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Lipid Solid Dispersions for the Aqueous Solubility and Bioavailability Enhancement of Entacapone

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The main objective of the present study is to increase the aqueous solubility and *in vivo* bioavailability of entacapone by preparing the solid dispersion by spray drying. The prepared spray dried dispersions are duly characterized for drug content, particle size distribution, drug morphological conversion *in vitro* dissolution and *in vivo* bioavailability. The drug content in the prepared dispersions is between 85.7 and 95.3 % (w/w) of the theoretical values and the mean volume diameter of the particles collected from drying chamber and cyclone are found to be 12.43 and 69.19 μm, respectively. The DSC thermograms have indicated the morphological conversion of entacapone to amorphous form. The saturation solubility for entacapone in the spray dried formulation is 4.6 and 1.8 times higher to the plain drug and spray dried drug, respectively. The dissolution of entacapone in acetate buffer pH 1.2 is 79 % in the solid dispersion formulation whereas it is only 11 % in the plain drug in 1 h. The release of entacapone in all the three pH conditions studied is instantaneous and complete indicating the pH independent release behaviour from the formulation. The formulations have demonstrated the significant improvement of bioavailability (AUC = 54048 ng/h/mL) compared to plain drug suspension (AUC = 9438 ng/h/mL). These results demonstrated the efficacy of solid lipid dispersions for the enhancement of entacapone bioavailability by increasing its aqueous solubility.

Key Words: Entacapone, Lipids, Bioavailability, Spray drying, Dissolution, Amorphous.

INTRODUCTION

With the implementation of high-through put screening in pharmaceutical industry, a significant number of poorly water-soluble drugs have been identified[1]. Such poorly water soluble drugs pose significant hurdles for drug bioavailability that in turn affect *in vivo* efficacy. Also poorly soluble drugs produce technical difficulties in formulation development[2]. Consequently innovative pharmaceutical technologies are being developed to improve the desired properties of such poorly soluble drugs to increase the aqueous solubility and/or the dissolution rate of drug substances such as formation of salts for ionizable compounds solutions in solvents[3], cosolvents and lipids micelle systems[4] including self-emulsifying drug-delivery

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systems (SEDDS)[5], particle size reduction[6] including the use of attrition-milled nanocrystalline form, complexations[7], prodrugs and solid dispersions[8]. A solid dispersion has traditionally been defined as "the dispersion of one or more active ingredients in an inert excipient or matrix" where the active ingredients could exist in finely crystalline, solubilized or amorphous states. Pharmaceutical solid dispersions have been studied for close to half a century as a means for increasing the dissolution rate and oral bioavailability of poorly water soluble drugs. However so far developed solid dispersions with polymers met with some challenges and created a path to use lipid excipients in the preparation of solid dispersions. These lipid formulations range from simple solutions of drugs in dietary triglycerides to the use of complex mixtures of triglycerides, partial glycerides, surfactants, co surfactants and co solvents to solubilizing the drugs[9]. However the work that was carried out in lipid mediated solid dispersions involves the dispersion of drug in the molten lipid and filled into hard gelatin or soft gelatin capsule[10]. To make a solid oral dosage forms the prepared solid dispersions shall have suitable flow property. Spray drying is widely used technique in developing free flowing powders from aqueous or non aqueous suspensions and solutions.

In the present paper, we discuss the development of free flowing entacapone loaded lipid solid dispersion (LSD) by spray drying. Entacapone is a new adjunct to levodopa/carbidopa therapy in the tratment of Parkinson's disease[11]. The drug is a potent, specefic and orally acting peripheral catechol-O-methyltransferase (COMT) inhibitor. The aqueous solubility of entacapone is very poor and the oral bioavailability of entacapone is very low (29-46 %) and is characterized by large inter individual variation[12]. The lipid excipients, polyglycolized glycerides (gelucire 50/13) and glyceryl dibehenate (compritol), have been utilized for the preparation of dispersions. The prepared dispersions are duly characterized by DSC for morphological conversion and *in vitro* release study was conducted. Finally the formulations are evaluated for their *in vivo* absorption by orally administering the formulations to the rats.

