DEVELOPMENT AND IN VITRO–IN VIVO EVALUATION OF GASTRORETENTIVE FLOATING TABLETS OF AN ANTIRETROVIRAL AGENT RITONAVIR

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ABSTRACT

Objective: The present research work concerns the development of the extended release of Ritonavir floating matrix tablets, designed to prolong the gastric residence time, increase the drug bioavailability, and diminish the side effects of irritating drugs.

Methods: The floating tablets of Ritonavir were prepared by direct compression method using different grades of hydroxypropyl methylcellulose (HPMC), crospovidone, Polyoxy WSR 303, and sodium bicarbonate, as gas generating agent. Evaluation parameters and in vivo radiographic studies were conducted in suitable model.

Results: Among all formulations, F21 was chosen as optimized formulation based on evaluation parameters such as floating lag time (33 s), total floating time (>24 h), and in vitro dissolution studies. From in vitro dissolution studies, the optimized formulation F21 and marketed product were shown 98.67% and 91.46±5.02% of drug release, respectively. The main appliance of medication discharge follows zero-order kinetics and non-Fickian transport by coupled diffusion and erosion. In vivo experiments maintained the potentials in extending the gastric residence time in the fastest state in beagle dogs. The mean gastric residence time of the optimized formulation found to be 330 min±40 in the stomach, where longer gastric residence time is an important condition for prolonged or controlled drug release and also for enhanced bioavailability.

Conclusion: From in vitro and in vivo radiographic studies, Ritonavir floating tablets estimated to provide novel choice for harmless, inexpensive, and extended release for the effective management of AIDS.

Keywords: Ritonavir AIDS, Floating tablets, Hydroxypropyl methylcellulose, Radiographic studies.

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INTRODUCTION

Oral route is foremost common route of administering the drug due to the ease of formulation, low-cost, convenient for the patient, and simple regulatory approval process. Based on the requirement, formulations can be changed from immediate release to extended release using several polymers [1]. Gastrorretentive drug delivery systems can valuate in the transport of medications that remain mainly engrossed in the duodenum and upper jejunum/those that need an absorption window in the gastrointestinal tract [2]. Gastrorretentive floating dosage forms remain constantly researched and advanced stomach is a main window in the gastrointestinal tract [2]. Gastroretentive floating dosage forms are maintained to buoyant in the stomach without disintegrating gastric emptying rate for a prolonged period of time [6]. While system remains afloat, drug released the desired rate from system [7]. Following drug release, residual system gets emptied from the stomach. However beside a minimal gastric content needed to allow proper achievement buoyancy retention principle, minimal equal of floating force is also required keep dosage form reliably buoyant on surface meal [8].

MATERIALS AND METHODS

Materials
Ritonavir was procured from MSN Labs Ltd., Hyderabad. Hydroxypropyl methylcellulose (HPMC) K4M, HPMC K15M, HPMC K100M, and Polyoxy WSR 303 existed were obtained from Granules India Ltd., Hyderabad. Sodium bicarbonate, citric acid, PVP K 30, talc, and magnesium stearate existed were procured from S D Fine-Chem Ltd., Mumbai, and all other chemicals used existed were of analytical grade.

Methods
Micromeritic properties of final blend
Final blend of all preparations was evaluated for BD, tapped density (TD), compressibility index (CI), Hausner ratio, and angle of repose [9].

Formulation method
Accurately weighed amounts of polymers were in a mortar and mixed geometrically, and the required quantity of Ritonavir was added to the polymers. Sodium bicarbonate was taken separately, and the powder passed through sieve no 40. The whole mixture was collected in a plastic bag and mixed for 3 min. Magnesium stearate added and mixed for 5 min, and later, Talc was added and mixed for 2 min [10–12]. The mixture equivalent to 300 mg was compressed into tablets with 10 mm round concave punches, and the composition is shown in Tables 1-3.
Table 1: Composition of floating matrix tablets of Ritonavir with HPMC K4M

<table>
<thead>
<tr>
<th>Ingredients (weight in mg)</th>
<th>Formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>100</td>
</tr>
<tr>
<td>HPMC K4M</td>
<td>50</td>
</tr>
<tr>
<td>Crospovidone</td>
<td>50</td>
</tr>
<tr>
<td>Polyox WSR 303</td>
<td>20</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>20</td>
</tr>
<tr>
<td>Citric acid</td>
<td>10</td>
</tr>
<tr>
<td>PVP K-30</td>
<td>46</td>
</tr>
<tr>
<td>Talc</td>
<td>2</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>2</td>
</tr>
<tr>
<td>Total weight</td>
<td>300</td>
</tr>
</tbody>
</table>

