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Research Article

DEVELOPMENT AND EVALUATION OF RITONAVIR MUCOADHESIVE MICROSPHERES

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ABSTRACT

Objective: The objective of this research was to formulate and evaluate guar gum and ethyl cellulose mucoadhesive microspheres in combination with sodium alginate for controlled release of ritonavir.

Materials and Methods: Microspheres were prepared by ionotropic gelation method using aluminum sulfate as a cross-linking agent. The developed ritonavir microspheres were characterized for micrometrics properties, morphology, drug entrapment efficiency, mucoadhesive property, *in vitro* drug release and interaction studies (Fourier transforms infrared spectroscopy [FTIR] and differential scanning calorimeter [DSC]).

Results: The ritonavir mucoadhesive microspheres were free-flowing and discrete. The mean particle size ranged from 802.40±3.90 to 962.77±3.16 μm and the entrapment efficiencies ranged from 75.21 to 94.00%. All the ritonavir microsphere batches showed good *in vitro* mucoadhesive property ranging from 12 to 49% in the *in-vitro* wash off test. FTIR studies indicated the lack of ritonavir-polymer interactions in the ideal formulation F8. There were no compatibility issues and the crystallinity of ritonavir was found to be reduced in prepared mucoadhesive microspheres, which were confirmed by DSC and X-ray diffraction studies. Among different formulations, the ritonavir microspheres of batch F8 had shown the optimum percent drug entrapment of microspheres and the controlled release of the ritonavir for about 12 hrs. Release pattern of ritonavir from F8 microspheres batch followed Korsmeyers-Peppas and zero-order release kinetic model. The value of 'n' was found to be 1.593, which indicates that the drug release was followed super Case-II transport type. Stability studies were carried out for F8 formulation at 4°C/ambient, 25±2°C/60±5%, 40±2°C/75±5% relative humidity revealed that the drug entrapment and mucoadhesive behavior were within permissible limits.

Conclusion: The results obtained in this present work demonstrate the potential use of ethyl cellulose polymer for preparation of controlled delivery ritonavir mucoadhesive microspheres and prolonged residence at the absorption site.

Keywords: Guar gum, Ethyl cellulose, Mucoadhesive microspheres, Ritonavir.

INTRODUCTION

Mucoadhesive microcarriers has been a topic of interest in the development of drug delivery systems to prolong the residence time at the site of application or absorption [1]. Mucoadhesive microspheres become adhesive on hydration, and hence can be used for localizing the drugs to a particular target site of gastrointestinal tract (GIT) for prolong periods of time [2,3]. Mucoadhesive microspheres have advantages like efficient absorption, enhanced bioavailability of the drugs, maximum utilization of drugs, much more intimate contact with intestinal cells, better patience compliance and targeting to specific absorption site can be achieved by using suitable mucoadhesive polymers on the surface of microcarriers [4-6].

Ritonavir is an antiretroviral bioactive used in treatment of HIV/AIDS and viral diseases. Belongs to Class II under biopharmaceutical classification system classification and exhibits low and variable oral bioavailability due to poor water solubility. Ritonavir is a peptidomimetic inhibitor of both the HIV-1 and HIV-2 proteases [7]. Ritonavir requires control release due to its short biological half-life (3-5 hrs), low bioavailability 65%, narrow therapeutic index and moreover it is primarily absorbed from stomach [8]. All the drawbacks necessitated the development of mucoadhesive microspheres for improving residence of dosage form in GIT, which could utilize all the efficacy of ritonavir, thereby reduced dosing frequency and enhance bioavailability. Therefore, mucoadhesive microspheres are promising candidate for delivery of ritonavir for treatment of HIV/AIDS patients.

MATERIALS AND METHODS

Materials

Ritonavir was a gift sample from Dr. Reddys Pharma Ltd., Hyderabad. Guar gum and ethyl cellulose polymers were received as gift sample from Hetro Pharma Ltd., Hyderabad. All other ingredients used were of analytical grade.

