International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Issue 1, 2022

Original Article

ENHANCEMENT OF SOLUBILITY AND BIOAVAILABILITY OF BCS CLASS-II AMBRISENTAN: *IN VITRO, IN VIVO AND EX VIVO* ANALYSIS

RAHUL RADKE*, NEETESH K. JAIN

Faculty of Pharmacy, Oriental University, Indore, M. P. India
***Email: rahul.radke@rediffmail.com**

Received: 06 Oct 2021, Revised and Accepted: 17 Nov 2021

ABSTRACT

Objective: The aim of this investigation was to enhance the solubility and bioavailability of the BCS class II poorly water-soluble drug ambrisentan by solid dispersion (SD) techniques using Gelucire 50/13 as a hydrophilic carrier.

Methods: Solid dispersion of ambrisentan was prepared by kneading method using different dug: carrier ratios. Prepared SD was characterized for solubility, drug content, percentage yield, *in vitro* dissolution, *ex vivo* permeation and bioavailability. Solid-state characterization was performed by differential scanning calorimetry (DSC), X-ray diffraction (XRD) and scanning electron microscopy (SEM).

Results: All the SDs formulations showed increase in drug solubility and dissolution when compared with its pure form. Aqueous solubility of the drug was found to be increased 8.23 fold in SD. DSC study showed that endothermic peak of the drug was disappeared in spectra of SD, confirming its amorphous conversion, XRD study revealed the reduction to almost absence of specific high-intensity peaks of drug which confirmed the reduction of crysatallinity of ambrisentan in SD. SEM of optimized SD formulation demonstrates the complete encapsulation and solubilization drug. *In vitro* dissolution study showed that optimized SD formulation (ASD4) gives the faster drug release of 101.5% in 60 min, as compare to its pure form and other SD formulations.

Conclusion: Solid dispersion ASD4 prepared with 1:4 drug to carrier ratio showed the highest drug solubility and *in vitro* dissolution. The *ex vivo and in vivo* studies performed on optimized formulation ASD4 showed enhancement in drug permeability and bioavailability in Gelucire 50/13 based SD formulation.

Keywords: Ambrisentan, Gelucire 50/13, Bioavailability, Solid dispersion, Kneading technique etc

DOI: https://dx.doi.org/10.22159/ijap.2022v14i1.43347. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Absorption of a drug from oral route majorly depends up on the dissolution of the drug from the formulations into GI fluids followed by its permeation. Bioavailability of drugs from oral route depends on their solubility as well as permeability. In order to achieved desired pharmacological response, the solubility of the drug is one of the important parameter [1, 2]. Low bioavailability of the drugs are generally associated with poor aqueous solubility which is turn required the higher dose and repeated administration of drug. Bioavailability of poorly water-soluble drugs can be increased by increasing its aqueous solubility and dissolution rate [3]. Solid dispersion is one of the widely utilized technology in the improvement of the solubility, dissolution and bioavailability of poorly water-soluble drugs [4, 5].

Solid dispersion prepared with novel surfactants and self-emulsifiers-based carrier is found to advantageous in the improvement drug solubility and dissolution rate. Gelucire 50/13 is a mixtures of monoesters, diesters, and triesters of glycerol and monoesters and diesters of polyethylene glycols was a specially designed polymer for the enhancement of poorly soluble drugs [6]. SD prepared with Gelucire 50/13 is supposed to reduce the crystallinity and convert the drug in to amorphous form so as to enhance the bioavailability of low water-soluble drugs [7].

Ambrisentan is endothelin receptor antagonist mostly used in the treatment of pulmonary arterial hypertension. Ambrisentan is BCS class II drug, which suggest that it has low aqueous solubility and high membrane permeability. Ambrisentan is practically insoluble in water and in aqueous solutions at low pH [8, 9]. Because of its very poor aqueous solubility, the absorption and bioavailability of ambrisentan is incomplete. There is very few literature available regarding solubility and bioavailability improvement of ambrisentan. So in order to improve the aqueous solubility and bioavailability of the drug, an attempt was made in this present study to increase solubility, dissolution rate and bioavailability of ambrisentan by developing its solid dispersion using carrier Gelucire 50/13.

MATERIALS AND METHODS

Marerials

Ambrisentan was obtained as a gift sample from Cadila Pharmaceuticals, Mumbai India. Gelucire 50/13 was supplied by Gattefosse India as a gift sample. All other chemical and reagents used were of analytical grade.

Method

Saturation solubility study of drug

Saturation solubility study of selected drug was determined in distilled water, acetate buffer pH 1.2, phosphate buffer pH 6.8 and phosphate buffer pH 7.4. Extra amount of drug was added to 10 ml study fluid in a glass vial. Samples were shaken on rotary shaker at constant speed at 25 °C±2 °C for 48 h. The resultant saturated solutions was then filtered using whatman filter paper no 1. Filtrates sample ware then estimated spectrophotometrically after suitable dilution [10].

Phase solubility study

In order to predict the effect carrier on the solubilization of drug, the phase solubility study of drug was carried out. Excess amount of drug were added to 10 ml glass vial containing 0.25%, 0.50%, 0.75%, 1% and 2% aqueous solution of carriers and shaken on rotary shaker for 48 h at a controlled temperature at 25 °C±2 °C. The solutions were filtered using no 1 whatman filter paper. Filtrate were analyzed by UV-spectrophotometer in order to determine the concentration of the dissolved drug [11].

Preparation of physical mixture

A physical mixture of Ambrisentan with Gelucire 50/13 in different ratio (1:1, 1:2, 1:3, 1:4, 1:5) and denoted as APM 1 to APM 5

respectively was prepared by mixing of drug and carrier using mortar and pestle. This mixture was then passed through sieve no 40 and store in desiccators. The composition was shown in table 1.

