediffmail.com; Mobile tel: 09052983544

INTRODUCTION

Ritonavir (RN) is a Protease Inhibitor widely prescribed in anti-retroviral regimen. It blocks the HIV protease, thereby reducing the viral load in the infected individual (1). In spite of its favorable clinical response, some specific problems associated with it are low oral bioavailability. This is due to degradation of RN by the cytochrome P45-3A4 (CYP3A4) isoenzymes in the distal intestine (2), efflux of the absorbed drug by counter transporter proteins (mainly P-glycoprotein) present in the distal intestine (3,4) and is unstable at alkaline pH (5). It shows pH dependent solubility and solution stability (solubility and solution stability decreases as pH increases) (6,7). It also shows high inter-individual variability in pharmacokinetics, with a greater than six fold variability in through concentrations among patients given 600 mg ritonavir every 12 hr. The major side effect associated with RN is gastro intestinal (GI) toxicity (dose-dependent) and is less common with lower doses (8). The short biological half life of drug (~3–5 hr) also favors development of extended release (ER) formulation (9).

Oral ingestion has long been the most convenient and commonly employed route of drug delivery for systemic effect. Indeed for ER systems, the oral route of administration has by far received the highest attention with respect to research on physiological drug constraints as well as design and testing of products. This is because there is more flexibility with regard to dosage form in case of oral route than in parenteral route (10).

ER dosage forms are now used with number of drugs due to following potential advantages such as reduction in dosing frequency leading to improved patient compliance, employ less total drug which minimizes or eliminates local side effects, obtain less potentiation or reduction of drug activity with chronic dosing, minimise loss of valuable drug substances which has high solubility and low permeability or those which utilise carrier mediated transport in gut, improve efficiency in treatment, improve control of disease condition by avoiding fluctuations in plasma drug level, improve bioavailability of some drugs (11,12). The popularity of simple ER Formulations is decreased due to certain drawbacks like decrease systemic availability due to incomplete release, poor *in vitro – in vivo* correlation, possibility of dose dumping, retrieval of drug is difficult in case of toxicity and high cost of formulation (13).

In addition to above, this simple pharmaceutical approach of ER could not be beneficial for oral delivery of certain drugs. For example: Drugs that have absorption window in upper gastrointestinal tract, drugs that are unstable in lower GI tract, either due to pH variation or enzymes present in intestinal brush border epithelium (P450 isoenzymes) or due to efflux proteins (P-glycoprotein), drugs that have poor solubility at higher pH e.g. RN, ofloxacin, drugs having adverse activity in colon, drugs given for local action in gastric region, drugs for acute conditions require greater physician adjustment of the dosage form than that provided by ER products and drugs with cumulative action and low therapeutic indices (e.g. Phenobarbital, Digoxin) are not suitable because of the technologic limitation of precise controls over release rates and the risk of dose dumping (14,15). Furthermore, the relatively short gastric emptying time can result in an incomplete release of drug from the drug delivery system, leading to a diminished efficacy of the administered dose (11). Therefore, an effective control of the placement of a delivery system in a specific region of the GI tract offers numerous advantages, especially for the drugs with specific absorption site in the GI tract or the drugs with stability problem. These considerations have led to the development of the ER dosage forms that possess gastric retention capability (16,17). Floating drug delivery system (FDDS) is one of gastro-retentive dosage forms that could prolong gastric residency time (GRT) to obtain sufficient drug bioavailability (12–15). The system basically floats in the gastric fluid because of its lower bulk density compared to that of the aqueous medium. Above system with a prolonged GRT are called Gastro Retentive Floating Drug Delivery System (GRFDDS) (18).

The object of the present study is to prepare FDDS of Ritonavir with four grades of HPMC (E15 LV, E50 LV, K100 LV and K4 M) along with gas-generating agent and PVP K30. The different tablet formulations were subjected to *in vitro* release, release profile comparision, drug release kinetics, scanning electron microscope (SEM) and stability study along with physical characterization.

MATERIALS AND METHODS

The drug, RN, was a gift sample of Cipla Ltd., Mumbai. HPMC E15 LV, HPMC E50 LV and HPMC K100 LV were obtained from Colorcon Asia Pvt. Ltd., Mumbai. HPMC K4 M and Cellulose Microcrystalline were purchased from Loba Chemie Pvt. Ltd., Mumbai and Thomas Baker (Chemicals) Pvt. respectively. Other chemicals were of analytical grade.

Preformulation Studies
Solubility study of the drug

The solubility of RN was studied in 0.1N HCl, at 37±0.5°C. An excess quantity of Ritonavir was taken in 25 ml of 0.1N HCl. The solutions were shaked in 'water bath shaker' for 24 hr at 37±0.5°C. The solution was then passed through a Whatmann filter paper (No.1) and the amount of the drug dissolved was analyzed spectrophotometrically (UV- 1170, Systronics) at 246.2 nm after suitable dilutions.

Powder flow property

The flow properties of powders were determined included the following: bulked density, tapped density, flow through an orifice, compressibility index (Carr's index), Hausner's ratio and angle of repose. All the above properties were measured according to USP XXXI (19).

Preparation of gastroretentive floating tablets for Ritonavir

Tablets were prepared by direct compression technique and the composition for each batch is shown in Table 1 . Accurately weighed all ingredients (except magnesium stearate and talc) were taken in a mortar, mixed well and sifted through 18 mesh screen. Moisture content of blend was controlled and maintained between 2-3 % by using IR lamp. Final blending of powder, magnesium stearate (30 mesh) and talc (30 mesh) was performed in mortar for 5 min with the help of spatula. Above blend with the compression weight of 400 mg was compressed by using 11 mm normal flat punches (Cadmach tablet punching machine).

Table 1. Compositions of different tablet formulation.

Composition (mg/tab)	FA1	FA2	FA3	FB1	FB2	FB3	FC1	FC2	FC3	FD1	FD2	FD3
Drug	100	100	100	100	100	100	100	100	100	100	100	100
HPMC E15LV	100	150	200	----	----	----	---	---	---	---	---	---
HPMC E50LV	---	---	---	100	150	200	---	---	---	---	---	---
HPMC K100LV	---	---	---	---	---	---	100	150	200	----	----	----
HPMC K4M	----	----	----	---	---	---	----	----	----	100	150	200
Microcrystal line cellulose	150	100	50	150	100	50	150	100	50	150	100	50

All the formulations contains 30 mg of Sodium biocarbonate, 10 mg of PVP K30 and 5 mg each of Magnesium stearate and talc. The total weight of all the formulations were 400 mg.

