Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(5):952-960

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Formulation and evaluation of ritonavir mucoadhesive microspheres

Sellappan Velmurugan[1,2]* and Mohamed Ashraf Ali[1]

[1]Department of Pharmaceutics, Sunrise University, Alwar, Rajasthan, India
[2]Department of Pharmaceutics, KLR Pharmacy College, Palvoncha, Andhra Pradesh, India

---

ABSTRACT

The aim of the present research was to formulate and evaluate Xanthan gum and Carbopol 940 mucoadhesive microspheres for controlled release of Ritonavir. The mucoadhesive microspheres were formulated by Ionotropic gelation technique, using sodium alginate, Xanthan gum and carbopol as mucoadhesive polymer in various proportions in combination. Further, the prepared Ritonavir mucoadhesive microspheres were characterized for particle size, morphology, entrapment efficiency, mucoadhesion, in vitro drug release, ritonavir release kinetics and compatability studies (FTIR &DSC). The Ritonavir Microspheres were free-flowing and discrete. The mean particle size ranged from 772.71 ±4.77 μm to 941.50 ±3.13 μm and the entrapment efficiencies ranged from 72.93 to 96.86 %. The Ritonavir entrapment efficiency was found to be dependent on type and concentration of mucoadhesive polymer used for formulation. Scanning electron microscopy revealed the surface morphology of microspheres .The FTIR & DSC study confirmed stable character of Ritonavir in the drug-loaded mucoadhesive microspheres. The crystallinity of ritonavir was found to be reduced in prepared mucoadhesive microspheres, which were confirmed by XRD studies. The mechanism of Ritonavir release from the mucoadhesive microsphere was found to be anomalous and super case-II transport type. Stability studies were done for the best formulation F8 indicates that there is no change in entrapment efficiency and percentage mucoadhesion of the formulation.

Keywords: Sodium alginate, Xanthan gum, Carbopol 940, mucoadhesive Microspheres, Ritonavir.

INTRODUCTION

The conventional formulation of anti HIV drugs is rapidly dissolved in upper gastric intestine and produces peak plasma concentration within few hours and then declines quickly. Consequently, multiple dosing is recommended for maintaining the effective plasma concentration. However, conventional dosage forms exhibited drawbacks due to their inability to retain and localize the system at gastro-intestinal tract [1]. All the drawbacks necessitate the development of an effective drug delivery system which could utilizes all the potential of anti HIV drugs. Last two decades the development of mucoadhesive microspheres has gained considerable interest in the design of drug delivery systems to prolong the gastric residence time of the dosage form at the site of absorption and to facilitate intimate contact of the dosage form with the underlying absorption surface to improve the bioavailability of bioactives [2-5]. Among the various methods developed for formulation of mucoadhesive microsphere, the ionotropic gelation method has gained much attention due to its easy, rapid fabrication and does not involve the use of toxic organic solvent [6,7].

Ritonavir is an antiretroviral agent used in treatment of HIV and viral diseases, belongs to class II under BCS and exhibits low & variable oral bioavailability due to poor aqueous solubility. Ritonavir is a peptidomimetic inhibitor of

both the HIV-1 and HIV-2 proteases [8]. Ritonavir having narrow therapeutic index, low bioavailability (65%) and short biological half life (3-5hrs). The usual dose of ritonavir is 100 mg twice daily; moreover it is primarily absorbed from stomach [9]. All the shortcomings necessitate the development of gastrorentensive mucoadhesive microspheres for enhancing retention of formulation in GIT which could utilize all the efficacy of Ritonavir, thereby reduced dosing frequency, improve the bioavailability and to enhance the quality of HIV infected patients.

EXPERIMENTAL SECTION

Materials

Ritonavir was a gift sample from Dr.Reddys Pharma Ltd, Hyderabad. Sodium alginate, Xanthan gum and Carbopol 940 polymers were received as gift sample from Hetro Pharma Ltd, Hyderabad. All other ingredients and solvents used were of analytical grade.

Formulation of Ritonavir mucoadhesive microspheres

The composition of the various Ritonavir mucoadhesive microspheres formulations were mentioned in Table1. Ritonavir and mucoadhesive polymers were individually passed through sieve ≠ 80. The required quantities of mucoadhesive polymers were dissolved in purified water to form a homogenous solution. Ritonavir was added to the polymer solution , mixed thoroughly with magnetic stirrer at 400 rpm to form a homogeneous dispersion and resulting dispersion was sonicated for 30 min to remove entrapped air bubbles. For the formation of mucoadhesive microspheres homogeneous dispersion was then extruded manually drop wise into 10% crosslinking (aluminum sulphate) using syringe (needle size 24 G). The extruded droplets were cured in the aluminium sulphate solution for 30 minutes to complete the reaction and to produce spherical rigid microspheres [10]. The obtained ritonavir microspheres were collected by decantation, washed continually with distilled water and dried at 45°C for12 hour. The final products were stored in well closed container for further use.

