SOLVENT MODIFICATION FOR THE ENHANCEMENT OF SOLUBILITY OF POORLY SOLUBLE DRUG: CARRAGEENAN AS CARRIER

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ABSTRACT

The aim of the work was to evaluate the effect of carrageenan on the solubility of poorly soluble, BCS class II, NNRTI drug Efavirenz for its solubility enhancement. Solid dispersions were formulated using naturally obtained polysaccharide carrageenan in the molecular ratio of 1:1, 1:3, 1:5 using trituration method (TM), solvent evaporation method (SE) and kneading method (KM). The formulated solid dispersions were manually filled in hard gelatin capsules (size 1) and evaluated for the physicochemical properties like weight variation (100-300 mg), disintegration time (3-4 min), drug content (99-103%) and dissolution using water and compendial media water with 1% sodium lauryl sulphate. The solid dispersions were subjected to instrumental analysis for confirming its purity using Fourier transform infrared spectroscopy (FTIR) and polymorphic changes using differential scanning calorimetry (DSC). The obtained data confirmed the enhancement of the solubility of Efavirenz using carrageenan by two folds.

Keywords: Solid dispersions, Carrageenan, Hot stage microscopy, HIV, Anti-retroviral Therapy (ART)

INTRODUCTION

Efavirenz (EFV) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and is used as a part of highly active anti-retroviral therapy (HAART) for the treatment of Human Immunodeficiency Virus (HIV) type I. Efavirenz was approved by the Food and Drug Administration (FDA) on September 21, 1998 making it the 14th approved antiretroviral drug. It is also used in combination with other anti-retroviral agents like reverse transcriptase inhibitors (RTI). The symptomatic IUPAC name is 8-chloro 5-[2-cyclo propyl ethenyl] 5-trifluoro methyl 4-oxa 2-aza tri cyclo (4.4.0) dec 7,11 trien 3-one. The molecular formula and molecular weight of the drug is C20H24ClF3NO2 and 315.6 g/mol respectively. The drug-protein binding and its bioavailability are reported in the range of 99.5% - 99.75% and 42% approximately. Efavirenz is practically insoluble in water (<10mcg/ml) and so it is categorized under BCS class II. Efavirenz has been used as a first line treatment for AIDS in preference to the protease inhibitor.

The natural carrageenan is isolated from the species of red seaweed, family of rhodophyceae. The major sources are the chordrus crispus, eucheuma spinosum and eucheuma cottonii. The former is usually found in cold climate territories and the other two are generally grown in temperature climate. It is made as high molecular weight linear polysaccharide comprising of galactose, sulfated and non-sulfated joined by α-(1-3) and β-(1-4) glycosidic linkage. Carrageenan acts as thickening, suspending and gelling agent for many industrial applications. There are three types of carrageenan such as kappa, iota and lambda which indicates the addition of potassium, calcium and sulfate molecules respectively. Solutions of carrageenan are more stable in slightly acidic, neutral and alkaline conditions, and in water it forms a weak gel at very low concentrations. It also allows solids to remain suspended in solution. General applications of carrageenan includes drug delivery systems for both oral and topical routes, wound dressing, cosmetics, contraceptive gels, dentofores and in humidity control as well as in biotechnology for cell immobilization.

MATERIALS AND METHODS

Materials

Efavirenz obtained as gift sample from ISP Hong Kong (P) Ltd., Hyderabad, India. Efavirenz procured from Himedia Lab (P) Ltd., Mumbai, India. Sodium lauryl sulphate was purchased from S.D Fine chemicals Ltd., Mumbai, India. All other reagents were of analytical grade.

Preparation of solid dispersion

In the molar ratios of 1:1, 1:3, 1:5 of drug to carrier (carrageenan), the solid dispersions of Efavirenz were prepared by the well established techniques (Table 1).

Trituration method

For the preparation of physical mixture, the previously sieved and weighed Efavirenz and carrageenan was taken and homogeneously blended in a mortar and pestle. The physical mixtures were stored in desicicators with anhydrous CaCl2 at ambient temperature until used, to prevent the moisture absorption and contamination.

Solvent evaporation method

The requisite amount of Efavirenz and carrageenan carrier was dissolved in easily evaporating organic solvent such as methanol and allowed to stand overnight. The so formed solid dispersions were subjected to complete dryness at 60°C, the solvent was removed under vacuum condition until it became dry. The pulverized dried mass was passed through 44-mesh sieve and stored in desiccators for future use.

Kneading Method

The Efavirenz and complexing carrier were accurately weighed in different drug-polymer ratio 1:1, 1:3, 1:5 and transferred to mortar for kneading (31) using hot water up to 45 minutes. Sufficient hot water was added to maintain paste like consistency. The resulting paste was then dried in hot air oven at 45°C for 24 hours. The dried dispersions were milled and passed through sieve no. 18. The prepared dispersions were stored in amber coloured glass vials and used for further studies.

Filling in Capsules

The formulated solid dispersion was accurately weighed for its equivalent weight as per the yield and filled in hard empty gelatin capsule shells by manual pour filling method. The filled capsules were utilized for the further studies.

