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FORMULATION AND CHARACTERIZATION OF RITONAVIR LOADED ETHYL CELLULOSE MICROSPHERES FOR ORAL DELIVERY

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ABSTRACT

The objective of the present study was to prepare and evaluate micro particles for the controlled release of Ritonavir using cellulose polymer. The micro particles were prepared by the solvent evaporation method (O/O) using ethyl cellulose as wall materials. In order to increase the encapsulation efficiency, a mixed solvent system comprising 1:1 proportions of ethanol and dichloromethane were used as a dispersed phase. The prepared micro particles were characterized for the percent drug content, entrapment efficiency, FTIR, DSC, scanning electron microscopy (SEM) and *in vitro* dissolution studies. The prepared micro particles were white, free-flowing, and almost spherical in shape. The drug-loaded micro particles showed 86-111% drug entrapment, mean particle size was in the range of 36-40µm. *In vitro* drug release studies were carried out up to 10h in two different pH media, i.e., acidic buffer (pH 1.2) and SLS solution (0.7%). FTIR and DSC thermo grams showed the stable character of Ritonavir in the micro particles. SEM showed that the micro particles were porous in nature. The release kinetics study revealed that the prepared micro particles were best fitted to the zero order. The release kinetics data and characterization studies indicated that drug release from microcapsules was diffusion – controlled and that the micro particles were stable.

Key words: Microspheres, solvent evaporation method, ethyl cellulose, release Kinetics.

INTRODUCTION

Acquired immune deficiency syndrome is one of the life threatening diseases of the world. Human Immunodeficiency Virus (HIV) is a retrovirus that causes AIDS by irreversible destruction of immune system, leading to occurrence of opportunistic infection. The HIV protease inhibitors have contributed to the improvement of life of many HIV-infected patients the last few years. Inhibition of the HIV protease leads to the production of non-infectious viruses. The result is declining levels of HIV RNA in plasma, which correlates to reduced morbidity and mortality[1].

Ritonavir is a human immunodeficiency virus (HIV) protease inhibiter and is mainly used for treatment of auto immune deficiency syndrome (AIDS). The protease inhibitors are usually administered as one or two in combination. Patients experience adverse reactions and because of this they sometimes have to stop treatment. Other patients do not respond to treatment and never achieve low plasma HIV RNA levels. One method to solve such problems is release the drug in controlled manner. There are various approaches in delivering a therapeutic substance to the target site in a controlled release fashion. One such approach is using microspheres as carriers for drugs.

Microspheres are one of the multiparticulate delivery systems and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability or stability and to 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.[2]

MATERIALS AND METHODS

Ritonavir is a gift sample from the strides arcolabs ltd., Bangalore. Ethyl cellulose, Dichloromethane and ethanol were also used in study. All the reagents and solvents used were of analytical grade satisfying pharmacopoeial standards.

Preparation of microspheres

Ritonavir microspheres were prepared by solvent evaporation technique[3]. Different ratios of polymer was dissolved in dichloromethane and ethanol (1:1ratio) by using a magnetic stirrer (Remi motors, Mumbi). Ritonavir were dispersed in the polymer solution. The resulting

dispersion was then poured into 100 ml water containing 0.01% Tween 80 maintained at a temperature of 30–40 °C with stirred at speed of 500 rpm. Stirring was continued for an hour, until solvent evaporated completely. After evaporation of solvent, the microspheres formed were collected by filtration, washed 4–5 times with distilled water and dried at room temperature for 24 h.

Encapsulation efficiency of the microspheres [4]

About 50mg of microspheres were weighed and added 100ml of 0.7% SLS solution and shaken in mechanical shaker for 24h. Then solution was filtered and made suitable dilution and analyzed spectrophotometrically at 240nm.

Scanning electron microscope analysis

Shapes and surface characteristics of the microspheres were investigated and photographed using scanning electron microscope (SEM; JEOL JSM T-330A, Japan).