Table 1: Composition of Ritonavir mucoadhesive microspheres

Formulation code	Drug: Polymer ratio	Polymer ratio
F1	1:0.5	0.25:0.25 (Sodium alginate: Xanthan gum)
F2	1:1	0.5:0.5 (Sodium alginate: Xanthan gum)
F3	1:1.5	0.75:0.75 (Sodium alginate: Xanthan gum)
F4	1:2	1:1 (Sodium alginate: Xanthan gum)
F5	1:0.5	0.25:0.25 (Sodium alginate: Carbopol 940)
F6	1:1	0.5:0.5 (Sodium alginate: Carbopol 940)
F7	1:1.5	0.75:0.75 (Sodium alginate: Carbopol 940)
F8	1:2	1:1 (Sodium alginate: Carbopol 940)

Percentage yield

The percentage yield was calculated by dividing weight of dried Ritonavir microspheres (W1) by initial weight of the ritonavir and polymers (W2) used for the formulation and converting the weight ratio into percent [11].

Particle Size

Particle size and size distribution of the ritonavir microspheres were measured by sieve analysis method. The ritonavir microspheres were separated into different size fractions (% weight fraction) by sieving for 10 min using standard sieves having nominal mesh aperture of 1.4 mm, 1.2 mm, 1.0 mm, 0.85 mm and 0.71 mm and the mean particle size of the ritonavir microspheres was determined [12].

Morphology of Microspheres

The surface morphology and shape of the Ritonavir mucoadhesive microspheres was confirmed by scanning electron microscopy using SEM Model – Philips-XL 20. The sample was mounted on to an aluminum stub and sputter-coated with platinum particles in an argon atmosphere [13].

Drug Entrapment Efficiency

Entrapment efficiency of prepared Ritonavir microsphere was estimated by method of extraction of drug present in microsphere. The dried mucoadhesive microspheres (100mg) were taken and extracted in 100 ml of 0.1N HCl for 24 hours in rotary shaker. The solution was filtered through a 0.45 μm filter and the concentration of Ritonavir present in filtrate determined spectro photometrically at 240 nm (LABINDIA UV-3092 PC) against 0.1 N HCl as a blank [14].

Mucoadhesive Test

The mucoadhesive property of Ritonavir microspheres was evaluated by *in vitro* wash off test . The freshly excised piece of goat intestinal mucosa was mounted on the glass slide using cyanoacrylate glue. About 100 microspheres were spread onto each wet rinsed intestinal mucosa and immediately the support was hung onto the arm of USP disintegration apparatus. Now intestinal mucosa was given a slow regular up and down movement in test fluid (0.1N HCL buffer at 37±0.5^{0}C) by operating the disintegration test apparatus. Every one hour intervals up to 8 hrs the equipment was stopped and the number of Ritonavir mucoadhesive microspheres still sticking onto the intestinal mucosa was counted and percent mucoadhesion was calculated [15].

***In Vitro* Dissolution**

Mucoadhesive Microspheres containing equivalent to 100 mg of Ritonavir were introduced into dissolution medium of 0.1N HCl (900ml) for 12 hrs at 37±0.5°C at a rotation speed of 50 rpm by using USP type II dissolution test (Electrolab Mumbai, India). Samples of 5ml were withdrawn through a filter (0.45 µ) at every one hour intervals up to 12th hrs and replaced with equal volume of 0.1N HCl buffer. The samples were analyzed at 240 nm for Ritonavir content using UV spectrophotometer. All dissolution runs were carried out in triplicate [16].

Release kinetic and mechanism of Ritonavir release

In order to understand the mechanism and kinetic of Ritonavir release from the prepared microspheres, formulation were analyzed by fitting the dissolution data into various kinetic models like zero order; first order, korsemeyer peppas and Higuchi's model and Coefficient of correlation (r) values were calculated for the liner curves by regression analysis of the above plots [17].

FTIR Studies

Compatibility study of Ritonavir with different mucoadhesive polymers was determined by I.R. Spectroscopy (FTIR) using Shimadzu FT-IR spectrometer model. The pellets were prepared with IR grade KBr using Ritonavir, mucoadhesive polymers, mucoadhesive microspheres formulations containing both Ritonavir & polymer and the scanning were done between wave numbers 4000 to 400 cm^{-1} at 4 cm^{-1} resolution.

Thermal Analysis (DSC)

Differential scanning calorimetries were carried out on pure drug Ritonavir and Ritonavir loaded microspheres using a Shimadzu DSC 60 to evaluate any possible Ritonavir - mucoadhesive polymers interaction. Samples (4mg each) were accurately weighed into aluminum pans and sealed. DSC run were conducted over a temperature range 40-300 °C at a heating rate of 10 °C / min under nitrogen atmospheres [18].

***X-Ray* Diffraction study (XRD)**

The crystallinities of ritonavir and ritonavir loaded mucoadhesive microspheres were evaluated by XRD measurement using an X-ray diffractometer. XRD studies were performed on the prepared samples by exposing them to Cuk α1 radiation (40 kV, 30 mA) and the scanning rate was 5° /min over a range of 4-90° and with an interval of 0.1 [19] .

