PREPARATION AND CHARACTERIZATION OF MICROSPHERES ENCAPSULATING RITONAVIR BY SOLVENT EVAPORATION TECHNIQUE

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ABSTRACT:
Aim: The aim of the present study is to prepare and characterize microspheres containing Ritonavir using Eudragit L100 as the polymer.
Methods: The Ritonavir loaded microspheres were prepared by solvent evaporation 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 calorimetry 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 32 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 nanoparticles 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 & was found to be diffusion controlled. According to the data obtained, this eudragit L100-based microspheres opens new and interesting perspectives as drug carriers for treating the AIDS.

KEY WORDS: Microsphere, Eudragit L100, Ritonavir, Solvent evaporation technique.

1. INTRODUCTION:
Human immunodeficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS) commonly referred to as HIV & AIDS have emerged as being amongst the most serious and challenging public health problems in the world. There are two species of HIV, namely, HIV 1 and HIV 2 with their respective subspecies. HIV 1 is the global common infection whereas the latter is restricted to mainly West Africa. HIV infection in the human body results mainly from the integration of the viral genome into the host cell for the purpose of cell replication.

The current clinical therapy, known as highly active antiretroviral treatment (HAART), is considered as one of the most significant advances in the field of HIV therapy. HAART is a lifelong necessity and any non-compliance leads to a rapid increase in the viral load. The reason for this relapse is related to the poor targeting ability of the antiretroviral agent to the latent sites of infection. The two main objectives of the antiretroviral therapy are virological control and restoration of immunity. Once these two objectives are achieved, it is possible to delay the progression of the disease, minimize opportunistic infections, malignancies and prolong the survival of the patient.

Currently the five different classes of antiretroviral drugs available are Nucleoside Reverse Transcriptase Inhibitors (NRTI’s), Nucleotide Reverse Transcriptase Inhibitors (NtRTI), Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI), Protease Inhibitors (PIs), and more recently, fusion and integrase inhibitors. NRTI’s are among the first agents to be used for the treatment of HIV/AIDS. These agents inhibit the reverse
transcriptase enzyme responsible for the conversion of viral RNA to DNA within the host cell. Ritonavir is the antiretroviral drug. It is manufactured as norvir by Abbvie inc. The food and drug administration (FDA) approved ritonavir on March 1, 1996 making it the seventh approved anti-retroviral drug, the second approved protease inhibitors in the United States. Ritonavir is used for the treatment of HIV and AIDS related conditions. However ritonavir has half-life 3-5 hours and low bioavailability 65% narrow therapeutic index, moreover it is primarily absorbed from stomach. Eudragit L100 is a white, free flowing powder with at least 95% of dry polymers. It is an anionic co-polymerization product of methacrylic acid and methyl methacrylate. It is soluble at pH > 6. The ratio of free carboxyl groups to the ester is approximately 1:1 in eudragit L100. It is readily soluble in neutral to weakly alkaline conditions (pH 6-7) and form salts with alkalis, thus affording film coats which are resistant to gastric media, but soluble in intestinal fluid. It is freely soluble in acetone, alcohol and sodium hydroxide. It is insoluble in dichloromethane, ethyl acetate, petroleum ether and water. Ritonavir and eudragit L100 were selected as core and coat material for the formulation of microspheres to achieve controlled drug release.

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 solvent evaporation method, evaluate its physicochemical characteristics such as particle size, shape, zeta potential, drug loading capacity and in vitro release characteristics.

2. MATERIALS AND METHODS:
Ritonavir is a gift sample from the strides arcolabs ltd., Bangalore. Eudragit L100 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 pharmacopoeial standards.

2.1. Preparation of microspheres:
Ritonavir loaded Eudragit L100 microspheres were prepared by solvent evaporation Technique. 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 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.

<table>
<thead>
<tr>
<th>Sl No</th>
<th>BATCH CODE</th>
<th>DRUG: POLYMER RATIO</th>
<th>% DRUG ENTRAPMENT EFFICIENCY</th>
<th>MEAN PARTICLE SIZE(µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>1:1</td>
<td>63.0</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>1:2</td>
<td>66.27</td>
<td>74.5</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>1:3</td>
<td>69.84</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>1:4</td>
<td>76.47</td>
<td>80.5</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>1:5</td>
<td>81.02</td>
<td>84</td>
</tr>
</tbody>
</table>
Characterization of prepared microspheres:  

2.2.1. Fourier transform infra-red spectroscopy (FTIR) analysis

The FT-IR spectra of pure Ritonavir and Eudragit L100 microspheres loaded Ritonavir were recorded to check drug polymer interaction and stability of drug (Fig. 1).

![FT-IR spectra](image)

Fig. 1 – FT-IR spectra of (A) pure Ritonavir, (B) Ritonavir microspheres, (C) Eudragit L100.