Preparation of solid dispersion

Solid dispersion of ambrisentan with Gelucire 50/13 in different weight ratio (1:1, 1:2, 1:3, 1:4, 1:5 and denoted as ASD 1 to ASD 5 respectively, was prepared by kneading method. A mixture of drug and carrier was placed in a mortar and was kneaded thoroughly with water and methanol (1:1) for 20 min. The kneaded mixtures were then dried in an oven at 40 °C until it reached the uniform weight and then pulverized and screened through 80-mesh and stored in desiccator for further study [12, 13]. The composition for solid dispersion is shown in table 1.

Table 1: Composition of Ambrisentan solid dispersion

Formulation	Formulation code	Ambrisentan: gelucire 50/13
Physical Mixture	APM1	1:1
	APM2	1:2
	APM3	1:3
	APM4	1:4
	APM5	1:5
Solid Dispersion	ASD1	1:1
	ASD2	1:2
	ASD3	1:3
	ASD4	1:4
	ASD5	1:5

Characterization of solid dispersion

Determination of saturation solubility of PMs and SDs

The saturation solubility of physical mixture and solid dispersion was determined in distilled water using shake flask method. Excess quantities of sample were added in 25 ml of distilled water and phosphate buffer in conical flask and shaken for 24 h at room temperature on rotary flask shaker. After shaking resultant samples containing undissolved solid suspended in the test medium were centrifuged at 10,000 rpm for 5 min, the clear supernatants obtained were filtered through whatman filter paper. Filtered sample ware analyzed by spectrophotometer at 263.5 nm after dilution [14].

Determination of percent yield of solid dispersion

The percent yield of ambrisentan solid dispersions was determined by using the following formula:

$$\% \text{ Yield} = \frac{\text{Weight of Prepared Solid Dispersion}}{\text{Weight of drug + carrier}} \times 100$$

Determination of drug content

Ambrisentan solid dispersion equivalent to 10 mg of drug was accurately weighed and dissolved in methanol (100 ml). The solution was filtered after vigorous shaken. The drug content was analyzed at 263.5 nm against blank by UV spectrometer after appropriate dilution [15].

Fourier transform infra-red spectroscopy

Compatibility studies of ambrisentan with carrier were performed using FTIR spectroscopy (Shimadzu FTIR-8700). Spectrum of pure drug, physical mixture and solid dispersion was recorded over the frequency range of 400 to 2000 cm-1 at 4 cm resolution.

Differential scanning calorimetry

The thermal analysis was carried out using Shimadzu Thermal analyzer DT 40 (Japan). The samples were placed in sealed aluminum pans and heated at a rate of 10 °C per min in the temperature range of 20-300 °C under a nitrogen flow rate of 40 ml/min [16].

Powder X-ray diffraction

X-ray powder diffraction patterns of drug, carrier and solid dispersion was recorded on an X-ray powder diffraction system (Rigaku, Mini Flex 600). The scanning was done over range of 5° to 60°. The position and intensities of diffraction peaks were considered for the comparison of crystallinity [17].

Scanning electron microscope analysis (SEM)

The surface morphology of pure ambrisentan and selected solid dispersion was studied using SEM. (ZEISS, EVO 18, Germany). The samples were mounted on a sample stub with double-sided adhesive tape and coated under vacuum with gold ion using sputtering device prior to study. SEM image at different magnifications were recorded to study the morphological and surface characteristics of the solid dispersions.

***In vitro* dissolution study**

In vitro dissolution study of pure ambrisentan and solid dispersions were determined using USP dissolution test apparatus II (Paddle type) (Esico International, Mumbai). Accurately weighted preparation equivalent to 10 mg of ambrisentan ware added to 900 ml of phosphate buffer pH 6.8 used as a medium of dissolution, which was maintained at 37±0.5 °C and rotation speed was selected at 50 rpm. 5 ml samples were withdrawn at time interval of 10, 20, 30, 40, 50, 60 min and the same volume was replaced with fresh media in order to maintain the sink condition. After suitable dilution, collected samples were analyzed at 263.5 nm using UV-visible spectrophotometer against the blank [18, 19].

***In vivo*/Bioavailability study**

Based on the solubility study and *in vitro* dissolution profile, an optimized ambrisentan SD formulation (ASD4) was selected for comparison of *in vivo* performance against plain ambrisentan. The pharmacokinetic study was carried out on wister rats as per the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), after approval of study, registration No. IAEC/2019-20/RP-09 dated 03/03/2021 at Oriental College of Pharmacy, Indore, (M. P.), India.

Study design

Healthy male wistar rats obtained from the animal house of Oriental college of Pharmacy, Indore, of weight (250–300 g) were used for the study. The rats were randomly divided into 2 groups containing 6 rats in each group. All rats were fasted overnight with free access to water prior to the experiment. The oral bioavailability of ambrisentan was determined at a dose of 10 mg/kg of body weight. The drug sample was prepared by suspending the drug in 1 ml of 1% w/v aqueous sodium carboxyl methylcellulose and further diluted with water to the concentration of 1 mg/ml. Based on the body weight, aqueous solution of pure ambrisentan was given orally to one group of animal, which was treated as control using oral feeding sonde. The second group of animal was administered with optimized formulation (ASD4) at the same dose and treated as test. 0.5 ml of blood sample were withdrawn from postorbital vein sinus into micro centrifuge tube treated with EDTA at time interval of 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 hr. The plasma was separated by centrifugation at 3000 rpm for 10 min and was treated with small quantity of acetonitrile and repeated for centrifugation for 10 min at 3000 rpm. The 50µl of plasma was mixed with 0.5 ml of mobile phase. The 20 µl of sample was injected with flow rate of 0.7 ml/min to C 18 column and analyzed at the wavelength of 263.5 nm. Non-compartment pharmacokinetic parameters such as T_{max}, C_{max} and AUC were estimated by PK Solver computer program. Results of *in vivo* experiments are reported as mean±SD Statistical tests of significance were performed using Graph Pad Prism 5.0 software. The variables were compared with a one-way ANOVA. P-value less than 0.05 were considered significant [20, 21].