Preparation of ritonavir mucoadhesive microspheres

The ritonavir mucoadhesive microspheres were prepared by Ionotropic external gelation technique. Ritonavir and mucoadhesive polymers were individually passed through sieve ≠ 60. The weighed quantity of the ritonavir was added to 50 mL of purified water containing the mucoadhesive polymers and thoroughly mixed with a stirrer at 400 rpm to form a homogenous polymer solution. The resulting homogeneous dispersion was sonicated for 30 minutes to remove any air bubbles. For the formation of microspheres the dispersion was extruded drop-wise from a needle of 22 G in diameter from a height of about 5 cm into aqueous aluminum sulfate solution (10%) and stirred at 400 rpm. The added droplets were retained in the aluminum sulfate solution for 30 minutes to complete the curing reaction and to produce spherical rigid ritonavir microspheres [9]. Then the solution containing formed microspheres was filtered by using Whatman filter paper. The mucoadhesive microspheres were allowed to dry at 45°C for 12 hrs and stored in well-closed container for further use. The composition of various formulations was mentioned in Table 1.

Percentage yield

The percentage yield of ritonavir microspheres of various batches were calculated by using the weight of final product after drying with respect to initial total weight of the ritonavir and polymer used for preparation of ritonavir mucoadhesive microspheres [10].

Particle size

Particle size and size distribution of the ritonavir mucoadhesive microspheres were calculated by sieve analysis method [11]. The ritonavir mucoadhesive microspheres were separated into different size fractions (percentage of weight fraction) by sieving for 5 minutes using standard sieves having nominal mesh opening of 1.4 mm, 1.2 mm, 1.0 mm, 0.85 mm and 0.71 mm and the mean particle size was determined (Table 2).

Morphology of microspheres

The surface morphology and shape of the ritonavir microspheres was determined by scanning electron microscopy (SEM). The sample was mounted on copper sample holder and sputter coated with platinum in an argon atmosphere [12].

Drug entrapment efficiency

To determine the amount of ritonavir entrapped in microspheres, a weighed amount (100 mg) of microspheres was suspended into 100 mL of 0.1 N HCl for 24 hrs in rotary shaker in order to extract the entrapped drug completely. The solution was filtered and the concentration of drug present infiltrate was measured after suitable dilution by ultraviolet (UV) spectrophotometer at 240 nm (LABINDIA UV-3092 PC) against 0.1 N HCl as a blank [13].

Mucoadhesive test

The mucoadhesive property of prepared ritonavir microspheres was evaluated by *in-vitro* wash off method. Briefly, a small portion of the goat intestinal mucosa was mounted on a glass slide and about 100 microspheres were spread onto wet rinsed tissue specimen and it was hung onto one of the grooves of a USP disintegration apparatus. Now operating the disintegration test apparatus, the goat intestinal mucosa was given regular up and down movement in test fluid (900 mL of 0.1 N HCL/pH7.4 phosphate buffer) at 37±0.5°C. At every 1 hr intervals up to 8 hrs the apparatus was stopped and the number of ritonavir microspheres still adhering to tissue was counted and percent mucoadhesion was calculated [14].

Table 1: Composition of ritonavir mucoadhesive microspheres

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

***In vitro* dissolution**

The release rate of ritonavir from mucoadhesive microspheres was determined using USP Type II (paddle) dissolution test apparatus. The dissolution test was performed using 900 mL of dissolution medium of 0.1 N HCl, at 37±0.5°C and a rotation speed of 50 rpm. 5 mL of aliquots were withdrawn through a filter (0.45 μ) from the dissolution apparatus hourly for 12 hrs, and replaced with an equal volume of fresh dissolution medium. The samples were analyzed at 240 nm for drug content using UV spectrophotometer (LABINDIA UV-3092 PC). The ritonavir release experiments were carried out in three replicate [15].

Release kinetic studies

The rate and the mechanism of release of ritonavir from the prepared mucoadhesive microspheres were analyzed by fitting the dissolution data into various kinetic models like zero order; first order, Korsemeyer-Peppas, Higuchi's model and coefficient of correlation (r) values were calculated for the linear curves by regression analysis of the above plots [16].

Fourier transforms infrared spectroscopy (FTIR) studies

FTIR spectra of the pure ritonavir and powdered ritonavir microspheres were produced using KBr disk method. The samples were analyzed between wave numbers 4000 and 400/cm resolution [17].

