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PREPARATION AND CHARACTERIZATION OF MICROSPHERES ENCAPSULATING RITONAVIR BY IONIC GELATION TECHNIQUE

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ABSTRACT

Aim: The aim of the present study is to prepare and characterize microspheres containing Ritonavir using Chitosan as the polymer. **Methods:** The Ritonavir loaded microspheres were prepared by Ionic gelation method. Microspheres of different core: coat ratio were prepared and characterize for process yield, loading efficiency, particle size, zeta potential, *in vitro* drug release, kinetic studies and stability studies. **Results:** The prepared microspheres were white, free flowing and spherical in shape. The infrared spectra and differential scanning colorimetry thermographs showed stable character of Ritonavir in the drug-loaded microspheres and revealed the absence of drug polymer interactions. The microspheres have a zeta potential 28 mV. The formulation with the initial ritonavir concentration of 0.5 mg/ml provided the highest loading capacity. The *in vitro* release behavior from all the drug loaded batches were found to follow first order and provided sustained release over a period of 24 h. No appreciable difference was observed in the extent of degradation of product during 90 days in which microspheres were stored at various temperatures. **Conclusion:** The best-fit release kinetics was achieved with First order followed by Higuchi plot. The release of Ritonavir was influenced by the drug to polymer ratio and particle size and was found to be diffusion controlled. According to the data obtained, this Chitosan-based microspheres opens new and interesting perspectives as drug carriers for treating the AIDS.

KEYWORDS

Microsphere, Chitosan, Ritonavir and Ionic gelation.

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INTRODUCTON

Drug discovery alone is insufficient in treating diseases; often correct dosing and targeting are equally important for clinical success. Researchers in the area of controlled or sustained drug delivery systems specifically concentrate in to these areas to enhance the efficacy of therapeutics for specific treatment regimens. Controlled drug delivery systems are aimed at controlling the release of the drug at a therapeutically effective rate, prolonging the duration

of drug delivery and therapeutic response and targeting the delivery of the drug to a tissue[1].

Ritonavir is a human immunodeficiency virus (HIV) protease inhibitor, mainly used for the treatment of acquired immunodeficiency syndrome (AIDS). HIV infects the human cells and uses the energy of the cells to grow and reproduce, so it is necessary to take medicines for prolonged period of time. This is the main problem for drugs with short half life which has to be administered frequently and also Ritonavir has a half-life of 3-5 h. Moreover, Ritonavir has dose related side effects, the most common of which are nausea, vomiting, diarrhoea, stomach pain 1. One of the approaches to solve such problems is release the drug in controlled manner. There are various approaches in delivering therapeutic substance to the target site in a controlled release fashion. One such approach is using microspheres as carriers for drugs[2].

Microspheres are one of the multiparticulate delivery systems and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability or stability and target drug to specific sites. Microspheres can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing dosing frequency and improving patient compliance.

Chitosan is a natural cationic biopolymer obtained from N-deacetylation of chitin. Chitin is the principal component of protective cuticles of crustaceans such as crabs, shrimps, prawns and cell walls of some fungi such as aspergillus. Chitosan is a weak base and is insoluble in water and organic solvents however it is soluble in dilute aqueous acid solution (pH 6.5). Chitosan is widely used in the formulation of particulate drug delivery systems to achieve control drug delivery. With this background, combination of chitosan and ritonavir were selected as core material for the formulation of microspheres to achieve controlled drug release[3, 4].

Hence, these Microspheres are being used to target drugs to a specific site only in the body, to improve oral bioavailability, to sustain drug effect in the target tissue, to solubilize drugs for intravascular delivery and to improve the stability of drugs against enzymatic degradation. The objective of the work was to formulate microspheres containing Ritonavir by Ionic gelation method, evaluate its physicochemical characteristics such as particle size, shape, zeta potential, drug loading capacity and in vitro release characteristics[5, 6].

