

RESEARCH AND REVIEWS: JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

Preparation and Evaluation of Floating Microspheres of Ritonavir.

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Research Article

Received: 13/09/2013
Revised : 14/12/2013
Accepted: 30/12/2013

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Keywords: Ritonavir, Floating
microspheres, Sodium alginate,
Sodium bicarbonate, Guar gum.

ABSTRACT

Floating microspheres of Ritonavir was prepared by ionic gelation method with an aim of increasing the gastric residence time and for controlled release. Sodium alginate, polymeric mixture of Sodium alginate and Guar gum were used as polymers. Sodium bicarbonate was used as the gas-forming agent. The prepared floating microspheres were evaluated with respect to particle size distribution, floating behaviour, drug content, entrapment efficiency, morphology and in vitro release study. These results indicated that the release rate was found to decrease with increase in concentration of coating material applied. The wall thickness of microspheres was found to be increased with the increase in concentration of coating material applied. The floating microspheres followed zero order kinetics and the mechanism of drug release was governed by Peppas model. For all the microspheres the exponential coefficient values were found to be in between 0.7664 and 0.8565, indicating non-Fickian diffusion controlled release mechanism.

INTRODUCTION

Oral controlled release dosage forms (OCRDFS) are being developed for the past three decades due to their advantages. The design of oral controlled drug delivery system is primarily aimed at achieving more predictable and increased bioavailability, thereby obtaining a maximum therapeutic effect. However, some of these systems don't work as planned due to several physiological difficulties, such as an inability to restrain and localize the drug delivery system within the desired region of the GI tract and highly variable nature of gastric emptying process. It can be anticipated that, depending upon the physiological state of the subject and the design of pharmaceutical formulation, the emptying process can last from a few minutes to 12 hours. Rapid GI transit can prevent complete drug release in the absorption zone and reduce the efficacy of the administered dose since the majority of drugs are absorbed in the stomach or upper part of the small intestine [1].

Thus, placement of a drug delivery system in a specific region of the GI tract offers numerous advantages, especially to the drugs having a narrow absorption window, stability problems in the intestine, poor solubility in alkaline pH, local activity in the stomach and property to degrade in the colon. Therefore, the design of a sustained release preparation requires both prolongation of gastrointestinal transit of the dosage form as well as controlled drug release. Recently, one of such systems has been reported as floating drug dosage systems (FDDS) [2,3]. FDDS have a lower density than gastric fluids and thus remain buoyant in the stomach, without affecting the gastric emptying rate for a prolonged period of time. While the systems are floating, the drug is released slowly from the system at a desired rate. Ritonavir is an antiretroviral agent used in the treatment of HIV and viral diseases; it has been taken as a model drug in the present investigation because of its low biological half-life (3-5h) and moreover, it is primarily absorbed from the stomach [4].

MATERIALS AND METHODS

Ritonavir was obtained as a gift sample from Aristo, Bhopal (MP). Sodium bicarbonate, calcium chloride, acetic acid, used was of analytical grade, purchased from Merck Specialties Pvt Ltd, Chemistry-Chem. Ltd, Loba Chemie Pvt Ltd, Mumbai respectively. Guar gum was purchased from Yarrow Chemical Products, Ambala, Sodium alginate was purchased from Sd.Fine Chemicals, Mumbai.

Preparation of Ritonavir floating microspheres^[5]

The floating microspheres containing Ritonavir were prepared by orifice ionic gelation technique. Sodium alginate alone or in combination with guar gum and the gas forming agent sodium carbonate were dispersed in the purified water to form a homogeneous polymer mixture. The drug, Ritonavir was added to the polymer dispersion and mixed thoroughly on a magnetic stirrer to form a homogeneous dispersion. The gelation medium was prepared by dissolving calcium chloride in 2% glacial acetic acid. The homogenous alginate solution was extruded using 21G syringe needle into the gelation medium. The distance between the edge of the needle and surface of gelation medium was about 10cms. The gel microspheres formed were left in the solution with gentle stirring for 30 min at room temperature to improve mechanic strength. After that, microsphere was collected and washed with distilled water twice, dried at room temperature for 24 hr and stored in desiccators. The composition and the conditions observed during the preparation of microspheres are showed in table no 1.

Table.1: Composition of Floating Microsphere of Ritonavir

Excipients	F1	F2	F3	F4	F5	F6
Ritonavir (mg)	500	500	500	500	500	500
Sodium Alginate (mg)	500	1000	1500	250	500	750
Guargum (mg)	-	-	-	250	500	750
Concentration of Sodium bicarbonate (% w/w)	50	50	50	50	50	50
Concentration of Calcium chloride(% w/v)	10	10	10	10	10	10
Acetic acid (%v/v)	1.5	1.5	1.5	1.5	1.5	1.5
Drug:polymer ratio	1:1	1:2	1:3	1:1	1:2	1:3

Measurement of Micromeritic properties ^[6]

The flow properties of prepared microspheres were investigated by measuring the bulk density, tapped density, Carr's index and packing factor. The bulk and tapped densities were measured in a 10 ml graduated measuring cylinder. The sample contained in the measuring cylinder was tapped mechanically by means of constant velocity rotating cam. The initial bulk volume and final tapped volume were noted from which, their respective densities were calculated.

Particle size Analysis ^[7]

Microspheres were separated into different size fractions by sieving for 10 minutes using a mechanical shaker (Labtech, Indore, Co. India) containing standard sieves # 16, # 24, # 30, # 44 and # 60 and mean particle sizes of microspheres were calculated.

Buoyancy test ^[8]

In vitro evaluation of floating behaviour studies were performed by placing 50 particles into 50 ml glass flask and subsequent addition of 50 ml 0.1 N HCl containing 0.02% w/v. Tween 20 was added to exclude floating due to non wetted surfaces followed by horizontal shaking (37 O, 75 rpm). At pre determined time intervals (2, 4, 6, 8 hrs) the flasks were allowed to stand to 5 mins without agitation and numbers of floating particles were counted. The % of floating microspheres was calculated by following equation.

$$\% \text{ floating microsphere} = \frac{\text{no.of floating microsp heres}}{\text{initial no.of floating microsp heres}} \times 100$$

Drug content and encapsulation efficacy of floating microspheres ^[9]

50 mg of formulations was dissolved in 50 ml of 0.1N HCl. The samples were assayed for drug content by UV- spectrophotometer (UV-1700) at 284 nm and the drug content was calculated.

In vitro drug release study ^[9]

The drug release rates from floating microspheres were carried out using Tablet dissolution test apparatus. A weight of floating microspheres corresponding to 100 mg of drug was filled into a capsule and placed in basket. Dissolution media was 500 ml 0.1N HCl maintained at $37 \pm 1^{\circ}$ and stirred at 100 rpm. Samples (5 ml) were withdrawn at suitable interval of time and volume was adjusted. It was then assayed spectrophotometrically at 284 nm.

Fourier Transform Infrared Spectroscopy [FTIR] Study ^[10]

Drug-polymer interactions were studied by FTIR spectroscopy. The spectra were recorded for pure drug, pure polymer and drug-loaded microspheres using FTIR. Samples were prepared in KBr disks [2mg sample in 200mg KBr]. The scanning range was 400-4000cm⁻¹ and the resolution was 2cm⁻¹.

