Formulation and Invitro Evaluation of Ambrisentan Solid Dispersions

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Abstract: **Ambrisentan is an endothelin receptor antagonist that is primarily prescribed to treat hypertension of the pulmonary arterial system. Ambrisentan is classified as a BCS class II drugs, meaning that it has a high membrane permeability and a low solubility in water. Ambrisentan has a low pH and is practically insoluble in water and aqueous solutions. Ambrisentan has insufficient absorption and bioavailability due to its extremely low water solubility. The aim of present study was preparation of solid dispersion of Ambrisentan for increasing the solubility and dissolution. Solid dispersion of Ambrisentan was prepared by using fusion methods. Prepared solid dispersions were evaluated for solubility, FTIR, drug content, entrapment efficiency and *in vitro* drug release studies. FTIR studies revealed that there are no interaction between drug and optimized solid dispersion, drug content and entrapment studies showed that within the limits and satisfactory. *Invitro* drug release studies showed that among all the formulations FF12 showed highest drug release that is 99.12 % at the end of 60 minutes, hence FF12 was best formulation.**

Key words: **Ambrisentan, solid dispersion, fusion method, PEG4000, PEG6000**

INTRODUCTION

Absorption of drug and its therapeutic effectiveness get affected by solubility which is a significant physicochemical factor. Poor aqueous solubility can leads to failure in formulation development process[1]. The main reason behind inadequate bioavailability of drug is its low dissolution rate and low solubility in aqueous medium. A large number of hydrophilic carriers are explored today which have shown significant results for solubility enhancement[2]. Nowadays, most of the drug substances were innovated but the venture to improve the solubility and dissolution of hydrophobic drug substances remain one of the trickiest tasks in drug development. Dissolution of drug in aqueous medium like gastric fluid is important to get better absorption and bioavailability for orally administered drug. Therefore, to progress bioavailability of poorly water soluble compounds like biopharmaceutical classification system class II and IV drugs, polymer matrix of various origins can be used[3].

One of the most promising and efficient techniques for solubility enhancement is solid dispersion formulation. Solid dispersion systems can be defined as 'the dispersion of one or more active ingredients in an inert carrier or matrix at solid state prepared by the melting [fusion], solvent, or melting-solvent method'. The drug is hydrophobic in nature whereas matrix is hydrophilic. Solid dispersion can be classified as simple eutectic mixtures, solid solutions, glass solutions and glass suspensions, amorphous precipitation in a crystalline carrier, compound or complex formations[4].

Ambrisentan is an endothelin receptor antagonist that is generally prescribed to treat hypertension of the pulmonary arterial system. Ambrisentan is classified as a BCS class II drugs, implying that it has a high membrane permeability and a low solubility in water. Ambrisentan has a low pH and practically insoluble in water and aqueous solutions[5, 6]. Ambrisentan has limited absorption and bioavailability due to its extremely low water solubility. Regarding the enhancement of ambrisentan's solubility and bioavailability, there is remarkably little published literature. Hence, an attempt was made in this study to enhance ambrisentan's solubility, dissolution rate, and bioavailability through the production of its solid dispersion utilizing carriers such polyethylene glycol and polyvinyl pyrrolidone in order to improve the drug's solubility in water and dissolution rate.

MATERIALS AND METHODS

Materials: Ambrisentan obtained gift sample from Dr Reddy labs, Hyderabad, India.PEG 4000, PEG6000, PVP K30 and mannitol obtained from SD fine chemicals Mumbai , India. All other reagents used are analytical grade.

Methods:

Saturation Solubility studies: a Saturation solubility study of Ambrisentan was conducted in distilled water, 0.1N HCl and pH 6.8 phosphate buffers. Excess amount of drug was added to 10 ml of study solutions in glass vial. Samples were shaken in Rotary shaker by maintaining constant temperature and speed for 24 hr. The resultant saturated solutions were then filtered using whatman filter paper no 1. Filtrates sample ware then estimated spectrophotometrically after suitable dilution[7].

λ_{max} scanning of Ambrisentan:

The stock solution is prepared upon addition of 10 mg of Ambrisentan to a 10 ml volumetric flask. It's then thoroughly combined with 5 mL of methanol to dissolve the drug. To reach a concentration of 1000 g/ml, 0.1 N HCl was added to the solution as a top-up.