Differential scanning calorimeter (DSC) studies

The thermal behavior of pure ritonavir and ritonavir loaded microspheres were studied using a DSC Shimadzu DSC 60 at a heating rate of 10°C/minutes. 5 mg samples were accurately weighed into aluminum pans and then sealed. The measurements were performed at a heating range of 50-300°C under nitrogen atmospheres [18].

X-ray diffraction study (XRD)

The crystallinities of ritonavir, physical mixture, and ritonavir loaded mucoadhesive microspheres were evaluated by XRD measurement using an X-ray diffractometer (Brucker). All samples were measured in the 2ø angle range between 1° and 90° and with an interval of 0.1 by exposing them to Cuk α1 radiation (40 kV, 30 mA) and the scanning rate was 5°/minutes [19].

Stability study

Stability studies were carried out for ritonavir formulation as per ICH guidelines. The best mucoadhesive microspheres formulation (F8) was sealed in high-density polyethylene bottles and stored at ent, 25±2°C/60±5%, 40±2°C/75±5% relative humidity (RH) for 90 days. The samples (F8) were evaluated for entrapment efficiency and percentage mucoadhesion for every 1 month up to 3 months [20].

RESULT AND DISCUSSION

Percentage yield and micrometric studies

The production yields of microspheres prepared by ionotropic gelation method were found to be between 88.53% and 94.69% as shown in

Table 2: Physicochemical properties of ritonavir mucoadhesive microspheres

Formulation code	Percentage yield[a]	Theoretical drug content (mg)	Practical drug content (mg)[a]	Entrapment efficiency[a]	Particle size (μm)[a]
F1	88.53±1.07	66.6	50.09±0.39	75.21±0.59	802.40±3.90
F2	91.62±2.12	50	41.20±0.58	82.41±1.17	840.20±1.85
F3	93.29±1.12	40	35.72±0.36	89.31±0.91	885.13±3.63
F4	94.69±1.02	33	30.41±0.42	92.14±1.26	926.22±3.67
F5	90.27±1.12	66.6	52.09±0.47	78.22±0.71	835.07±3.99
F6	92.50±2.14	50	42.74±0.36	85.48±0.72	885.73±2.77
F7	94.65±1.17	40	36.02±0.39	90.05±0.98	921.38±3.62
F8	93.36±2.11	33	31.02±0.56	94.00±1.69	962.77±3.16

[a]Mean±SD, n=3, SD: Standard deviation

Table 2. It was found that production yield of microspheres prepared by ethyl cellulose was greater than guar gum. All ritonavir microspheres formulations were evaluated for micromeritic properties. Results are shown in Table 3. Angle of repose of all microspheres batch varied from 20.34 to 33.72. Compressibility index varies from 9.103 to 13.37%. Hausner's ratio varies from 1.100 to 1.154. All formulations results revealed excellent flow property and compressibility.

Particle size

The average particle size of ritonavir microspheres ranged from 802.40±3.90 to 962.77±3.16 µm. The mean particle size was significantly increases with increasing mucoadhesive polymer concentration this may be attributed to high viscosity of mucoadhesive polymer solution (Table 2).

Morphology of microspheres

The morphology of the mucoadhesive microspheres of best formulation F8 was examined by SEM. The SEM photographs revealed that ritonavir microspheres were discrete and spherical shape with a rough surface morphology (Fig. 1).

Entrapment efficiency

The percentage entrapment efficiency ranged from 75.21 to 94.00% (Table 2). The entrapment efficiency of the ritonavir microspheres prepared with ethyl cellulose was higher than those of microspheres prepared with guar gum. This may be attributed to higher molecular weight of ethyl cellulose than guar gum. Increase in the molecular weight of the polymer increases the entrapment efficiency of the microspheres due to the formation of more intact matrix network.