Stability Study

To assess the Ritonavir and mucoadhesive formulation stability, stability studies were carried out as per ICH guidelines. The best mucoadhesive microspheres formulation (F8) was selected for stability study on the basis of *in vitro* drug dissolution studies; drug entrapment efficacy and *invitro* wash off test. In the investigation, selected formulations were stored at 4^{0}C ± 1^{0}C / Ambient, 25 ± 2^{0}C/ 60 ± 5 % RH, 40 ± 2^{0}C/ 75 ± 5 % RH in closed high density polyethylene bottles for 90days. The samples were periodically evaluated for entrapment efficiency and percentage mucoadhesion [20, 21].

RESULTS AND DISCUSSION

Percentage yield and Micromeritics studies

The purpose of this study was to formulate mucoadhesive microspheres of Ritonavir by ionotropic gelation method, using sodium alginate, Xanthan gum and Carbopol 940 as a polymer, Carbopol microspheres are used to provide controlled release of Ritonavir and to enhance the uptake of drug across epithelial layer.

Table 2 : Physico chemical properties of Ritonavir mucoadhesive microspheres

Formulation code	Percentage yield [a]	Theorital drug content (mg)	Practical drug content (mg) [a]	Entrapment efficiency [a]	Particle size [µm] [a]
F1	86.11 ± 1.54	66.6	48.57 ± 0.76	72.93 ± 1.14	772.71 ± 4.77
F2	87.93 ± 2.11	50	39.43 ± 0.50	78.85 ± 1.00	804.50 ± 3.50
F3	90.41 ± 1.51	40	34.24 ± 0.70	85.60 ± 1.75	845.85 ± 6.37
F4	91.96 ± 1.62	33	29.65 ± 0.85	89.84 ± 2.57	875.46 ± 5.54
F5	88.69 ± 1.80	66.6	52.94 ± 0.56	79.50 ± 0.83	809.60 ± 8.17
F6	90.20 ± 2.51	50	44.04 ± 0.70	88.07 ± 1.40	856.13 ± 3.63
F7	93.11 ± 1.96	40	37.56 ± 0.73	93.89 ± 1.82	896.00 ± 7.25
F8	95.00 ± 1.77	33	31.96 ± 0.70	96.86 ± 2.12	941.50 ± 4.13

[a] *Mean ±SD, n = 3.*

The prepared Ritonavir microsphere gave good percentage yield. The percentage yield of ritonavir mucoadhesive microspheres ranged from 86.11 % to 95.00 %. All Ritonavir microspheres formulations were evaluated for micrometric properties and results are shown in Table 2. Angle of repose of all microspheres batch varied from 24.47 to 35.02.Compressibility index varies from 10.42 % to 16.06 %. Hausner's ratio varies from 1.096 to 1.76. Here all these formulations results revealed good flow property and compressibility.

Table 3: Micromeritics properties of ritonavir mucoadhesive microspheres

Formulation code	Bulk density [a]	Tapped density [a]	Compressibility index [a]	Hausner's ratio [a]	Angle of Repose [a]
F1	0.400 ± 0.012	0.453 ± 0.016	10.94 ± 1.56	1.123 ± 0.020	24.47 ± 0.983
F2	0.374 ± 0.009	0.431 ± 0.018	13.12 ± 1.63	1.151 ± 0.022	26.59 ± 1.043
F3	0.353 ± 0.006	0.412 ± 0.012	14.32 ± 1.19	1.167 ± 0.016	28.64 ± 1.096
F4	0.336 ± 0.006	0.401 ± 0.011	16.06 ± 0.93	1.176 ± 0.013	31.62 ± 0.656
F5	0.333 ± 0.007	0.365 ± 0.012	10.42 ± 1.91	1.096 ± 0.015	25.49 ± 1.061
F6	0.328 ± 0.009	0.365 ± 0.016	10.61 ± 0.53	1.119 ± 0.011	28.64 ± 1.096
F7	0.306 ± 0.006	0.343 ± 0.011	10.75 ± 1.01	1.191 ± 0.013	31.79 ± 1.223
F8	0.279 ± 0.005	0.325 ± 0.009	13.89 ± 0.75	1.161 ± 0.010	35.02 ± 1.347

[a] *Mean ±SD, n = 3.*

Particle Size

The average particle size of Ritonavir mucoadhesive microspheres ranged from 772.71 ± 4.77 to 941.50 ± 4.13 µm, and such particles are considered to be suitable for oral administration. The results also revealed that with the increase in the Ritonavir: polymer ratio there was an increase in the size of mucoadhesive microspheres (Table 2) [22].

Morphology of Microspheres

The morphology of the Ritonavir microspheres of optimized formulation F8 was examined by scanning electron microscopy and depicted in the Figure 1. The SEM photographs revealed that microspheres were discrete and spherical shape with a rough surface morphology which could be due to the surface association of the ritonavir with mucoadhesive polymer [23].