Particle size analysis

Measurements of the particle size distribution of microspheres were carried out with an optical microscope. Stage micrometer was used to calculate calibration factor. The particle size was calculated by multiplying the number of division of the ocular disc occupied by the particle with calibration factor. Hundred randomly chosen spheres were taken to measure their individual size.

Differential Scanning Colorimetry

The physical state of Ritonavir in the microspheres was analyzed by Differential Scanning Colorimeter (Mettler-Toledo star 822^{e} system, Switzerland). The thermo grams of the Ritonavir, physical mixture of Ritonavir and polymer and Ritonavir microspheres were obtained at a scanning rate of 10°C/min conducted over a temperature range of 25–220°C, respectively.

Drug polymer interaction (FTIR) study

FTIR spectroscopy was performed on Fourier transform infrared spectrophotometer. The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 3 min on KBr-press and the spectra were scanned in the wave number range of 4000- 600 cm^{-1}. FT-IR study was carried on Ritonavir, physical mixture of Ritonavir and Ritonavir microspheres.

***In vitro* dissolution studies**[5]

Dissolution studies were carried out by using USP type II dissolution test apparatus by rotating basket method in stimulated gastric fluid pH 1.2 for 2 h and in 0.7% SLS solution for 8h. The dissolution media were maintained at a temperature of 37 ± 5^0 C. The speed of rotation of basket maintained was 100 rpm.

Ritonavir microspheres were placed in basket in each dissolution vessel and covered with nylon cloth to prevent floating. 1 ml of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were analyzed and the amount of Ritonavir released was determined by UV absorption spectroscopy at 240 nm.

RESULTS AND DISCUSSION

Microspheres of Ritonavir were prepared by solvent evaporation techniques by using polymer like Ethyl cellulose. The prepared Ritonavir microspheres were then subjected to FT-IR, SEM, particle size, size distribution, % yield, drug content, entrapment efficiency, *in vitro* dissolution, release kinetics, and DSC.

First, trials were made to prepare microspheres by using a solvent evaporation technique in the acetone/ liquid paraffin system was used but no spherical particles could be obtained. Then water phase is used and various formulations with different polymer: drug ratios were tried, stirring speed was also changed to obtain spherical particles. These microsphere formulations are shown in table 1.

Loading efficiency of the drug depended on solubility of drug in solvents and continuous phase and physic chemical properties of drug and polymer[6]**.** High encapsulation efficiency was observed in different formulation of Ritonavir Encapsulation efficiency is given in table: 2. as it was evident from the results, drug loading increases as the polymer concentration increases.[7]

Table: 1 Formulation details of Ethyl cellulose microspheres of Ritonavir

Ingredients	Formulation codes		
	A-1	A-2	A-3
Ritonavir (mg)	200	200	200
Ethyl cellulose(mg)	200	400	600
Ethanol: Dichloromethane (1:1) in ml	10	10	10
Tween 80	0.02 %	0.02 %	0.02 %
Water (ml)	100	100	100

Table: 2 Drug loading and % Entrapment efficiency

Formulation	Percentage yield	Drug content (%)	Entrapment Efficiency (%)
A1	57.5	74.8	86.03±0.16
A2	63.33	55.6	105.7±0.36
A3	71.25	39.2	111.64±0.14

The mean particle size of Ritonavir microspheres was in range of 37-40 µm. As it was evident from the results that particle size increases as polymer concentration was increased. The data showing particle size analysis is given in table: 3.

Table: 3 Particle size analyses of Ritonavir microspheres

Formulation	Average size (µm)
A1	37.34±13.54
A2	38.87±6.65
A3	39.13±9.11

From Scanning electron microscopy, it was observed that particles were spherical. The surface of the drug-loaded Ethyl cellulose microspheres shown in Figure: 1. Ethyl cellulose microspheres showed smooth surface. All the microspheres had small pores on their surfaces, which will be responsible for release.