***Ex vivo* permeation study**

Ex vivo permeation study was performed on pure drug and optimized SD formulation (ASD4) using an everted chicken intestine model. The study was performed using modified apparatus and USP type II dissolution test apparatus according to the method reported by Tekade *et al.* [22]. A freshly procured chicken intestine from local slaughterhouse was used for the study. The selected intestinal

portion was rinsed with phosphate buffer solution (pH 6.8, Krebs-Ringer solution). A 6 cm segment of the intestine was taken and everted using glass rod and then clamped to arm B of modified apparatus. The total volume of absorption compartment was 55 ml. The apparatus was then placed in dissolution apparatus containing 1000 ml of phosphate buffer solution (pH 6.8) as a dissolution medium at 37±0.5 °C. The pure drug and optimized solid dispersion was transferred to the dissolution medium and apparatus was rotated at a speed of 75 rpm. The amount of drug diffused from dissolution medium (mucosal side) to the absorption compartment side was measured by withdrawing 5 ml sample from absorption compartment (arm B) at 5, 10, 20, 30, 40, 50 and 60 min and analyzed spectrophotometrically at 263.5 nm. The experiment was carried out in triplicate (n = 3)

RESULTS AND DISCUSSION

Saturation solubility study of drug

Saturation solubility study indicates that ambrisentan was poorly soluble in water, showing 7.347±0.003 µg/ml of solubility in distilled water. Ambrisentan shown pH dependent solubility, solubility of ambrisentan increases as the pH of solvent increases. Solubility of drug in acetate buffer pH1.2, phosphate buffer pH 6.8 and pH 7.4 was found to be 5.173±0.006 µg/ml, 28.507±0.012 µg/ml and 35.391±0.004 µg/ml, respectively. Solubility profile of the drug is shown in fig. 1.

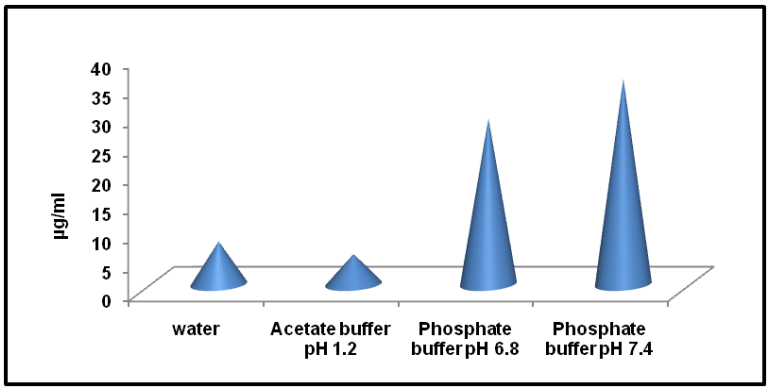

Fig. 1: Solubility profile of ambrisentan in different solvent

Phase solubility study

In order to determine the possible solubilizing effect of Gelucire 50/13 on drug solubility, phase solubility study of ambrisentan was studied using an increasing concentration of carrier. A linear increase in the solubility of drug was seen with an increasing concentration of hydrophilic carriers in water. The solubility of ambrisentan at 0.25, 0.5, 0.75, 1 and 2% aqueous solution of Gelucire 50/13 was found to be 16.89±2.13, 21.30±1.26, 27.18±0.94, 32.32±3.21, 48.49±1.86 µg/ml, respectively. Increased solubility may be due solubilization effect of Gelucire 50/13 that increased the wettability of the drug. At 2% w/v concentration of carrier, the aqueous solubility of ambrisentan was increased by 6.6 fold, indicating good affinity between drug and polymer. The phase-solubility diagram investigated for Gelucire 50/13 in distilled water was linear giving A_L type solubility curve. The apparent stability constant (Kc) calculated from the linear plot of the phase solubility diagram, was found to be 143.60 M^{-1}. The Kc values were falls in the ideal range of (50 to 2000 M^{-1}) indicate stronger interactions between the drug and carrier [23]. The phase solubility curve of ambrisentan is shown in fig. 2.

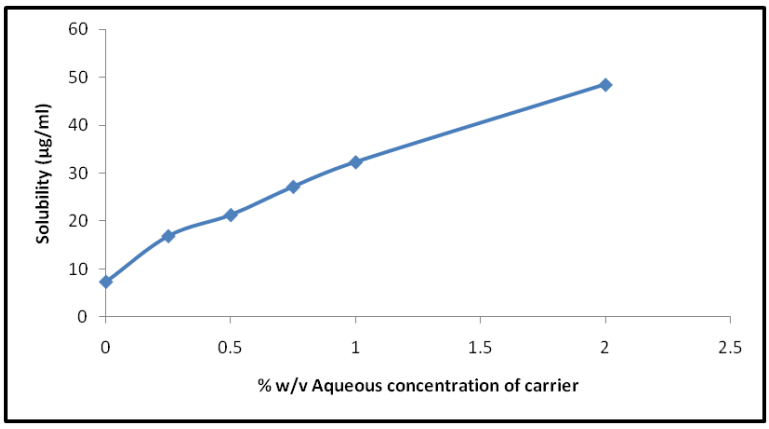

Fig. 2: Phase solubility study of ambrisentan with gelucire 50/13

Saturation solubility study of solid dispersion and physical mixture

The solubility of ambrisentan physical mixture and solid dispersion was determined in distilled water and phosphate buffer 6.8. Prepared physical mixture showed improved solubility as compare to pure drug in both solvent. Solubility study of solid dispersion showed multi-fold increase in solubility of the drug when compare with pure and physical mixture of drug. It was observed that solubility of the drug increases with increase in carrier concentration up to 1:4 ratio, but after that no significant increase in drug solubility was observed by increasing the carrier ratio. Solid dispersion ASD4 (1:4 ratio) showed maximum solubility of the drug, giving 8.23 fold increase in water solubility of ambrisentan. Higher