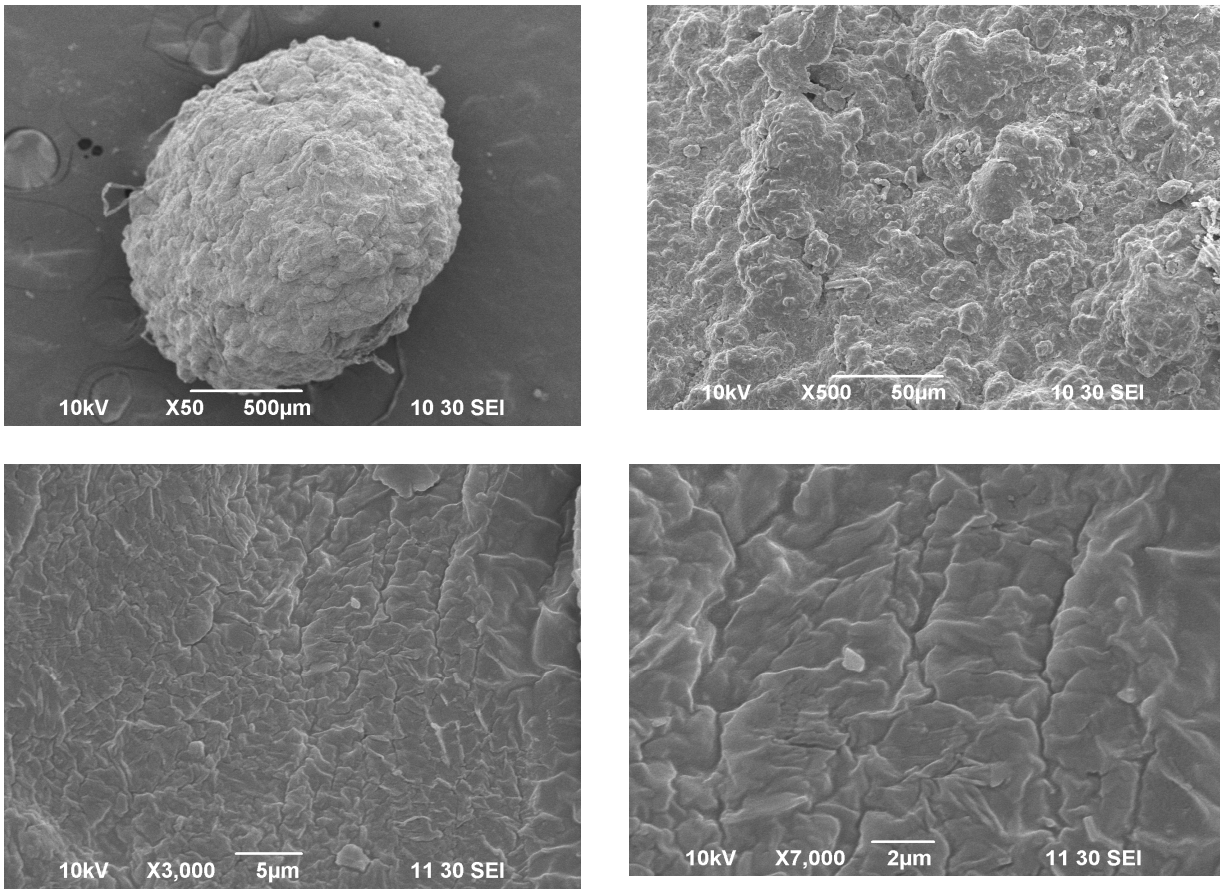

Figure 1: Scanning electron photomicrographs of the Formulation F8: a) 50 X, b) 500 X, c) 3000 X, d) 7000 X

Entrapment Efficiency

The percentage entrapment efficiency ranged from 72.93 to 96.86%. (Table 2). The entrapment efficiency of the ritonavir microspheres prepared with Carbopol was higher than those of microspheres prepared with Xanthan gum. The results revealed that increase in the concentration of the mucoadhesive polymer increase the entrapment efficacy of ritonavir. This can be due to increase in the viscosity of the mucoadhesive polymeric solution, which increases the strength of formed matrix [24].

Table 5: Results of *in vitro* wash off test

	In 0.1 M HCL (pH 1.2) [a]					In Phosphate buffer (pH 7.4) [a]				
Hours	**1**	**2**	**4**	**6**	**8**	**1**	**2**	**4**	**6**	**8**
F1	100	95 ± 1.53	80 ± 2.65	56 ± 2.08	37 ± 0.58	96± 0.58	92 ± 1.53	73 ± 1.73	50 ± 100	33 ± 2.52
F2	100	98 ± 1.53	84 ± 2.52	71 ± 1.15	49 ± 1.73	98 ± 1.15	95 ± 0.58	80 ± 1.53	65 ± 1.00	45 ± 1.73
F3	100	99 ± 1.00	86 ± 0.58	73 ± 2.08	60 ± 1.73	99 ± 0.58	97 ± 1.00	83 ± 1.53	68 ± 2.00	53 ± 2.52
F4	100	99 ± 0.58	91 ± 1.53	80 ± 2.31	67± 2.89	100	98 ± 0.58	89 ± 1.15	74 ± 2.52	66± 1.53
F5	100	98 ± 1.53	88 ± 1.73	73 ± 2.31	54 ± 2.52	97 ± 1.53	96 ± 1.15	85 ± 0.58	71 ± 1.00	48 ± 2.52
F6	100	100	94 ± 1.15	80 ± 2.52	61 ± 0.58	98 ± 0.58	96 ± 2.52	89 ± 2.00	75 ± 1.53	54 ± 1.00
F7	100	100	95 ± 1.53	83 ± 2.52	64 ± 2.08	100	98 ± 0.58	91 ± 1.53	78 ± 2.52	60 ± 2.08
F8	100	100	98 ± 1.53	87 ± 1.53	73 ± 2.08	100	99 ± 1.00	93 ± 2.52	84 ± 1.73	70 ± 0.58