MATERIAL AND METHODS

Ritonavir is a gift sample from the strides arcolabs ltd., Bangalore. Chitosan was obtained from Rohm Pharma, GmbH, Germany. Dichloromethane and ethanol were purchased from Spectro chem Pvt. Ltd. Mumbai. All the reagents and solvents used were of analytical grade satisfying pharmacopoeia standards.

Preparation of microsphere

Chitosan microspheres were prepared by ionic cross linkage of chitosan solution with TPP anions. Chitosan was dissolve dinaqueo us solution of acetic acid 0.25% V/V at various concentration, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/ml under magnetic stirring at room temperature, 25ml of 0.84% w/v TPP aqueous solution was added drop wise to 100 ml of chitosan solution containing 100mg of Ritonavir. The stirring was continued for about 20min and pH was adjusted by 0.1N NaOH. Microspheres formed immediately and were left into the original solution for 1hr to ensure internal gellification. Then they were filtered, washed with alcohol and dried at room temperature.

CHARACTERIZATION OF PREPARED MICROSPHERES

Fourier transforms infra-red spectroscope (FTIR) analysis

The FT-IR spectra of pure Ritonavir and Chitosan microspheres loaded Ritonavir were recorded to check drug polymer interaction and stability of drug (Figure No.1).

Differential scanning colorimetry (DSC)

The DSC analysis of pure drug and drug loaded microspheres were carried out using a Diamond DSC (PerkinElmer, USA) to evaluate any possible drug-polymer interaction. The analysis was performed at a rate 5.00℃min^{-1}from 10℃ to 400℃temperature range under nitrogen flow of 25 ml min^{-1} (Figure No.2).

Drug entrapment efficiency of microspheres[7]

For this 50mg of microspheres were weighed and added into 100ml of 0.7% SLS solution. This was shaken in mechanical shaker for 24h. The solution was filtered and analyzed spectro photometrically for drug content at 240nm (Table No.1). The drug entrapment efficiency was determined using the relationship.

$$\text{Drug entrapment efficiency} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100$$

Surface morphology study

Scanning electron microscopy (SEM) of the microsphere was performed to examine the particle size and surface morphology (Figure No.3). The microspheres were mounted on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument. The photographs were taken using a Jeol scanning electron micro scope under magnification of 7500 - 20,000×.

Particle size distribution[8]

The particle size distribution of the microspheres was determined by laser particle size analyzer using n-hexane as dispersant. The Microsphere dispersions were added to the sample dispersion unit containing stirrer and stirred to reduce the aggregation between the microspheres. The average volume-mean particle size was measured after performing the experiment in triplicate.

Zeta potential[9]

The zeta potential of drug loaded microspheres was measured by Zeta meter. To determine the zeta potential, micro spheres samples were diluted with KCL (0.1 Mm) and placed in electro phoretic cell where an electrical field of 15.2 V/cm was applied. Each sample was analyzed in triplicate.

***In vitro* release studies**[10]

In vitro release study of Ritonavir from various formulations was conducted for 24hrs by using USP basket type dissolution test apparatus. Cumulative % drug release was plotted against time. All the formulation showed more than 20 % in the first 1 hr due to the presence of un-entrapped drug and the drug entrapped on the surface of microspheres which released faster showing slight dose dumping. It has been found that from the microspheres of formulation FC1-FC5 prepared by ionic gelation method shows FC1-86.86%, FC2-84.03%, FC3-82.62%, FC4-80.08% and FC5-77.68 were shown in Figure. The increase in Chitosan ratio from FC1 to FC5 causes decrease in the drug release.

Kinetic modeling

In order to understand the kinetic and mechanism of drug release, the result of *in vitro* drug release study of microspheres were fitted with various kinetic equation like zero order (cumulative % release vs. time), first order (log % drug remaining vs. time), Higuchi's model (cumulative % drug release vs. square root of time), Peppas plot (log of cumulative % drug release vs. log time). R^2 and 'n' values were calculated for the linear curve obtained by regression analysis of the above plots (Table No.2).