value of solubility was shown by all SD formulations, this may be due to conversion of drug in amorphous form or by the increased wet ability of drug by hydrophilic carrier [24]. All SD formulations showed higher solubility of the drug in phosphate buffer solution than distilled water. The solubility data of all the PMs and SDs formulations are presented in table 2.

Table 2: Solubility analysis of ambrisentan-gelucire 50/13 physical mixture and solid dispersion

Formulation code	Distilled water (µg/ml)	Phosphate buffer pH 6.8 (µg/ml)	Formulation code	Distilled water (µg/ml)	Phosphate buffer pH 6.8 (µg/ml)
APM1	19.41±1.30	91.43±1.16	ASD1	32.14±0.94	153.93±0.66
APM2	22.12±1.41	108.86±0.46	ASD2	40.25±0.47	173.89±0.41
APM3	25.61±0.84	124.42±0.62	ASD3	48.74±0.81	210.95±0.56
APM4	28.58±1.14	140.32±1.21	ASD4	60.46±1.63	250.86±1.24
APM5	31.41±0.81	154.52±1.63	ASD5	62.24±1.24	258.64±0.12

Data given in mean±SD, n=3

Fourier transform infrared spectroscopy

IR spectra of ambrisentan and its SD with Gelucire 50/13 are presented in fig. 2. Sharp characteristic peaks showed in pure ambrisentan ware also appears in the spectra of SD, indicating no interaction between the drug and the carrier (Gelucire 50/13). FTIR spectra of pure ambrisentan, Gelucire 50/13 and SD are shown in fig. 3.

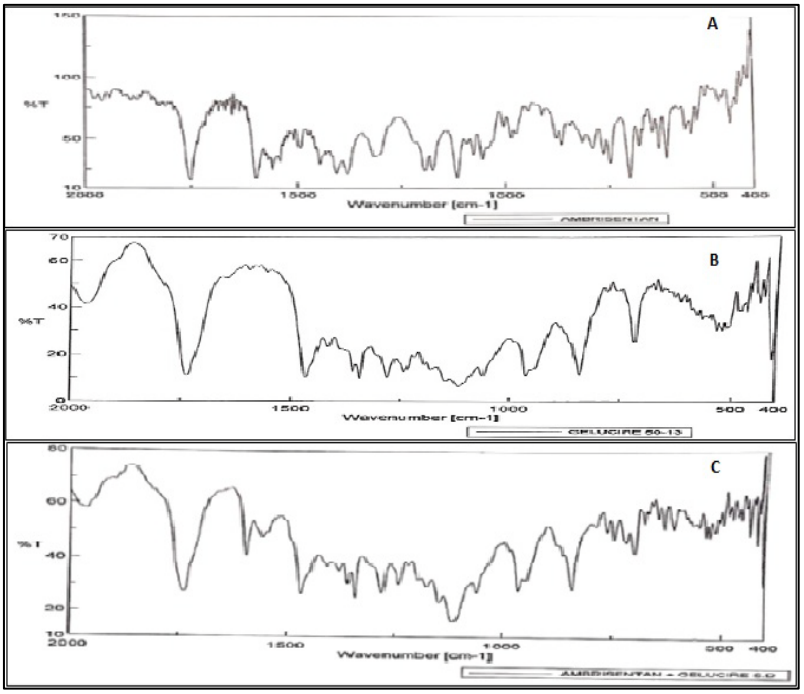

Fig. 3: FTIR Spectra (A) Ambrisentan (B) Gelucire 50/13 (C) Ambrientan Gelucire SD

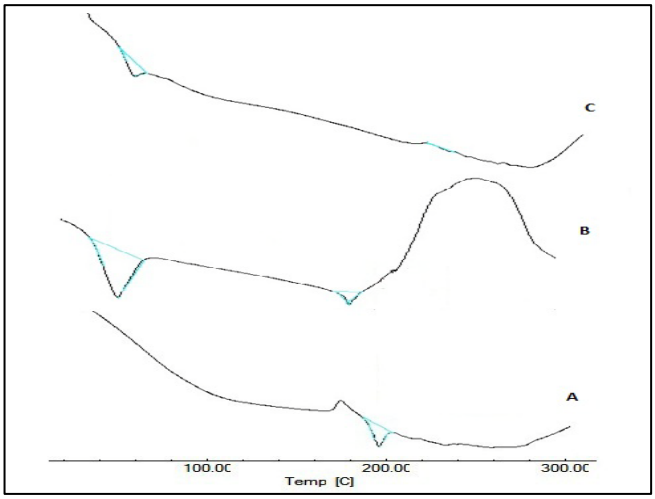

Fig. 4: DSC thermogram of (A) Pure ambrisentan, (B) Gelucire 50/13 and (C) ambrisenatn Gelucire SD

Differential scanning calorimetry

The DSC thermogram of pure ambrisentan, Gelucire 50/13 and SD formulation (ASD4) were presented in fig. 4. Pure ambrisentan showed a single sharp endothermic peak at 194.16 °C with an enthalpy of fusion (ΔH)-10.20 J/g corresponding to its melting point, indicates its crystalline nature. Gelucire 50/13 had showed main sharp endothermic peak at 56.78 °C with enthalpy of fusion (ΔH)-72.76 J/g and another short endothermic peak at 185.78 °C. The optimized solid dispersion formulation (ASD4) showed only single less intense endothermic peak at 51.37 °C, corresponding to peak of Gelucire 50/13 and does not showed an characteristic endothermic peak of ambrisentan, which indicates the complete conversion of crystalline form of drug in to amorphous form, [25] which results in enhanced solubility and dissolution rate of drug.