Tablet properties

In-process samples were tested for weight variation, hardness (Monsanto hardness tester), friability (Roche friabilator), thickness (Vernier caliper) and drug content. For drug content, tablets

were powdered in a mortar and powder equivalent to 100 mg of RN was taken in a 100 ml volumetric flask. The powder was dissolved in a 70 ml of methanol, vortexed for 10 minutes and then volume was further adjusted with methanol. The solution was filtered through Whatmann filter paper (No.1). Then the solution was further diluted as per requirement and analyzed spectrophotometrically at 239.40 nm (UV-1170, Systronics).

Floating behavior of tablets

The floating lag time and total time of floating was determined in the USP Dissolution Apparatus II in an acid environment (0.1 N HCl). Volume of the medium was 900 ml and the temperature was maintained at 37 ± 0.5 °C. The rotation speed was 50 rpm. The time interval between the introduction of the tablet into the dissolution medium and its buoyancy to the top of dissolution medium is taken as floating lag time and the total time of floating and dimensional stability was observed visually (15).

Swelling studies

The extent of swelling was measured in terms of percent weight gain by the tablet. Three tablets from each formulation were kept in petri dishes containing 0.1 N HCl. Periodically, tablets were withdrawn, blotted with absorbent tissue to remove any excess medium on the surface and weighed. Process was continued till the end of 12 hr (20). Swelling Index (% weight gain) was calculated by using the Eq. 1:

$$\text{Swelling Index (SI)} = \{(M_t-M_o)/M_o\} \times 100 \qquad \text{Eq. 1}$$

where M_t is the weight of tablet at time t, and M_o is the weight of tablet at time t=0

In-vitro dissolution studies

After verification of the drug content of the tablets, *in vitro* dissolution testing was performed by the rotating paddle USP apparatus II, (TDT-08 L, Electrolab) at 37 ± 0.5°C and 50 rpm, using 900 ml of 0.1N HCl for 12 hours. Sink conditions were maintained throughout the test. Ten ml aliquots were collected at 0.5 , 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 hr and same volume of fresh medium maintained at same temperature was replaced into the dissolution vessel. The samples were filtered through Whatmann filter paper (No.1) and analyzed spectrophotometrically (UV-1700, Shimadzu, Japan) at 246.2 nm for RN content after suitable dilution.

Comparison of drug release profiles

Release profiles were compared using dissolution efficiency (DE) and mean dissolution time (MDT), which were calculated using following equations (21). The Dissolution Efficiency of a dosage from is defined as the area under the dissolution curve upto a certain time (t) and expressed as a percentage of the area under the rectangle described by 100% dissolution in the same time. Both DE and MDT are presented as Eq. 2 and Eq. 3.

$$\text{DE (\%)} = \frac{\int_0^t y \times dt}{y_{100} \times t} \times 100 \qquad \text{Eq. 2}$$

where y is drug percent dissolved at time t.

$$\text{MDT} = \frac{\sum_{j=1}^{n} i_j \Delta M_j}{\sum_{j=1}^{n} \Delta M_j} \qquad \text{Eq. 3}$$

where j is the sample number, n is the number of dissolution sample times, i_j is the time at the midpoint between i_j and i_{j-1} [easily calculated with the expression $(i_j + i_{j-1})/2$] and ΔM_j is the additional amount of drug dissolved between i_j and i_{j-1}. One-way analysis of variance test (ANOVA) was performed with $P < 0.05$ as the level of significance.

Drug release kinetic modelling

To investigate the effect of viscosity and concentration of polymer on the mechanism of Ritonavir release from tablets, data were subjected to various mathematical kinetic models like Zero order, First order, Higuchi model and a semi-empirical model, known as the power law (22), which is given by the Eq. 4.

$$M_t / M_\infty = kt^n \hspace{3cm} \text{Eq. 4}$$

where M_t is the mass of drug released at a time t, M_∞ is the total mass of released drug, k is a constant incorporating morphologic characteristics of the delivery system, and n is the release exponent which characterizes the mechanism of drug release, i.e. for cylindrical systems n = 0.45 for purely Fickian diffusion, 0.45<n<0.89 for anomalous (non-Fickian transport) controlled by both diffusion and swelling, n = 0.89 for zero-order release (swelling controlled drug release) systems, and values >0.89 indicate super case-II transport (23-25).

Fourier-transform infrared spectroscopy (FT-IR)

The FT-IR absorption spectra in the range 4000-400 cm^{-1} for pure drug, drug with different excipients in 1:1 ratio and the final formulation were obtained using KBr disc method (Shimadzu, Japan, FTIR-8400 S) and observed for characteristic peaks of drug.

Scanning electron microscopy of tablets

The morphology of intact tablet surface of formulation FD2 and at 3rd, 6th, 9th, 12th hr of dissolution was evaluated by a Scanning Electron Microscopy (SEM, JSM-6390LV, JEOL). Samples were coated with platinum using auto fine coater for 75 seconds with thickness 25 nm at a 40 mA operating current prior to viewing.

Stability study

For the convenience of the study, only the final formulation batch was stored in room temperature in desiccator (28-32°C), at 40°C and at 50°C for one month (26). The parameters studied were appearance, hardness, drug content and FT-IR.

RESULTS AND DISCUSSION

Preliminary studies were conducted in order to optimize lag time i.e. the time required for the dosage form for its buoyancy by taking various parameters into consideration such as concentration of sodium bicarbonate at three levels (20 mg, 25 mg and 30 mg per tablet), nature of diluents (microcrystalline cellulose and lactose at equal concentration) and effect of hardness at three levels i.e. 1.25, 2.5 & 5 kg.

The batch with highest concentration (30 mg per tablet) of sodium bicarbonate shows an acceptable lag time (16 sec) for floating and floats over desired period of time (11 hr). The tablets with MCC as diluent demonstrates dimensional stability (without disperson of tablets) for a longer period of time. This may be attributed to the insoluble nature and high crosslinking structure of MCC. In case of hardness, the medium hardness (2.5 kg) yields highest floating time (12 hr) and an acceptable lag time. Therefore, the highest level of SB, MCC as diluent and 2.5 kg hardness were selected for further formulation development

Flow property of precompression blend of different batches were determined by calculating Carr' Index, Hausner Ratio & Angle of Repose. The observed values are presented in Table 2. The observed values of Carr's Index, Hausner Ratio & Angle of repose were found to be in the range of powder having fair flow property.

Table 2. Powder flow property parameters of formulation blends.

F. No.	Bulk Density (g/mL)	Tapped Density (g/mL)	Carr's Index	Hausner Ratio	Angle of Ripose
FA1	0.345	0.430	19.8	1.247	37.747
FA2	0.334	0.404	17.3	1.209	37.919
FA3	0.315	0.380	17.1	1.206	37.890
FB1	0.320	0.394	18.7	1.230	37.253
FB2	0.333	0.409	18.5	1.227	39.611
FB3	0.317	0.394	19.5	1.242	38.876
FC1	0.322	0.390	17.4	1.211	39.396
FC2	0.325	0.397	18.2	1.222	39.183
FC3	0.315	0.390	19.3	1.239	38.719
FD1	0.328	0.395	16.9	1.203	38.474
FD2	0.322	0.392	17.7	1.215	37.427
FD3	0.339	0.414	18.1	1.221	38.806

Table 3. Physical characteristics & Drug Content of formulations (FA1-FD3).