Stability study

The stability study was carried out using the batch FC-5. Formulation FC-5 was divided into 3 sets of samples and stored at 5 ± 3 ℃ in refrigerator, room temperature and 45 ℃ $\pm$ 2℃, 75% RH in humidity control ovens. After 90 days drug content of all samples were determined by the method as in drug content (Figure No.7). *In vitro* release study of formulation FC-5 was also carried out after 90 days of storage (Table No.3 and Figure No.5).

RESULTS AND DISCUSSION

Microspheres prepared by Ionic gelation technique were found to be discrete and through SEM analysis. The drug entrapment efficiency of microspheres containing drug: polymer in various ratios of 1:1, 1:2, 1:3, 1:4 and 1:5 were found to be 70.55%, 74.96%, 81.22%, 86.88% and 90.61%. Thus there was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulation. The interaction study between the drug and polymer was evaluated using FT-IR spectrophotometer. There was no significant difference in the IR spectra of drug loaded microspheres. Differential scanning calorimetry study thermo gram of pure Ritonavir showed a sharp endothermic peak at 126.17℃. The thermo grams of formulations FC-5 of Figure No.2, showed the same endothermic peak at the similar temperature. This further confirmed that there is no drug to polymer interaction. This indicates that they are stable. Cumulative percentage drug released for FC-1, FC-2,

FC-3, FC-4 and FC-5 after 24 h were found to be 86.86%, 84.03%, 82.62%, 80.08% and 77.68% respectively. Zeta potential for FC-5 was found to be28mV and it shows good stability. It was apparent that *in vitro* release of Ritonavir showed a very rapid initial burst and then followed by a very slow drug release. An initial, fast release suggests that some drug was localized on the surface of the microspheres. In order to describe the release kinetics of all five formulations the corresponding dissolution data were fitted in various kinetic dissolution models like zero order, first order, and Higuchi respectively.

As indicated by higher R^2 values, the drug release from all formulations follows first order release and Higuchi model. Since it was confirmed as Higuchi model, the release mechanism was swelling and diffusion controlled. The Peppas model is widely used to confirm whether the release mechanism is Fickian diffusion, Non-fickian diffusion or zero order. 'n' value could be used to characterize different release mechanisms. The 'n' values for all formulations were found to be greater than 0.50. This indicates that the release approximates Non-fickian diffusion mechanism.

Table No.1: Formulation and physicochemical characterization of Ritonavir microspheres

S.No	Batch Code	Drug: Polymer Ratio	% drug Entrapment Efficiency	Mean particle Size(µm)
1	FC1	1:1	70.55	95
2	FC2	1:2	74.96	101
3	FC3	1:3	81.22	115
4	FC4	1:4	86.88	151
5	FC5	1:5	90.61	180

Table No.2: Correlation coefficients according to different kinetic equations

S.No	Formulation	% Cumulative drug release	Zero order	First order	Higuchi plot	Peppas plot	'n' Values
1	FC1	86.86	0.5963	0.9191	0.8728	0.4192	0.8285
2	FC2	84.03	0.6570	0.9245	0.9088	0.4393	0.8334
3	FC3	82.62	0.6392	0.8956	0.8961	0.4222	0.8157
4	FC4	80.08	0.7025	0.9081	0.9354	0.4817	0.8582
5	FC5	77.68	0.7641	0.9236	0.9584	0.5558	0.9080

Table No.3: Stability studies – *in vitro* release study of a selected formulation FC-5 after three months storage at 5±2℃, Room temperature, 45℃±2℃/75%RH