Mucoadhesive test

To assess the mucoadhesive property of ritonavir mucoadhesive microspheres, *in-vitro* wash-off test was carried out for all batches, and the results are shown in Table 4. Percentage mucoadhesion increased with the increase in concentration of mucoadhesive polymer. The higher mucoadhesion of ethyl cellulose microspheres may be attributed to the higher molecular weight of ethyl cellulose than guar gum. The rank order of percentage mucoadhesivity of all the microsphere formulations after 8 hrs was found to be as follows: F8 > F7 > F4 > F6> F3 > F5> F2 > F1.

***In vitro* dissolution studies**

The *in vitro* Ritonavir release profiles for all batches were shown in Fig. 2. Drug release from these mucoadhesive microspheres were slow, controlled release and dependent upon the nature and concentration of mucoadhesive polymers used. Among all the formulations F8 showed good dissolution profile with 75.33% of drug release in 12 hrs. Hence it is considered as the best microsphere formulation, which seems to be a good candidate for controlled release of ritonavir.

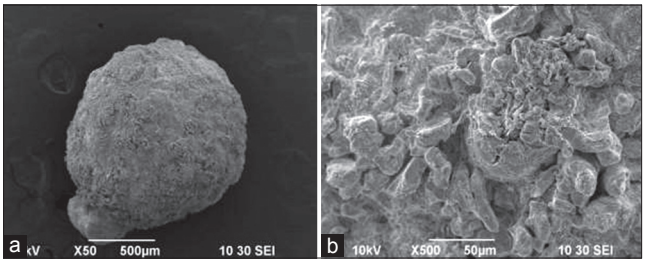

Fig. 1: Scanning electron photomicrographs of the formulation F8: a) ×70, b) ×500

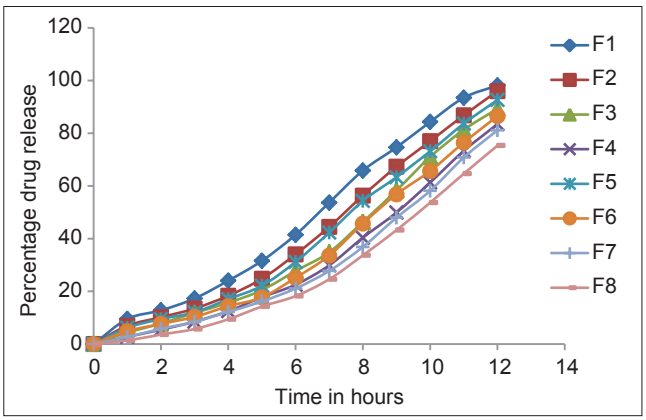

Fig. 2: Comparative release profile of formulation F1-F8

Table 3: Micrometrics 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	0.345±0.009	0.380±0.015	9.103±1.42	1.100±0.017	20.34±0.692
F2	0.332±0.003	0.371±0.004	10.57±0.43	1.118±0.005	22.95±0.792
F3	0.329±0.002	0.368±0.005	10.64±0.78	1.119±0.010	24.94±1.076
F4	0.317±0.001	0.358±0.001	11.57±0.69	1.131±0.009	26.76±1.227
F5	0.340±0.004	0.379±0.005	10.42±1.91	1.117±0.024	24.47±0.983
F6	0.338±0.003	0.380±0.004	11.21±1.39	1.126±0.018	27.58±1.023
F7	0.328±0.002	0.370±0.007	11.34±1.06	1.128±0.014	30.60±1.142
F8	0.316±0.002	0.365±0.005	13.37±0.84	1.154±0.011	33.72±1.261