[a] *Mean ±SD, n = 3.*

Mucoadhesive Test

The *invitro* wash-off of ritonavir microspheres was faster at simulated intestinal fluid (pH 7.4) than that at simulated gastric fluid (pH 1.2). Our results are supported by the report of Robinson et al. [25]. The solubility, hydration and mucoadhesiveness of the polymers depend on the pH of the *in- vitro* wash off medium. The faster *in- vitro* wash-off

results observed at simulated intestinal fluid may be owing to the ionization of carboxyl acid group and other functional groups in the mucoadhesive polymers, which increase their solubility and reduce mucoadhesive strength. The results of the *in- vitro* wash-off test indicated that the ritonavir microspheres had fairly good mucoadhesive properties. The developed ritonavir mucoadhesive microspheres would adhere to the Gastric mucosa, thus resisting gastric emptying and extend residence time at the absorption site thereby enhance the bioavailability of drug [26, 27].

***In Vitro* Dissolution studies**

The *invitro* Ritonavir release profiles for all batches were shown in Figure 2. The Ritonavir release behaviors depend upon the nature and concentration of mucoadhesive polymers in polymer matrix [28,29].Xanthan gum-alginate microspheres (F1 and F4) were able to control the Ritonavir release up to 12 hours whereas Carbopol microspheres were able to control the drug released more than 12 hours. It has been observed that Xanthan gum based mucoadhesive microsphere showed comparatively rapid ritonavir release as compared to Carbopol based formulations.

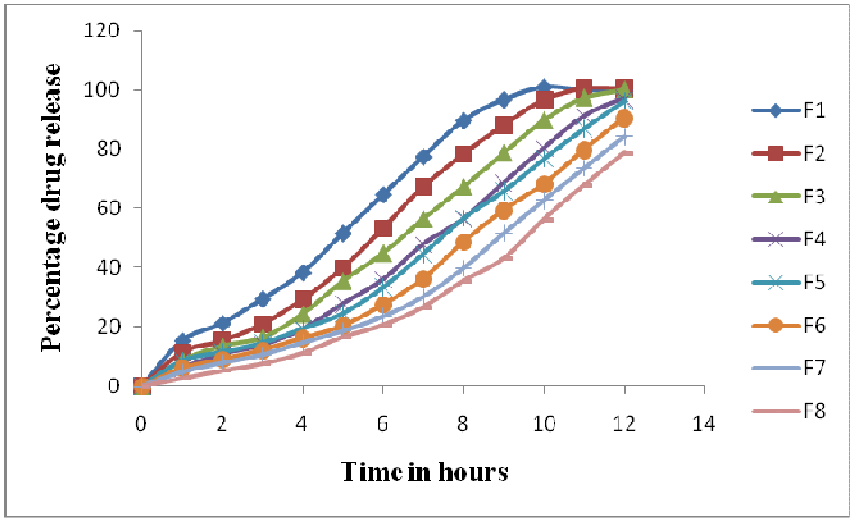

Figure 2: Comparative release profile of formulation F1 to F8

Release kinetic and mechanism of ritonavir release

Drug release kinetic data for Ritonavir microspheres was shown in Table No. 3. All the formulations (F1 to F8) follow zero order release kinetics with regression values ranging from 0.938 to 0.986. Korsmeyer-Peppas plots 'n' value ranges from 0.867 to 1.387 indicating that the Ritonavir release mechanism followed anomalous and super case-II transport mechanism.

The results of the in-vitro mucoadhesion studies of all ritonavir formulations were shown in Table 4. Percentage mucoadhesion of batches increased with the increase in amount of mucoadhesive polymers. The higher mucoadhesion of Carbopol based mucoadhesive microspheres may be attributed to the higher molecular weight of Carbopol than Xanthan gum based microspheres.