S.No	Time in hrs	%Cumulative Drug Release		
		5ºC±3ºC	30ºC±2 ºC/65% 5%RH	40ºC±2ºC/75% ± 5%RH
1	0	0	0	0
2	1	19.95	18.09	15.56
3	2	26.75	25.46	23.01
4	3	31.99	30.93	27.26
5	4	39.89	38.81	36.02
6	6	46.13	45.66	43.27
7	8	59.06	58.25	56.35
8	12	67.78	66.82	63.18
9	24	77.08	75.22	73.09

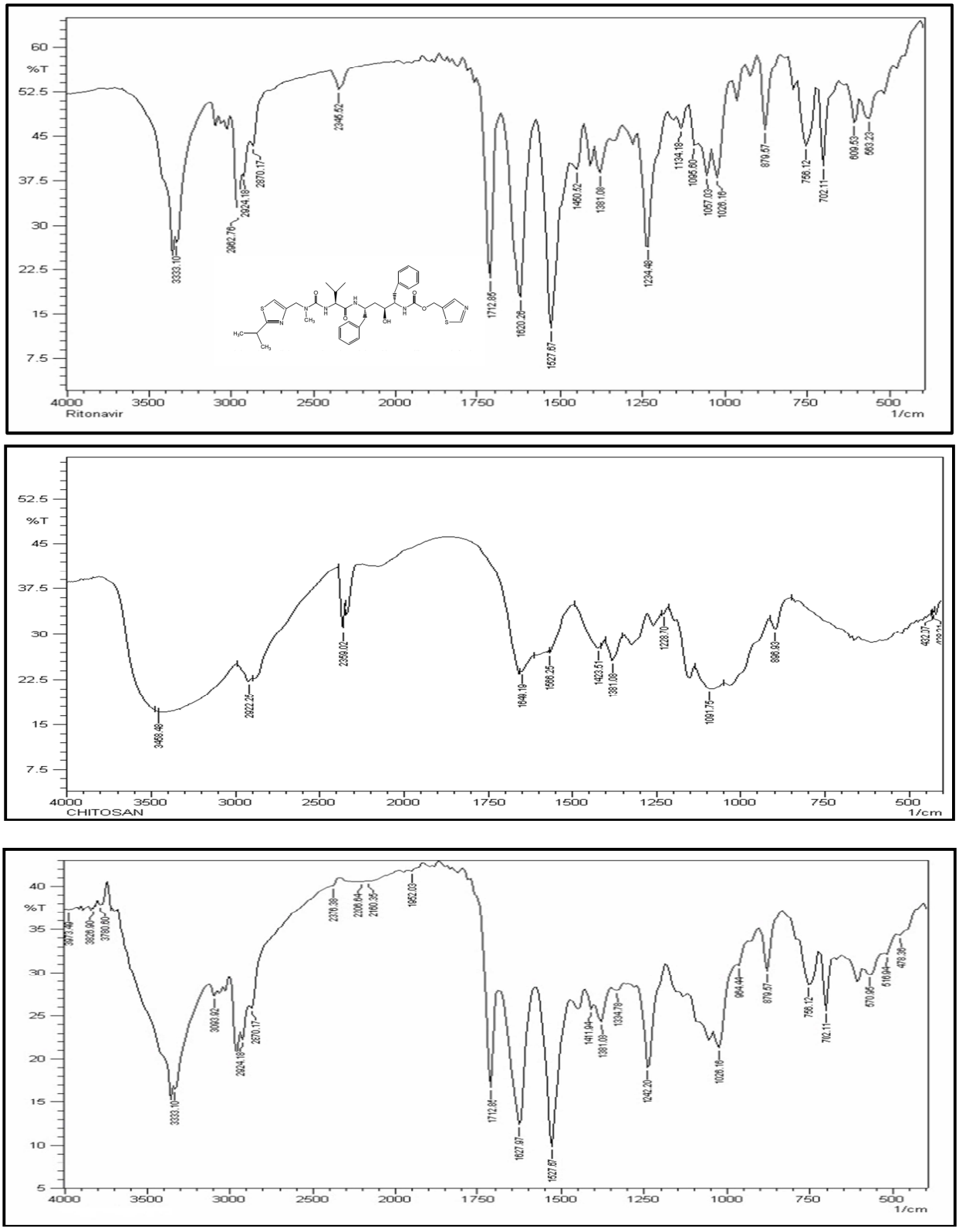
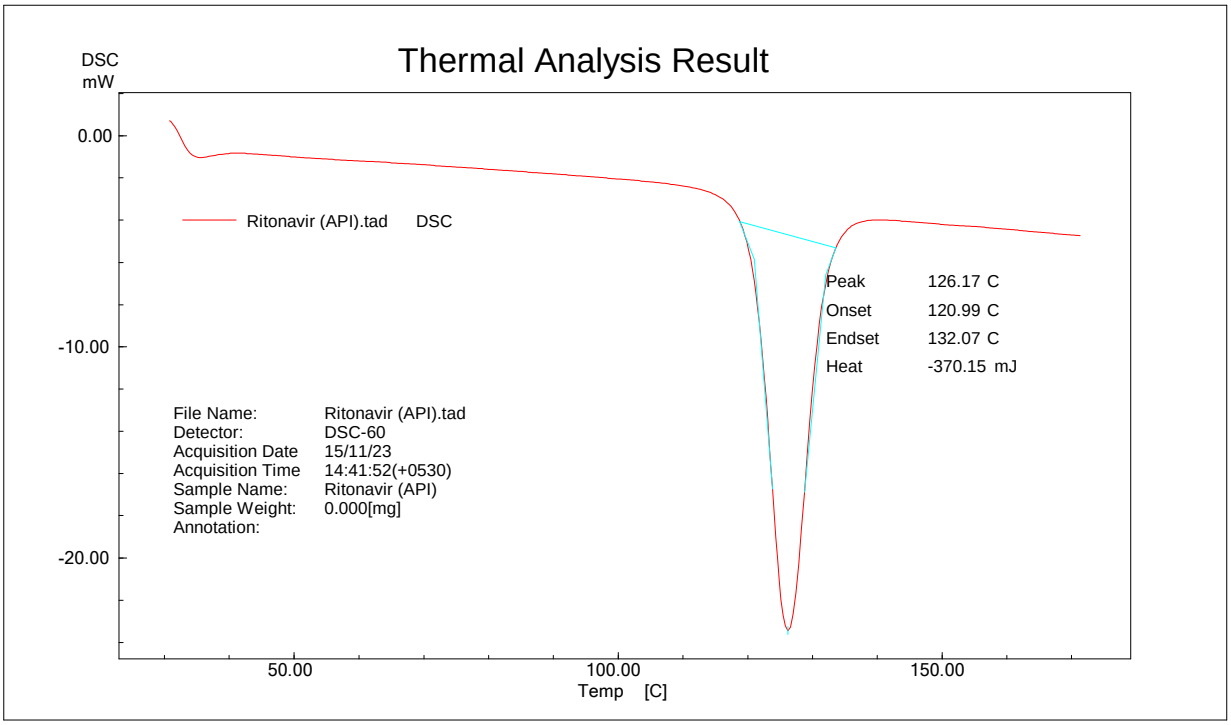