[a]Mean±SD, n=3, SD: Standard deviation

Table 4: Results of *in vitro* wash off test

	In 0.1 M HCL (pH 1.2)[a]					In phosphate buffer (pH 7.4)[a]				
Hrs	1	2	4	6	8	1	2	4	6	8
F1	88±0.58	66±1.00	46±0.58	35±1.53	17±1.53	83±2.52	63±1.53	41±2.00	30±2.52	12±1.00
F2	92±1.53	73±2.52	56±1.00	45±2.52	21±0.58	88±1.53	68±1.00	49±2.50	37±2.08	17±1.53
F3	96±2.08	77±2.00	62±2.08	49±2.00	30±1.00	90±2.00	72±2.00	57±1.50	43±1.73	24±1.53
F4	98±1.53	88±0.58	71±1.53	57±3.06	47±2.65	94±1.53	73±2.52	66±0.60	51±1.53	42±2.52
F5	93±2.08	86±2.00	65±2.58	52±1.53	33±1.53	92±2.52	72±2.08	58±1.50	46±2.00	28±1.53
F6	97±0.58	91±1.00	73±2.00	62±2.08	40±2.52	96±1.53	86±1.53	67±±2.00	54±2.08	34±2.52
F7	98±1.53	96±1.53	81±1.00	68±2.52	49±2.00	98±0.58	91±1.00	76±1.50	61±2.52	43±1.53
F8	100	100±0.58	90±3.06	74±1.00	57±1.52	100	95±1.15	83±2.50	57±1.52	49±2.00

[a]Mean±SD, n=3, SD: Standard deviation

Release kinetic studies

Drug release kinetic data for ritonavir mucoadhesive microspheres was shown in Table 5. All the microsphere formulations (F1-F8) followed Korsmeyers-Peppas model and zero-order release kinetic with regression values ranging from 0.940 to 0.985. Korsmeyer-Peppas plots, "n" value ranges from 1.049 to 1.593 indicating that the ritonavir release mechanism followed super Case-II transport mechanism.

FTIR studies and DSC studies

Infrared (IR) spectra of pure drugs sample of ritonavir were compared with IR spectra of ritonavir loaded microspheres, as there was no significant change in the pattern of peaks of pure drug and ritonavir loaded microspheres (Fig. 3). Hence, there was no interaction seen in between ritonavir and polymers. The thermal behavior of prepared ritonavir microspheres was studied in comparison with thermo grams of pure ritonavir as shown in Fig. 4. The thermogram of pure ritonavir showed a sharp endothermic peak at 125.1°C, which corresponds to its melting point. The characteristic peak of ritonavir was well-recognized in the ritonavir-loaded microspheres. Thus, there was no incompatibility between ritonavir and mucoadhesive polymers used in the formulation of microspheres.

XRD study

The XRD spectra's were recorded for ritonavir, physical mixtures and ritonavir loaded microspheres for investigating the crystalanity of the ritonavir in the polymeric microspheres. The X-ray diffractogram of ritonavir showed sharp peaks at diffraction angle 21.85° depicting a typical crystalline pattern. Physical mixtures showed less intense peaks, however ritonavir loaded mucoadhesive microspheres showed peaks, but of low intensity, revealing that some amount of ritonavir was changed to amorphous form (Fig. 5).

Stability study

Stability studies of the prepared ritonavir mucoadhesive microspheres were carried out by storing the optimized formulation F8 at 4°C/ambient, 25±2°C/60±5%, 40±2°C/75±5% RH for 3 months. The optimized batch F8 show negligible change in entrapment efficiency and percentage mucoadhesion as shown in Table 6. Hence, it can be said that ritonavir mucoadhesive microspheres prepared with ethyl cellulose is stable.

CONCLUSION

In this study, controlled release ritonavir microspheres were prepared successfully using the ionotropic gelation method. This study has been a satisfactory attempt to formulate a mucoadhesive microparticulate system of an anti-retroviral drug ritonavir with a view of controlled delivery of the drug. Interaction studies (FTIR and DSC) data revealed that there was no interaction between mucoadhesive polymers and ritonavir, hence they are compatible. The prepared ritonavir microspheres gave good micrometrics properties, percent yield, drug entrapment, mucoadhesive property and *in vitro* release. SEM analysis of the ritonavir microspheres revealed that F8 formulation was spherical shape with rough surface morphology. Among different formulations, the ritonavir microspheres of batch F8 had shown the optimum percent drug entrapment of microspheres, mucoadhesive properties and the controlled release of ritonavir for about 12 hrs. Thus, the results demonstrate the potential use of ethyl cellulose polymer for preparation of controlled delivery ritonavir mucoadhesive microspheres and prolonged residence at the absorption site.