Table 4: Release Kinetic parameter of Ritonavir from mucoadhesive microspheres

Formulation code	Zero order	First order	Higuchi	Korsmeyer peppas	n-value	Hixson crowel
F1	0.954	0.902	0.961	0.974	0.867	0.747
F2	0.976	0.853	0.952	0.965	1.007	0.639
F3	0.986	0.794	0.941	0.969	1.089	0.530
F4	0.979	0.783	0.916	0.972	1.178	0.686
F5	0.969	0.775	0.897	0.937	1.061	0.688
F6	0.957	0.810	0.878	0.947	1.152	0.763
F7	0.942	0.823	0.856	0.952	1.188	0.792
F8	0.938	0.829	0.850	0.973	1.387	0.804

FTIR Studies & DSC studies

FT-IR spectra of pure ritonavir and ritonavir loaded microspheres were compared and shown in Figure 4. The FT-IR spectra of ritonavir loaded Microspheres showed the characteristic peaks of the pure ritonavir indicating that there was no interaction between the drug and mucoadhesive polymers. The thermogram of ritonavir exhibited a sharp endothermic peak at 125.1°C shown in (Fig.5), which corresponds to its melting point. The characteristic peak of ritonavir was well recognized in the drug-loaded mucoadhesive microspheres. Thus, there was no interaction between ritonavir and mucoadhesive polymers.

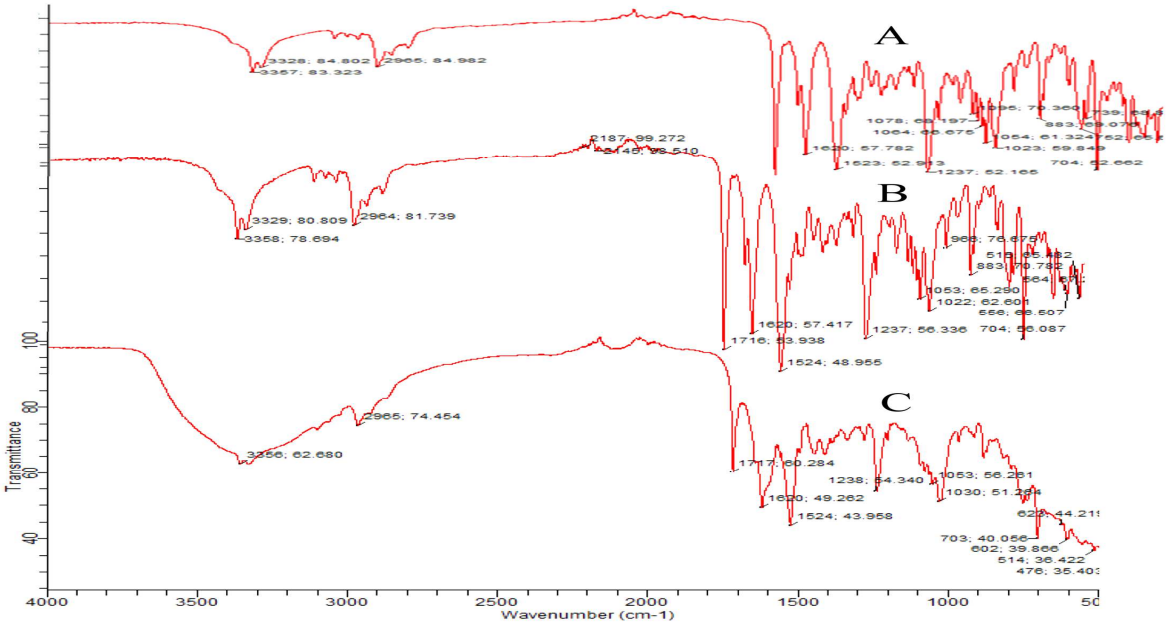

Figure 4 : FTIR spectra of, (A): Pure ritonavir; (B): Formulation containing Xanthan gum (F4) ; (C): Formulation containing Carbopol 940 (F8)