F. No.	Average weight (mg)	Hardness of tablet (Kg/cm^2)	Friability (%)	Thickness (mm)	Drug Content (%)
FA1	398.74 ± 2.37	4.0 ± 0.5	0.45	4.26 ± 0.24	100.07
FA2	398.77 ± 2.13	3.5 ± 0.5	0.34	3.91 ± 0.02	101.82
FA3	399.22 ± 4.9	4.0 ± 0.5	0.38	3.81 ± 0.07	99.76
FB1	398.27 ± 1.09	5.0 ± 0.5	0.29	3.93 ± 0.05	99.44
FB2	399.41 ± 2.44	4.5 ± 0.5	0.27	4.01 ± 0.02	101.66
FB3	400.25 ± 0.47	3.5 ± 0.5	0.32	3.93 ± 0.07	101.03
FC1	401.45 ± 0.73	4.0 ± 0.5	0.23	4.08 ± 0.07	99.28
FC2	400.11 ± 1.22	3.5 ± 0.5	0.22	4.03 ± 0.05	100.23
FC3	401.89 ± 1.17	3.5 ± 0.5	0.17	4.41 ± 0.24	100.55
FD1	401.33 ± 0.86	4.5 ± 0.5	0.23	3.96 ± 0.05	101.19
FD2	401.69 ± 1.54	4.5 ± 0.5	0.20	4.13 ± 0.05	98.96
FD3	401.95 ± 1.49	4.0 ± 0.5	0.17	4.06 ± 0.05	99.92

All values are expressed as Mean ± S.D, n=3.

The IR absorption spectra of the pure drug, drug with different excipients and final formulation (not presented) were taken in the range of 4000-400 cm^{-1} using KBr disc method (Schimadzu FT IR 8400S) and observed for characteristic peaks of drug. After thorough interpretation of the IR spectra,

it was found that the major peaks of pure drug were preserved in the drug with individual excipients and formulations, which concludes that there was no chemical interaction.

Various physical characteristics and drug content of the prepared batches were determined and the results obtained are presented in Table 3. Physical parameters and drug content of all the batches are within tolerance limits (27).

Floating behavior of tablets

The observed floating lag time, total floating time are given in the Table 4. The floating matrix tablets were prepared with sodium bi carbonate which produces carbon dioxide bubbles in acidic environment. Different grades of HPMC were evaluated for their carbon dioxide bubble entrapment efficiency as well as ability to maintain matrix integrity.

Table 4. Floating lag time and Total floating time for floating matrix tablets .

F. No.	Floating lag time (sec)	Total floating time (hr)	Dimensional Stability
FA1	12.88 ±1.02	10	Disperse in 2 separate layers in 10th hr
FA2	15.63 ± 2.17	10	Formation of floating flakes in 10th hr
FA3	23.42 ± 2.28	10	Formation of floating flakes is less
FB1	19.02 ± 1.92	> 12	Flake formation is very less
FB2	15.35 ± 7.05	>12	Flake formation is less
FB3	25.27 ± 9.90	>12	Flake formation is absent
FC1	17.12 ± 3.76	>12	Flake formation is absent
FC2	28.63 ± 1.87	>12	Swelled tablet floats without any flakes
FC3	14.58 ± 2.28	>12	Swelled tablet floats without any flakes
FD1	10.08 ± 1.98	>12	Much more swelling is observed
FD2	9.74 ± 1.08	> 24	More swelling
FD3	9.18 ± 1.58	> 24	Maximum swelling

All values are expressed as Mean ± S.D, n=3.

Effect of amount of HPMC on floating property

The effect of different concentration (100 mg, 150 mg and 200 mg/tablet) of various grades of HPMC on floating property of tablets were evaluated. In case of FA and FB series, increasing amount of HPMC showed a significant effect on dimensional stability of the floating tablets. This may be attributed to the fact that with increasing amount of HPMC, more stable matrix was formed. As the concentration of K4M (FD series) increases, the floating lag time was delayed. Furthermore, K4M demonstrate highest floating time (>24 hr) and lowest floating lag time (9.187 ± 1.581 s).

Effect of viscosity of HPMC grade on floating property

HPMC was a hydrocolloid gelling agent. Upon contact with aqueous medium, HPMC takes up water and swell, which retarded the drug release. As the increase in volume is greater as comparison to the increase in mass during swelling, the density decreases and the systems start to float (28, 29).

For FA series containing very low viscosity HPMC grade (E15 LV) were found incapable of retaining the matrix integrity for a desired time span. With increase in HPMC viscosity (E50 LV), the FB series showed desired floating lag time, total floatation time and improved dimensional characteristics. Same trend were seen for next two series having higher viscosity grades of HPMC (K100 LV & K4M), probaly owing to to more polymer entanglement and more gel strength and also

to the smaller effective molecular diffusion area at higher viscosity as compared with lower viscosity grades of HPMC.

Swelling behaviors of floating matrix tablets

In all the batches of FA, FB & FC series swelling index increased with time upto a maximum level and then it started decreasing (Figure 1). But in case of FD series swelling index did not start reducing even at 12th hour.
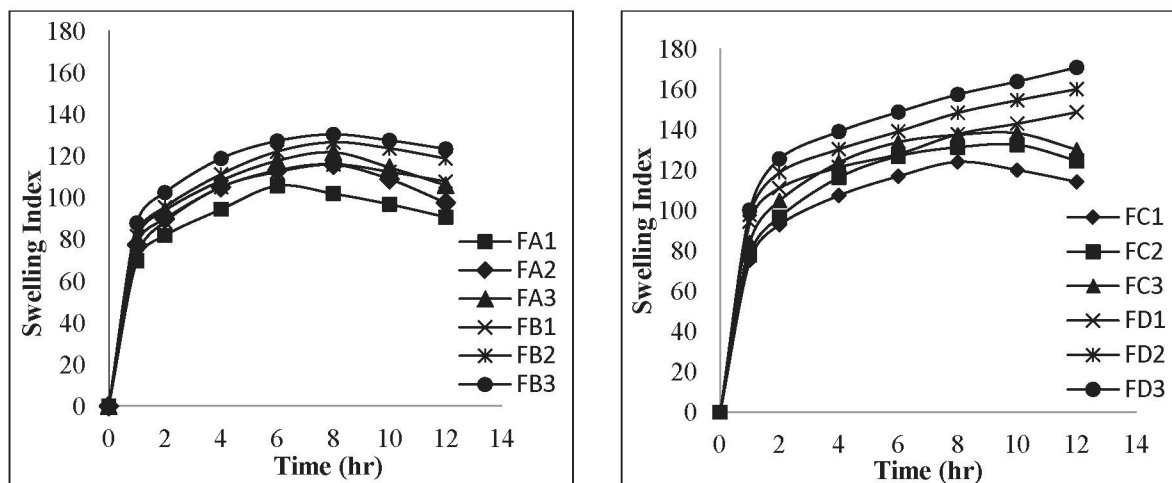

Figure 1. Average swelling index of all final formulations.