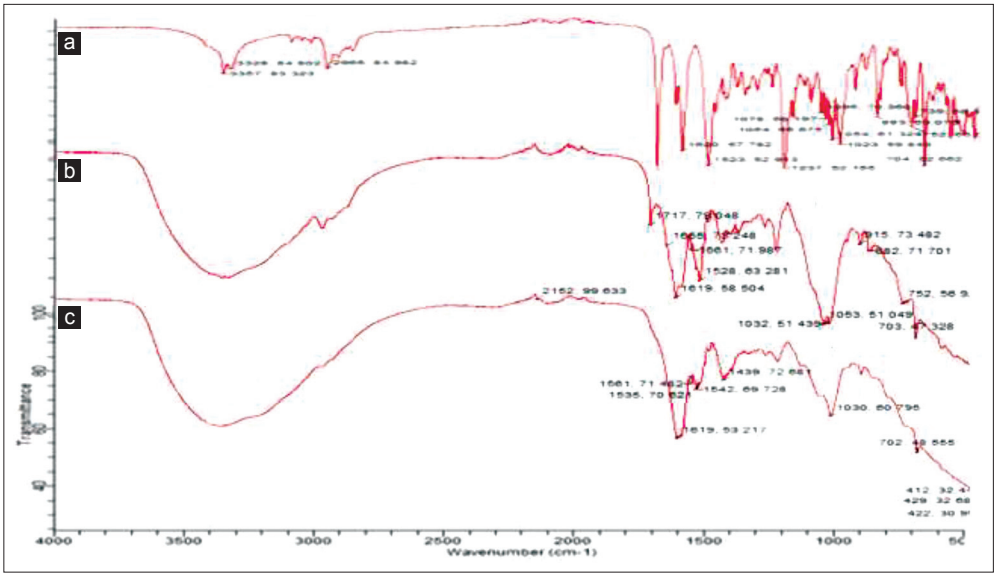

Fig. 3: Fourier transforms infrared spectroscopy spectra of, (a): Pure ritonavir; (b): Formulation containing guar gum (F4); (c): Formulation containing ethyl cellulose (F8)

Table 5: Release kinetic parameter of ritonavir from mucoadhesive microspheres

Formulation code	Zero order	First order	Higuchi	Korsmeyer-peppas	n-value	Hixson crowel
F1	0.985	0.798	0.934	0.961	1.049	0.840
F2	0.974	0.790	0.906	0.956	1.153	0.828
F3	0.958	0.830	0.881	0.973	1.275	0.792
F4	0.950	0.882	0.866	0.972	1.178	0.869
F5	0.970	0.828	0.900	0.954	1.183	0.773
F6	0.954	0.839	0.873	0.950	1.237	0.806
F7	0.940	0.827	0.853	0.976	1.396	0.800
F8	0.940	0.882	0.852	0.989	1.593	0.872