1 ml of the aforementioned solution is to 10 ml with 0.1 N HCL in order to get a concentration of 100 g/ml. To produce a concentration of 10 g/ml, take 1 mL of the aforementioned solution and dilute it to 10 mL with 0.1 N HCl. The generated solution, which had a concentration of 10 g/ml, was scanned in a Ultraviolet-visible spectrophotometer for maximum wavelengths between 200 to 400 nm

Ambrisentan's calibration curve in 0.1 N HCl:

Stock solution preparation:

To attain the concentration of 1000 g/ml, 10 mg of Ambrisentan was put into the volumetric flask of 10 ml and the flask was topped off to the mark with 0.1 N HCl. 100 g/ml concentration will be attained by taking 1 ml of the above solution and diluting it to 10 ml with 0.1 N HCL. The dilutions containing 5-30 g/ml solutions are created from the aforementioned stock solution. Ultraviolet-visible spectroscopy is utilized in the determination of the absorbance of each test solution at maximal wavelength of Ambrisentan (i.e., 263 nm).

Preparation method of solid dispersions of Ambrisentan:

b. Fusion/Melting Method

A physical mixture of Ambrisentan and different carriers (PEG 4000, Mannitol, PVPK30,PEG6000) were taken in china dish with different ratios(1:1,1:2,1:3, that is denoted as FF1 to FF12)This mixture is heated to generate the molten mixture, this is then cooled and hardened while vigorous stirring is performed. To reach the desired particle size, the solid mass is crushed, pulverized, and sieved then stored in dessicator until further study[8]. Composition of Ambrisentan solid dispersions are shown in table 1

Table 1: Formulation of solid dispersion of Ambrisentan by fusion method

Formulation code)	FF1	FF2	FF3	FF4	FF5	FF6	FF7	FF8	FF9	FF10	FF11	FF12
Drug: polymer ratio	1:1	1:2	1:3									
Drug: polymer ratio				1:1	1:2	1:3						
Drug: polymer ratio							1:1	1:2	1:3			
Drug: polymer ratio										1:1	1:2	1:3

* FF1 to FF3: PEG4000. FF4 to FF6: Mannitol, FF7 to FF9: PVPK30, FF10 to FF12:PEG6000

Solid Dispersions Evaluation:

The drug-polymer conjugates were evaluated by

1) Estimation of drug content
2) Efficiency of Entrapment
3) *In-vitro* dissolution studies

Drug content Estimation:

An exact 10 mg dose of the drug was measured out and put into a 100 ml volumetric flask. Following that, 0.1 N HCl buffer was added to the volume, and it was shaken vigorously for 10 minutes to ensure that the drug was thoroughly dissolved[9]. After that, the remedy was filtered. Similar concentration of the standard solution was made by dissolving 10 mg of the reference drug in 0.1 N HCl buffer. The sample and the standard solutions both are measured in aUltraviolet-visible spectrophotometer with the Ambrisentan's absorbance at 263nm.

Entrapment efficiency:

The solid dispersion's entrapment efficacy was a crucial factor in evaluating the amount the amount of material entrapped inside solid dispersions prior to the study of the behavior of this entrapped drug in

physical and biological systems because the effects observed experimentally are frequently dose-related. Because greater lipid dosages might be harmful and result in non-linear (saturable) pharmacokinetics of formulation, solid dispersions formulation of pharmaceuticals can only be made if the encapsulation efficiency of therapeutic doses can be given with a reasonable amount of drug[10]. A well-designed filling method might produce 90% or greater trapping efficiency. Because loading dosages of 10% or less of free medication can often be tolerated, there is no longer a requirement to remove non-entrapped material. Procedures to remove non-entrapped material, such as dialysis and passing through an exclusion column, are frequently time-consuming, tiresome, and expensive, and recovery of non-entrapped material is typically challenging.