Figure No.1: FT-IR spectra of (A) pure Ritonavir, (B) Eudragit L100, (C) Ritonavir microspheres



Drug+Chitosan

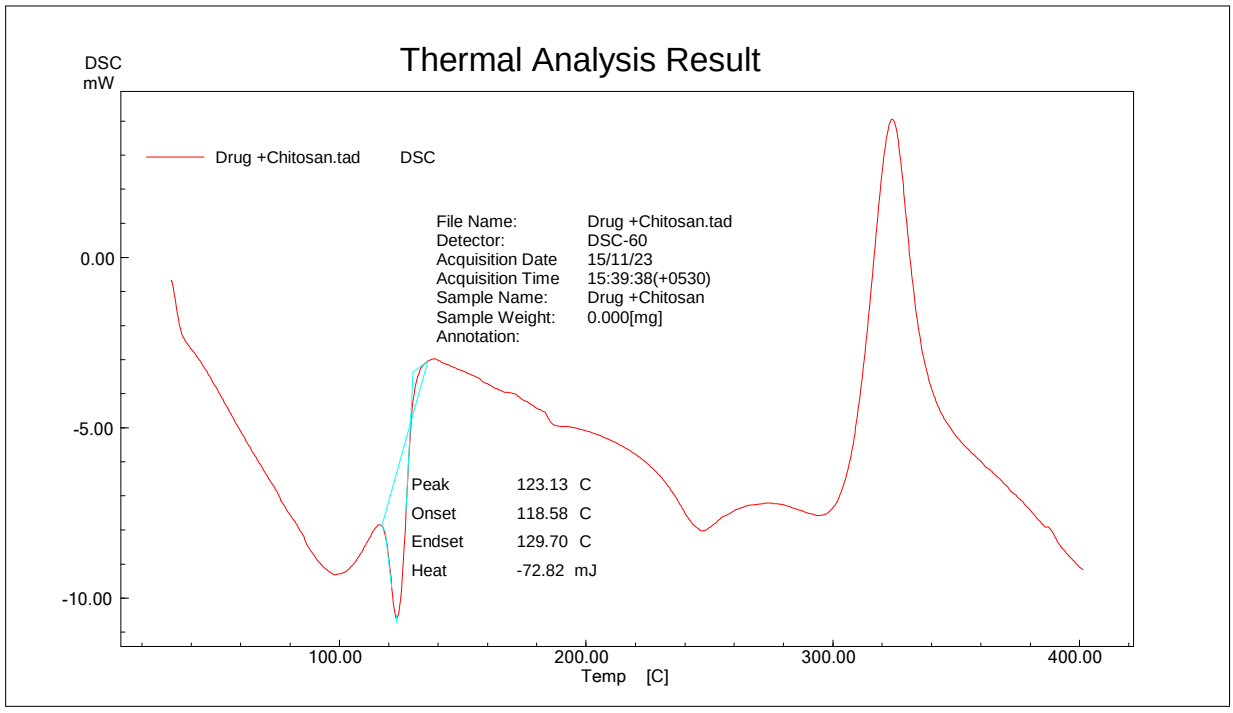


Figure No.2: DSC thermo grams of pure Ritonavir and Ritonavir loaded Chitosan microspheres

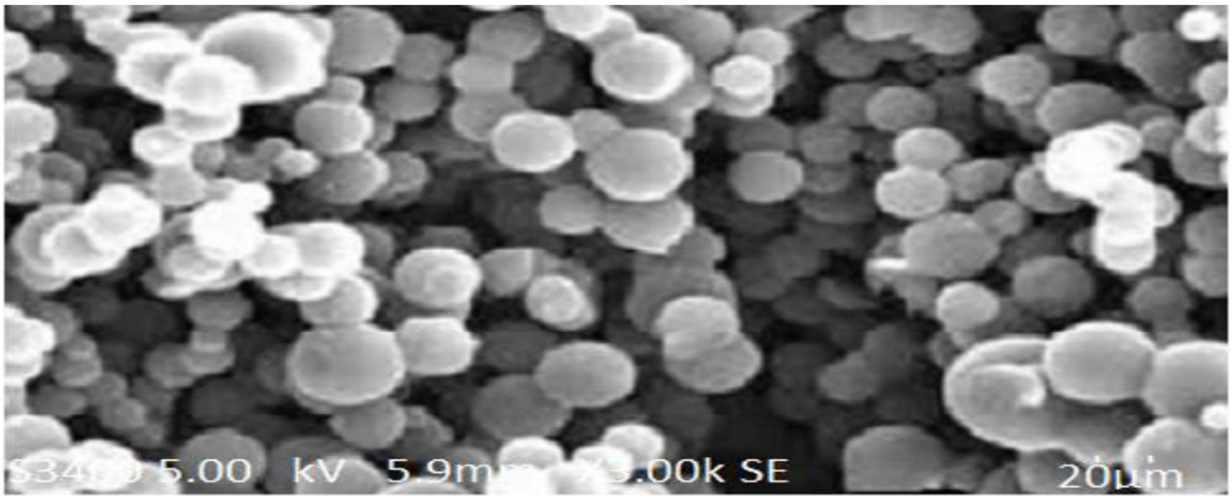


Figure No.3: SEM of formulation FC-5

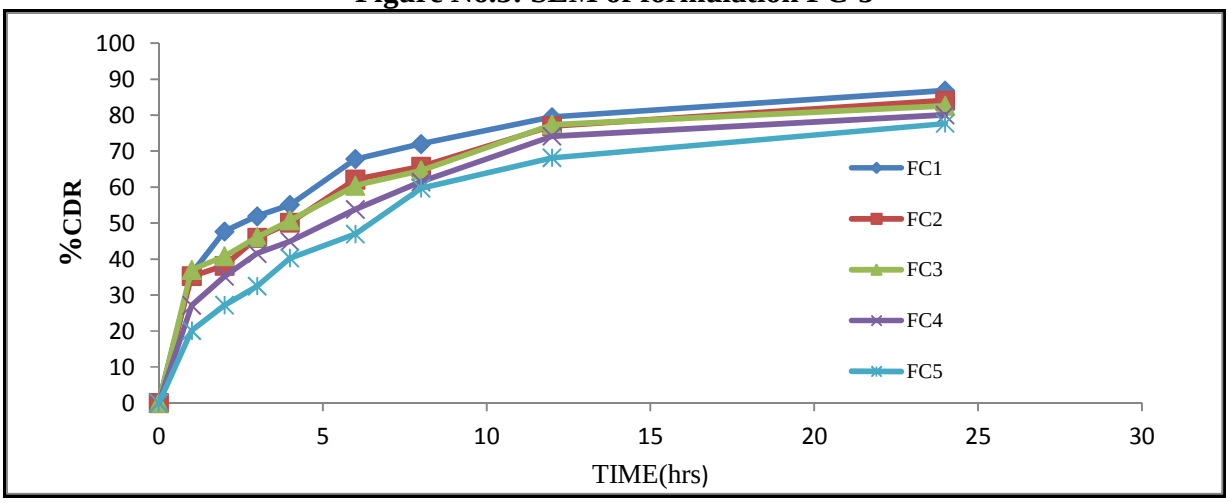


Figure No.4: % cumulative drug release of Ritonavir microspheres

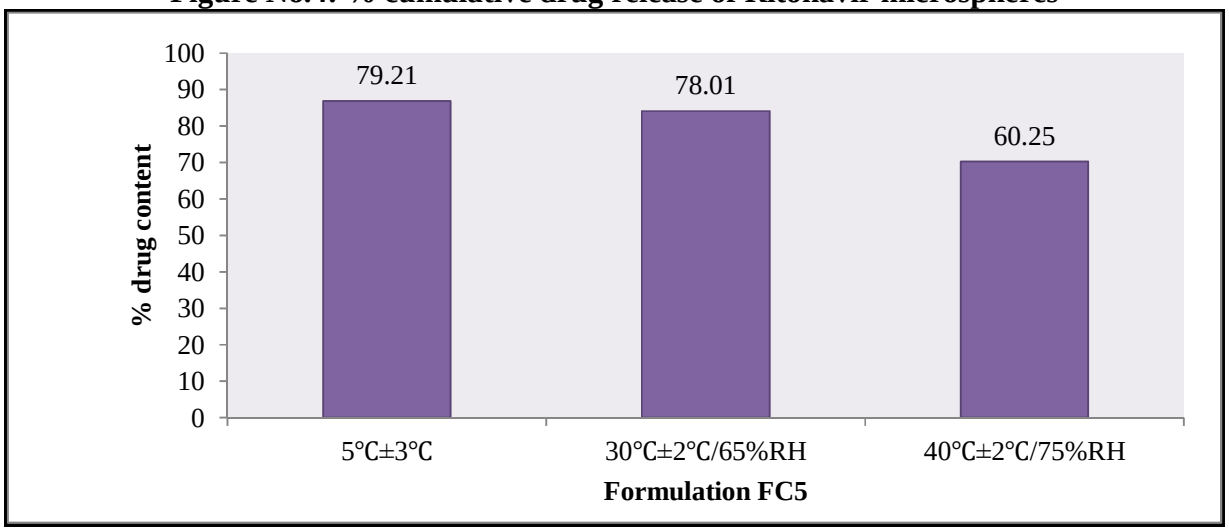


Figure No.5: Stability study: comparison of % drug content of formulation FC-5 at 5±3℃, room temperature and 45℃ ± 2℃/75% RH

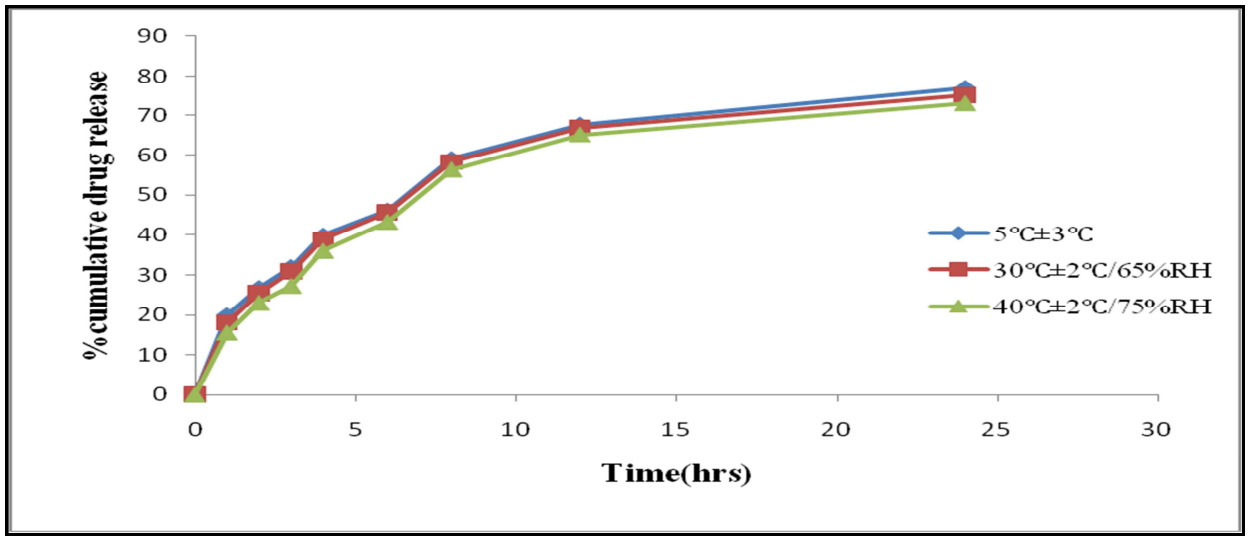


Figure No.6: Stability study: comparison of *in vitro* drug release profile for formulation FC-5 at 5±3℃, room temperature and 45℃ ± 2℃/75% RH after three months storage

CONCLUSION

Ritonavir microspheres were prepared by ionic gelation technique were found to be suitable for controlled release. The microspheres prepared by using Chitosan as a polymer show prolonged release rate when compared with other formulations.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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