2.2.2. 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°C min⁻¹ from 10°C to 400°C temperature range under nitrogen flow of 25 ml min⁻¹(Fig. 2).
Figure 1: Ritonavir

Thermal Analysis Result

File Name: Ritonavir (API).tad
Detector: DSC-60
Acquisition Date: 15/11/23
Acquisition Time: 14:41:52(+0530)
Sample Name: Ritonavir (API)
Sample Weight: 0.000[mg]
Annotation: Thermal Analysis Result

Fig. 2 – DSC thermo grams of pure Ritonavir and Ritonavir loaded eudragit L100 microspheres.
2.2.3. Drug entrapment efficiency of microspheres:

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 spectrophotometrically for drug content at 240nm. The drug entrapment efficiency was determined using the relationship:

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

2.2.4. Surface morphology study:

Scanning electron microscopy (SEM) of the microsphere was performed to examine the particle size and surface morphology (Fig. 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 microscope under magnification of 7500 - 20,000×.

2.2.5. Zeta potential

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

2.2.6. In vitro release studies:

In vitro release study of Ritonavir from various formulations was conducted for 24 hrs 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 FE1-FE5 prepared by solvent evaporation method shows FE1-88.62%, FE2-85.91%, FE3-83.06%, FE4-80.93% and FE5-78.80 were shown in Figure No.6. The increase in Eudragit L100 ratio from FE1 to FE5 causes decrease in the drug release.
2.2.7. 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 2).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>%Cumulative drug release</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi plot</th>
<th>Peppas plot</th>
<th>‘n’ Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>FE1</td>
<td>88.62</td>
<td>0.5931</td>
<td>0.9605</td>
<td>0.8653</td>
<td>0.3945</td>
<td>0.8056</td>
</tr>
<tr>
<td>FE2</td>
<td>85.61</td>
<td>0.6252</td>
<td>0.9531</td>
<td>0.8874</td>
<td>0.4092</td>
<td>0.8100</td>
</tr>
<tr>
<td>FE3</td>
<td>83.06</td>
<td>0.6584</td>
<td>0.9278</td>
<td>0.9058</td>
<td>0.4265</td>
<td>0.8177</td>
</tr>
<tr>
<td>FE4</td>
<td>80.93</td>
<td>0.7120</td>
<td>0.9305</td>
<td>0.9419</td>
<td>0.4865</td>
<td>0.8629</td>
</tr>
<tr>
<td>FE5</td>
<td>78.80</td>
<td>0.7769</td>
<td>0.9462</td>
<td>0.9699</td>
<td>0.5531</td>
<td>0.9053</td>
</tr>
</tbody>
</table>

Table 2 – Correlation coefficients according to different kinetic equations.

FE-1, FE-2, FE-3, FE-4 and FE-5 represent formulations 1-5 respectively, etc.

2.2.8. Stability study

The stability study was carried out using the batch FE-5. Formulation FE-5 was divided into 3 sets of samples and stored at 4 °C in refrigerator, room temperature 45 °C, 75% RH in humidity control ovens. After 90 days drug content of all samples were determined by the method as in drug content (Fig. 7). In vitro release study of formulation FE-5 was also carried out after 90 days of storage (Table 3 and Fig. 8).
Fig. 7 - Stability study: comparison of % drug content of formulation FE-5 at 4°C, room temperature and 45°C ±2°C/75% RH.

Table 3 – stability studies – in vitro release study of a selected formulation FE-5 after three months storage at 4°C, Room temperature, 45°C 2°C/75%RH.

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>% Cumulative drug release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4°C</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>20.01</td>
</tr>
<tr>
<td>2</td>
<td>27.19</td>
</tr>
<tr>
<td>3</td>
<td>32.04</td>
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<tr>
<td>4</td>
<td>39.91</td>
</tr>
<tr>
<td>6</td>
<td>46.84</td>
</tr>
<tr>
<td>8</td>
<td>58.17</td>
</tr>
<tr>
<td>12</td>
<td>66.97</td>
</tr>
<tr>
<td>24</td>
<td>77.89</td>
</tr>
</tbody>
</table>
Fig. 8 - Stability study: comparison of in vitro drug release profile for formulation FE-5 at 4℃, room temperature and 45℃ ± 2℃/75% RH after three months storage.

3. RESULTS AND DISCUSSION:

Microspheres prepared by solvent evaporation 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 63.05%, 66.27%, 69.84%, 76.47%, and 81.02%. Thus there was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulation. The formulation FE-5 registered highest entrapment of 81.02%. The interaction study between the drug and polymer was evaluated using FT-IR spectrophotometer. There was no significant difference in the IR spectra of pure and drug loaded microspheres. Differential scanning calorimetry study thermogram of pure Ritonavir showed a sharp endothermic peak at 126.17℃. The thermo grams of formulations FE-5 of Fig. 2, showed the same endothermic peak at the similar temperature. This further confirmed that there is no drug to polymer interaction. Zeta potential of all formulated microspheres was in the range of 30-32mV, which indicates that they are moderately stable. Cumulative percentage drug released for FE-1, FE-2, FE-3, FE-4 and FE-5 after 24 h were found to be 88.62%, 85.61%, 83.06%, 80.93% and 78.80% respectively. Zeta potential for FE-5 was found to be 32 mV 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 first order, and Higuchi respectively. As indicated by higher R² 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.

References: