Development and evaluation of ritonavir hollow microballoons for floating drug delivery

Abstract

Ritonavir is human immunodeficiency virus (HIV) proteaseinhibitor used as the antiretroviral agent. The objective of the present investigation was to formulate and evaluate Ritonavir gastro-retentive floating microballoons for controlled release. Different batches of microballoons were prepared by the emulsion solvent diffusion method. The resultant microballoons were evaluated for percentage yield, entrapment efficiency, particle size, and *in vitro* drug release, stability study. The densities of floating microspheres were found to be less than the density of gastric fluid (1.004 g/cm^3). The entrapment efficiency of preparedfloating microspheres was satisfactory (68.37 to 88.52%). Among all formulations, FM1 prepared with polymer HPMC was found to be the best as it exhibited highest drug release (89.07%) in 12 hrs and was stable for three months at ambient conditions.

Key words: Hollow microballoons, floating drug delivery,Ritonavir, in vitro drug release, gastro-retentive.

INTRODUCTION

The oral route is being used for the delivery of therapeutic agents because of different advantages including the low cost of the therapy and ease of administration lead to high levels of patient compliance. More than 50% of the drug deliverysystems available in the market are oral drug delivery systems[1]. The purpose of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to attain promptly andthen maintain desired drug concentration[2].

Drugs that are easily absorbed from the gastrointestinal tract and have a short half-life are eliminated quickly from the blood circulation, so there is a need of frequent dosing to maintain therapeutic concentration of drug. To eliminate this limitation, the oral sustained controlled release formulations have been developed in an attempt to release the drug slowly into the gastro-intestinal tract and maintain an effective drug concentrationin the blood over long period of time[3]. Floating systems or dynamically controlled systems are low-density systems that have sufficient buoyancy to float over the gastric contents and remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. While the system floats over the gastric contents, the drug is released slowly at the desired rate. Conventional oral dosage forms do not offer any control over drug delivery and cause greatfluctuations in plasma drug concentrations[4].

Ritonavir is human immunodeficiency virus (HIV) protease inhibitor primarily absorbed from stomach used as the antiretroviral agent[5,6].

MATERIALS AND METHODS

Materials

Ritonavir was obtained from Silva Hill Pharma Limited, Nigeria city, Eudragit L 100 and hydroxypropyl methylcellulose (HPMC) were purchased from the Jagal Pharmaceutical, Lagos, Nigeria. All other chemicals used were of analyticalreagent grade.

Preparation of floating microballoons

Floating microballoons were prepared by the emulsion solvent diffusion method. Ritonavir, Eudragit L100 and HPMC were dissolved in a mixture indifferent ratio in ethanol and dichloromethane (table 1). The resulting solution was added slowly to stirred 250 mL of aqueous solution of 0.50% (w/v) PVA at room temperature. The stirring was done for 2 hrs at 1000-1200

rpm by mechanical stirrer equipped with four bladed propellers, to evaporate the volatile solvent. The floating microballoons formed were screened (#12), washed with water and dried at roomtemperature in a desiccator for 24 hrs[7].

Table-1: Composition of floating microballoons formulations of Ritonavir

Batch code	Eudragit L100 (mg)	HPMC (mg)	Di-chloromethane :Ethanol ::1:1
FM1	-	300	10
FM2	300	-	10
FM3	150	150	10
FM4	100	200	10
FM5	200	100	10

EVALUATION OF MICROBALLOONS

a. The percentage yield

It was determined by weighing the Ritonavir hollow microballoons after drying. The percentage yield was calculated as follows[8]:

% Yield =(Total weight of hollow microballoons)/(Total weight of drug and polymer) X 100

b. Micromeritic properties

1. Tapped density[9]

Tapped density of Ritonavir hollow microballoons was determined by the tapping method. Accurately weighed quantity of hollow microballoons wastransferred in to a 10 ml measuring cylinder. After observing the initial volume of floating microballoons, the tapping was continued on a hard surface until no further change in volume was noted and the tapped density was calculated according to following formula:

Tapped density =(Mass of hollow microballoons)/(Volume of hollow microballoons after tapping) X 100

2. Angle of repose[10]

The angle of repose of Ritonavir hollow microballoons was determined by fixed funnel method. The hollow microballoons were allowed to fall freelythrough a funnel until apex of conical pile just touched the tip of the funnel.

Angle of repose is determined by following formula:

$\tan \theta = h/r$

Where,

θ = angle of repose, h = height of the cone, r = radius of the cone base

3. Carr's Index[11]

It indicates the ease with which a material can be induced to flow and powder compressibility. It is expressed in percentage and is given by

Carr,s Index= (Tapped density-Bulk density)/(Tapped density) x100

4. Hausners ratio[12]

Hausner ratio (Hr) is an indirect index of ease of powder flow. It is calculated by the following formula:

Hausner^' s ratio= (Tapped density)/(Bulk density)

c. Buoyancy study[13]

Ritonavir microballoons (100 mg) were placed in 0.1 N HCI (300 ml) containing 0.02% Tween 20 and stirred at 100 rpm. The layer of buoyantmicroballoons was pipetted and separated by filtration at 1, 2, 4 and 6 h. The collected microballoons were dried in a desiccator over night. The percentage of microballoons was calculated by the following equation:

% Buoyancy =(Weight of floating microballoons at time t)/(Initial weight of microballoons) X 100

d. Drug entrapment efficiency[14]

Ten mg of hollow Ritonavir microballoons from all batches were accurately weighed and crushed. The powdered microballoons were dissolved with 10 ml ethanol in 100 ml volumetric flask and volume was made up with 0.1 N HCl. The resultingsolution is then filtered (Whatmann filter paper No. 44), suitably diluted and the absorbance was measured at 246 nm against 0.1 N HCI as blank. The percentage drug entrapment was calculated as follows:

% DEE =(Calculated drug concentration)/(Theoretical drug concentration) X100

e. *In vitro* release studies[15]

A 12 hrs study of drug release rates from floating Ritonavir microballoons was carried out using USP type II dissolution paddle assembly. Floating microballoons equivalent to 100 mg drug were dispersed in 900 ml of 0.1 N HCI pH 1.2 maintained at 37±0.5°C and stirred at 100 rpm. Five ml sample was withdrawn at predetermined intervals while replacing equal amount of fresh dissolution medium. The samples were filtered,suitably diluted and analyzed spectrophotometrically at 246 nm to determine the concentration of drug present in the dissolution medium.

Drug release kinetic data analysis

The dissolution data of all the formulations was fitted to zero order, Higuchi matrix and Korsemeyer-Peppas to ascertain the kinetic modeling of drug release.The value of 'n' gives an indication of the release mechanism. When n = 1, the release rate is independent of time (typical zero order release / case II transport); n = 0.5 for Fickian release (diffusion/ case I transport); and when 0.5<n<1, anomalous (non-Fickian or coupled diffusion/ relaxation) are implicated. Lastly, when n>1.0 super case II transport is apparent[16].

f. Stability study

From the prepared Ritonavir microballoons, best formulationwas selected on basis of buoyancy and the percentage drug released. The selected formulation was placed in borosilicate screw capped glass containers and stored at different temperatures (27±2°C), oven temperature (40±2°C) and in the refrigerator (5-8°C) for a period of 3 months. The samples were assayed for drug content at regular intervals[17].

RESULTS AND DISCUSSION

The hollow microballoons of Ritonavir were successfully prepared using Eudragit L100 and HPMC as a polymer by emulsion solvent diffusion method.

Mean particle size range was varied from 543 to 928 mm and was found to be affected by change in drug and polymer ratio. In general if sizes of microballoons areless than 500 mm, release rate of drug will be high and floating ability will reduce, while if size lies in the range of 500-1000 mm, the floating ability will be more and release rate will be in sustained manner. So the prepared microballoons are having particle size range suitable for floating[18].

Density values for all formulations were less than that of gastric fluid (1.004 g/cm^3), suggesting that they exhibit good buoyancy. The floating ability pattern differed according to the

formulation tested and medium used. Microballoons formulation of batch FM1 showed the best, 85.23% floating ability in 0.1 N HCl. This can be mainly due to its low bulk density value obtained before and after tapping respectively. Themicroballoons remain buoyant for prolonged time over the surface of the dissolution medium without any apparent gelation, which might be responsible for good floating property.