HPMC: Hydroxypropyl methylcellulose

Table 2: Composition of floating matrix tablets of Ritonavir with HPMC K15M

<table>
<thead>
<tr>
<th>Ingredients (weight in mg)</th>
<th>Formulations</th>
</tr>
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<tbody>
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<td>F8</td>
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<tr>
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<tr>
<td>HPMC K15M</td>
<td>50</td>
</tr>
<tr>
<td>Crospovidone</td>
<td>50</td>
</tr>
<tr>
<td>Polyox WSR 303</td>
<td>20</td>
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<tr>
<td>Sodium bicarbonate</td>
<td>20</td>
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<tr>
<td>Citric acid</td>
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<tr>
<td>PVP K-30</td>
<td>46</td>
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<tr>
<td>Talc</td>
<td>2</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>2</td>
</tr>
<tr>
<td>Total weight</td>
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</tr>
</tbody>
</table>

HPMC: Hydroxypropyl methylcellulose

Table 3: Composition of floating matrix tablets of Ritonavir with HPMC K100M

<table>
<thead>
<tr>
<th>Ingredients (weight in mg)</th>
<th>Formulations</th>
</tr>
</thead>
<tbody>
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<tr>
<td>Ritonavir</td>
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<tr>
<td>HPMC K100M</td>
<td>50</td>
</tr>
<tr>
<td>Crospovidone</td>
<td>50</td>
</tr>
<tr>
<td>Polyox WSR 303</td>
<td>20</td>
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<tr>
<td>Sodium bicarbonate</td>
<td>20</td>
</tr>
<tr>
<td>Citric acid</td>
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<tr>
<td>Talc</td>
<td>2</td>
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<tr>
<td>Magnesium stearate</td>
<td>2</td>
</tr>
<tr>
<td>Total weight</td>
<td>300</td>
</tr>
</tbody>
</table>

HPMC: Hydroxypropyl methylcellulose

Evaluation of floating matrix tablets of Ritonavir
Parameters such as weight variation, thickness, hardness, and ability were evaluated according to the reported method [13].

In vitro buoyancy studies
The in vitro buoyancy was determined by floating lag time. The tablets existed placed in a 100 ml beaker containing 0.1 N hydrochloric acid (N HCl). Time required for tablet to rise to surface and float was determined as floating lag time [14].

Drug content
Twenty tablets were taken and powdered. Powder equivalent to one dose each transferred to a 100 ml volumetric flask and 0.1 N HCl was added; volume was then made up to mark with 0.1 N HCl. Solution was filtered and diluted suitably and drug content in samples was estimated using ultraviolet (UV)-spectrophotometer at 238 nm 15].

In vitro drug release studies
In vitro drug release study was performed for prepared tablets using USP Type II dissolution apparatus using 900 ml of 0.1 N HCl at a temperature of 37±0.5°C at 50 rpm. 5 ml of sample was collected at 0, 2, 4, 6, 8, 12, 16, 20, and 24 h and the same volume of fresh media was replenished. Drug content in samples was estimated using UV visible spectrophotometer at 238 nm [16].

Release order kinetics Ritonavir tablets
In vitro release data from several formulations containing Ritonavir were determined kinetically using different mathematical models such as zero-order, first-order, Higuchi, and Korsmeyer–Peppas model [17].

Drug-exciptent compatibility studies
Fourier-transform infrared spectroscopy (FTIR)
The spectral analysis can be used to identify the functional groups in the pure drug and drug-exciptent compatibility. Pure Ritonavir FTIR spectra, physical mixtures, and optimized formulation were recorded using FTIR (SHIMADZU) [18].

Differential scanning calorimetry (DSC)
DSC studies carried out using DSC 60, having TA60 Software, Shimadzu, Japan. DSC thermograms were recorded for pure drug and optimized formulations. Accurately weighed samples were placed on aluminum plate, sealed with aluminum lids, and heated at a constant rate of 5°C/min, over a temperature range of 0–250°C.

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

Stability studies
Stability testing was conducted at 40°C±2°C/75% RH±5% RH 3 months using stability chamber (Thermolab, Mumbai). Samples were withdrawn at predetermined intervals of 0, 30, 60, and 90 days’ period according to the ICH guidelines [19]. Various in vitro parameters such as percentage yield, entrapment efficiency, and in vitro release studies were evaluated.