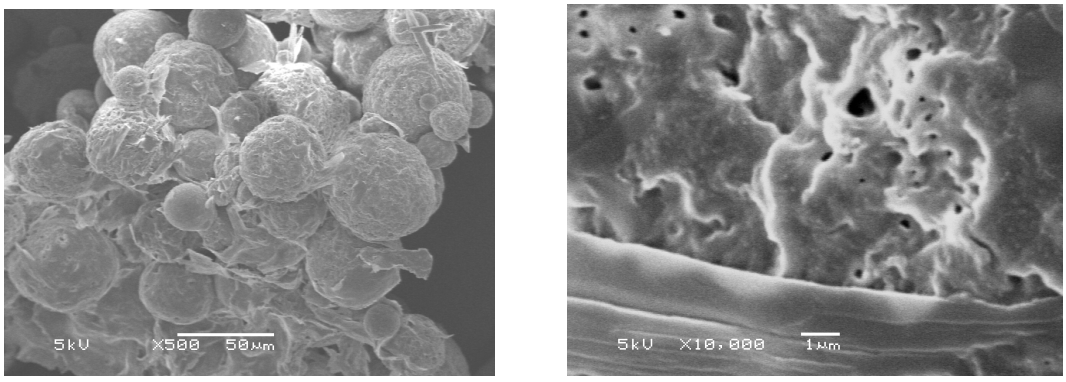

Fig:1 SEM images of Ethyl cellulose microspheres of Ritonavir.

Drug polymer interaction can be studied by FT-IR analysis. Figure: 2 show the IR spectra of pure Ritonavir, drug loaded microspheres and physical mixture. The characteristic CH stretching, NH stretching of secondary amine, C=C stretching and C=O stretching of pure drug was observed at 2960 cm-1, 3356 cm-1, 3030 cm-1 and 1714 cm-1. The characteristic peaks confirmed the structure of Ritonavir. The same peaks were also reported in all drug loaded microspheres and in physical mixture. 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.

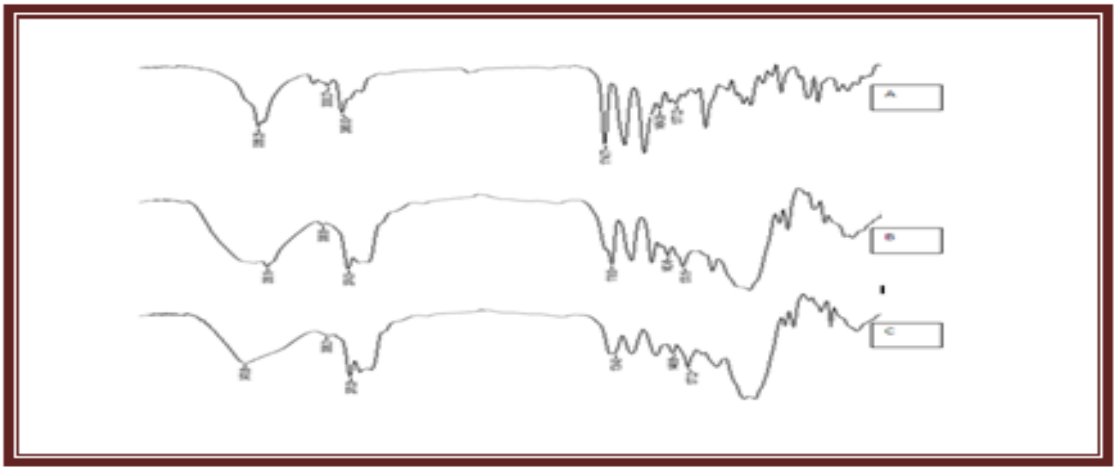

Fig. 2 FT IR spetra of pure ritonavir(A) physical mixture(B) Ethyl cellulose microspheres(C)

Any possible drug polymer interaction can also be studied by thermal analysis. DSC studies were performed on pure drug and drug-loaded microspheres. DSC thermo grams of pure Ritonavir (figure: 3) showed a sharp endothermic peak at 121.59°C. The themo grams of

formulation also showed a similar endothermic peak at 118.25°C. This further confirms that there is no drug polymer interaction.