 Characterization of Solid dispersion

FT-IR Spectroscopy

KBr pellet technique was used to determine the compatibility between drug and polymer. The modified procedure of Fourier Transform Infra-Red Spectrophotometer (Perkin-Elmer system 200 FT-IR spectrophotometer) was used for the measurement of pure drug, polymer and drug-loaded solid dispersion formulation spectra. The pellets were prepared on KBr-press under hydraulic
pressure of 150 kg/cm²; the samples were scanned over the wave number range of 3600 to 400 cm⁻¹ at the ambient temperature.

**Differential Scanning Calorimeter (DSC)**

DSC is used to characterize the presence of water in hydration in pharmaceuticals. Thermograms of formulated solid dispersions and pure drug were obtained by the modified procedure of DSC instrument equipped with an intra cooler. Indium / Zinc standards were used to calibrate the DSC temperature and enthalpy scale. The sample preparations were hermetically sealed in an aluminium pan and heated at a constant rate of 10°C / min; over a temperature range of 25⁰C - 200⁰C. Inert atmosphere was maintained by purging nitrogen gas at the flow rate of 50ml / min.

**Evaluation of Capsules**

The capsules were evaluated for the weight variation test and uniformity of weight as per the standard pharmacopoeial method. The disintegration time of the capsules were evaluated using the Tablet / Capsule Disintegration test apparatus (Lab India model DT 1000).

**Drug content analysis**

An accurately weighed quantity of formulation equivalent to 50 mg of EFV was taken into a 100 ml volumetric flask and dissolved in compendial media of distilled water with 1% sodium lauryl sulphate (SLS) and sonicated for 5min using bath sonicator. 1 ml of the filtrate was diluted to 25 ml using water with 1 % SLS and assayed for drug content using UV/Visible spectrophotometer (Perkin Elmer, USA) at 247 nm.

**Hot stage microscopy**

The hot stage microscopy (HSM) is used to study the changes taking place in morphology of samples due to heat flux. The pure drug and solid dispersions (Kneading method 1:5 ratio) of Efavirenz samples were subjected for the HSM by using hot plate (SEMDO Ltd.) and LEICO S8 TAPO Stereo Zoom Microscope (LEICA Micro Systems, Switzerland Ltd.). About 10-50 mg of samples were placed on glass slides and covered with cover slips and heated on hot plate. At various temperatures, the changes were noticed and images were captured.

**In-Vitro release**

In-vitro dissolution studies were conducted in 900 ml of water with 1% sodium lauryl sulphate solution using USP XXII dissolution type I (basket) apparatus (Electrolab) at 37± 0.5°C at the speed of 50 rotations per minute for the different ratios of solid dispersions. At the specific predetermined time intervals 5ml samples were withdrawn and the equivalent quantity of fresh medium was replaced to maintain the sink condition. The aliquots were diluted suitably and analyzed by using UV/Visible spectrophotometer (Perkin Elmer, USA) at 247 nm.

**RESULTS AND DISCUSSION**

**Formulation of solid dispersion complex**

The solid dispersions formulated by trituration, solvent evaporation and kneading methods at different ratios of drug and carrier (1:1, 1:3, 1:5) were compared for its physicochemical properties. The equivalent quantities of the drug were calculated and accurately weighed for each solid dispersion formulation and then filled in hard empty gelatin capsule shells by manual method. It was also subjected to drug content study, in-vitro release studies, FTIR and DSC.

**Evaluation of Capsules**

The average weight of the capsules for various solid dispersion formulations are shown in the table 1. The individual weight of the tablets was found to lie within the pharmacopoeial limits. The disintegration time of the capsules was obtained to be less than 5 minutes.

**Fourier Transform Infra red spectrosocpy (FT-IR)**

The FTIR spectrum of the pure drug and the solid dispersion formulation (1:3 ratio) are comparatively shown in Fig 1. The spectrum shown from the range of 4000 cm⁻¹ to 400 cm⁻¹ showed no significant changes in the functional groups of specific interaction between the drug and polymer, which provided the evidence about the compatibility between the drug and excipients used in the formulation. But in the formulation (1:3 ratio, solvent evaporation method) the wave number of around 3240 – 3430 cm⁻¹ it gave a broader peak which may be due to the hydrogen bonding / complex formation of the drug with polymer.