Effect of concentration of HPMC on swelling behavior

With increase in HPMC concentration, swelling index increased in all the batches. This may be attributed to rapid hydration and gel layer formation by HPMC around the surface of the tablet. As the proportion of the HPMC increases, proportion of the diluent decreases. The diluent MCC is very porous and weakly swellable polymer. Therefore MCC does not form gel layer around the surface. In the batches FA1, FB1, FC1 & FD1 the proportion of MCC is higher, therefore observed swelling index is much less in these cases as compared to other batches in the same series.

Effect of viscosity of HPMC on swelling behavior

With increase in viscosity of HPMC, swelling index increased in all the batches. In FA & FB series swelling index increased upto a maximum level in 8th hr and then onwards it started decreasing. This may be due to the low viscosity grades of HPMC used in these formulations, which results in loss of matrix integrity and hence rapid erosion. The FC series is formulated with higher viscosity grade, therefore the swelling index showed increments upto 10th hr except in the case of FC1 batch. This may be attributed to the low concentration of HPMC used in the formula. In FD series the viscosity of HPMC grade is much higher, therefore resulting in more hydration and gel formation around the surface of the tablet, attributing to high swelling index in this series. Due to high viscosity, matrix integrity is maintained for a longer duration leading to least erosion. Therefore swelling index in FD series did not start decreasing even at 12th hr.

In-vitro drug release study

From the Figure 2 below, it is evident that there is significant difference in drug release profile between batches containing different HPMC grades at different concentrations but there is no significant difference in the drug release profile within the replicate batches. The permeability decreased with increasing viscosity grade of HPMC. With increasing viscosity grade the average molecular weight of the polymer increased, resulting in an increased entanglement of the macromolecules. Thus, the mobility of the polymer chains decreased, reducing the free volume available for diffusion. Hence, the probability for a drug molecule to jump from one cavity to another is decreased, leading to a decreased mass transfer rate (30).

76

In case of polymer with very low viscosity (HPMC E15LV & E50LV), at 1:1 drug & polymer ratio i.e. FA1 & FB1 batches, drug release is more uniform with time indicating that erosion of the matrix is the main mechanism of drug release.

With increase in polymer concentration, significant difference in release pattern between formulation containing drug & polymer ratio 1:1 and 2:3 was observed. But in case of 2:1 drug and polymer ratio, release pattern is comparable with 2:3 ratio. This may be due to optimum formation of polymeric matrix in 2:3 ratio, which does not show any further increase in matrix rigidity on increasing the polymer concentration, for low viscosity grades of HPMC.

Initial burst release of drug was observed with formulations containing comparatively high viscosity grade HPMC K4M. With increase in polymeric concentration this burst effect and the drug release in later hrs of dissolution were reduced significantly. This may be attributed to greater gel formation resulting in increased diffusional path length for drug release (31).

Each grade of polymer has a decreasing effect on the cumulative drug release with increase in their ratio in the formulations (Figure 2). But the major decrease is observed in case of the lowest viscosity grade polymer (FA series), especially in the later half of the dissolution study i.e. after 6 hr.

It is also clear that HPMC K4M can not control the initial drug release as much as the lower viscosity grades at least up to initial 3 hr (Figure 3). Then, it starts releasing drug in a more sustained manner than its counter parts irrespective of the polymer concentrations. This may be attributed to the inceasing swelling index of HPMC K4M over the entire dissolution time and at the same time decrease in swelling index was seen after a certain point of time in case of lower viscosity grades. It may also be assumed that increase in amount of lower grade polymers delays the onset of matrix erosion of the formulation, since higher the low grade polymer concentration, longer would be the time taken to obtain the optimum swelling index. The permeability increased at higher HPMC concentrations as upon hydration of the film water channels within the film become the major pathway for drug release (32).