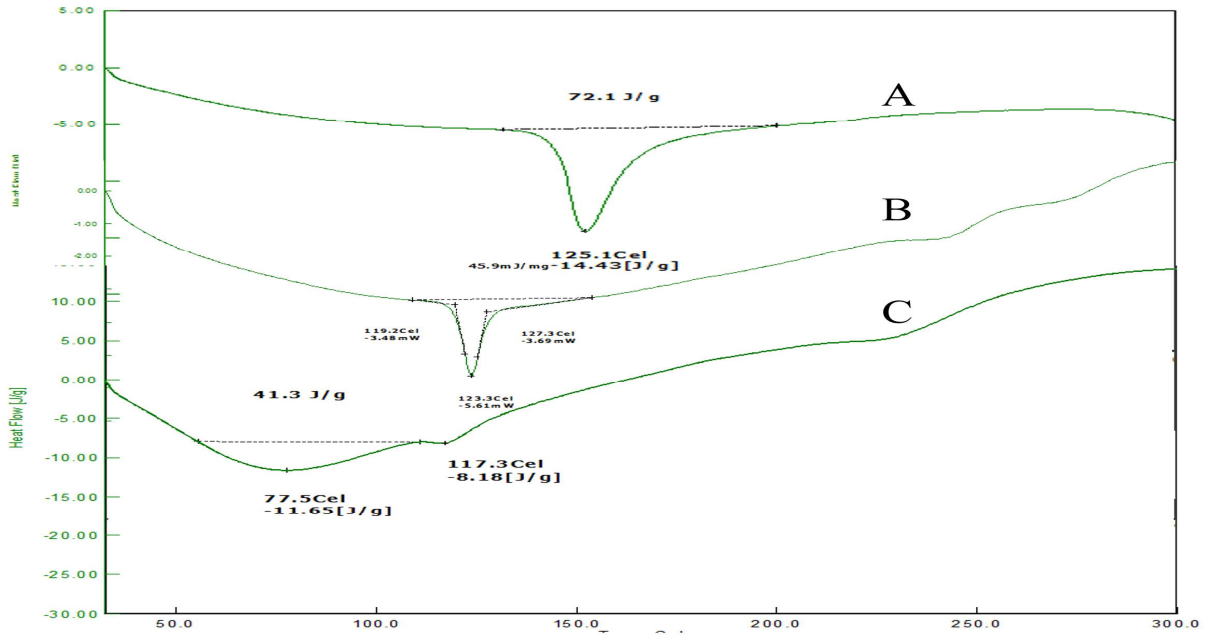

Figure 5: DSC Thermograms of,(A): Pure ritonavir; (B):Formulation containing Xanthan Gum (F4) (C): Formulation containing Carbopol 940 (F8)

***X-Ray* Diffraction study (XRD)**

The X-ray diffractograms of ritonavir and formulation F8 are shown in Figure 6. Pure ritonavir has shown characteristic intense peaks due to its crystalline nature. Whereas, in case of formulation F8 showed less intense peak of low intensity, revealing amorphous dispersion of the ritonavir after entrapment into mucoadhesive microspheres [30].

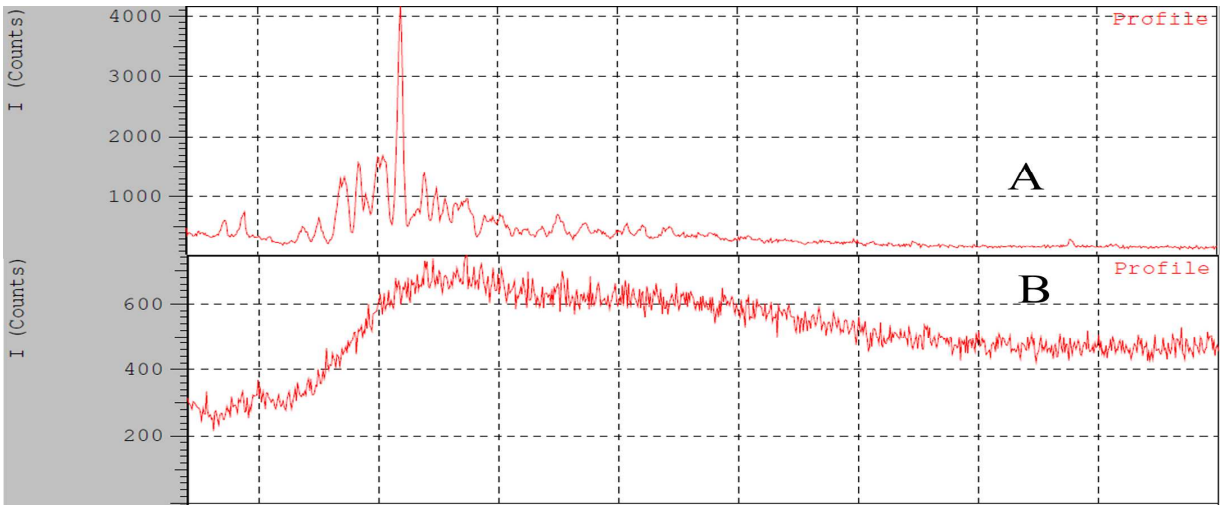

Figure 6 : XRD pattern of, (A): Pure ritonavir; and (B): Best formulation F8

Stability Study

Stability studies of the prepared Ritonavir microspheres were carried out by storing the best formulation F8 at 4 ^{0}C± 1^{0}C / Ambient ,25 ± 2^{0}C/ 60 ± 5 % RH, 40 ± 2^{0}C/ 75 ± 5 % RH for 90 days. The best formulation F8 show insignificant change in entrapment efficiency, percentage mucoadhesion and physical appearance as shown in table 6. So it can be said that ritonavir mucoadhesive microspheres prepared with Carbopol 940 is stable.

Table: 6 Percentage entrapment efficiency and mucoadhesion of the F8 formulation

Stability condition	Sampling Day	Percentage Entrapment efficiency	Percentage mucoadhesion
4 ^{0}C / Ambient	30	96.80 ± 1.78	73.33 ± 1.15
	60	96.35 ± 1.10	71.33 ± 1.52
	90	95.90 ± 0.64	70.33 ± 1.15
25°C/ 60 RH	30	96.52 ± 0.76	73.00 ± 1.73
	60	96.30 ± 0.61	70.33 ± 0.57
	90	96.07 ± 0.64	68.67 ± 0.58
40°C/ 75RH	30	96.80 ± 0.61	73.33 ± 2.08
	60	96.41 ± 0.59	69.00 ± 1.00
	90	95.85 ± 0.54	66.33 ± 1.15