All formulations showed excellent flowability as represented in the terms of angle of repose (<40°). All the formulations showed satisfactory entrapment efficiency ranging in 68.37 to 88.52%.

Percentage drug release for the formulations FM1, FM2, FM3 and FM4 was found to be 89.07%, 66.14%, 58.43% and 77.43% respectively in 12 hrs. It was observed that drug release rate increased by increasing the ratio of HPMC respectively.

FM1 formulation showed appropriate balance betweenbuoyancy and drug release rate of which is considered as a best formulation.

Table 2: Micromeritic properties of Ritonavir microballoons formulations.

Code	Mean particle size (mm)	Bulk density (gm/cm^3)	Tapped density (gm/cm^3)	Hausners ratio	Carr's Index	Angle of repose
FM1	928±0.56	0.741±0.24	0.801±0.42	1.08	7.49	19.32 ^{o} ±0.13
FM2	734±0.31	0.763±0.09	0.825±0.08	1.08	7.51	20.12 ^{o} ±0.24
FM3	627±0.25	0.863±0.36	0.920±0.13	1.06	6.19	15.21 ^{o} ±0.09
FM4	543±0.38	0.792±0.47	0.840±0.51	1.06	5.71	18.35 ^{o} ±0.08

Table-3: Different evaluation parameters of Ritonavir microballoons formulations.

Code	Particle Size (µm)	% Yield	Entrapment Efficiency (%)	% Buoyancy
FM1	255.03±0.57	87.34±0.86	88.52±0.08	85.23±0.08
FM2	268.56±0.48	82.32±0.48	74.64±0.22	68.46±0.36
FM3	270.52±0.52	75.46±0.08	68.37±0.43	80.84±0.46
FM4	300.37±0.35	79.22±0.33	71.37±0.63	78.53±0.33

(Mean± S.D., n=3)

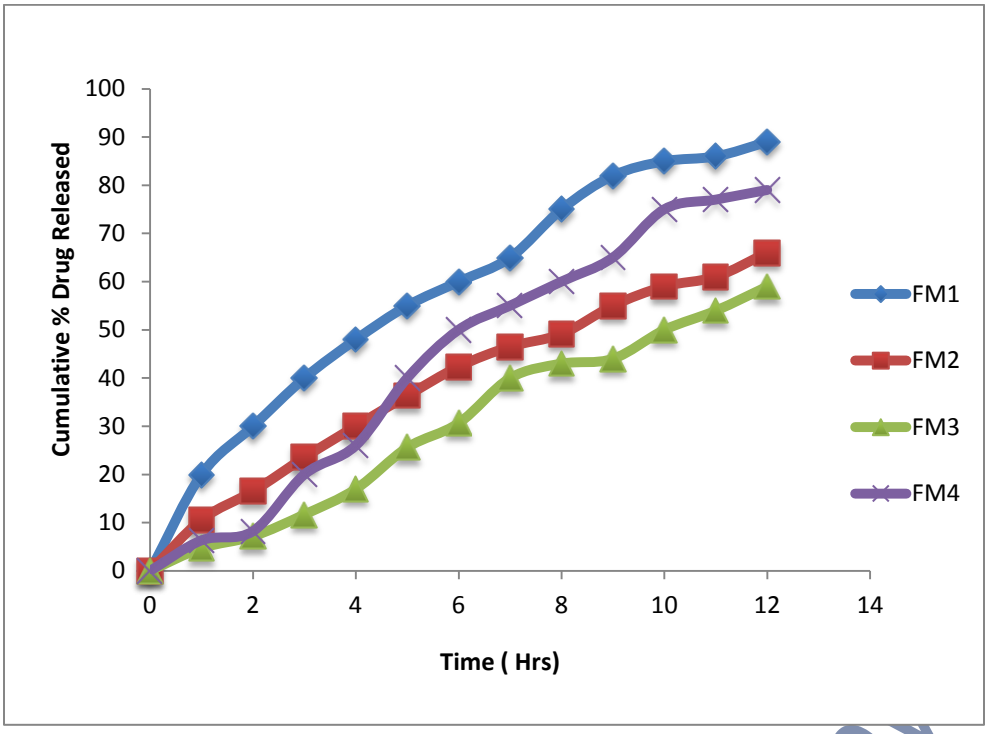

Figure 1: *In-vitro* release profile of different Ritonavirmicroballoons formulations

Table 4- Kinetic models applied on Ritonavir microballoons formulations.