EXPERIMENTAL

Gelucire 50/13 (stearoyl macrogoglycerides) and glyceryl dibehenate (compritol 888 ATO) were generous gifts from gateefosse, India. Entacapone was obtained from Suven Life Sciences, Hyderabad, India. All HPLC and analytical grade chemicals were purchased from Merck, India.

Preparation of entacapone loaded solid dispersions (LSD): The lipid matrix consisting of gelucire and compritol at a weight ratio of 1:3 was dissolved in dichloromethane (DCM) and entacapone is dissolved in this solution at a final concentration of 1:3, drug to lipid ratio. The lipid and drug dispersed solution was fed into a spray drier (Labultima, Mumbai) with a co-axial nozzle with co current flow. The total concentration of the solution was 5 w/v %. The conditions, that are maintained during spray drying are as follows: Inlet temperature 50 ºC, outlet temperature 40 ºC, feed rate 3 mL/min, atomization pressure 2.5 kg/cm^2 and aspiration of 25 m^3/h. The

dried drug loaded dispersions (F2) are collected from drying chamber and cyclones and stored in desiccated environment until further study. As a control, plain drug alone dispersed in dichloromethane and spray dried with the above described conditions (F1).

HPLC method development for entacapone estimation: Entacapone is estimated using a validated RP-HPLC method by Agilent make model 1200 series. The mobile phase is a filtered and degassed mixture of 0.1 % orthophosphoric acid and acetonitrile in the ratio 65:35 (v/v) and the separations were performed with L1-Packing (Zorbax XDB, C_{18}-150 mm × 4.6 mm, 5 µm) column. The chromatographic conditions were as follows: The nanometers is set to 305 nm, flow rate is 1.5 mL/min, injection volume 20 µL with a run time of 10 min. System is found to be suitable after injecting blank followed by standard (6 times) and evaluating the RSD % (< 2 %) and USP tailing factor (< 2). The LOD is identified as 97.02 ng/mL and the LLOQ is found to be 294.01 ng/mL.

Drug content estimation: The content of entacapone in the solid dispersion F2) sample was determined using HPLC. A 10 mg sample was dissolved in 10 mL of acetonitrile and vortexed well. The solutions were filtered through a membrane filter (0.45 mm) and suitably diluted with mobile phase before injecting to the HPLC.

Saturation solubility: To evaluate the increase in solubility of entacapone after the preparation of solid dispersion, saturation solubility measurements were conducted for formulations F1 and F2. The impact of spray drying on the solubility enhancement was studied by taking plain entacapone as a control. The known excess amount of entacapone was added to 10 mL of pH 1.2 acetate buffer. Samples were rotated at 20 rpm in a water bath (37 ± 0.5 ºC) for 48 h. The samples were then filtered, suitably diluted and analyzed by HPLC.

Laser diffraction particle size analysis: Particle size distribution was measured using a laser diffraction size analyzer (HELOS/BF-R5, Sympatech, Germany). Samples were suspended in water and 2 to 3 drops of isopropyl alcohol added to the disperse the particles and treated by ultra sonication at 50 % amplitude. The particle size and distribution was measured at a measurement range of 10 s in 500 ms time base and at the optimum concentration of 10 %.

Differential scanning calorimetry (DSC): DSC studies were conducted using a TA instrument, model Q200 equipped with a with RCS-90 (-90-450 °C) cooling unit. DSC was performed with 2 mg sample in T zero pan-aluminium, encapsulated with T zero lid-aluminium by T zero press. Inert atmosphere was maintained by purging nitrogen gas at a flow rate of 50 mL/min. Samples are heated at a temperature range of 0-300 °C with ramping at 10 °C.