RESULTS AND DISCUSSION

Floating microspheres of Ritonavir were prepared by ionic gelation technique. This process produced uniform microspheres. Microspheres were developed with 1:1, 1:2, 1:3 ratios of core:coat to determine the affect of coating material concentration on the release rate of Ritonavir. These microspheres were characterized for flow properties and the results are given in Table 2. All the formulations offered good flow property. The microspheres also evaluated for size analysis, Drug Content and % Encapsulation Efficiency. The results are given in Table 3. The technique also showed good entrapment efficiency. The microspheres were subjected to *In-vitro* release studies by employing 0.1N Hydro chloric acid and the data was shown in Figure 1&2. When the amount of drug release values were plotted against time straight lines were obtained in all the cases indicating that the rate of drug release from these microspheres followed zero order kinetics (Fig 3&4). To ascertain the mechanism of drug release from various microspheres, plot of log %Released vs log time (peppas plots) were drawn. The plots were found to be linear (Fig 5&6). For all the microspheres the exponential coefficient values were found to be in between 0.7664 and 0.8565, indicating non fickian diffusion controlled release mechanism. These results indicated that the release rate was found to be decrease with increase in concentration of coating material applied. The wall thickness of microspheres was found to be increased with the increase in concentration of coating material applied. There exists a good correlation ship in between wall thickness and release rate constant. Drug polymer interactions were studied by FT-IR analysis. Figure: 7 showed the IR spectra of pure Ritonavir. The characteristic CH stretching, NH stretching of secondary amine, C=C stretching and C=O stretching of pure drug was observed at 2964.47 cm⁻¹, 3357.73 cm⁻¹, 3025.35cm⁻¹ and 1714.67 cm⁻¹. The characteristic peaks confirmed the structure of Ritonavir. The same peaks were also reported in all drug loaded microspheres. There was no change or shifting of characteristic peaks in drug loaded microspheres suggested that there was no significant drug polymer interaction which indicates the stable nature of drug in all formulations.

Table 2: Flow properties of Ritonavir floating microspheres prepared with sodium alginate alone and incombination with guar gum

Formulation	Bulk density (g/ml)	Tapped density (g/ml)	Angle of repose (θ)	Compressibility index (%)	Hausner's ratio
F ₁	0.52	0.62	27.14°	13.88	1.16
F ₂	0.53	0.68	29.14°	13.91	1.16
F ₃	0.57	0.66	25.14°	12.70	1.14
F ₄	0.53	0.63	29.26°	15.07	1.17
F ₅	0.54	0.62	29.37°	12.82	1.14
F ₆	0.51	0.61	26.23°	15.08	1.17

Table 3: Particle size of Drug content, Entrapment efficiency, Wall thickness of Ritonavir microspheres prepared with sodium alginate alone and incombination with guar gum,

Formulation	Mean Diameter (µm)	% Drug Content	Entrapment Efficiency	Wall Thickness (µ M)	Invitro Buoyancy
F ₁	322.49	49.12	98.24	27.42	84.15±1.23
F ₂	441.94	65.45	99.16	38.29	86.24±1.74
F ₃	561.66	74.56	99.41	45.43	87.12±1.13
F ₄	340.48	49.24	98.48	28.52	86.36±1.45
F ₅	463.99	65.58	99.33	39.44	87.76±1.53
F ₆	584.64	74.69	99.58	46.57	88.16±1.26

Table 4: Dissolution kinetics data of Ritnovir floating microspheres prepared with sodium alginate alone and incombination with guar gum

Formulation	Correlation coefficient values					
	Zero order	First order	K ₀	T ₅₀	T ₉₀	n
F ₁	0.9855	0.8881	13.26	3.770	6.787	0.8406
F ₂	0.9976	0.7811	11.42	4.37	7.880	0.7689
F ₃	0.9943	0.8108	8.47	5.303	10.625	0.8121
F ₄	0.9916	0.8094	17.8161	4.029	7.2522	0.8565
F ₅	0.9943	0.7903	14.3497	4.5167	8.1300	0.7664
F ₆	0.9957	0.7956	13.8115	5.5066	9.9118	0.7862

Figure 1: Invitro dissolution profiles of Ritnovir floating microspheres prepared with sodium alginate