Entrapment efficacy was calculated by following the formula:

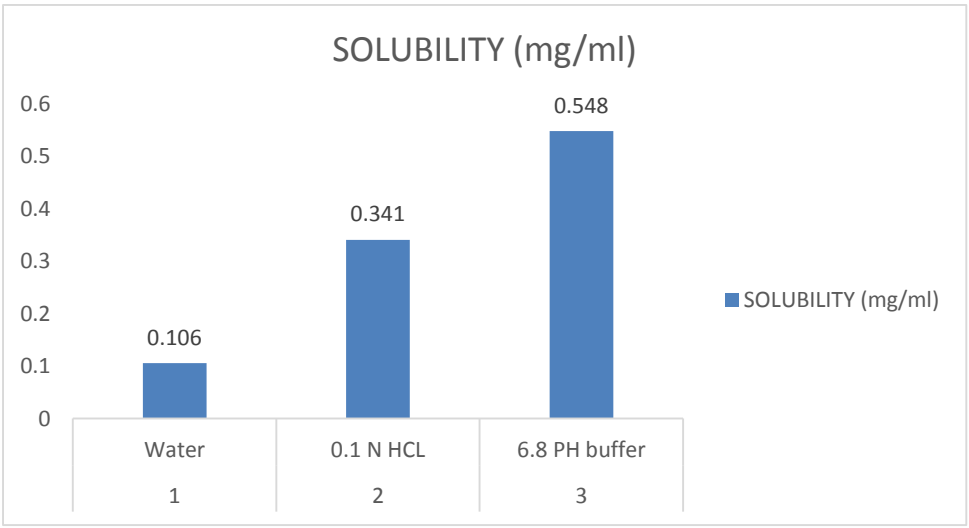
%Entrapment efficacy= Drug content X 100/Drug added in each Preparation.

In-vitro dissolution study:

In-vitro dissolution was done on the generated solid dispersions. The USP type II paddle technique and equipment II were utilized in the dissolution studies. 0.1 N HCL buffer was utilized as the dissolving medium, 50 rpm was utilized for stirring, and 37°C was maintained as the dissolution medium temperature. Ambrisentan was detected at 263 nm using a UV-visible spectrophotometer after 5 mL samples were regularly collected, filtered, and replaced with 5 ml of fresh dissolving medium. As required, dilutions were created[11].

RESULTS AND DISCUSSIONS

Solubility studies:



Solubility studies of Ambrisentan

Based on the results of the solubility tests performed in various buffers, we can conclude that compared to the other buffer solutions, 6.8 pH buffer solution has more solubility.

Determination of ƛ max of Ambrisentan:

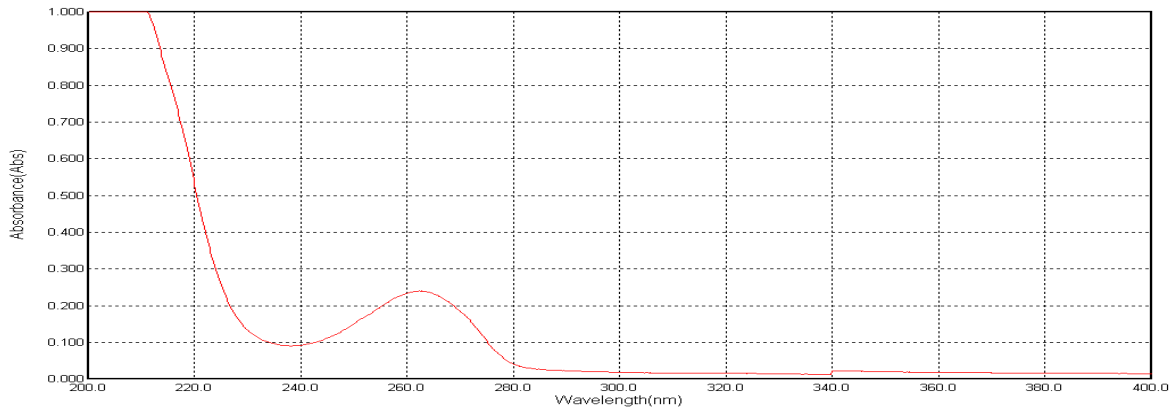


Figure 1: UV Spectrum of Ambrisentan pure drug

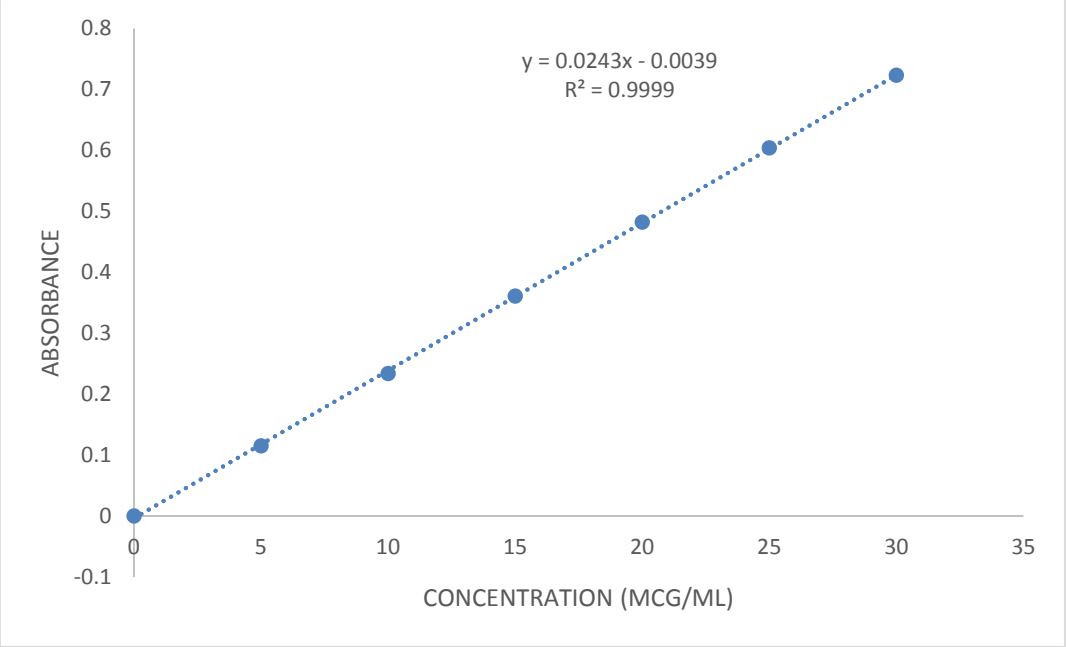


Figure 2: Calibration curve of Ambrisentan

3 Drug excipient compatibility:

The compatibility of the drug & excipients was established by comparing the FT-IR spectra of the of excipient employed in the formulation with the spectra of pure drug.