Increasing the amount of K4M decreases the initial burst release which may be due to increased swelling of the high viscosity polymer with increasing amount forming greater matrix integrity and longer diffusional path length.
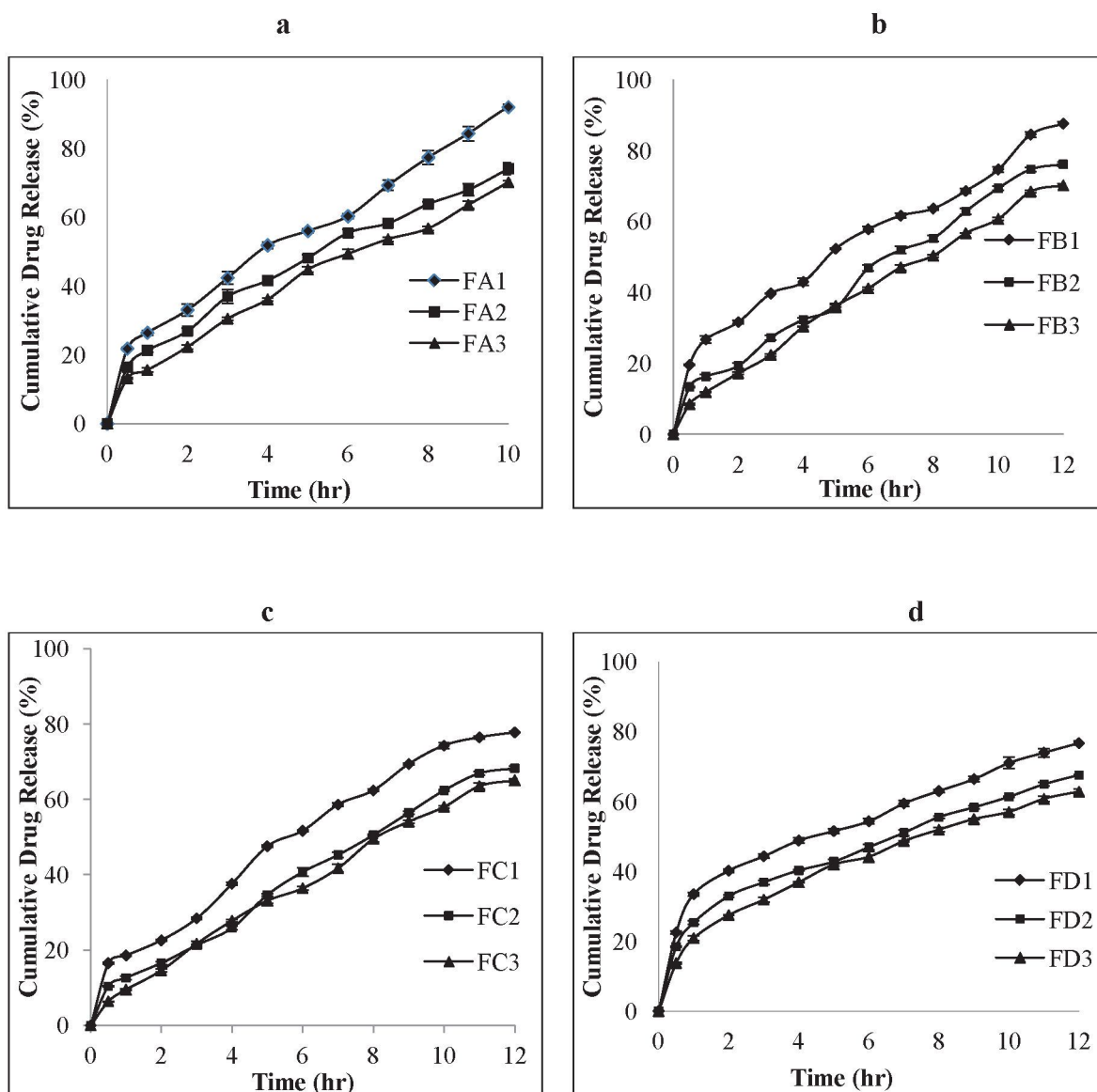
Figure 2. Effect of HPMC concentration on *in-vitro* release of Ritonavir containing (a) E15LV (b) E50LV (c) KL100V (d) K4M.
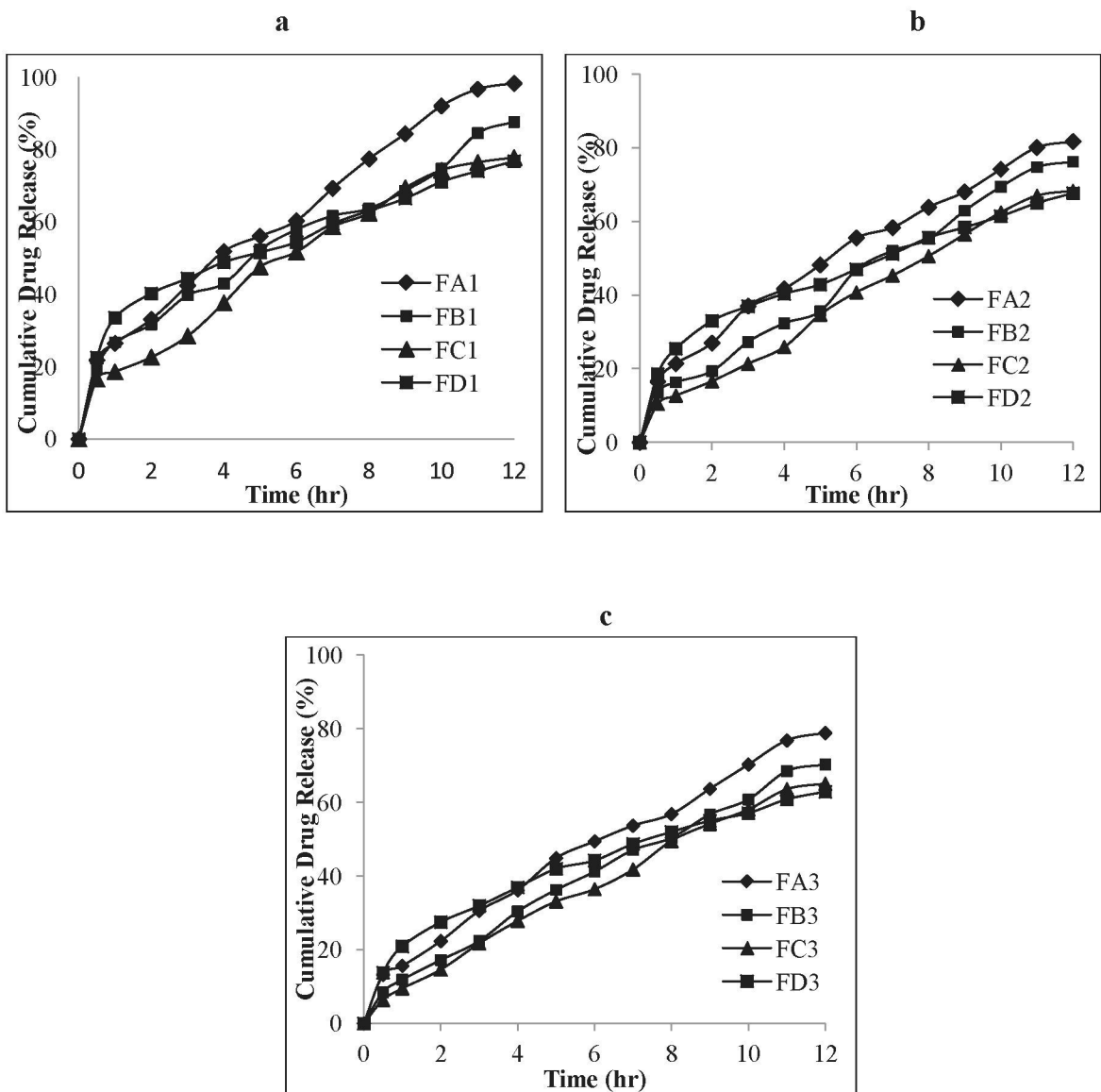
a

b

c

Figure 3. Effect of HPMC viscosity on *in-vitro* release of Ritonavir (a) at 1:1 Drug & Polymer ratio (b) at 2:3 Drug & Polymer ratio (c) at 1:2 Drug & Polymer ratio.

Table 5. Dissolution efficacy and mean dissolution time of different formulations.