Powder X-ray diffraction

XRD patterns of ambrisentan, Gelucire 50/13 and ambrisentan: Gelucire 50/13 SD are shown in fig. 5. The x-ray diffractograms of pure ambrisentan showed characteristic sharp high-intensity diffraction peaks at 2θ values of 12.26°, 14.09°, 18.21°, 20.48°, 22.77° and 24.16° indicates the highly crystalline nature of drug. XRD pattern of Gelucire 50/13 showed sharp high-intensity peak at a diffraction angle at 2θ value of 19.30° and 23.35°. XRD diffractograms of solid dispersion (ASD4) showed only two prominent sharp peak at 2θ values of 19.02° and 23.11°, corresponding to peak of Gelucire 50/13, with marked reduction in intensity or almost absence of the numerous distinctive peaks of ambrisentan. This indicates complete conversion of crystalline drug in to amorphous form in solid dispersion [26, 27].

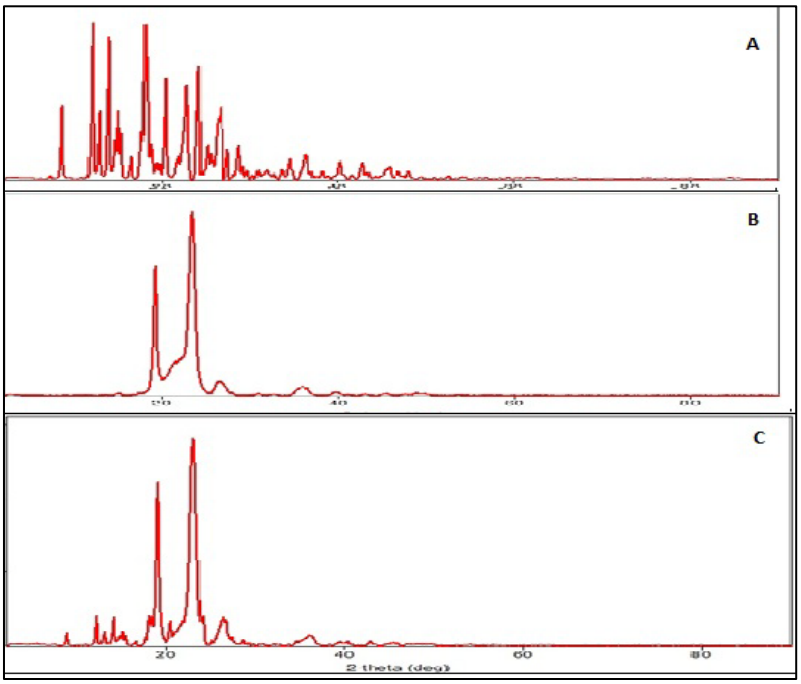

Fig. 5: XRD spectra (A) Ambrisentan, (B) Gelucire 50/13, (C) Ambrisentan Gelucire SD

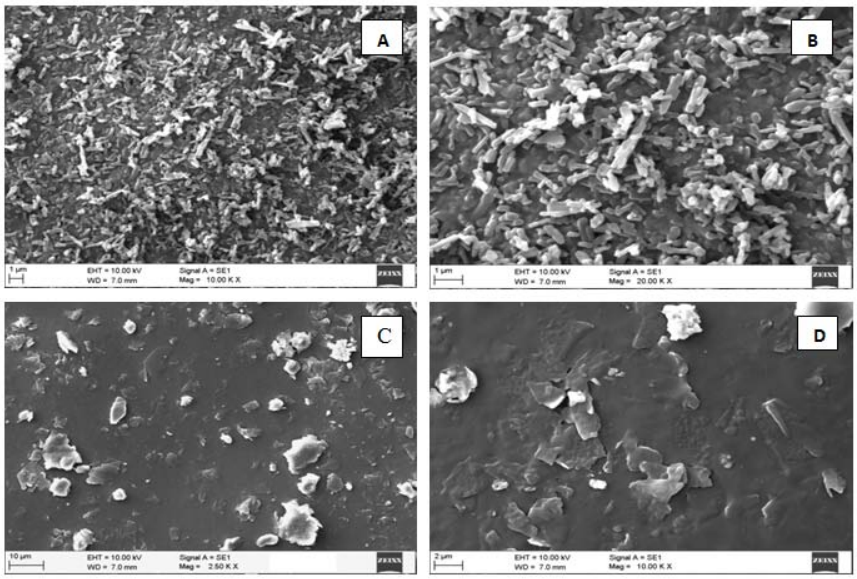

Fig. 6: SEM image of pure ambrisentan (A and B) and selected ambrisentan gelucire SD (ASD4) (C and D) at different magnification

Scanning electron microscopy (SEM)

Scanning electron micrograph of pure ambrisentan shows rod shaped crystals indicating the crystalline nature of the drug (fig. 6A and 6B). The SEM images of selected solid dispersions (ASD4) clearly showed disappearance of rod shape crystals and formation of homogeneous smooth surface indicating complete entrapment, encapsulation or solubilization of drug on a molecular level into the polymeric matrix, which suggest the conversion of drug in amorphous form (fig. 6C and 6D) [25]. This reduced crystallinity of ambrisentan in solid dispersion was further supported by the results of XRD and DSC studies.