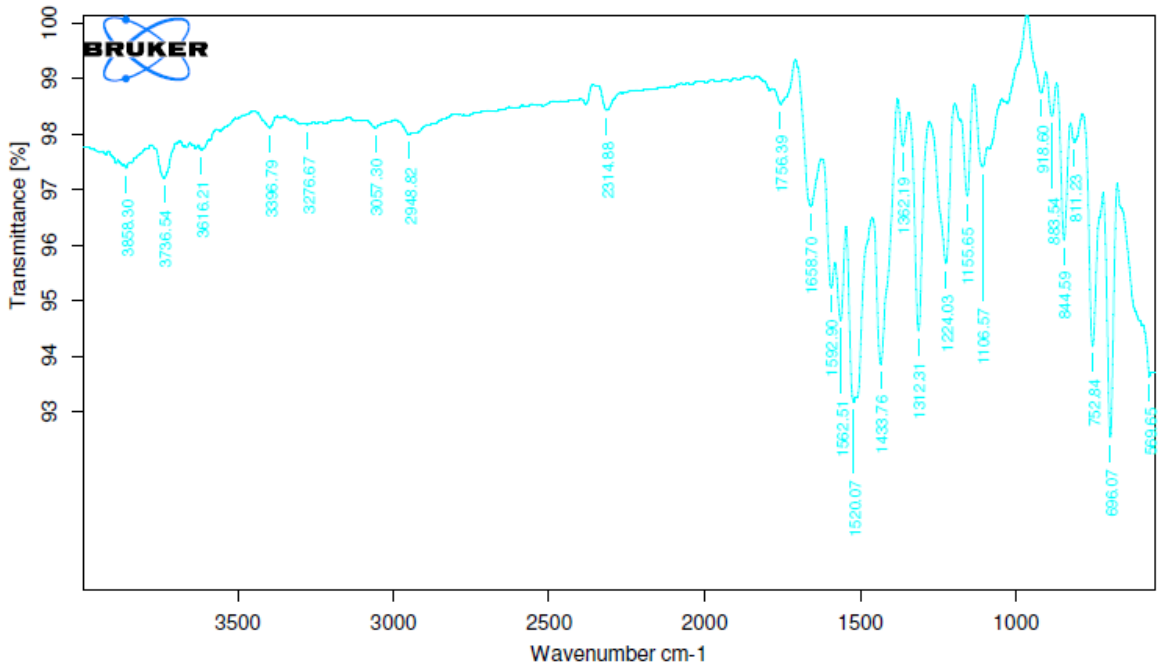


Figure 3: FT IR spectrum of pure Ambrisentan

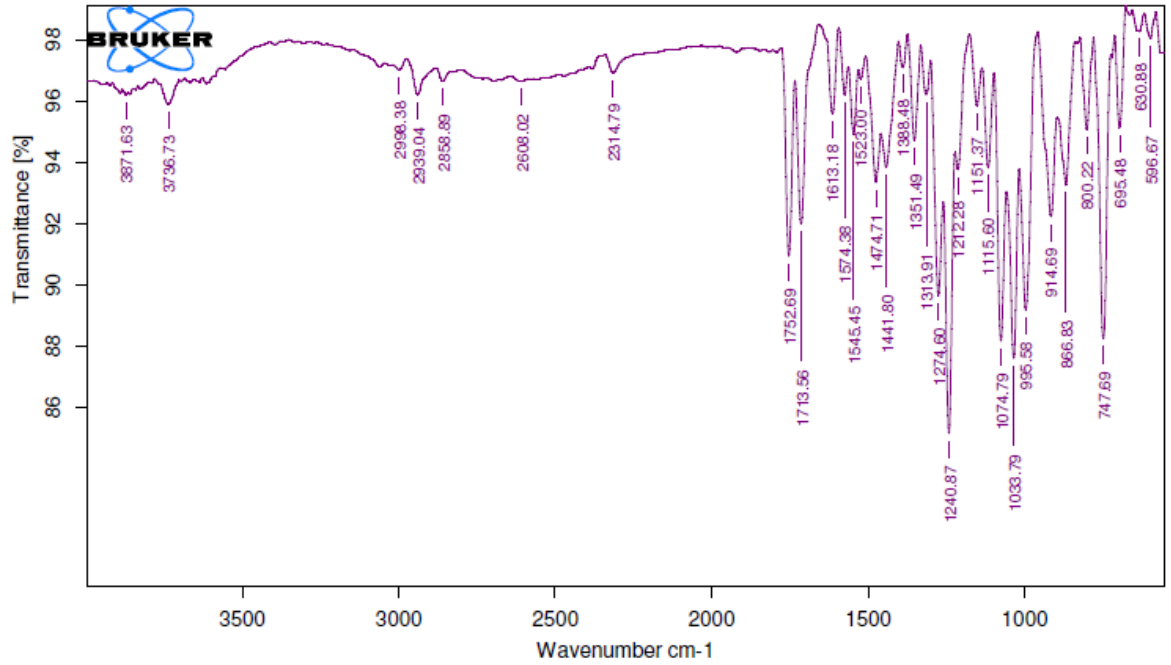


Figure 4: FT IR spectrum of Optimized Ambrisentan solid dispersion

FT-IR studies of pure drug Ambrisentan and optimized solid dispersions are shown in figures 3.4 respectively , it revealed that there was no interaction between the optimized preparation (Ambrisentan: Excipient) and the pure drug (Ambrisentan).

Table 2: Results of Drug content and entrapment efficacy of Ambrisentan solid dispersions:

Formulation code	Drug content	Entrapment efficiency
FF1	88.34	79.83
FF2	86.52	87.21
FF3	81.69	80.62
FF4	71.83	64.82
FF5	75.38	68.97
FF6	79.63	71.35
FF7	83.91	84.16
FF8	80.63	76.82
FF9	87.81	72.39
FF10	93.18	86.28
FF11	95.72	91.79
FF12	97.96	95.64

Results of percentage drug content and entrapment efficiency of all the solid dispersion are shown table 2.The percent drug content values of all the formulations (FF1-FF12) were found to be in the range of 71.83 – 97.96%.Entrapment efficiency of all the solid dispersions were found to be in the range of 64.82- 95.64%. Among all the formulations FF12 formulation showed highest drug content and entrapment efficiency

