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Formulation, Characterization and *in vitro* Evaluation of Entacapone Solid Dispersions with Lipid Carriers by Using Spray Drying Method

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ABSTRACT

In present study, immediate release solid dispersion formulation of Entacapone with various lipids was developed using spray drying method. Entacapone is indicated for the treatment of Parkinson's disease (PD) as an adjunct to levodopa/carbidopa therapy. Based on the process feasibility and solubility of resulting spray dried powder, formulation ENSD10 was selected for characterization and analyzed for *in vitro* dissolution profiles in three different pH media. The particle size of Entacapone in spray dried formulation ENSD10 was drastically reduced down to d_{90} of 25.86μ against d_{90} of as such drug substance, i.e., 124.74μ . Analysis by differential scanning calorimetry showed that the percentage of crystallinity has been significantly reduced in formulation ENSD10. The optimized solid dispersion formulation (ENSD10) by spray drying method comprising drug to lipid to lipid carrier ratio of 0.5:1:3 (Entacapone: Gelucire 50/13: Compritol) enhanced solubility nearly 6.31 fold and 3.75 fold as compared to pure drug and spray drug alone, respectively. The rate of drug release from spray dried powder was significantly improved and found to be on higher side as compared to the drug release from as such drug and spray dried drug alone. The obtained results suggested that developed Entacapone along with lipid carrier mixture by spray drying method has promising potential for oral delivery and might be an efficacious approach for enhancing the therapeutic potential of active substance.

Keywords: Entacapone, Spray drying, Gelucire, Compritol, Dissolution.

INTRODUCTION

Many of the drugs that are discovered in recent times are poorly water soluble and highly permeable and classified under BCS or IV drugs. Due to limitation in dissolution they have low and variable bioavailability. [1] Lipid based formulations of these drugs is an alternative approach to increase their solubility and

thereby bioavailability. [2-3] Lipid based drug delivery systems such as solid dispersions and microemulsions gained more because of their ease of preparation and other advantages. [4]

Therefore, to test the applicability of lipid based systems in the improvement of oral bioavailability of poorly water-soluble lipophilic drug, entacapone (anti Parkinson drug) was selected. It is poorly soluble in water, lipophilic in nature and the mean absolute bioavailability of entacapone was found to be 25%.

It has been focused on enhancing the solubility of entacapone and improving the bioavailability by administering through oral route resulting to increase

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its clinical efficacy. Poor solubility and bioavailability of an existing or newly synthesized drug always pose challenge in the development of efficient pharmaceutical formulation. [5] Numerous technologies can be used to improve the solubility and among them amorphous solid dispersion based spray drying technology can be successfully useful for development of product from lab scale to commercial scale with a wide range of powder characteristics. [6-7]

Spray drying is an efficient technology for solid dispersion manufacturing since it allows extreme rapid solvent evaporation leading to fast transformation of a drug-carrier solution to solid drug-carrier particles. [6,8] Entacapone (ENT) is an inhibitor of catechol-O-methyltransferase (COMT), used in the treatment of Parkinson's disease (PD) as an adjunct to levodopa/carbidopa therapy. Entacapone is in clinical use as an adjunct of L-dopa therapy in PD. The bioavailability of entacapone after oral administration is low and is characterized by large inter individual variation. [7] The reason for its poor bioavailability is unknown, but may be caused by 1) poor aqueous solubility of entacapone in the GI-tract, 2) poor membrane penetration or 3) first-pass metabolism.

Entacapone is a weak acid with a pKa value of 4.5. Entacapone is practically insoluble in water, but slightly soluble in organic solvents. The aqueous solubility of entacapone is very low at acidic pH, but increases strongly with increasing pH, but slightly soluble in organic solvents.

The main objective of the current research work was to prepare free flowing solid dispersion particles of Entacapone suitable for the development of solid dosage forms and to improve the rate of dissolution for solid oral dosage form. Various surface active lipid excipients were selected and evaluated for the suitability in the development of lipid solid dispersions by spray drying process. [5,8]

MATERIALS AND METHODS

Materials

Stearoyl macroglycerides (Gelucire 50/13) and Glyceryl dibehenate (Compritol 888 ATO), Diethylene glycol monoethyl ether (Transcutol) were generous gifts from Gatefosse, India. Caprylic/Capric Triglyceride (Miglyol 812) received as gift sample from SASOL. Entacapone was obtained from Suven Life Sciences, Hyderabad, India. Povidone (Kollidon 30) obtained from BASF, Mumbai as a gift sample. Mannitol (Pearlitol SD 200) from Signet, Mumbai as a gift sample. All HPLC and analytical grade chemicals were purchased from Merck, India.

Methods

Selection and optimization of excipients composition

The main objective of the current research work was to prepare free flowing solid dispersion particles of Entacapone suitable for the development of solid dosage forms. Various surface active lipid excipients were selected and evaluated for the suitability in the

development of lipid solid dispersions by spray drying process. [9-11] Different experiments were conducted with various ratios of drug to lipid excipient (s). Most of the lipid excipient (s) were liquid or semi solid in nature and requires use of adsorbents to prepare free flowing powder particles upon spray drying. In the selection of lipid composition for the development of lipid solid dispersions, with the optimized process parameters obtained of solid particles with high drug content upon spray drying. The compositions, their physical ratios and the physical behavior are given in the Table 1.