Tablet preparation for in vivo radiographic studies
Ritonavir tablets of 300 mg in weight existed were prepared for in vivo radiographic studies in beagle dogs. To make tablets X-ray opaque incorporation of BaSO4 necessary. For in vivo radiographic studies, 40 mg of Ritonavir and 5 mg of PVP K 30 were replaced with BaSO4 to ensure visibility by X-ray. Amount of X-ray opaque material in these tablets sufficient to ensure visibility by X-ray. Analyses confirmed that these tablets like tablets for in vitro testing, i.e. mechanical strength, floating properties, controlled drug release [20].

Ritonavir in vivo radiographic studies
Three healthy beagle dogs, weighing approximately 16 kg, existed were used throughout the study. In each experiment, an unanesthetized animal was fasted for 24 h and first radiograph was made to ensure the absence of radiopaque material in the stomach. Dogs swallowed one of the tablets and immediately afterward drank 100 ml of water. During experiment, dogs were not allowed to eat, but water existed available ad libitum. The study protocol was approved by the institutional animal ethics committee with No. CPCSEA/1657/IAEC/CMRC/PhD-15/37. For radiographic imaging, animal was positioned in a right lateral or ventrodorsally recumbency, and after determined time intervals, the radiographs of the abdomen were taken using an X-ray machine (General Electric Corporation, USA). Distance between the source of X-rays and the object was maintained similar for all images [21]. This allowed us to see tablet in the body of the stomach. At different time intervals of 0.5, 3, 4, and 6 h post-administration of tablets, dogs were exposed to abdominal X-ray imaging [22].
RESULTS AND DISCUSSION

Micromeritic properties of prepared powder blend Ritonavir

The results of BD formulations bearing F1 to F21 were reported in the range of 0.48±0.08 g/cc–0.54±0.12 g/cc. The findings of TD formulations F1 to F21 were reported in the range of 0.56±0.15 g/cc–0.62±0.22 g/cc. The angle of repose of all the formulations was found to be satisfactory. The formulation F21 was having an angle of repose value of 10.01±0.01, and CI values existed found to be in range of 10.01–15.38%. These findings indicated that all batches of formulations exhibited good flow properties. Hausner’s ratio values were in the range of 1.10–1.26% and values are shown in Table 4.

Physicochemical properties of Ritonavir floating tablets

The Ritonavir floating tablets were prepared, and the compositions of different formulations existed are shown in Tables 1-3 and the tablets are shown in Fig 1.
The micromeritic parameters and evaluation parameters such as weight variation, thickness, hardness, friability, and drug content existed were found to be within limits and summarized in Tables 4 and 5, respectively.

**In vitro buoyancy study**

All the prepared Ritonavir dosage forms showed floating lag time of <1 min and floated in 0.1N HCl intended for additional than 12 h (Fig. 2). Sodium bicarbonate content controls the floating behavior including the lag time. Increased sodium bicarbonate content caused rapid formation and entrapment of CO\(_2\) resulted in reduction floating lag time. Optimized formulation F21 showed minimum floating lag time of 33 s and total floating time for formulations >24 h (Fig 3).

**In vitro dissolution studies**

From the results, it can be observed that the polymer HPMC K 100M has a controlling effect on release drug from floating matrix tablet of Ritonavir compared to HPMC K15 M and HPMC K 4M. The concentration of polymer was added in increasing order to check its drug release retarding ability, and F21 was considered as best one among the all the formulations, drug release was found to be higher 98.67±5.40, when compared with marketed product of 91.46±5.02 within 24 h (Figs. 4-6).

**Mathematical modeling of optimized formula (F21) of Ritonavir floating tablets**

From the results, it is apparent that the regression coefficient value closer to unity in case of zero-order plot, i.e., 0.990 indicates that drug release follows zero-order mechanism. The drug release mechanism of optimized Ritonavir tablets F21 was best fitting to zero-order and Higuchi model because of the regression coefficient, thus, mechanism of drug release by diffusion. Further, the n value obtained from the Korsmeyer-Peppas plots, i.e., 0.62 indicates non-Fickian (anomalous) transport. Thus, active ingredient is being released by coupled diffusion and erosion. The reference standard (marketed product) regression coefficient value is closer to unity in case of zero-order plot, i.e., 0.958, specifying that drug release follows zero-order mechanism. These data indicate lesser amount linearity once plotted by the first-order equation. Hence, it can be concluded that, in case of marketed product also, the major mechanism of drug release follows zero-order kinetics. However, the linearity of the optimized formulation is more when compared to a marketed product. Results are summarized in Table 6 and graphs are depicted in Fig. 7-14.
Drug-excipient compatibility studies

There is no alteration in the peaks of Ritonavir pure drug and optimized formulation, suggesting that there was no interaction takes place between drug and excipients and the spectrums are depicted in Figs. 15-17.