Code	Zero order	Higuchi	Korsemeyer-peppas
FM1	0.9214	0.9763	0.9867 n=0.4073
FM2	0.8860	0.9565	0.9835 n= 0.5138
FM3	0.8761	0.9825	0.9836 n=0.5836
FM4	0.9213	0.9235	0.9937 n=0.5367

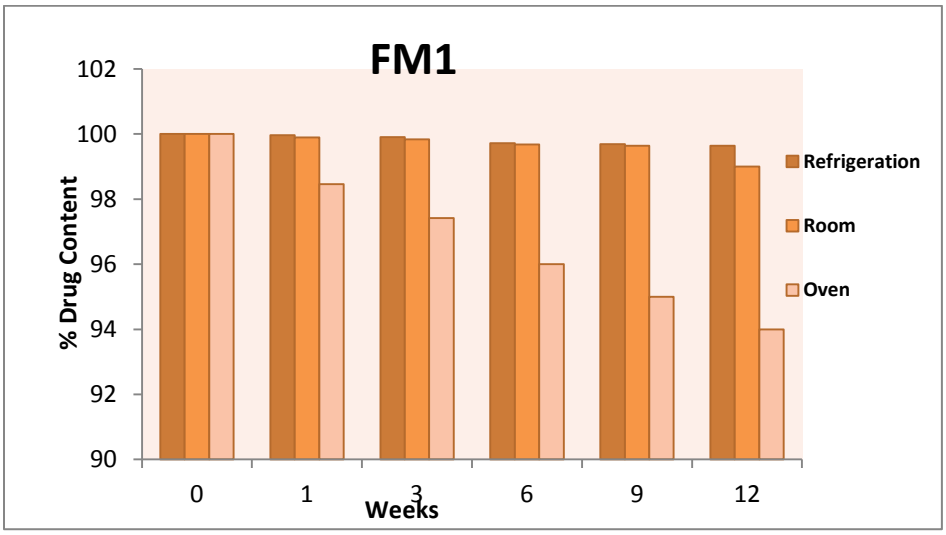

Figure 2: Stability study of Ritonavir microballoons formulations of batch FM1.

The *in vitro* release data was applied to various kinetic models to predict the drug release kinetic mechanism. Kinetics and mechanism of drug release from allformulation was evaluated on the basis of zero order, Higuchi equation and Peppas model.

Zero order plots for all formulations were found to be linear. Higuchi plot was found to be linear, which indicates diffusion may be the mechanism of drug release for each formulation. Correlation coefficient (r^2) and slope value for each equation in the range of (0.9835-0.9937 and n in the range of 0.4073- n=0.5836 for Peppas model. Peppas plot was found with good linearity, its n>0.5 for all formulations, indicating that drug release may follow anomalous diffusion. Stability study was carried out for the FM1 formulation by exposing it to 5-8°C, 27°C. There was no remarkable change in content of FM1 formulation during 3 months/ 12 weeks.

CONCLUSION

Floating hollow microballoons of Ritonavir were successfully prepared using Eudragit L100 and hydrophilic polymer HPMC by emulsion solvent diffusion method.

Floating hollow microballoons of different size and drug content could be obtained by varying the formulation variables. Prepared hollow microballoons of Ritonavir showed excellent micromeritic properties, good buoyancy and prolonged drug release for 12 hrs.

Thus the prepared floating microballoons may prove to bepotential candidates for multiple-unit delivery devices adaptable to any intra gastric condition.