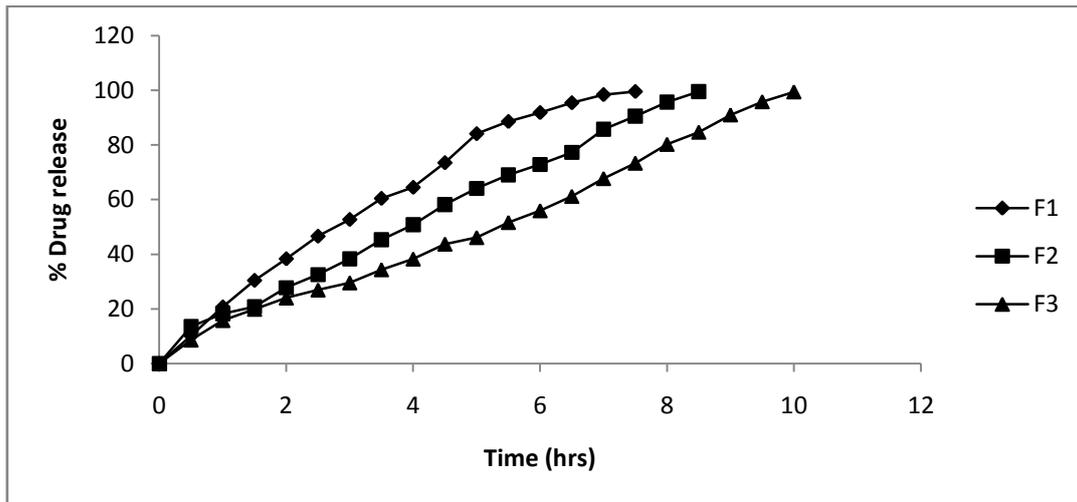


Figure 2 : Invitro dissolution profiles of Ritnovir floating microspheres prepared with sodium alginate and guar gum :

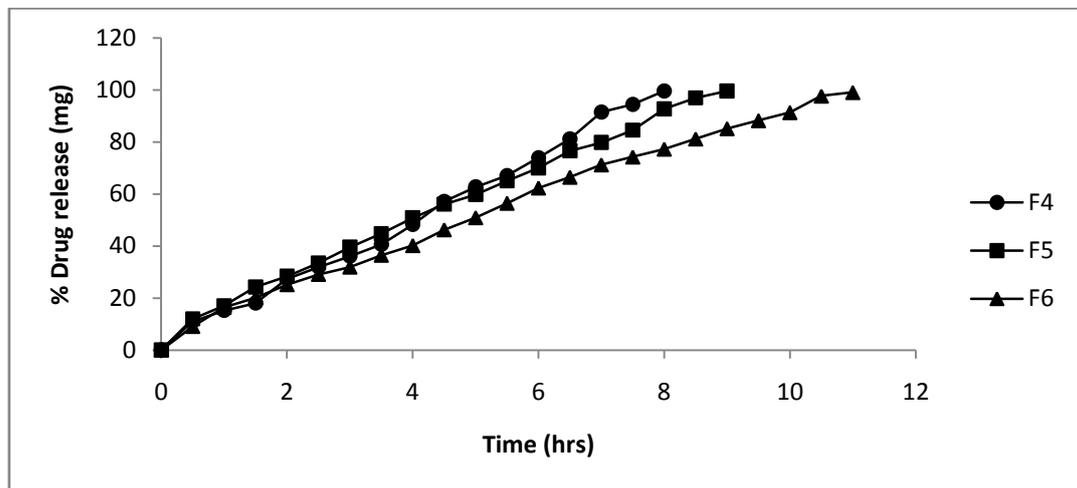


Figure 3: Zero order Plots of Ritonavir Floating Microspheres Prepared with Sodium alginate :