Figure 4: *In vitro* drug release profile of Ritonavir floating tablets F1–F7

Figure 5: *In vitro* drug release profile of Ritonavir floating tablets F8–F14

Figure 6: *In vitro* drug release profile of Ritonavir floating tablets F15–F21

Figure 7: Zero-order plots for the optimized formulation (F21)

Figure 8: First-order plots for the optimized formulation (F21)

Figure 9: Higuchi plots for the optimized formulation (F21)

Figure 10: Korsmeyer–Peppas plots for the optimized formulation (F21). *In vitro* drug release order kinetics for marketed product

Figure 11: Zero-order plots for the marketed product

Figure 12: First-order plot for the marketed product

Figure 13: Higuchi plot for the marketed formulation

Figure 14: Korsmeyer–Peppas plot for the marketed product

Drug-excipient compatibility studies

There is no alteration in the peaks of Ritonavir pure drug and optimized formulation, suggesting that there was no interaction takes place between drug and excipients and the spectrums are depicted in Figs. 15-17.
DSC studies
Pure powdered Ritonavir showed melting endotherm at 122.10°C. DSC thermogram of floating tablet showed melting peak drug at 119.36°C. There was no significant difference in melting point of drug and optimized formulation. It is compatible with excipients present in the tablet and there was no major interaction of the drug with the excipients; it is depicted in Figs. 18-21.

SEM studies of Ritonavir floating tablets
The SEM of optimized floating tablet shows a rough surface morphology and dented surface structure, but they showed good floating ability on medium, indicating that intact surface it is shown in Fig. 22. The shell of tablet also showed some porous structure and it may be due to the release of carbon dioxide.
Stability studies

There were no changes observed in percentage drug content, in vitro drug release studies, and floating lag time during storage of the optimized formulation, and the results are tabulated in Table 7. Hence, the optimized formulation was found to be stable.

In vivo radiographic studies of Ritonavir optimized formulation (F21)

The behavior of the tablet in the dog stomach was observed in real time using a radiographic imaging technique. On radiographic images made 0.5 h after the administration, the tablets were observed in the animal’s stomach. In the next picture taken at 1.5 h, significant changes were detected, and the tablet had altered its position and turned around. This provided evidence that the tablets did not adhere to the gastric mucus but, on the contrary, floated on the gastric fluid. In addition, the swelling of the tablet is visualized very well together with the white dry core and translucent swelling layer around it. As the swelling continued, the results have shown, in Fig. 23, that the mean gastric residence time for the developed floating tablets was 330 min±40. The comparison of gastric motility and stomach emptying between humans and dogs shows no big differences. Therefore, it might be speculated that experimentally proven increased GRT in beagle dogs can be compared to known literature data for humans.

CONCLUSION

In the present work, it can be concluded that the Ritonavir floating tablets can be an innovative and promising approach for the delivery and the treatment of HIV. The optimized formulation F21 contains HPMC K100M, Polyox WSR 303, and gas generating agent. In vitro release profile of Ritonavir and marketed product when compared the optimized formulation F21 showed drug release of 98.67±5.40%, whereas 91.46±5.02% of the drug was released from the marketed product within 24 h. The major mechanism of drug release follows zero-order kinetics and non-Fickian transport by coupled diffusion and erosion. This means that water diffusion and the polymer rearrangement have an essential role in the drug release. The release rate constant of optimized formulation F21 was low enough prolonging drug delivery. In vivo experiments supported the expectations in prolonging the gastric residence time in the fasted state in beagle dogs. This result is encouraging because a longer gastric residence time is an important condition for higher bioavailability of the drugs included in the prolonged or controlled release dosage form.

AUTHORS’ CONTRIBUTIONS

R. Shireesh Kiran: Drafted the complete manuscript. B. Chandra Shekar: Supervisor of the research work, provided the guidance in the research work. B. Nagendra Babu: Advised the scholar to get quality of work.

CONFLICTS OF INTEREST

No conflicts of interest were raised by the authors.

REFERENCES