Based on different parameters i.e. micromeritic properties, entrapment efficiency, drug content, *in-vitro* release study and stability study floating hollow microballoons of batch FM1 were found to an optimum formulation.

REFERENCES

1. Yadav A, Jain DK. Formulation and characterization of gastroretentive floating microballoons of metformin. *Int J PharmSci Res*. 2010; 1:38-43.
2. Wangsomboonsiri W, Mahasirimongkol S. Association between HLA-B4001 and lipodystrophy among HIV-infected patients from Thailand who received a stavudine-containing antiretroviral regimen. *Clin Infect Dis*. 2010, 50 (4): 597–604.
3. Nayak AK, Maji R, Das B. Gastroretentive drug delivery systems: a review. *Asian J. Pharm. Clin. Res*. 2010; 3(1): 2-10.
4. Abrol S, Trehan A, Katare OP. Formulation, characterization, and *in vitro* evaluation of silymarin-loaded lipid microballoons. Drug Deliv. 2004;11:185-91.
5. Henry JA, Hill IR. Fatal interaction between ritonavir and MDMA". Lancet. 1998, 352 (9142): 1751–1752.
6. Dashamukhi R, Kanagala V, Chittimalla AK. Formulation development of ritonavir tablets containing solid dispersions employing montmorillonite: dissolution rate enhancement. *Asian J Pharm Clin Res*. 2013; 6(2):206-208.
7. Prakash K, Raju PN, Shanta KK, Lakshmi MN. Preparation and characterization of lamivudine microcapsules using various cellulose polymers. Trop. J. Pharm. Res. 2007; 6(4):841-47.
8. Singh B, Kanoujia J, Pandey M, Saraf S. Formulation and Evaluation of Floating Microspheres of Famotidine. *Int J of Pharm Tech Research*. 2010, 2(2), 1415-1420.
9. Dinarvand R, Mirfaahi S, Atyabi F. Preparation characterization and *in vitro* drug release of isosorbide dinitrate microballoons. *J Microencapsul*. 2002;19:73-81.
10. Singh S, Joshi V, Barpete PK. Gastroretentive drug delivery system: current approaches. *J. Pharm. Res*. 2009; 2(5): 881-86.

11. Yusuf FS. Formulation and *in-vitro evaluation* of floating microballoons of stavudine**,** *Univ J of Pharm Res*. 2016, 1(1), 13-19.
12. Kulkarni RV, Sreedhar V, Mutalik S, Setty CM. Interpenetrating network hydrogel membranes of sodium alginate and polyvinyl alcohol for controlled release of prazosin hydrochloride through skin. *Int J Biol. Macromol*. 2010, 47, 520–527.
13. Murthy TEGK, Sravanthi P. Study of formulation variables affecting pioglitazone loading and its release from floating microspheres. *Invent Rapid Pharma Tech*. 2011; 2(1):156-160.
14. Kumar K, Pant NC, Ahmad S, Fateh MV, Rai AK, Verma B, Chaurasia H. Development and evaluation of floating microspheres of curcumin in alloxan induced diabetic rats, *Trop J of Pharm Res*. 2016, 15 (9), 1819-1825.
15. Hrsoliya MS, Patel VM, Pathan JK, Ankit C, Meenakshi P, Ali M. Formulation of floating microspheres of Ritonavir by cross linking-effect of $NaHCO_3$ as gas forming agent. *Int J Pharm, Bio Arch*. 2012;3(1);108-111.
16. Gadad AP, Naik SS, Dandagi PM, Bolmal UB. Formulation and evaluation of gastroretentive floating microspheres of lafutidine. Indian J of Pharm Ed and Res. 2016, 50 (2), S76-S81.
17. Kawashima Y, Niwa T, Takeuchi H, Hino T, Ito Y. Preparation of multiple unit hollow microspheres (microballoons) with acrylic resins containing tranilast and their drug release characteristics (*in vivo*). J Control Release. 1991; 16:279–90.
18. Lohithasu D, Midhun kD, Hemasundara R. Design and evaluation of Lafutidine floating tablets for controlled release by using semi-synthetic and natural polymer. *J. Drug Disc Ther*. 2014; 2(24):01-08.