Trial	Dissolution Efficiency (%)				Mean Dissolution Time (hr)			
	1	2	3	Avg.	1	2	3	Avg.
FA1	62.781	61.126	61.942	61.950	4.359	4.468	4.487	4.438
FA2	51.504	52.119	51.059	51.561	4.475	4.366	4.429	4.423
FA3	47.177	46.550	46.752	46.826	4.847	4.830	4.932	4.870
FB1	54.849	54.104	53.920	54.291	4.537	4.557	4.605	4.566
FB2	44.490	43.989	44.716	44.398	5.033	5.014	4.974	5.007
FB3	39.879	39.903	39.954	39.912	5.233	5.144	5.165	5.181
FC1	49.156	49.015	49.374	49.182	4.389	4.437	4.414	4.413
FC2	39.063	39.212	39.110	39.128	5.179	5.037	5.143	5.120
FC3	36.855	36.890	37.062	36.936	5.254	5.188	5.112	5.185
FD1	53.628	54.150	54.308	54.029	3.644	3.458	3.564	3.555
FD2	45.957	46.207	46.228	46.131	3.850	3.792	3.806	3.816
FD3	42.445	42.234	42.785	42.488	3.902	3.826	3.935	3.888

From the Table 5, it is evident that there is significant difference in drug release profile between different batches containing different HPMC grades but there is no significant difference in the drug release profile within the replicate batches.

From the kinetic data mentioned in Table 6, zero order kinetics were followed by the batches of FA, FB & FC series, whereas, FD series is mostly following Higuchi kinetics. This is attributed to the difference in viscosity of HPMC used in the formulations. For high viscosity grades of HPMC, the release of drug occurs mainly by diffusion through pores created on the surface gel layer of the matrix

Table 6. Dissolution Kinetics for Different Formulations of Ritonavir

| Triala No.	Drug Release Kinetic Model				
	Zero order	First order	Higuchi	Korsmeyer-Peppas	
	r^2	r^2	r^2	r^2	n
FA1	0.922	0.859	0.978	0.973	0.505
FA2	0.988	0.981	0.99	0.987	0.526
FA3	0.992	0.974	0.982	0.982	0.602
FB1	0.988	0.931	0.977	0.979	0.466
FB2	0.995	0.970	0.960	0.954	0.595
FB3	0.993	0.984	0.978	0.988	0.696
FC1	0.980	0.987	0.974	0.952	0.556
FC2	0.993	0.982	0.962	0.959	0.650
FC3	0.994	0.990	0.979	0.994	0.764
FD1	0.965	0.987	0.988	0.984	0.406
FD2	0.977	0.993	0.992	0.990	0.443
FD3	0.966	0.994	0.998	0.997	0.465

tablet, which is the basis of Higuchi release kinetics and in case of low viscosity HPMC grades, main mechanism of drug release is erosion of the matrix.

For further corroboration, release exponent (n) values were obtained by fitting data into Power equation. Average release rate exponent (n) values for FA, FB, FC and FD are 0.54, 0.585, 0.65 and 0.44, respectively. All the values of 'n', except the FD series, are within the range of 0.45 to 0.89 which corresponds to diffusion and swelling mechanism or mixed order kinetics. For FD series the calculated 'n' value is close to 0.45 value, so, it is assumed that it follows diffusion mechanism of drug release.

Scanning Electron Microscopy of intact tablet of formulation FC3 was done before dissolution and at 3rd, 6th, 9th, 12th hr of dissolution. The morphological characters of these scans were compared to hypothesize the mechanism of drug release kinetics from the polymer matrices. The intact tablet surface of final formulation shows irregular texture. The overall morphology is clear from the photograph of 100X magnification whereas 250 X magnifications confirm the absence of any pore or fracture. All the SEM represented in Figure 4.

At 3rd hr of dissolution study, surface morphology has changed significantly. The texture confirms, swelling of the polymeric masses and simultaneous erosion of the matrix to a less extent, leading to formation of very few pores. At the 6th hr of dissolution study, more irregular texture of the tablet surface is indicative of continuous erosion. With increasing time the number of pores and pore size was increased. SEM photographs at 9th hr of dissolution showed polymeric masses in fully swollen condition. After 12th hr of dissolution, photographs showed highly porous surface with big sized pores. Therefore, SEM study gave an idea about the drug release mechanism from the floating matrix tablet. In the first 6 hrs, drug release is mainly due to the erosion of the polymeric matrix with little diffusion through pores and in the later part of dissolution study, drug release occurs mainly by diffusion through pore created on the swollen matrix. This observation is in accordance with the results obtained from the drug release kinetic study.
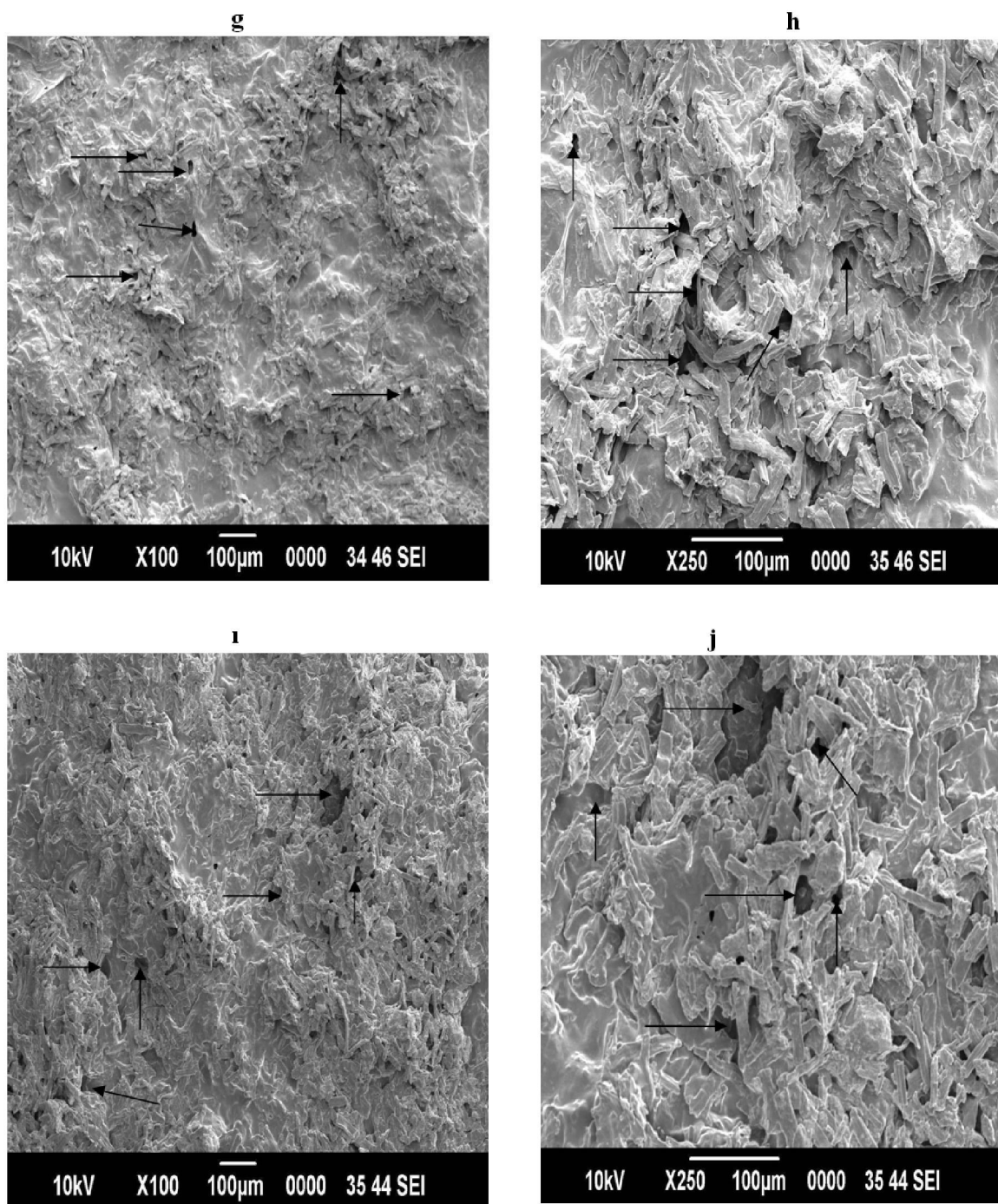
c