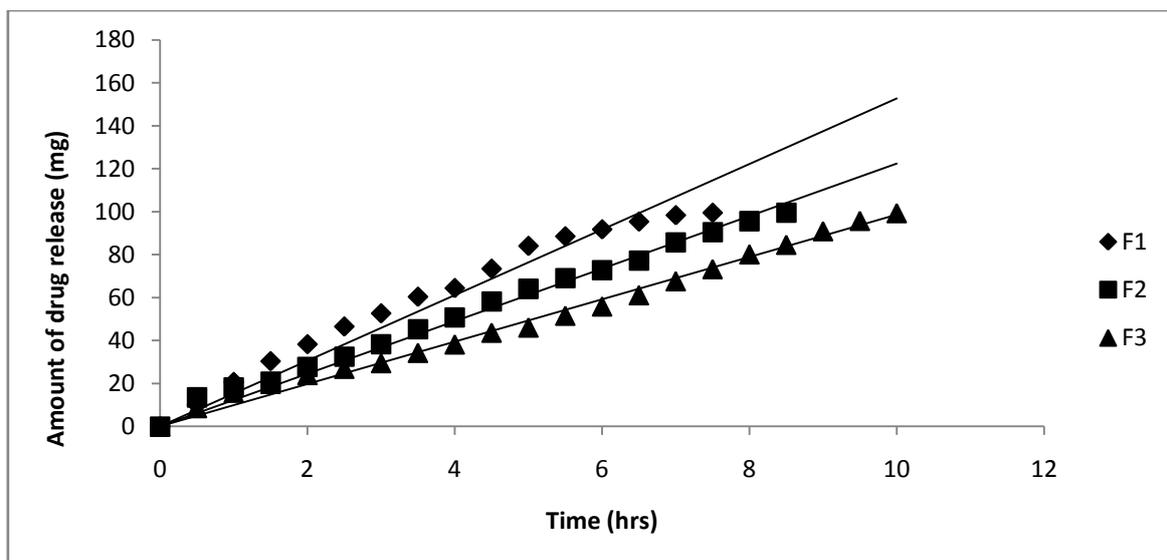


Figure 4: Zero order Plots of Ritonavir Floating Microspheres Prepared with Sodium alginate and guar gum

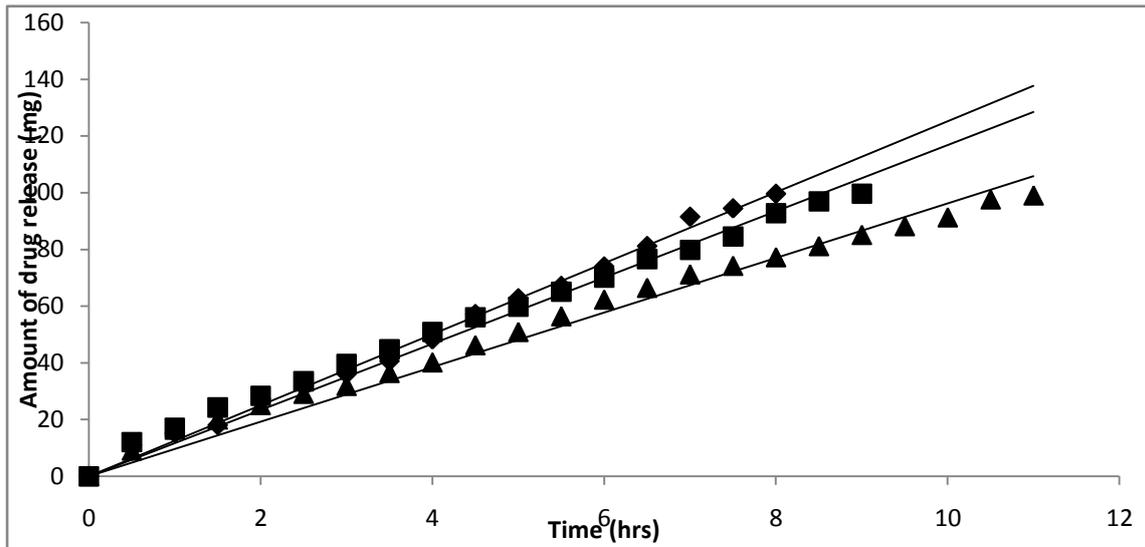


Figure 5: Peppas Plots of Ritonavir Floating Microspheres Prepared with Sodium alginate