***In vitro* dissolution:** The dissolution rate of entacapone from the prepared dispersion (F2) was measured in a Disso-2000 mode dissolution test system (Labindia, India) using simulated gastric fluid (SGF) without pepsin at pH 1.2 and USP apparatus II (paddle) method. The drug dispersed dispersions are filled into hard gelatin capsule

equivalent to 30 mg of entacapone. The equivalent plain entacapone was filled into capsules along with mannitol as an inactive excipient (F3). In each dissolution vessel, drug filled capsules were added to 900 mL dissolution medium. Bath temperature and paddle rotation speed were maintained at 37 °C and stirred at 100 rpm. Samples were collected periodically and replaced with a fresh dissolution medium. After collection of 90 min sample, recovery study is conducted by stirring the paddle at 200 rpm for 5 min and sample is collected. Samples are filtered through filters (10 µm) and analyzed using HPLC.

In another experiment the dissolution test was performed in 3 different pH media to evaluate the effect of pH in the release of entacapone from the formulations. The pH 1.2 acetate buffer, pH 4.5 acetate buffer and pH 6.8 phosphate buffer were used as dissolution media and other conditions are maintained as described above.

***In vivo* bioavailability study:** Male wistar rats (Suven Life Sciences, Hyderabad, India) weighing 220-250 g are used for the study. The rats were housed in stainless steel cages and kept on a 12 h light/dark cycle. On the day of experiment, animals were randomized and divided into groups of four and kept for fasting for 12 h. All animal studies conducted are approved by local animal ethical committee.

The formulations (F2) are prepared by dispersing the entacapone solid dispersion in potassium biphthalate buffer (pH 3; 50 mM) and in phosphate buffer solution (pH 7.4; 0.16 M) and administered orally at a dose of 5.7 mg/kg body weight. The plain entacapone is administered as a suspension (F4) in potassium biphthalate buffer (pH 3; 50 mM) and at a dose of 5.7 mg/kg body weight. Blood samples were withdrawn from the animals after a period of 15, 30, 60, 90, 120 min post administration of the dose. The blood samples were collected into glass tubes containing disodium-EDTA and plasma was separated by 1500 g centrifugation at 4 ºC. The plasma samples were transferred into plastic tubes and stored at -80 ºC until further analysis was done. The drug levels in plasma are estimated by HPLC with the slight modifications to the method reported earlier[13]. Briefly, the drug is extracted from plasma by adding 300 µL of plasma with 100 µL of 10 % orthophosphoric acid and vortexing for 30 s and further added with 4 mL of mixture of *n*-hexane, ethyl acetate (7:3). The mixture is vortexed for 2 min and centrifuged at 2000 rpm for 10 min and 3 mL of supernatant is separated. The solvent is evaporated by purging nitrogen gas and the residue is reconstituted with 500 µL of HPLC mobile phase and injected into HPLC. The HPLC conditions used are Zobrax XDB (C_{18}, 250 mm × 4.6 mm, 5 µm) column, flow rate of 1 mL/min, injection volume is 100 µL and at a wavelength of 315 nm. The column is maintained at 25 ºC. The mobile phase used is acetonitrile and pH 2.75 buffer at a ratio of 38:62 % v/v.

RESULTS AND DISCUSSION

The lipid solid dispersion is an amorphous molecular dispersion of drug in lipid matrix. It is a solid solution and prepared by spray drying the mixture of drug and lipid matrix after dissolving in a suitable solvent. Process conditions are chosen so that the solvent rapidly evaporates from the droplets to rapidly solidify the lipid

and drug mixture, trapping the drug in amorphous form. In the present study, gelucire 50/13 and glyceryl dibehenate are taken as lipid matrix. The gelucire 50/13 is a surface-active excipient that can solubilize poorly soluble drugs[14]. However spray drying of gelucire alone is problematic since it forms a sticky and tacky mass in the drying chamber because of its low melting point. Hence gelucire in combination with high melting lipids such as glyceryl dibehenate is designed. The lipid matrix along with entacapone are dissolved in dichloromethane to form a clear solution and spray dried. Upon spray drying solid powder with good flow property was obtained with a yield of 75 %. Selection of the suitable solvent and spray drying conditions results in the formation of homogenous solid dispersion.