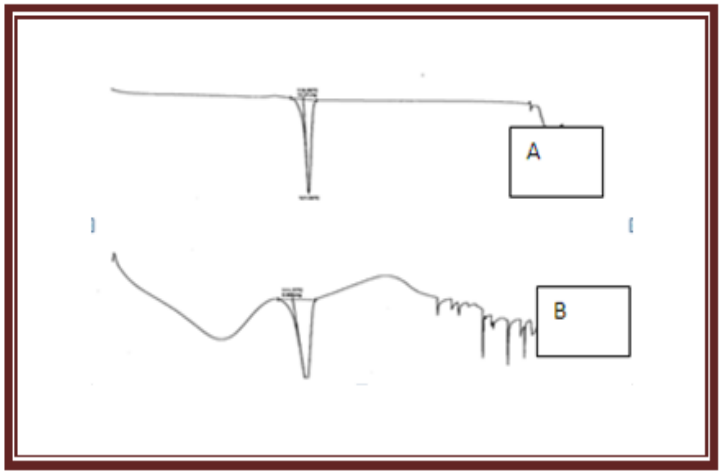

Fig. 3 DSC Thermo grams of Ritonavir (A) Ritonavir microspheres (B)

Drug release from microspheres was sustained up to 10 hours. Different formulation showed different degree of release (figure: 4). the cumulative release of Ritonavir significantly decreased with increasing Ethyl cellulose concentration. The increased density of the polymer matrix at higher concentrations results in an increased diffusion path length[8]. After 10 hours drug release from different formulation was in range of 75 to 95 %.

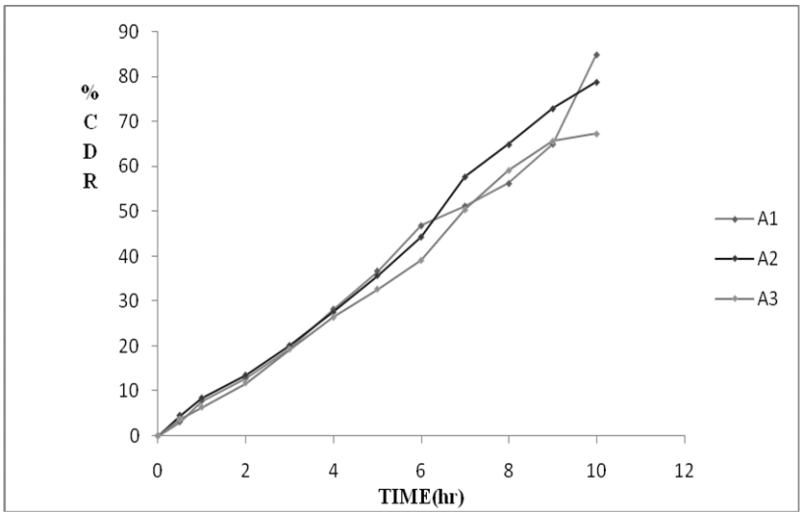

Fig. 4 %CDR of Ritonavir microspheres (A1, A2 &A3)

The in-vitro release data was fitted to various kinetic models in order to find out the drug release mechanism. The ‘n’ value obtained from Korsemeyer - Peppas equation for Ethyl

cellulose was in range of 0.956 to 0.982 suggested that the drug release from microspheres followed non-fickian diffusion mechanism[9].

CONCLUSION

The Ritonavir loaded microspheres were prepared by solvent evaporation method using Ethyl cellulose. Polymer: drug ratio and stirring speed of the system were important to obtain spherical particles with smooth surfaces. The yields of preparation and encapsulation efficiencies were very high for all microspheres obtained. The prepared microspheres were free flowing and discrete. The FT-IR and DSC suggested no drug polymer interaction during encapsulation process. The drug release was studied up to 10 hours and results indicate that release of drug from microspheres followed non-fickian diffusion mechanism.

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