Polyoxy glycerides are surface active agents and semi solid in nature. To develop a free flowing fine particle by spray drying different adsorbents were selected and evaluated. [11-12] The flow properties of resulting spray dried powder from the trails Gelucire 50/13 in combination with Compritol was found to be satisfactory. The lipid mixture of Gelucire 50/13 and Compritol was fixed at 1:3 and the drug concentrations were changed. The optimum drug to excipient (s) ratio was identified on the basis of drug content, drug solubility and physical form. The preparation process of Entacapone loaded lipid solid dispersions includes the solubilizing the drug and lipid excipients in a common solvent to have a clear solution and spray drying the solution to obtain a dried particles.

Upon spray drying the dried powder were collected from the both drying chambers and the cyclones and was evaluated for physical form, drug content and saturation solubility.

Table 1: Table showing composition with different drug/ excipient ratios and their physical nature

S. No.	Formulation	Composition	Drug/ Excipient(s) ratio	Nature of dispersion
1.	ENM	ENT + Migloyl 812	1:1	Sticky mass
2.	ENMM	ENT + Miglyol 812 + Mannitol	1:1:2	Wet particles
3.	ENT	ENT + Transcutol	1:1	Wet particles
4.	ENTM	ENT + Transcutol + Mannitol	1:1:2	Wet particles
5.	ENG	ENT + Gelucire 50/13	1:1	Sticky mass
6.	ENGP	ENT + Gelucire 50/13 + Povidone	1:1:2	Sticky mass
7.	ENG C	ENT + Gelucire 50/13+Compritol ATO 888	1:1:3	Solid dried particles

Optimization of spray drying process

The prepared drug dispersed lipid solutions was fed into a spray drier (Labultima, Mumbai) with a co-axial nozzle with co-current flow. The majority of the spray dried powder was collected in the drying chamber cylinder with aspiration below 90 % and it was found to be coarser powder as compared to spray dried powder, which was collected in Extraction cyclone cylinder where aspiration above 90% to 100 %. Spray dried powder was found to be coarser with nozzle size

more than 0.4 mm, coarser grade powder was collected in drying chamber cylinder. [13] The optimized spray drying process parameters are depicted in Table 2.

Table 2: Parameters during spray drying

Atomizer Qualifications	
Nozzle tip	0.2 mm
Nozzle diameter	0.4 mm
Cap diameter	1.4 mm
Spray drying parameters	
Inlet temperature	50 – 60°C
Pump rate for spraying dispersion	25 – 35 %
Nitrogen gas pressure	30 – 40 mm Hg

The drug was dispersed in the lipid matrix consisting of Gelucire 50/13 and Compritol at a ratio of 1:3. The drug dispersed lipid matrix was dissolved in the dichloromethane (DCM) to obtain a clear solution. The drug to lipid mixture was taken in 0.1:1, 0.25:1, 0.5:1, 0.75:1 and 1:1 ratio (ENSD 8 to ENSD 12, respectively). The spray drying of organic solution of drug dispersed lipid matrix was carried out by using lab model spray dryer. The organic solution was spray dried with a co-axial nozzle and co-current flow. The total concentration of the solids in the solution was maintained at 5% w/v. The spray dried powder was collected from drying chamber and the cyclones and placed in 60cc child resistant heavy weight HDPE container and then it was sealed properly and stored in controlled environment until further study. As a control, plain drug alone dispersed in dichloromethane (DCM) and spray dried with the above described conditions (ENSD).

Characterization

Drug content estimation

The content of Entacapone in the lipid solid dispersions formulation was determined by using the HPLC method after extracting the drug from the lipid matrix. The extraction method includes weight of spray dried powder equivalent to 200 mg of Entacapone was taken in 200 mL of acetonitrile and vortexed well. The solutions were filtered through a membrane filter (0.45 µm) and were suitably diluted with mobile phase before injecting to the HPLC.

Saturation Solubility

To evaluate the solubility of Entacapone after the preparation of solid dispersion, saturation solubility measurements were conducted for formulations ENSD8 to ENSD12 and ENSD. The impact of spray drying on the solubility enhancement was studied by taking plain Entacapone as a control (ENT). The known excess amount of Entacapone was added to 25 mL of pH 1.2 (0.1 N HCl). Samples were rotated at 20 rpm in a water bath (37 ± 1°C) for 48 hours. The samples were then filtered, suitably diluted, and analyzed by HPLC.

In-vitro dissolution

The dissolution rate of Entacapone from the prepared solid dispersion (ENSD 10) was analyzed by using Disso-2000 mode dissolution test system (Labindia, India) using USP apparatus II (paddle) method. The

spray dried powder was filled into hard gelatin capsule equivalent to 200 mg of Entacapone. The equivalent plain Entacapone was filled into same size hard gelatin capsule shell along with Mannitol (Pearlito SD200) as an inactive excipient (ENT1); spray dried Entacapone with Mannitol (Pearlito SD200) was filled into same size hard gelatin capsule shell (ENT2) for the comparative evaluation. To evaluate the effect of pH on the release of Entacapone from the formulations, the *in vitro* dissolution analysis was carried out for ENT1, ENT2 and ENSD10 in 900 mL of three different pH media. The pH 1.2 (0.1 N HCl), pH 4.5 acetate buffer and pH 6.8 phosphate buffer were used as dissolution media, bath temperature and paddle rotation speed were maintained at 37 ± 0.5°C and stirred at 100 rpm, respectively. Samples were collected periodically and replaced with a fresh dissolution medium. After collection of 90 minutes sample, recovery study was conducted by stirring the paddle at 200rpm for 10 minutes and sample was collected. Samples were filtered through filters (10µm) and were diluted suitably with mobile phase and then analyzed by using HPLC.

In the present investigation, samples of Entacapone were estimated using a Validated HPLC method developed in laboratory.

Instrument details

A HPLC system (Agilent 1200 series) selected, it was equipped with in-line four channels Degasser, Quaternary pump, thermostated Column compartment, Diode Array (DAD) detector and thermostatted auto sampler. The HPLC system uses EZChrome Elite C/s software (Version 3.3.1) as Chromatography Data System (Software Control). Samples were chromatographed on a reversed phase C₁₈ column (Zorbax XDB C18, 250 × 4.6mm, 5µm or equivalent).

Chromatographic conditions

Acetonitrile and pH 2.75 Phosphate buffer (2.7 grams of Potassium di hydrogen Phosphate and 1.7 grams of Di Potassium hydrogen Phosphate were taken in 500 mL of HPLC grade water and made up to 1000 mL with HPLC grade water. The pH was adjusted to 2.75 with ortho-phosphoric acid) in the ratio of 62:38% v/v were used as mobile phase. The mobile phase components were degassed and filtered through a 0.45µm Nylon 66 membrane filter and pumped from the respective solvent reservoirs at a flow rate of 1.0 mL/min. Eluents were monitored using UV detection at a wavelength of 315 nm. The injection volume was 10µL with run time of 20 minutes.

Preparation of stock solution and standard solutions

Stock solution of Entacapone was prepared by weighing about 50 mg of Entacapone in a 50 mL volumetric flask, 30 mL of methanol was added to dissolve and the volume was made up to 50 ml with mobile phase. The stock solution of Entacapone was subsequently diluted with diluent solution (50:50 of pH

2.75 buffers and Acetonitrile) to obtain a series of standard solutions containing 0.025, 0.05, 0.1, 0.25, 0.5, 1, 1.5, 2, 2.5 and 3 µg/mL of Entacapone. All standard solutions were filtered through 0.45 µm Nylon 66 membrane filter and 10 µL of the sample is injected on to the HPLC column.

Laser Diffraction Particle Size Analysis

One of the reasons for the solubility improvement from the lipid solid dispersion formulations assumed to be due to the reduced particle size of the drug. The particle size and particle size distribution of the spray dried powder were measured using a laser diffraction size analyzer (HELOS/BF-R5, Sympatech, Germany). Samples were suspended in water and two to three drops of isopropyl alcohol added to the dispersed particles and treated by ultra sonication at 50% amplitude. The particle size and distribution was measured at a measurement range of 10 seconds in 500ms time base and at the optimum concentration of 10%.

Differential Scanning Calorimetry (DSC)

The enhanced solubility of the Entacapone from the spray dried powder formulations can be attributed to the morphological conversion of the drug upon spray drying. To prove the morphological conversion of the Entacapone DSC studies were conducted for the formulations using a TA instrument, Model Q200 equipped with RCS-90(-90°C to 450°C) cooling unit. DSC was performed with 2 mg sample in Tzero pan-Aluminium, encapsulated with Tzero lid-Aluminium by T zero press. Inert atmosphere was maintained by purging nitrogen gas at a flow rate of 50 mL/min. Samples were heated at a temperature range of 0 to 300°C with ramping at 10°C/min.

Infrared Spectroscopy (IR)

The chemical compatibility of the Entacapone with the lipidic materials was evaluated by using the Infrared spectroscopy. The IR spectras of Entacapone, Gelucire 50/13, Compritol and optimized formulation (ENSD10) were obtained by using FTIR spectrometer (JASCO). Spectras were taken after preparing the pellet with 2-3 mg of sample with potassium bromide using and the sample is scanned from 4000 - 400cm⁻¹.

RESULTS AND DISCUSSION

In the present study various lipid excipients were evaluated in the preparation of Entacapone entrapped lipid-solid dispersions. Miglyol alone and in combination with Mannitol produced a sticky mass after spray drying. Miglyol which is a liquid, was not resulted into a dried solid particles even after the addition of an adsorbent, Mannitol. Similarly, the spray drying of Entacapone dispersions with Transcutol alone or in combination with Mannitol resulted into a wet particles, since the lower (less than 50°C) inlet temperature used for drying, this could be due to the low temperature might not sufficient for melting materials to obtain a dry powder. The Gelucire 50/13 is

a surface-active excipient that can solubilize poorly soluble drugs. [9-11] However spray drying of Gelucire 50/13 alone is problematic since it forms a sticky and tacky mass in the drying chamber because of its low melting point². Hence Gelucire 50/13 in combination with high melting lipids/polymers were attempted to prepare the solid dispersion. Gelucire 50/13 in combination with Povidone also resulted in incompletely dried particles. Alternatively when Gelucire 50/13 was used in combination with Mannitol or any other adsorbents the amount of drug loading was very low and required the use of high quantity of lipid materials. The combination of Gelucire and Compritol (Glyceryl dibehenate) in 1:3 ratio has given Entacapone solid dispersions with high drug loading (at an inlet temperature of 50°C). Entacapone dispersed lipid matrix was dissolved in dichloromethane to form a clear solution and then subjected to spray drying.