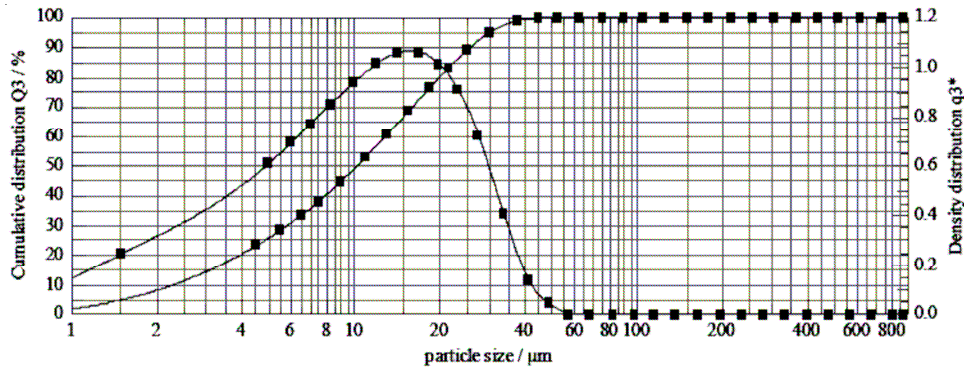
Solid state characterization: In the successful development of solid dispersions for the enhancement of aqueous solubility several solid state characters such as physical form of the solid dispersion, morphology of drug and particle size of the dispersion are very critical.

The size of the particle significantly influences the dissolution. It is generally regarded as lower the particle size the higher surface area and results in higher dissolution[15]. The particles are separated based on their size and collected from drying chamber and cyclone in spray dryer. The size of particle is evaluated by laser diffraction and was represented in Table-1 and Fig. 1. The mean volume diameter (VMD) of the particles collected from drying chamber and cyclone are found to be 12.43 and 69.19 μm, respectively. And the 90 % of particles collected from cyclone and drying chamber are below 25.86 and 152.2 μm, respectively. The size of the particle is precisely controlled by atomization pressure and feed rate during the spray drying process.

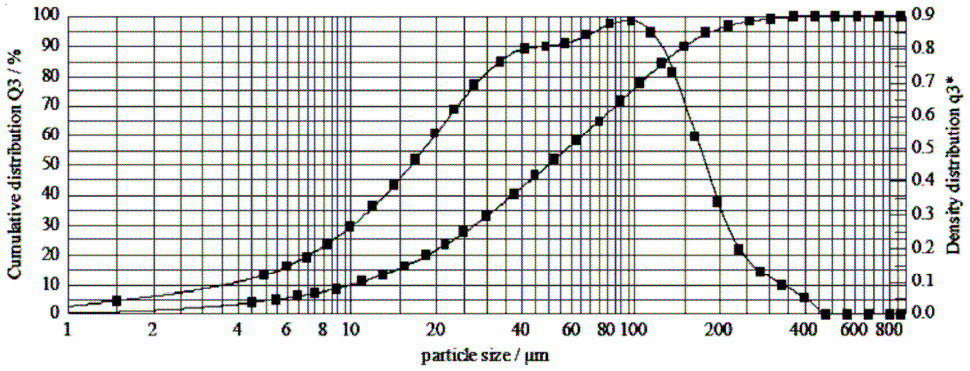
TABLE-1
PARTICLE SIZE ANALYSIS DATA OF THE LIPID DISPERSIONS COLLECTED FROM CYCLONE AND DRYING CHAMBER AFTER SPRAY DRYING

Fraction	X10	X50	X90	VMD (μm)
Cyclone	2.24	10.27	25.86	12.43
Drying chamber	10.35	49.99	152.20	69.19

Differential scanning calorimetry (DSC): Differential scanning calorimetry thermograms are obtained for entacapone, gelucire, compritol and for solid dispersion (F2) and are displayed in Fig. 2. Pure entacapone has shown well defined endothermic peak at 164.9 ºC corresponding to the melting point of crystalline drug. Likewise the lipid excipients have shown endothermic peaks at 43.42 and 71.82 ºC for gelucire and compritol, respectively, representing the melting points. However in the thermogram of the spray dried solid dispersion, the endotherm peak of drug disappeared and instead new peak was observed at 153.3 ºC. However the endothermic peaks of gelucire and compritol remains same. The significant reduction in the melting point of the entacapone can be attributed to the morphological conversion of entacapone from crystalline to amorphous form.