***In vitro* dissolution study**

All the SD formulations and the pure ambrisentan were subjected to a dissolution study. Phosphate buffers pH 6.8 was used as the dissolution media to performed the study. Percent drug release of pure ambrisentan showed 41.15 % in 60 min, indicating its poor solubility and dissolution rate. SD formulation ASD1 to ASD5 prepared with Gelucire 50/13 showed an improved dissolution rate of 91.18%, 94.95%, 97.58%. 101.5% and 98.88% drug release respectively in 60 min. It was observed that rate of drug dissolution increases with an increased in carrier concentration.

All SD formulations showed improved and faster drug dissolution rate as compare to pure drug; this may be due to combined effect of dispersibility, improved wettability and reduction of particle size in kneading technique [28]. All SD formulations showed more than 50% drug release in 10 min showing its significant improvement in drug dissolution. Among all SD formulations, ASD4 containing drug and Gelucire 50/13 in 1:4 drug to polymer ratio showed highest drug release of 101.5%. The drug release profile of all SD formulations and pure ambrisentan are shown in fig. 7.

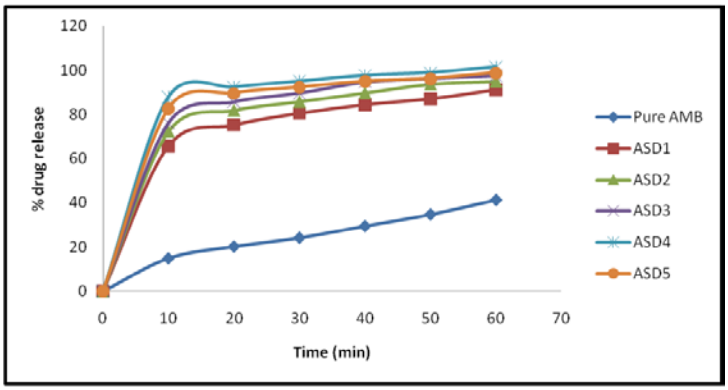

Fig. 7: *In vitro* dissolution profile of pure ambrisentan and ambrisentan gelucire 50/13 SDs in 6.8 pH phosphate buffer

Table 3: % Drug content and practical yield of SD formulations (ASD1 to ASD5)

S. No.	Formulation code	% Practical yield	% Drug content*
1	ASD1	80.43	98.15±1.39
2	ASD2	81.27	97.26±0.38
3	ASD3	81.61	97.56±0.26
4	ASD4	83.58	98.62±1.18
5	ASD5	83.54	98.12±0.24

*Data given in mean±SD, n=3

Drug content and percentage practical yield

Drug content for solid dispersion formulations ASD1 to ASD5 was found to be in the range of 97.26% to 98.62 %. The percentage drug content for all SDs formulation was found within pharmacopoeial limit which indicate uniform distribution of drug in solid dispersion.

The percentage practical yields calculated for all SDs formulation was shown in table 3. SDs formulation prepared with Gelucire 50/13 gives low yield, this may be due to sticky nature of Gelucire 50/13 which get adhere during formulation process.

***In vivo* pharmacokinetic study**

Based on the solubility study and *in vitro* dissolution profile of drug, an optimized formulation, (ASD4) containing Ambrisentan: Gelucire 50/13 (1:4 ratio) was selected for comparison of *in vivo* performance against plain ambrisentan. The non-compartmental pharmacokinetic parameters of drug were determined after oral administration of pure ambrisentan (Control) and solid dispersion of ambrisentan (ASD4) (Test) to wistar rats by HPLC method. The peak plasma concentration (C_{max}) of pure ambrisentan and its SD formulation was found as 4.12±0.021 µg/ml in 2 h, 8.04±0.100 µg/ml in 1.5 h respectively. From the peak plasma concentration data it was observed that absorption of drug in plasma from SD formulation is more and rapid as compare to pure drug, which suggest the reduction in crystallinity and amorphous conversion of ambrisentan in solid dispersion. The AUC_0-t for pure and SD formulation of drug (ASD4) was found to be 17.77±0.148 and 45.72±0.397 µg/mlh, while AUC_0-∞ was found to be 20.37±0.420 and 62.94±1.75µg/mlh, respectively. The pharmacokinetic parameter indicated the maximum plasma drug concentration and area under the curve was achieved by ambrisentan SD formulation. Pharmacokinetic parameter clearly suggested the enhanced bioavailability of amabrisentan in solid dispersion (ASD4) as compared to pure form. Increased wettability of drug in the presence of hydrophilic carrier Gelucire 50/13 and SD preparation by kneading method, that causes reduction in particle size, can be a another reason in bioavailability enhancement of drug [20, 29]. The obtained pharmacokinetic values were analyzed using one-way ANOVA and the values were found to be significant ($p<0.05$). Different pharmacokinetic parameters of pure ambrisentan and ambrisentan SD (ASD4) is shown in table 4 and plasma concentration time profile was shown in fig. 8.

Ex vivo permeation study

The ex vivo drug absorption study was perform in order to predict the dissolution and absorption rate of pure ambrisentan and its SD formulation (ASD4) through everted chicken intestine. Pure ambrisentan showed 32.52 % drug absorption, while the solid dispersion formulation (ASD4) showed 71.43% of drug absorption in 60 min. The rate of drug absorption from solid dispersion was

significantly higher than the pure drug. This improvement in absorption rate of drug was might be due to the presence of Gelucire 50/13, which increased wettability drug, resulting faster dissolution [30]. The result of ex vivo permeation of drug is shown in fig. 9.