**Table 1: Composition and Evaluation of capsules filled with solid dispersion**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Batch Code</th>
<th>Drug :Carrier ratio (Molar)</th>
<th>Drug (mg)</th>
<th>Carrier (mg)</th>
<th>% Drug content</th>
<th>Weight variation (mg)</th>
<th>Disintegration time (Min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TR</td>
<td>1:1</td>
<td>100</td>
<td>100</td>
<td>99.8</td>
<td>100.3 ± 2.0</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>TR</td>
<td>1:3</td>
<td>100</td>
<td>300</td>
<td>100.2</td>
<td>202 ± 3.3</td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td>3</td>
<td>TR</td>
<td>1:5</td>
<td>100</td>
<td>500</td>
<td>101</td>
<td>305 ± 2.9</td>
<td>3.5 ± 0.4</td>
</tr>
<tr>
<td>4</td>
<td>SE</td>
<td>1:1</td>
<td>100</td>
<td>100</td>
<td>100.5</td>
<td>104.8 ± 3.1</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>5</td>
<td>SE</td>
<td>1:3</td>
<td>100</td>
<td>300</td>
<td>98.3</td>
<td>205.4 ± 5.1</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>6</td>
<td>SE</td>
<td>1:5</td>
<td>100</td>
<td>500</td>
<td>100.3</td>
<td>302 ± 3.3</td>
<td>3.2 ± 1</td>
</tr>
<tr>
<td>7</td>
<td>KM</td>
<td>1:1</td>
<td>100</td>
<td>100</td>
<td>101.2</td>
<td>104.83 ± 4.7</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td>8</td>
<td>KM</td>
<td>1:3</td>
<td>100</td>
<td>300</td>
<td>103.1</td>
<td>203.6 ± 2.3</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>9</td>
<td>KM</td>
<td>1:5</td>
<td>100</td>
<td>500</td>
<td>100.9</td>
<td>305.5 ± 7.8</td>
<td>3.4 ± 0.5</td>
</tr>
</tbody>
</table>
Differential Scanning Calorimetry (DSC)
The DSC analysis of the pure drug showed sharp melting point at 139.4 °C but the solid dispersions made with carrageenan showed the shift in the melting point from 139 to 95 and the broader peak reveals that there is a change in solid state i.e., crystalline to slight amorphous state. The carrageenan complex helps in conversion of Efavirenz crystalline state to transition in the molecular level. The data was supported by the dissolution studies (Fig.2 a, b).

**Fig. 2: DSC studies of pure drug (a) compared with Optimized solid dispersion 1:5 kneading method (b) formulation**

**Drug content**
The drug content estimation is the evidence for the presence of required quantity of drug in the final formulations. The solid dispersions of Efavirenz at different ratios with the carrier were noticed to confirm the encapsulation of exact amount of drug within the standard range of 90–110%. (Table 1)

**Hot stage microscopy**
The melting characteristics of the drug and formulation were observed using hot plate and optical microscope. It revealed that the pure drug became glassy at 70°C and melted at 140°C. But in the formulation it appeared crystalline in structure and became charred at 140°C which may be due to the complexation with carrageenan (Fig.3).
EFV at room temp

EFV Pure at 70°C

EFV Pure at 140°C

KM at room temp

KM 1:5 at 70°C

KM 1:5 at 140°C

Fig. 3: Hot stage microscopic images of solid dispersions compared with pure drug

In-Vitro drug release

Drug release in Distilled Water as dissolution media

In order to prove the aqueous solubility enhancement of the poorly soluble drug, the in-vitro dissolution of solid dispersions was carried out using distilled water as medium. The formulated solid dispersions of Efavirenz with Carrageenan have resulted in enhanced solubility of the drug compared to the pure drug solubility and release. The percentage of drug release from pure API encapsulated in the hard gelatin capsule showed only 6% at 5 min and gradually increased upto 28% in 1 hour. The solid dispersions formulated by trituration method had shown more than 7% drug release at 5 min which was found to be higher than 40-50% in 1 hour, for all the three different compositions (Fig 4). Similarly the formulation done by solvent evaporation method also showed more than 8-16% at 5 min and 55-65% at 1 hour (Fig 5).

The same effect was identified in solid dispersions formulated by kneading method, in which the release of drug was 19-29% in 5 min and became 76-86% at 60 min (Fig 6). It is evident that the solubility of the drug had been increased more than three times in the solid dispersions than the pure drug release.

Fig. 4: In-vitro release of SD prepared by trituration method in distilled water as media
Fig. 5: *In-vitro* release of SD prepared by solvent evaporation method in distilled water as media.

Fig. 6: *In-vitro* release of SD prepared by kneading method in distilled water as media.

Fig. 7: *In-vitro* release of SD prepared by trituration method in water with 1% SLS as media.
Drug release in water with 1% SLS media

Sodium lauryl sulphate (SLS) was added in the media which enhanced the solubility of poorly soluble drugs. The release studies for the pure drug, carried out using SLS in the dissolution media showed more than 50% drug release in 5 min and reached the maximum of 99% at 60 min. But the solid dispersions prepared by trituation method and solvent evaporation method showed less release than the pure drug in this media via, < 20% and < 90% in 5th minute and 60th minute, respectively. This may be due to the stronger complexation between the drug and polymer in the solvent evaporation method than the kneading technique. The improper miscibility of carrageenan in the SLS media can also cause less drug release from such complexed dispersions. However, the solid dispersion prepared by kneading method with five times higher concentration of the carrier (1:5 ratio), the drug release was greater than 75% at 5 min and achieved the maximum of 106% at 1 hour. The mechanism behind this is that, the addition of higher concentration of the carrier helps in enhancement of molecular level solubility of the drug dispersed in it (Fig. 7-9).

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REFERENCES