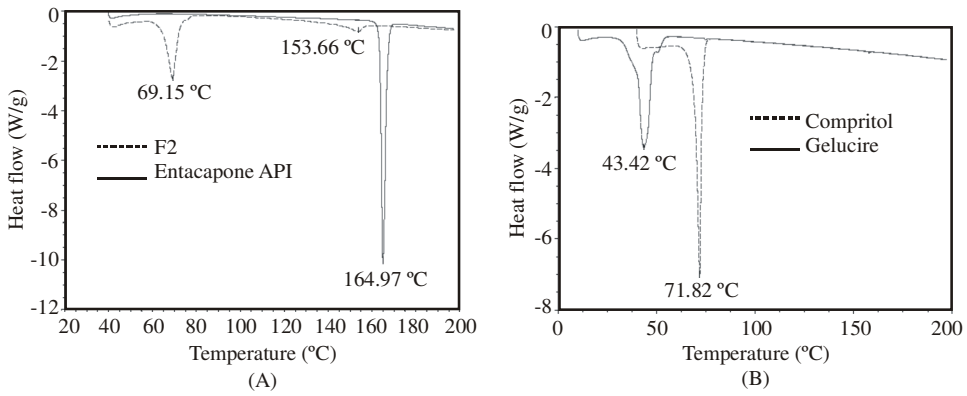


(A)



(B)

Fig. 1. Particle size distribution of lipid solid dispersion prepared by spray dried process. (A) Particles collected from cyclone (B) Particles collected from drying chamber



(A)

(B)

Fig. 2. Differential scanning calorimetry (DSC) thermograms of entacapone, gelucire, compritol and entacapone loaded solid dispersion. (A) Thermgrams of entacapone API and F2 (B) Thermograms of gelucire and compritol

Drug content and saturation solubility: HPLC analysis was used to estimate the drug content in the formulations. The obtained values were between 85.7 and 95.3 % (w/w) of the theoretical values.

The saturation solubility study is conducted for the plain drug, spray dried drug and spray dried formulation in pH 1.2 acetate buffer. After 48 h of incubation the solubilized drug is evaluated by HPLC and values represented in the Table-2. The saturation solubility for entacapone in the F2 is 280 µg/mL and is 4.6 and 1.8 times higher to the plain drug and F1, respectively. The higher solubility in the formulation is because of the morphological conversion of the drug and also attributed to the improvement of wetting of the drug, reduced particle size and localized solubilization by lipid carriers.

TABLE-2
SATURATION SOLUBILITY STUDY OF ENTACAPONE FORMULATIONS TESTED IN pH 1.2 ACETATE BUFFER AT 37 ± 0.5 °C. (MEAN ± SD)

Formulation	Saturation solubility (µg/mL)
Plain entacapone	60 ± 2.5
F1	150 ± 6.6
F2	280 ± 7.3

***In vitro* dissolution:** To assess the dissolution kinetics of entacapone from the developed formulations an *in vitro* dissolution test was performed under sink conditions. The dissolution profile of the F2 and F3 was shown in the Fig. 3. The release of entacapone from the F2 is steepest initial slope and the dissolution rate was higher compared to F3 in all time points. The enhancement of dissolution was approximately 10 times higher until 30 min compared to F3. The dissolution was 79 % in the F2 where as it is 11 % in the F3 in 60 min. And even after the recovery study the dissolution is only 17 % whereas it is 88 % for F2. It is assumed that there