Upon spray drying, the resulting spray dried powder was found to be fine powder with a yield in the range of 84% - 89% w/w. Selection of the suitable solvent and spray drying conditions results in the formation of homogenous solid dispersion.

In-vitro dissolution analysis by HPLC method

The concentrations of Entacapone and the corresponding peak area are presented in Table 3. The calibration curve was plotted between concentration of Entacapone and corresponding peak area shown in Figure 1. Sample chromatograms of Entacapone standard solutions are shown in Figure 2.

Table 3: Concentration versus peak area values for the estimation of Entacapone

Concentration (ng/mL)	Peak area (mean, n=5)	% C.V.
0	0	0
25	181374	1.21
50	367660	2.34
100	692060	1.22
250	1754737	1.54
500	3383828	0.15
1000	5906609	2.54
1500	8689922	2.22
2000	11693827	3.11
2500	14799431	2.11
3000	17690535	5.85

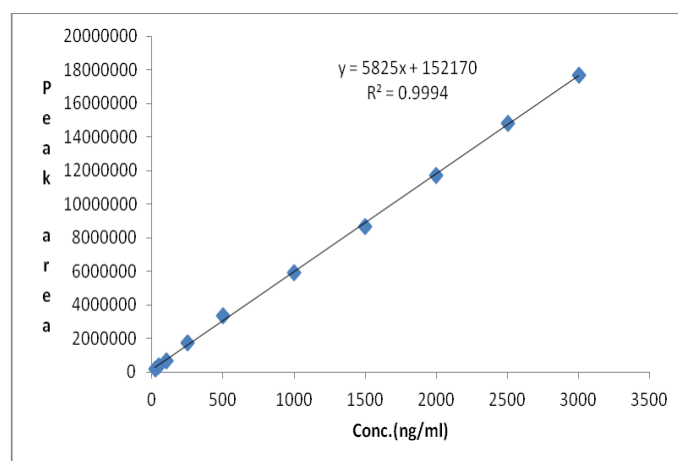


Fig. 1: Calibration curve for the estimation of Entacapone

The retention time of all the chromatograms was found to be 9.5 minutes (Figure 2). Each of the samples was injected five times and the same retention times were observed in all the cases with a variation of ± 0.05 minute. The peak area was reproducible as indicated by low coefficient of variation ($<3.2\%$).

A good correlation coefficient ($r = 0.999$) between the concentration of Entacapone and peak area values was observed indicating the linearity of the method. There is no precipitation of the drug during the dilution with the mobile phase used for the analysis of the Entacapone.

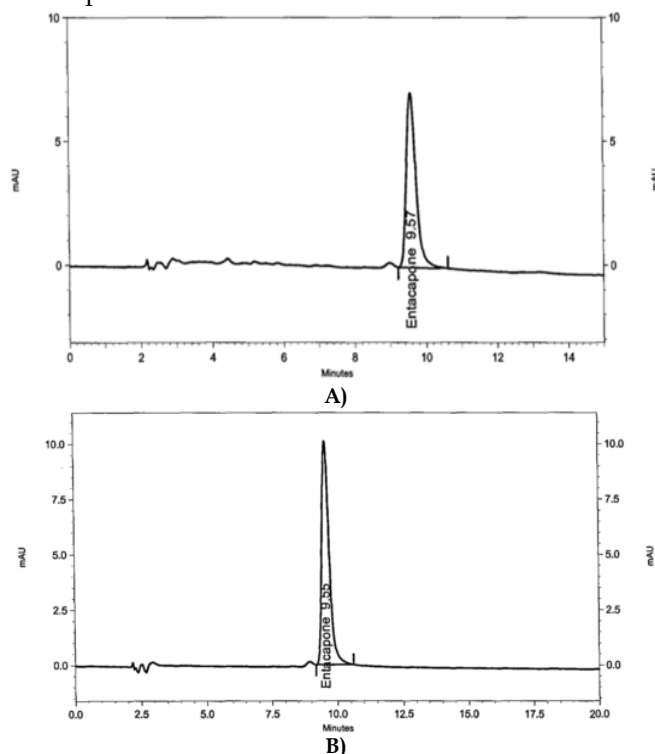


Fig. 2: HPLC chromatograms for Entacapone a) Test sample b) Standard.