d

e

f
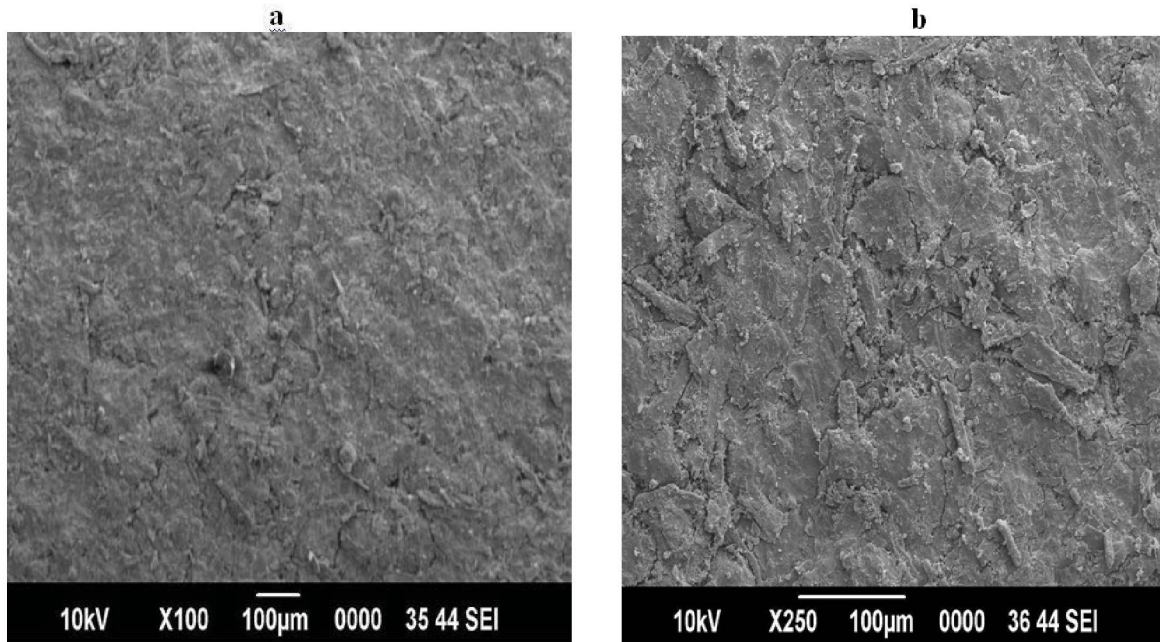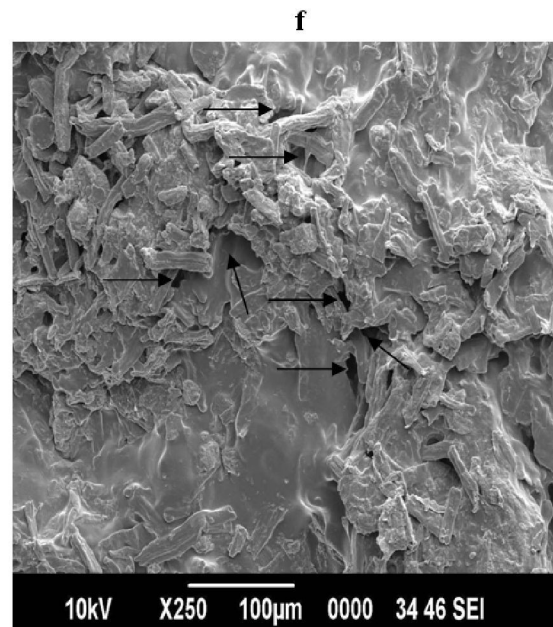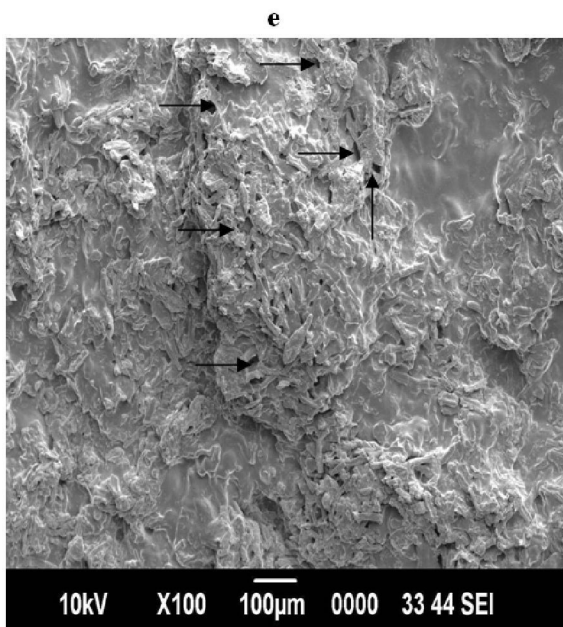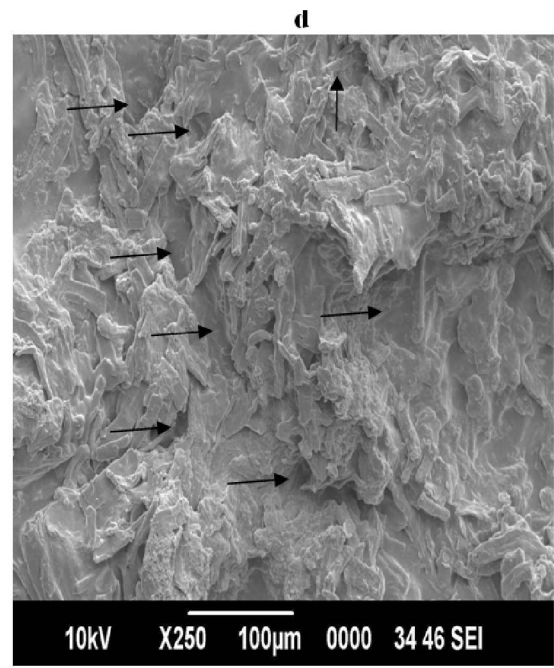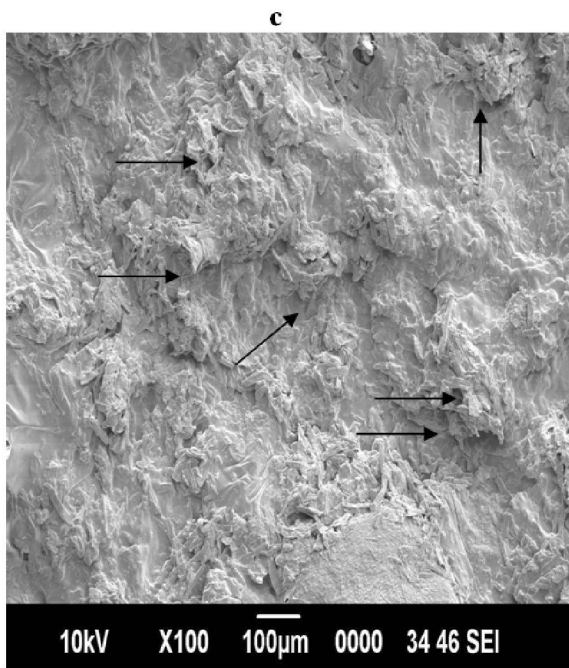
Figure 4. SEM observations of Gastroretentive tablet surface (a,c,e,g and I at 100X and b,d,f,h and j at 250X). Surface morphology at Zero hr (a,b), at 3rd hr (c,d), at 6th hr (e,f), at 9th hr (g,h) and at 12th hr (I,j). Arrow indicates the formation of pores on the surface.