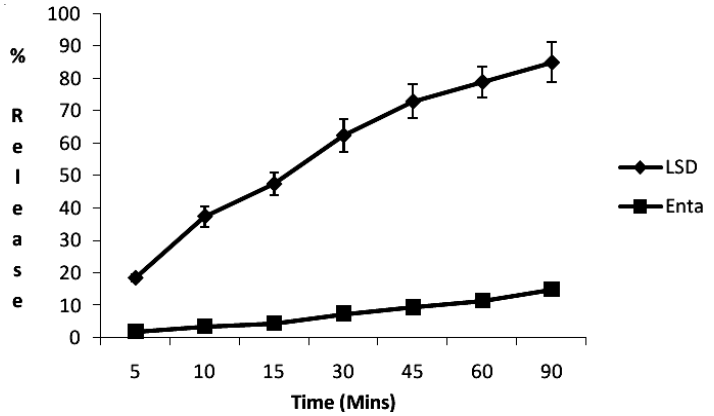


Fig. 3. Dissolution profile of spray dried formulation (F2) and plain entacapone (F3) carried in acetate buffer pH 1.2. Each point refers to mean ± SD

are two mechanisms responsible for dissolution of entacapone. They are drug controlled and carrier controlled dissolution. Because the entacapone in solid dispersion is in amorphous form and hence the dissolution was more compared to plain drug. And by means of spray drying more precise particles can be prepared and the produced smaller particles enhance the dissolution by increased surface area. The spray dried particles could improve the wettability of the drug and localized solubilization by the lipid materials in the diffusion layer more efficiently[16].

Entacapone has faster dissolution rate at pH 6.8 than at pH 1.2 and pH 4.5. At pH 6.8 the dissolution is 90 % by 60 min whereas it is only 60 and 63 % for pH 1.2 and 4.8, respectively (Fig. 4). And at pH 6.8, the dissolution is completed within 90 min, but in other conditions the release is below 80 %. This can be attributed to the fact that entacapone is having a pK_a 4.5 and is completely in the ionized form at pH 6.8. The main reason for lower bioavailability of entacapone is its slow dissolution rate at an acidic pH. This may hinder its absorption from the upper GI tract, which has been suggested to be the main site for absorption for entacapone[12]. Solid dispersion preparation with lipids increased the dissolution of entacapone even at pH 1.2, pH 4.5 and 6.8 indicates pH independent release. This clearly shows the preparation of solid dispersions for entacapone with lipid excipients is suitable tool to overcome the incomplete dissolution as a rate limiting step in its absorption process.

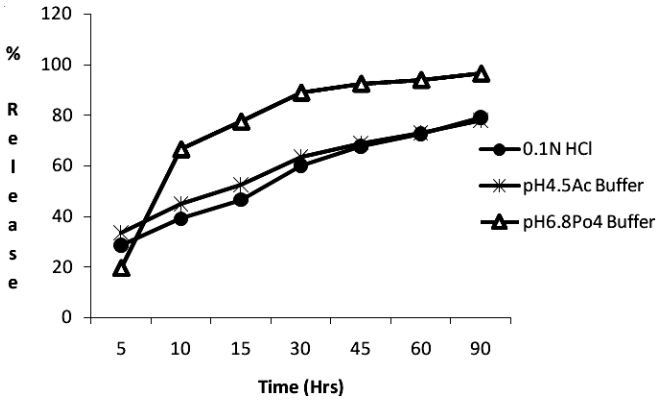


Fig. 4. Release (%) of entacapone from formulation (F2) in three different pH media

***In vivo* bioavailability study:** The efficacy of the F2 in the improvement of oral bioavailability of entacapone was evaluated after administering the dose to the rats. The plain drug suspension was prepared (F4) and administered to the rats for comparison purpose. The F2 was dispersed in pH 3 potassium biphthalate buffer and in pH 7.4 phosphate buffer. The idea of using two buffers, pH 3.0 and 7.4, is to prove that developed formulation can improve the bioavailability of entacapone by increasing its solubility and dissolution properties irrespective of pH condition. All the dosage forms were well tolerated and no obvious side effects were observed.