Table 4: Table showing formulations with different drug/ lipid mixture ratios and their assay values and saturation solubility values

S. No.	Formulation	Drug/lipid mixture ratio	Assay (%) [*]	Saturation Solubility ($\mu\text{g/ml}$) [*]
1.	ENSD8	0.1:1	94.2 $\pm$ 3.42	63.4 $\pm$ 2.52
2.	ENSD9	0.25:1	93.4 $\pm$ 2.68	143.6 $\pm$ 6.84
3.	ENSD10	0.5:1	94.7 $\pm$ 2.14	263.2 $\pm$ 5.44
4.	ENSD11	0.75:1	95.1 $\pm$ 3.24	173.1 $\pm$ 9.16
5.	ENSD12	1:1	92.2 $\pm$ 4.16	127.5 $\pm$ 4.62
6.	ENT	Plain drug	99.4 $\pm$ 1.20	41.7 $\pm$ 1.54
7.	ENSD	Spray dried drug	94.8 $\pm$ 3.62	70.3 $\pm$ 5.32
8.	ENT1	ENT + Mannitol	--	--
9.	ENT2	SNSD + Mannitol	-	-

^{*} mean $\pm$ SD, n = 3

Table 5: Particle size analysis data of the spray dried powder (ENSD10), entacapone and spray dried entacapone (ENSD)

Raw material/ Formulation	d ₁₀	d ₅₀	d ₉₀	VMD
	μm			
Entacapone	95.72	380.86	124.74	403.71
ENSD	6.41	19.91	44.11	23.34
ENSD10	2.24	10.27	25.86	12.43

Drug content and Saturation solubility

HPLC analysis was used to estimate the drug content in the formulations. The obtained values were between 92.2% and 95.1% w/w of the theoretical values.

The saturation solubility study was conducted for the plain drug, spray dried drug and spray dried formulation in pH 1.2 (0.1 N HCl). After 48 hours of incubation, the solubilized drug was evaluated by HPLC and values represented in the Table 4.

The saturation solubility of entacapone was the highest in the ENSD10 as compared to all other formulations. It was observed that the solubility of drug substance was increased with increasing proportion of drug substance in drug substance to lipid mixture from 0.1:1 to 0.5:1, beyond this ratio a negative trend was observed in the solubility. This could be due to the fact that with increasing the drug concentration the precipitation of drug and/or insufficient coating of drug by the excipients. The formulations ENSD10 have shown 263 $\mu\text{g/ml}$ and were 6.41 and 3.55 times higher than the plain drug (ENT) and spray dried drug (ENSD) respectively. The higher solubility in the formulation can be attributed to the morphological conversion of the drug, the improvement of wetting of the drug, reduced particle size and localized solubilization by lipid carriers. [5]

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 were very critical. [11-12]

The size of the particle significantly influences the dissolution. And it is generally regarded as lower the particle size the higher surface area and results in higher dissolution. [3] The sizes of particle were evaluated by laser diffraction and are represented in Table 5 and Figure 3. The mean volume diameter (VMD) of the lipid dispersion particles, spray dried entacapone and plain entacapone were found to be 12.43, 23.34 and 403.71 μm , respectively. The size of the particle was precisely controlled by atomization pressure and feed rate during the spray drying process. And it was observed that by spray drying the size of particles has come down drastically. Whereas the spray drying of entacapone alone has resulted in higher particle size as compared to formulations. This may be due to the fact that upon spray drying the plain entacapone particles have agglomerated because of static charge and resulted in higher size particles. In the case of lipid dispersion, the particles were stabilized by the lipid materials.

IR Spectroscopy [13]

The FTIR spectra of entacapone and spray dried powder are shown in Figure 4. The FTIR spectra of pure entacapone showed characteristic peaks at 1300 cm^{-1} (C=N stretching vibration), 1753 cm^{-1} (C=O stretching), 1598 cm^{-1} (NO₂ stretching) and 3392 cm^{-1} (O-H stretching).