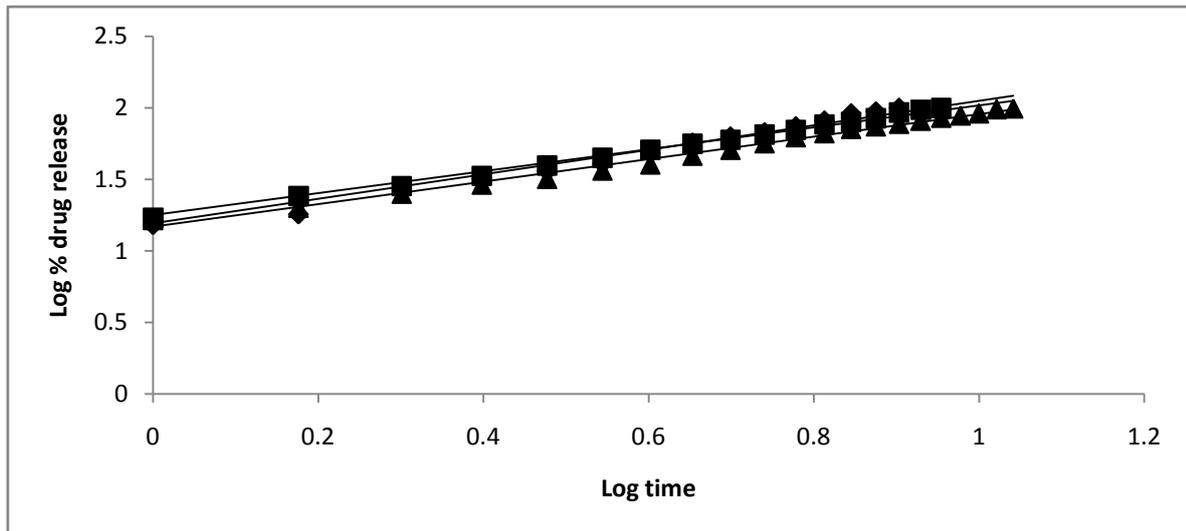


Figure 6 : Peppas plots of Ritonavir Floating Microspheres prepared with Sodium alginate and Guar gum