CONCLUSION

The Carbopol mucoadhesive microspheres containing ritonavir can be successfully prepared by ionotropic technique. The present method was quite simple, rapid and does not imply the use of toxic organic solvent. The method also achieves good micrometric properties and better encapsulation efficiency. The prepared mucoadhesive microspheres were spherical and free flowing. The entrapment efficiencies ranged from 72.93 to 96.86 % and mean size was in the range of 772.71 ± 4.77 µm to 941.50 ± 3.13 µm. The Ritonavir release depends upon the mucoadhesive polymer type and concentration in the polymer matrix. Thus the results demonstrate the potential use of Carbopol 940 polymer for preparation of controlled delivery Ritonavir mucoadhesive microspheres and prolonged residence at the absorption site. Further in-vivo activities are required to confirm the claim of beneficial effect of ritonavir in the form of Carbopol mucoadhesive microspheres.

REFERENCES

[1] Garg R; Gupta GD, *Trop J Pharm Res.,* **2008**, 7(3), 1055-1066.
[2] Vasir JK; Tambwekark; Garg S, *Int J Pharma*., **2003**, 255, 13-32.
[3] Patel JK; Bodar MS; Amin ; Patel, *Indian J. Pharm. Sci.*, **2005**, 66, 300-305.
[4] Upadhye K; Bakhle S; Dixit G; Wadetwar R; Deshpande S; Nagulwar V, *Indian Drugs.*, **2004**, 41 (11), 665-669.
[5] Kurana S; Madav NV, *Int J Pharm Bio Sci*., **2011**, 2 (1), 458-67.
[6] Dey NS; Majumdar S; Rao MEB, *Tropical Journal of Pharmaceutical Research.*, **2008**, 7(3), 1067-1075.
[7] Chowdary KPR ;Srinivas Rao Y, *AAPS Pharm. SciTech.,* **2003**, 4(3),87-92.
[8] Sellappan Velmurugan; mohamed ashraf ali; praveen kumar, *Int J Pharm Pharm Sci.,* **2014**, 6(2),31-39.
[9] Nelson Kenneth; Varadarajan Parthasarathy; Rajappan Manavalan; Chikkanna Narendra, *Am. J. PharmTech Res*., **2012**, 2(6),231-244.
[10] Sellappan Velmurugan; Mohamed Ashraf Ali, *Int J Pharm Pharm Sci.,* **2013**, 5,294-302.
[11] Sellappan Velmurugan ; Mohamed Ashraf Ali, *Int. J. Drug Dev. & Res.,* **2013**, 5, 229-233.
[12] Gohel MC; Amin AF, *J Control Release*., **1998**, 51,115-122.
[13] Lehr CM; Bouwstra JA; Schacht EH; Junginger HE, *International Journal of Pharmaceutics.,* **1992**, 78,43-48.
[14] Ahuja A and Khar RK. Ali J, *Drug. Dev.Ind. Pharm.*, **1997**, 23(5),489-515.
[15] Lehr CM; Bowstra JA; Tukker JJ; Junginger HE, *J Control Release* .,**1990**,13, 51-62.
[16] Singh B; Kaur T; Singh S, *Indian J Pharm. Sci.*, **1997**, 59, 196-199.
[17] Korsmeyer RW; Gurny R; Doelker EM; Buri P; Peppas NA, *Int J Pharm*., **1983**, 15,25-35. [18] HH Gangurde; NV Chavan; AS Mundada; DV Derle and S Tamizharasi, *J Young Pharm*., **2011**, 3(1), 9–14.
[19] L Pachuau; S Sarkar and B Mazumder, *Tropical Journal of Pharmaceutical Research.,* **2008**, 7 (2), 995-1002.
[20] Saraf S; Dashora K; Saraf S, *Pak J Pharm Sci.,* **2006**, 19,1-6.
[21 Shah P; Rajshree M; Rane Y, *Journal of Pharmacy Research.,* **2007**,6(1),1-9.
[22] Wong SM, Kellaway IW, Murdan S, *Int J Pharm.,* **2006**, 317,61–68.
[23] M. K. Das ; P. C Senapati, *Indian J Pharm Sci.,* **2008**, 70(1), 77–84.
[24] Belgamwar V; Shah V; Surana SJ,*Curr Drug Deliv.,* **2009**, 6, 113-21.
[25] Robinson J.R; Kelly P; Park H; Ching H.S, *J. Pharm. Sci*., **1985**, 74,399-405.
[26] Zheng J;Liu C; Bao D; Zhao Y, *J. Appl. Polym. Sci*., **2006**, 102, 2226–2232.
[27] Umamaheswari R; Jain S; Tripathi P; Agrawal G; Jain N, *Drug Deliv.*, **2002**, 9, 223–231.
[28] Xingna Zhao; Guofei Li; Lili Zhang, *Int. J. Pharm.,* **2010**, 400,42-48.
[29] Monica Rao; Yogesh Mandage, *Dissolution Technologies.,* **2010**, 61,27-34.
[30] Xu H; Zhong H; Liu M; Xu C; Gao Y ,*Pharmazie.,* **2011**, 66(9),654-61.