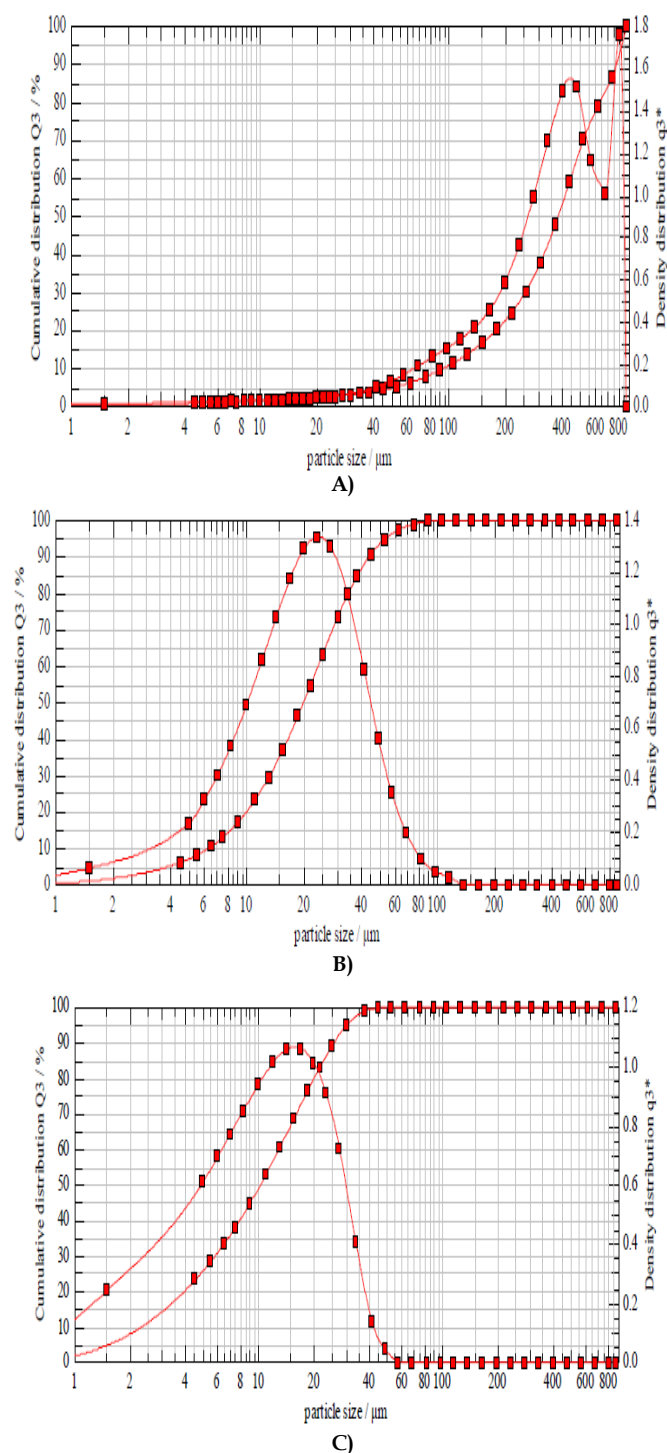


Fig. 3: Particle size distribution of entacapone - (A); Spray dried entacapone (ENSD) - (B); and spray dried powder (ENSD10) - (C).

The FTIR spectrum of spray dried powder comprising entacapone with Gelucire 50/13 and Compritol has shown a significant broadening O-H stretching vibrations peak characteristic for lipid carriers (large band between $3,500\text{cm}^{-1}$ and $3,100\text{cm}^{-1}$ for free O-H stretching vibration of the COOH groups) and C=O stretching vibration (1753cm^{-1}) characteristic of entacapone. The slight changes and shifts of the characteristic peaks of entacapone in the solid dispersion reveals the possibility of intermolecular hydrogen bonding via the -OH group of entacapone and -OH group of lipid carriers.

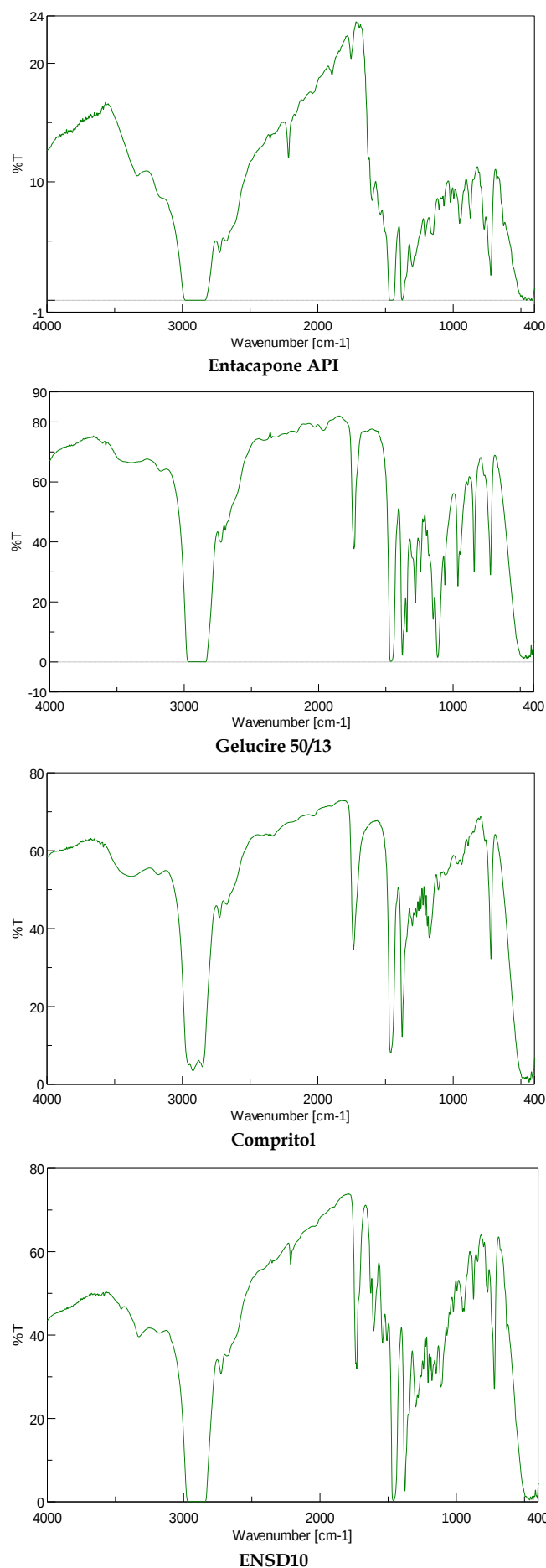


Fig. 4: FTIR spectra of excipients and spray dried formulation (ENSD10)