In-vitro drug release studies of solid dispersions:

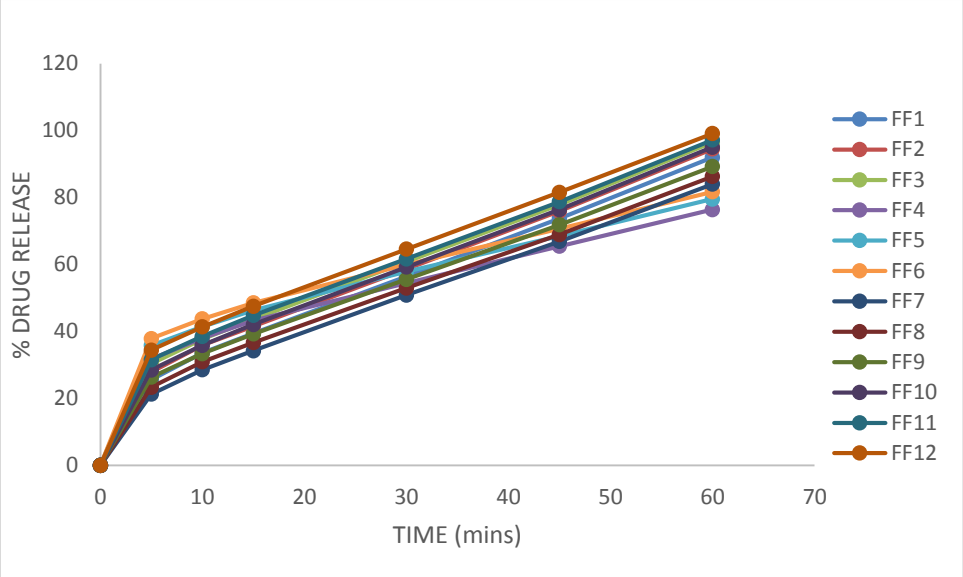


Figure 3: in vitro drug release studies of Ambrisentan solid dispersions by physical (FE1-FF12)

Fusion Method:

In-vitro drug release studies for formulations (FF1-FF12) are shown in figure 3. *In-vitro* release of the drug (Ambrisentan) solid dispersions with PEG 4000 in different ratios was observed, with the formulation FF1 releasing 92.02 %, the formulation FF2 releasing 94.52 %, and the formulation FF3 releasing 96.48 % at the end of 60 minutes. *In-vitro* release of the drug (Ambrisentan) solid dispersions with Mannitol in different ratios was observed, with the formulation FF4 releasing 76.42 %, the formulation FF5 releasing 79.58 %, and the formulation FF6 releasing 81.72 % at the end of 60 minutes.

In-vitro release of the drug (Ambrisentan) solid dispersions with PVP K30 in different ratios was observed, with the formulation FF7 releasing 84.01 %, the formulation FF8 releasing 86.31 %, and the formulation FF9 releasing 89.27 % at the end of 60 minutes.

In-vitro release of the drug (Ambrisentan) solid dispersions with PEG 6000 in different ratios was observed, with the formulation FF10 releasing 95.09 %, the formulation FF11 releasing 97.22 %, and the formulation FF12 releasing 99.12 % at the end of 60 minutes, hence all the formulations FF12 was best formulation due to highest drug release among all the formulations.

CONCLUSION

The main objective present research is formulation and evaluation of Ambrisentan solid dispersion for increasing solubility and dissolution of the drug. Ambrisentan solid dispersions were prepared by fusion method using PEG 4000, Mannitol, PVPK 30 and PEG 6000.Prepared solid dispersions were evaluated for drug and excipient compatibility studies by FTIR, drug content, entrapment efficiency and i*nvitro* drug release studies. FTIR studies revealed that there were no interaction between drug and optimized formulation. Drug content and entrapment efficiency within in the limits satisfactory. *In vitro* release studies revealed that , among all the formulation FF12 solid dispersion containing PEG 6000 in a 1:3 ratio showed 99.12 % at the end of 60 minutes, hence this formulation is optimized formulation.

Acknowledgements

Not applicable

Conflicts of interests:

The authors declare that there are no conflicts of interest regarding publication of this manuscript.

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