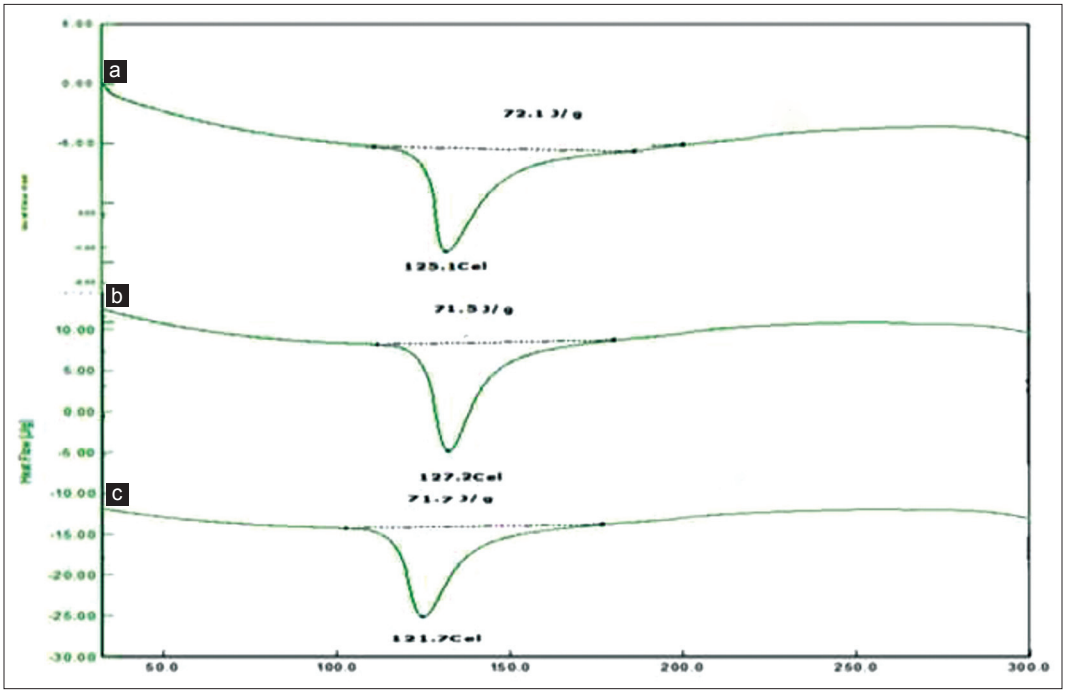

Fig. 4: Differential scanning calorimeter thermograms of, (a): Pure ritonavir; (b): Formulation containing guar gum (F4) (c): Formulation containing ethyl cellulose (F8)

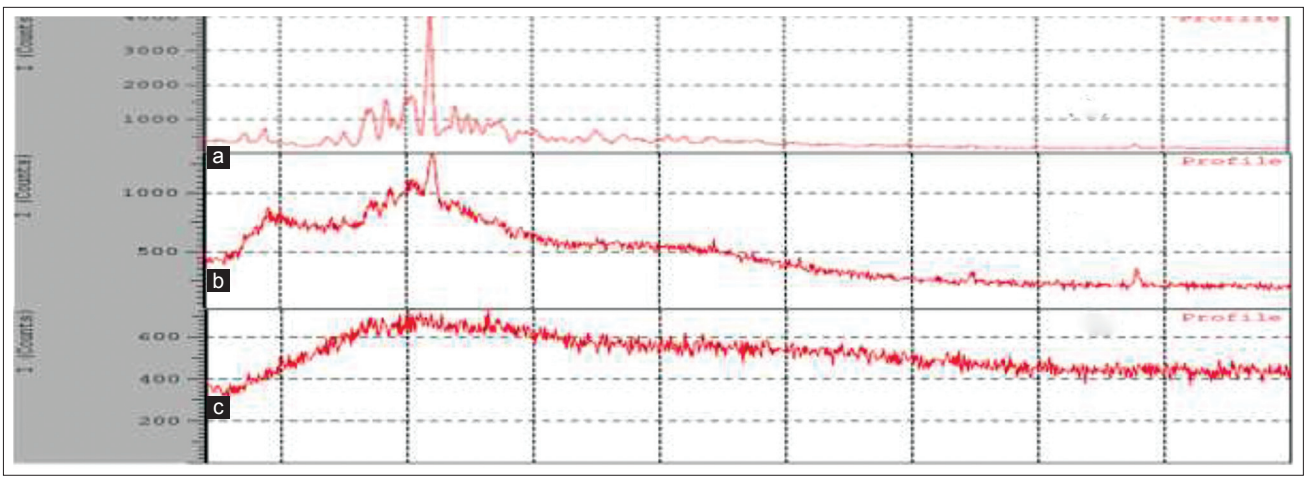

Fig. 5: X-ray diffraction pattern of, (a): Pure ritonavir, (b): Physical mixtures (c): Best formulation F8

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

Stability condition	Sampling day	Percentage entrapment efficiency	Percentage mucoadhesion
4°C/Ambient	30	93.88±1.10	56.33±0.58
	60	93.49±0.42	54.33±1.55
	90	92.99±0.26	53.33±1.153
25°C/60 RH	30	93.71±0.42	56.33±1.53
	60	93.43±0.34	53.67±2.08
	90	93.27±0.51	51.33±1.53
40°C/75 RH	30	93.93±0.35	56.67±2.08
	60	93.32±±0.51	52.67±1.15
	90	92.93±0.34	49.33±2.52

RH: Relative humidity

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