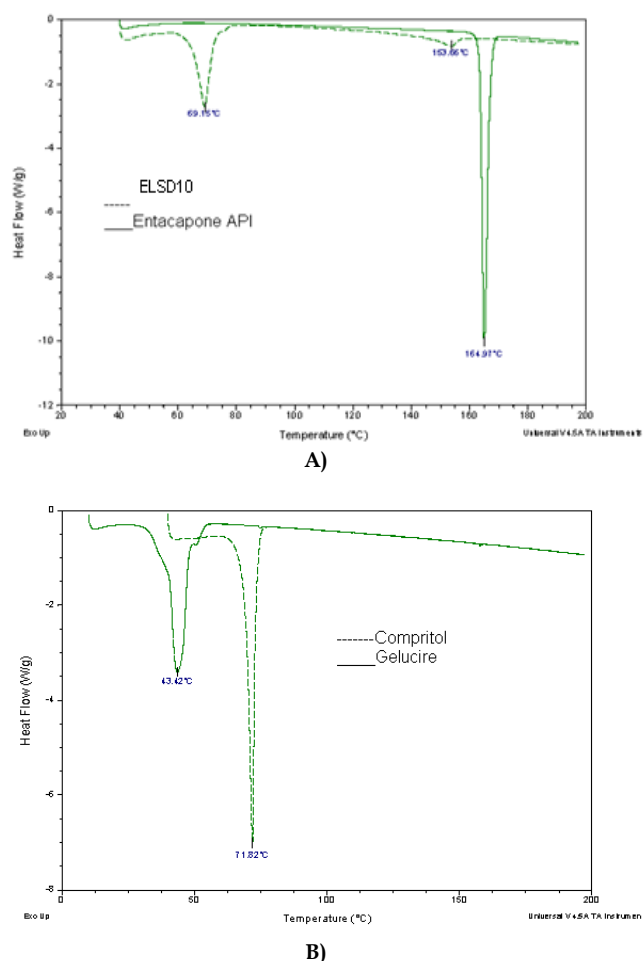


Fig. 5: Differential Scanning Calorimetry (DSC) thermograms of A) Entacapone API and spray dried powder (ELSD10); B) Gelucire 50/13 and Compritol

Differential Scanning Calorimetry (DSC)

DSC thermograms are obtained for entacapone, Gelucire 50/13, Compritol and for spray dried solid dispersion (ELSD10) and are displayed in Figure 5. Pure entacapone has shown well defined endothermic peak at 164.9°C corresponding to the melting point of crystalline drug. Likewise the lipid excipients have shown endothermic peaks at 43.42°C and 71.82°C for Gelucire 50/13 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°C. However the endothermic peaks of Gelucire and Compritol were 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.

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 comparative dissolution profiles of ENT1 Vs. ENT2 and ENT2 Vs. ENSD10 in 3 different pH media is presented in tabular form and in graphically shown in the Table 6 / Figure 6 and Table 7 / Figure 7 respectively.

The rate of drug release from the ENSD10 was on higher side as compared to the drug release from ENT1 and ENT2 at all time points. The drug release from the spray dried formulation was improved drastically can be attributed to the amorphous form of the drug as

Table 6: Comparative *in vitro* dissolution profiles of ENT1 Vs. ENT2 in 3 different pH media

Time (minutes)	Entacapone Capsules 200 mg					
	pH 1.2 (0.1N HCl)		pH 4.5 Acetate buffer		pH 6.8 Phosphate buffer	
	ENT1	ENT2	ENT1	ENT2	ENT1	ENT2
	Cumulative % drug release*					
0	0	0	0	0	0	0
5	1.2 ± 12.6	7.8 ± 14.2	2.1 ± 13.4	10.4 ± 16.4	8.4 ± 10.6	20.8 ± 8.4
10	2.4 ± 10.1	10.2 ± 12.4	4.2 ± 11.5	13.8 ± 11.4	14.2 ± 7.2	25.2 ± 8.0
15	3.8 ± 8.8	12.4 ± 9.3	7.8 ± 10.2	16.2 ± 9.6	19.6 ± 6.3	28.6 ± 6.8
30	6.6 ± 6.2	18.1 ± 10.1	11.2 ± 9.3	22.3 ± 8.4	27.4 ± 5.4	36.1 ± 7.4
45	9.2 ± 5.4	20.3 ± 7.6	16.6 ± 6.2	25.6 ± 5.4	33.2 ± 4.1	39.4 ± 4.9
60	10.4 ± 4.1	22.4 ± 3.4	18.8 ± 4.4	28.9 ± 2.1	36.4 ± 3.8	44.4 ± 3.2
90	11.6 ± 3.2	26.1 ± 4.1	20.2 ± 3.3	32.4 ± 1.8	38.2 ± 2.9	48.4 ± 1.3
Recovery [#]	18.3 ± 2.6	35.4 ± 2.0	32.4 ± 1.7	41.6 ± 2.2	49.0 ± 3.1	58.2 ± 1.8

*Mean ± SD, n=3; # at 200 rpm for additional 10 minutes

Table 7: Comparative *in vitro* dissolution profiles of ENT2 Vs. ENSD10 in 3 different pH media