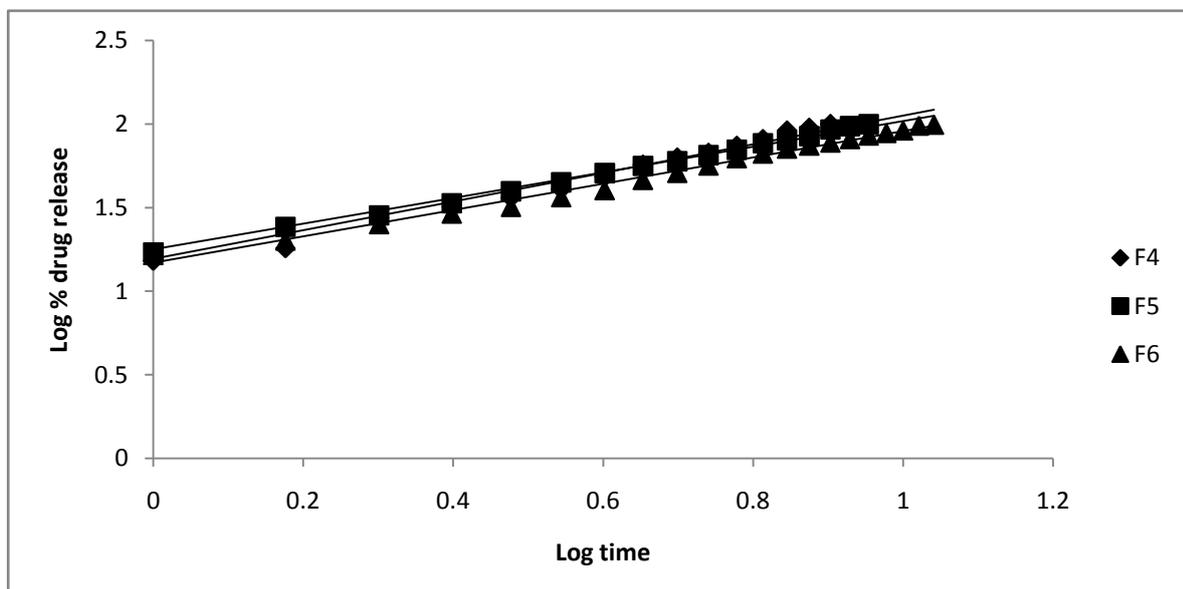


Figure 7: FTIR Spectrum of Ritonavir

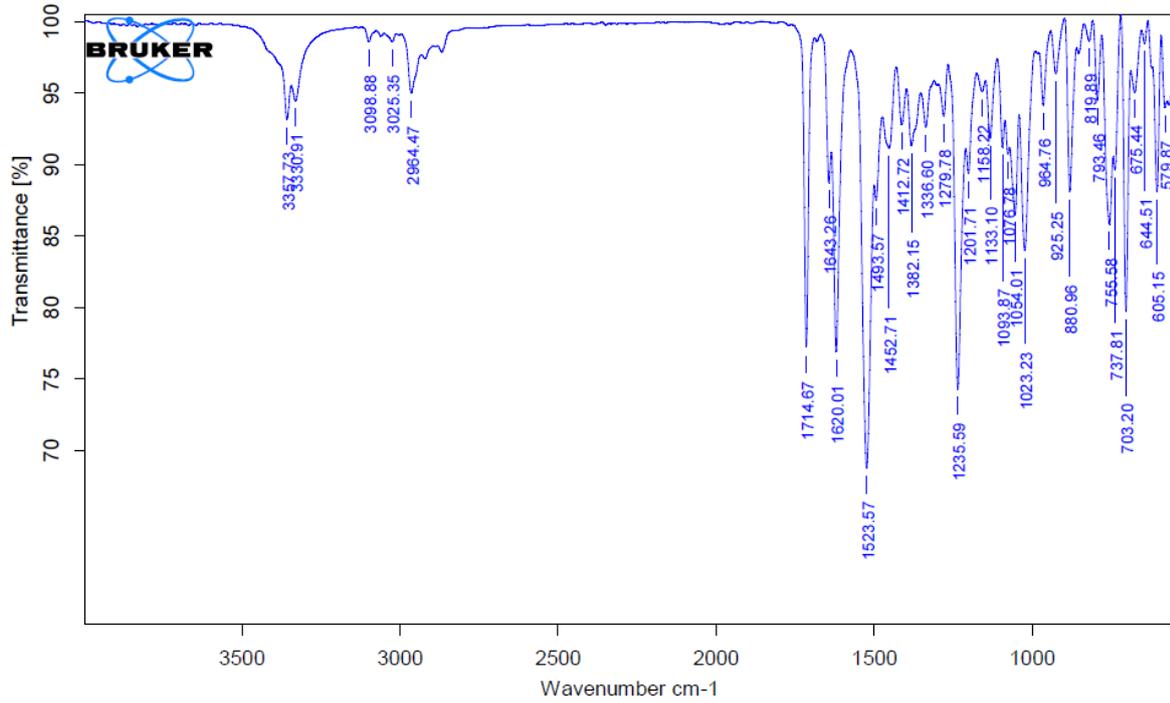


Figure 8: FTIR Spectrum of Ritonavir Floating Microspheres Prepared with Sodium Alginate