Table 4: Pharmacokinetic parameters of pure ambrisentan and solid dispersion of ambrisentan after oral dose in wistar rat

Pharmacokinetic parameter	Pure ambrisentan	Ambrisentan solid dispersion (ASD4)
T_{max} (h)	2±0.31	1.5±0.54
C_{max} (µg/ml)	4.12±0.021	8.04±0.100
$t_{1/2}$ (h)	3.35±0.112	6.37±0.335
AUC $_{0\text{-}t}$ (µg/mlh)	17.77±0.148	45.72±0.397
AUC $_{0\text{-}inf.}$ (µg/mlh)	20.37±0.420	62.94±1.75
MRT (h)	6.28±0.191	9.40±0.387

mean±SD (n=6)

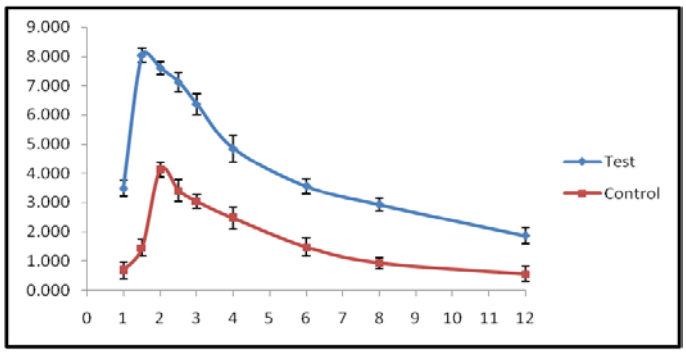

Fig. 8: Plasma concentration profile of pure ambrisentan and SD of ambrisentan (ASD4) in rats (Data are mean±SD (n=6))

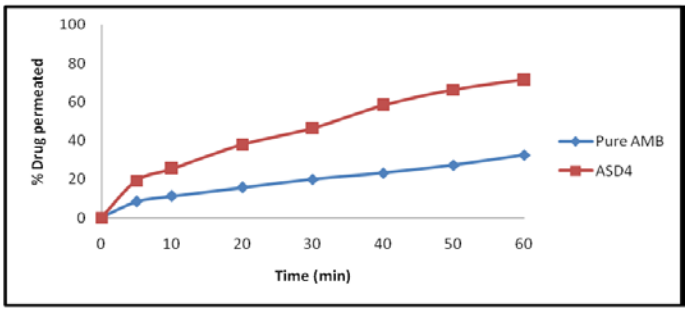

Fig. 9: Ex vivo permeation study of pure ambrisentan and its solid dispersion (ASD4)

CONCLUSION

In the present investigation, an attempt was made to develop Gelucire 50/13 based solid dispersion of poorly water-soluble ambrisentan using kneading method. The solubility and dissolution study of ambrisentan was increased multifold in solid dispersion using carrier Gelucire 50/13. DSC, XRD and SEM study confirmed the reduction in crystallinity and amorrphus conversion of drug in SD. *In vivo* and *Ex vivo* study suggests the rapid absorption and appearance of drug in plasma from SD formulation than its pure form. From this study, it was concluded that the solid dispersion prepared using Gelucire 50/13 is a good approach of solubility and bioavailability enhancement of poorly soluble drugs such as ambrisentan.

ACKNOWLEDGMENT

Authors are thankful to Cadila Pharmaceuticals for providing a gift sample of Ambrisentan to conduct this research. Also, authors are thankful to Gattefosse, India for supplying Gelucire 50/13 for completion of this research.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

1. Naseem A, Olliff CJ, Martini LG, Lloyd AW. Effects of plasma irradiation on the wettability and dissolution of compacts of griseofulvin. Int J Pharm. 2004;269(2):443-50. doi: 10.1016/j.ijpharm.2003.09.029, PMID 14706255.
2. Singh G, Kaur L, Gupta GD, Sharma S. Enhancement of the solubility of poorly water soluble drugs through solid dispersion: a comprehensive review. Pharm Sci. 2017;79(5):674-87. doi: 10.4172/pharmaceutical-sciences.1000279.
3. Aulton ME. Pharmaceutics: the science of dosage form design. 1st ed. New York: Churchill Livingstone; 1996.
4. Vo CL, Park C, Lee BJ. Current trends and future perspectives of solid dispersions containing poorly water-soluble drugs. Eur J Pharm Biopharm. 2013;85(3 Pt B):799-813. doi: 10.1016/j.ejpb.2013.09.007, PMID 24056053.
5. Sareen S, Mathew G, Joseph L. Improvement in solubility of poor water-soluble drugs by solid dispersion. Int J Pharm Investig. 2012;2(1):12-7. doi: 10.4103/2230-973X.96921, PMID 23071955.
6. Raymond CR, Paul JS, Marian EQ. Handbook of pharmaceutical excipients. 6th ed. Pharmaceutical Press; 2020. p. 557-60.