Stability study

The formulated floating tablets of FC3 batch were subjected to stability study. The samples were stored in desiccator, at 40° C and 50° C for one month period.

No significant change was observed in physical parameters and drug content of the final formulation stored at desiccator (room temperature), at 40 °C and 50 °C after one month. Therefore it can be concluded that drug is stable in final formulation at room temperature, at 40 °C and 50 °C for one month. This was further confirmed by FTIR analysis (Figure 5) of the stability batch after one month storage at 40 °C.
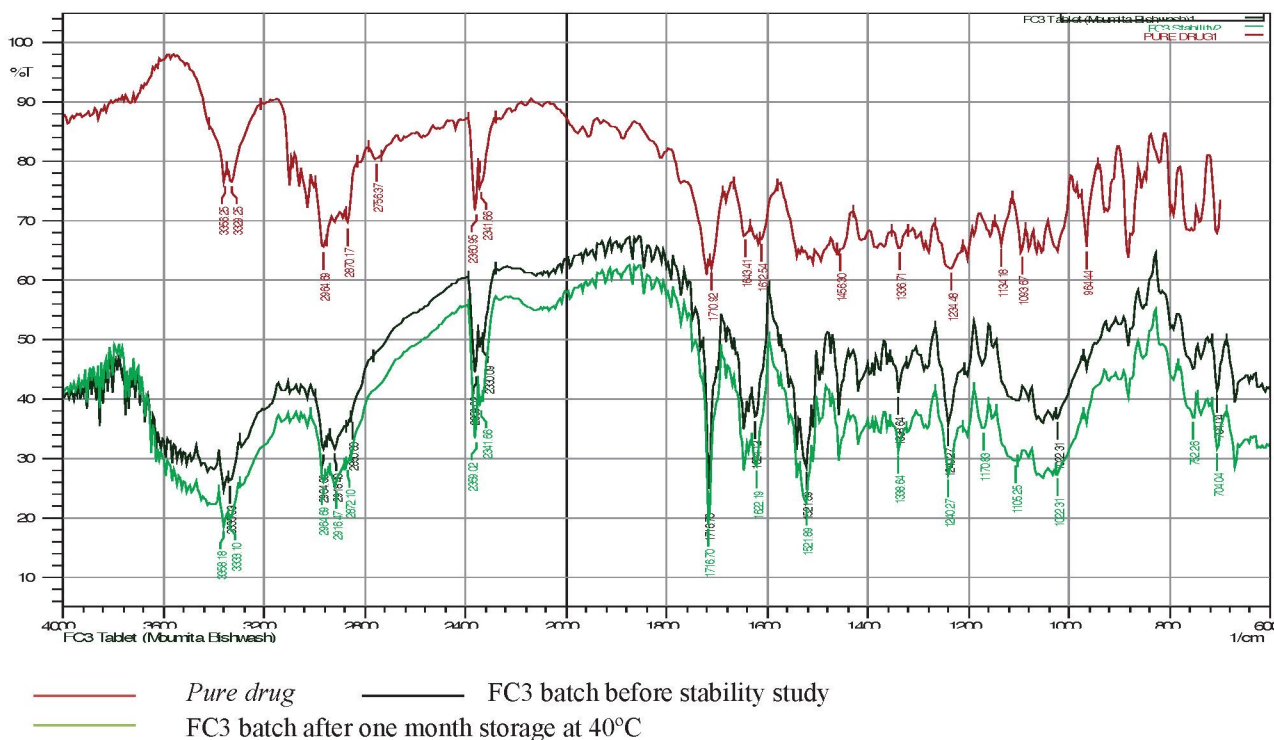

	Pure drug		FC3 batch before stability study
	FC3 batch after one month storage at 40°C		

Figure 5. IR Spectrum of batch FC3 after one month.

CONCLUSIONS

This project work was based on formulation and *in vitro* evaluation of floating matrix tablet for ER of RN. For this purpose different viscosity grades of HPMC were incorporated at different concentration in the formulations and the effect of polymer concentration & viscosity on floating behavior and drug release kinetics was evaluated. FC3 was the most succesful batch considering the fact that the r^2 value for zero order plot is highest (0.994) containing 25% of drug, 50% of HPMC K100LV, SB and other excipient. FC3 formulation took 14.58 ± 2.28 sec to become buoyant and float for more than 12 hr, which was sufficient for ER of RN. It has swelling index of 129.93 ± 0.5. Above two studies revealed that both are highly dependent on the polymer concentration and viscosity. Anomalous transport were confirmed from these tablets which corresponds to diffusion of water and swelling (polymer arrangment) mechanism or mixed order kinetics have an essential role in drug release. SEM study confirmed that the drug release was mainly due to the erosion of the polymeric matrix with little diffusion through pores initially and in the later part of dissolution study, drug release occurs mainly by diffusion through pore created on the swollen matrix. This result is encouraging, because a longer gastric residence time is an important condition for higher bioavailability of the drugs included in the ER release dosage forms.

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