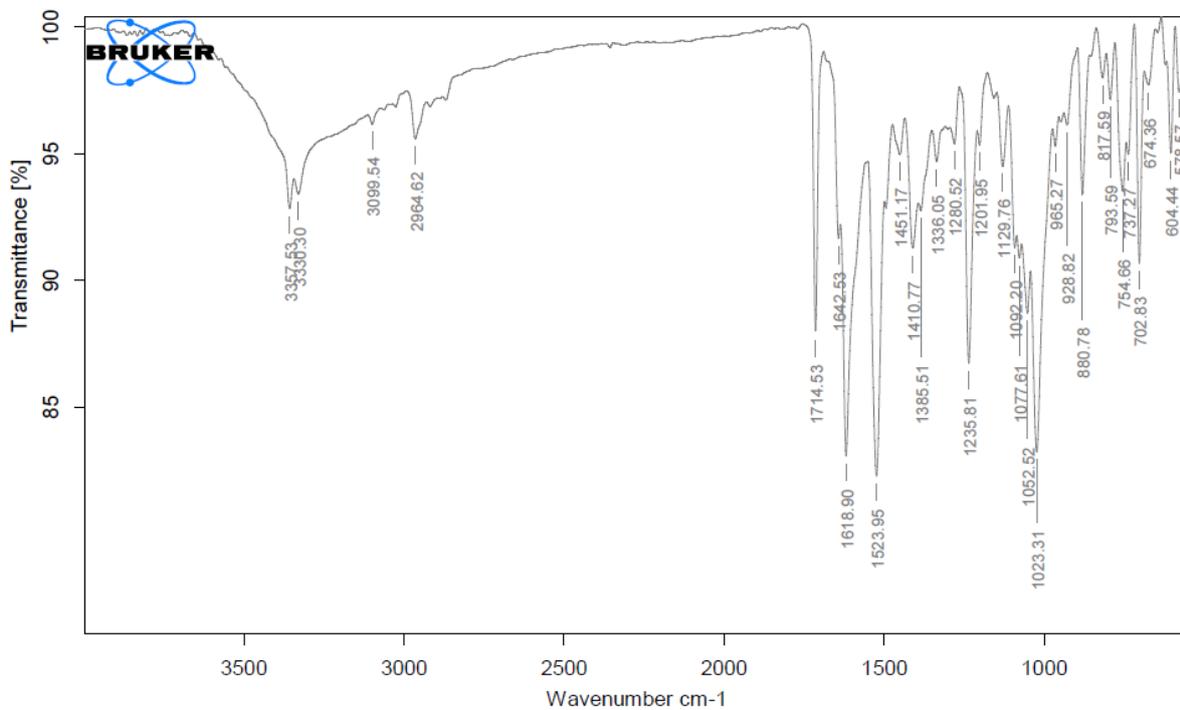
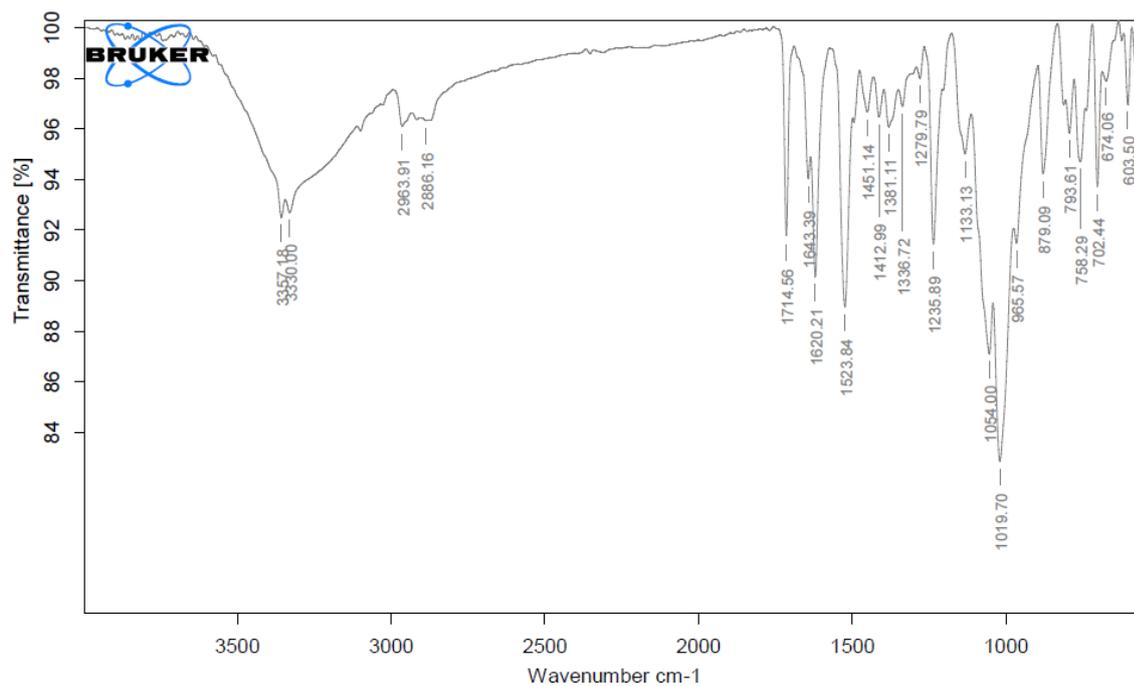


Figure 9: FTIR Spectrum of Ritonavir Floating Microspheres Prepared with Sodium Alginate and Guar gum :



CONCLUSION

Floating microspheres of Ritonavir prepared by ionic gelation technique were found to be suitable for controlled release. The floating microspheres prepared with sodium alginate and guar gum in 1: 3 ratio show prolonged release rate when compared with other formulations.

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