7. Yu DG, Yang JM, Branford White CB, Lu P, Zhang L, Zhu LM. Third generation solid dispersions of ferulic acid in electrospun composite nanofibers. Int J Pharm. 2010;400(1-2):158-64. doi: 10.1016/j.ijpharm.2010.08.010, PMID 20713138.
8. European medicine agency. Evaluation of medicines for human use, Assessment report for volibris Doc. Ref.: EMEA/123999/2008; 2008. p. 1-44.
9. Barst RJ. A review of pulmonary arterial hypertension: role of ambrisentan. Vasc Health Risk Manag. 2007;3(1):11-22. PMID 17583171.
10. Higuchi T, Connors KA. Phase-solubility techniques. Adv Anal Chem Instr. 1965;4:117-212.
11. Sharma A, Jain CP. Preparation and characterization of solid dispersions of carvedilol with PVP K30. Res Pharm Sci. 2010;5(1):49-56. PMID 21589768.
12. Modi A, Tayade P. Enhancement of dissolution profile by solid dispersion (Kneading) technique. AAPS PharmSciTech. 2006;7(3):68. doi: 10.1208/pt070368, PMID 17025249.
13. Iqbal A, Hossain S, Shamim A, Islam M, Siddique AT. Formulation, *in vitro* evaluation and characterization of atorvastatin solid dispersion. Trop J Pharm Res. 2020;19(6):1131-8. doi: 10.4314/tjpr.v19i6.2.
14. Sathali AAH, Jayalakshmi J. Enhancement of solubility and dissolution rate of olmesartan medoxomil by solid dispersion technique. J Curr Chem Pharm Sci 2013;3(2):123-34.
15. Yunoos M, Lavanya NSL, Sravani G, Madhuri Rao P, Krishna CH. Development of a validated UV spectrophotometric method for the estimation of ambrisentan in pure and marketed formulations. Sch Acad J Pharmacol. 2014;3(6):427-31.
16. Yang M, Wang P, Huang CY, Ku MS, Liu H, Gogos C. Solid dispersion of acetaminophen and poly (ethylene oxide) prepared by hot-melt mixing. Int J Pharm. 2010;395(1-2):53-61. doi: 10.1016/j.ijpharm.2010.04.033, PMID 20435110.
17. Adahalli SB, Talluri M. Formulation and evaluation of tablet prepared by coamorphous system containing anti-hypertensive and anti-hyperlipidemic drug. Int J Pharm Pharm Sci. 2016;8(9):182-93. doi: 10.22159/ijpps.2016.v8i9.12895.
18. Varma MM, SK RB. Formulation, physicochemical evaluation, and dissolution studies of carbamazepine solid dispersions. IJPSN 2012;5(3):1790-807. doi: 10.37285/ijpsn.2012.5.3.7.
19. Avachat A, Raut V. Solubility and dissolution enhancement of nebivolol hydrochloride using hydrophilic carriers. Asian J Pharm Sci. 2012;7(5):337-45.
20. Deshmane S, Deshmane S, Shelke S, Biyani K. Enhancement of solubility and bioavailability of ambrisentan by solid dispersion using daucus carota as a drug carrier: formulation, characterization, *in vitro*, and *in vivo* study. Drug Dev Ind Pharm. 2018;44(6):1001-11. doi: 10.1080/03639045.2018.1428339, PMID 29382236.
21. Srinivas I, Bhikshapathi DVRN. Preparation and *in vivo* evaluation of solid dispersions using repaglinide. Int J Pharm Sci Drug Res. 2018;10(5):362-71.
22. Rodde MS, Divase GT, Devkar TB, Tekade AR. Solubility and bioavailability enhancement of poorly aqueous soluble atorvastatin: *in vitro*, ex vivo, and *in vivo* studies. BioMed Res Int. 2014;2014:463895. doi: 10.1155/2014/463895, PMID 24995297.
23. Maulvi FA, Dalwadi SJ, Thakkar VT, Soni TG, Gohel MC, Gandhi TR. Improvement of dissolution rate of aceclofenac by solid dispersion technique. Powder Technol. 2011;207(1-3):47-54. doi: 10.1016/j.powtec.2010.10.009.
24. Daravath B, Tadikonda RR, Vemula SK. Formulation and pharmacokinetics of gelucire solid dispersions of flurbiprofen. Drug Dev Ind Pharm. 2015;41(8):1254-62. doi: 10.3109/03639045.2014.940963, PMID 25039470.
25. Mehanna MM, Motawaa AM, Samaha MW. In: sight into tadalafil-block copolymerbinary solid dispersion: mechanistic investigation of dissolution enhancement. Int J Pharm. 2010;402(1-2):78-88.
26. Murali Mohan Babu GV, Prasad ChD, Ramana Murthy KV. Evaluation of modified gum karaya as carrier for the dissolution enhancement of poorly water-soluble drug nimodipine. Int J Pharm. 2002;234(1-2):1-17. doi: 10.1016/s0378-5173(01)00925-5, PMID 11839433.
27. Deshkar S, Satpute A. Formulation and optimization of curcumin solid dispersion pellets for improved solubility. Int J Appl Pharm. 2020;12(2):36-46. doi: 10.22159/ijap.2020v12i2.34846.
28. Ghareeb MM, Abdulrasool AA, Hussein AA, Noordin MI. Kneading technique for preparation of solid binary dispersion of meloxicam with poloxamer 188. AAPS PharmSciTech. 2009;10(4):1206-15. doi: 10.1208/s12249-009-9316-0, PMID 19862626.
29. Liu C, Wu J, Shi B, Zhang Y, Gao T, Pei Y. Enhancing the bioavailability of cyclosporine a using solid dispersion containing polyoxyethylene (40) stearate. Drug Dev Ind Pharm. 2006;32(1):115-23. doi: 10.1080/03639040500388573, PMID 16455610.
30. Bhalekar MR, Upadhaya PG, Reddy S, Kshirsagar SJ, Madgulkar AR. Formulation and evaluation of acyclovir nanosuspension for enhancement of oral bioavailability. Asian J Pharm. 2014;8(2):110-8. doi: 10.4103/0973-8398.134942.