After dosing, plasma samples were analyzed by HPLC for entacapone levels and drug plasma concentrations were as a function of time was shown in Fig. 5. The plasma profiles were analyzed by non compartmental analysis for extravascular administration to determine the appropriate pharmacokinetic parameters of administered formulations and represented in Table-3. Statistically significant differences were observed for both AUC (0-inf) and T1/2 values indicating that developed solid dispersion formulation has improved the oral bioavailability of entacapone and suggested that large concentrations of drug were available for absorption throughout the GI tract.

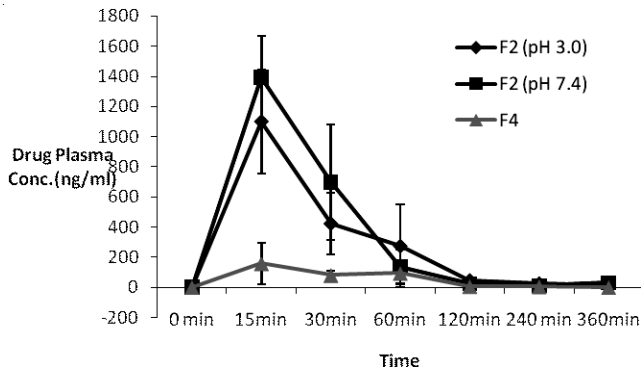


Fig. 5. Entacapone plasma concentrations (mean ± SD) in rats after oral administration of formulations (F2) and plain drug suspension (F4)

TABLE-3
MEAN PHARMACOKINETIC PARAMETERS FOR ENTACAPONE FORMULATIONS IN PLASMA AFTER ORAL ADMINISTRATIONS TO THE RATS

Parameter	F2 (pH 3.0)	F2 (pH 7.4)	F4
C_{max}	1100.6	1392	158.6
T_{max}	< 15	< 15	<15
AUC (0-t)	46115.25	47598.75	9500.25
AUC (0-inf)	47150.73	54048.26	9834.69
T1/2	71.05	51	48.28

The F2 dispersed in both pH 3 and pH 7.4 solutions have shown increased C_{max} value compared to F4. The C_{max} values found to be 1100 ng/mL in pH 3 buffer and 1392 ng/mL in pH 7.4 buffer. Whereas the F4 has shown only 158.6 ng/mL which is 6.9 and 8.8 times lesser to the formulation dispersed in pH 3 and 7.4, respectively. The AUC (0-inf) values are 4.7 and 5.4 times higher in F2 dispersed in pH 3.0 and pH 7.4 solutions compared to F4, respectively (47150 *versus* 9834 ng/h/mL; 54048 *versus* 9834 ng/h/mL). However in all the administered formulations the T_{max} found to be below 15 min. The increase in the C_{max} and AUC (0-inf) in the F2 compared to F4 can be mainly attributed to the enhancement of aqueous solubility and dissolution

properties. Thus the entacapone is known to be susceptible for first pass metabolism and developed formulations can be assumed to minimize the metabolism by promoting the lymphatic transport. This can also be believed to be the reason for elevation in the bioavailability of entacapone.

Conclusion

In the recent years the utilization of lipid excipients in the enhancement of oral bioavailability of poorly soluble drugs has become prominent. In the present work the solid dispersion with lipid excipient for an aqueous insoluble drug, entacapone, is prepared by spray drying. The *in vitro* dissolution tests, *in vivo* bioavailability studies proved the efficacy of the formulation in the aqueous solubility and oral bioavailability enhancement compared to plain drug. The enhancement of aqueous solubility and oral bioavailability can be attributed to the factors such as reduced particle size, amorphous form of drug, increased solubility of drug by lipids and minimization of hepatic metabolism by lymphatic transport. Hence it is concluded that the oral bioavailability of poorly soluble drugs can be increased by preparation of solid dispersions by spray drying using lipid excipients.

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