Time (minutes)	Entacapone Capsules 200 mg					
	pH 1.2 (0.1N HCl)		pH 4.5 Acetate buffer		pH 6.8 Phosphate buffer	
	ENT2	ENSD10	ENT2	ENSD10	ENT2	ENSD10
	Cumulative % drug release*					
0	0	0	0	0	0	0
5	7.8 ± 14.2	30.4 ± 11.5	10.4 ± 16.4	36.1 ± 15.2	20.8 ± 8.4	42.2 ± 9.5
10	10.2 ± 12.4	38.2 ± 10.2	13.8 ± 11.4	44.3 ± 13.3	25.2 ± 8.0	46.6 ± 8.4
15	12.4 ± 9.3	46.3 ± 9.4	16.2 ± 9.6	50.6 ± 11.1	28.6 ± 6.8	55.8 ± 7.1
30	18.1 ± 10.1	56.6 ± 8.4	22.3 ± 8.4	62.4 ± 8.1	36.1 ± 7.4	64.1 ± 6.8
45	20.3 ± 7.6	65.3 ± 6.1	25.6 ± 5.4	69.8 ± 6.2	39.4 ± 4.9	71.7 ± 5.2
60	22.4 ± 3.4	75.6 ± 1.9	28.9 ± 2.1	78.2 ± 3.5	44.4 ± 3.2	80.2 ± 2.4
90	26.1 ± 4.1	80.8 ± 2.4	32.4 ± 1.8	83.1 ± 2.4	48.4 ± 1.3	85.8 ± 0.8
Recovery [#]	35.4 ± 2.0	89.4 ± 2.1	41.6 ± 2.2	92.6 ± 1.2	58.2 ± 1.8	95.1 ± 1.4

*Mean ± SD, n=3; # at 200 rpm for additional 10 minutes

compared to the drug release from as such drug and spray drug alone. It was assumed that there are two mechanisms responsible for dissolution of entacapone. They are drug controlled and carrier controlled dissolution. The spray drying of non aqueous solution comprising drug substance dispersed lipid mixture resulted into more precise and smaller particles with higher surface area, thus enhancing the rate of drug release. The spray dried solid-lipid dispersions could have also improved the wettability of the drug and the localized solubilization by the lipid materials in the diffusion layer.

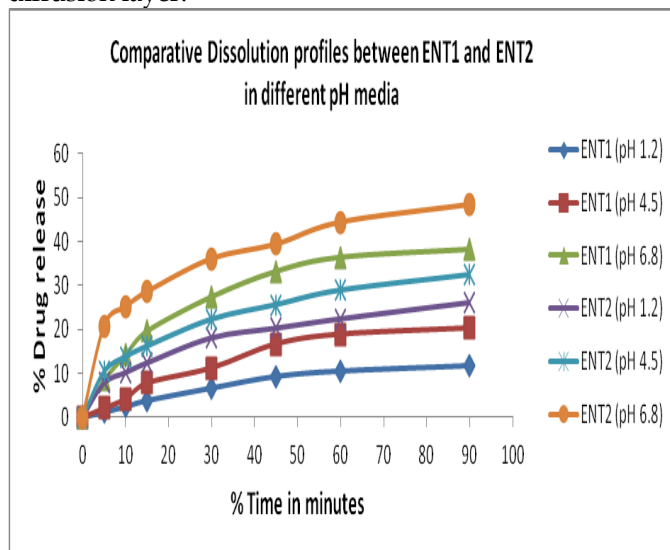


Fig. 6: Comparative Dissolution profiles of Entacapone (ENT1) and spray dried entacapone (ENT2) in 3 different pH media

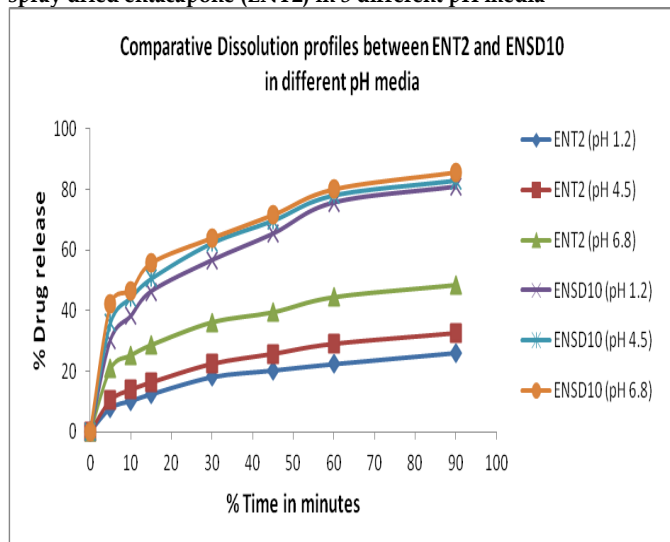


Fig. 7: Comparative Dissolution profiles of spray dried Entacapone (ENT2) and spray dried formulation (ENSD10) in 3 different pH media

From the *in vitro* dissolution profiles, it was clearly evident that the drug release from ENT1 and ENT2 has shown pH dependent dissolution profiles and found to be significantly on higher side in pH 6.8 phosphate buffer, but there was no complete drug release even after recovery in either of 3 media. There was no significant difference in the drug release profiles from spray dried powder formulation ENSD10 in 3 different media, but the drug release in pH 6.8 phosphate buffer

was approximately 10% on higher side as compared to the drug release in pH 1.2 (0.1 N HCl) up to 30 minutes. The reason can be attributed to the fact that entacapone has a pKa of 4.5 and is completely in the ionized form at pH 6.8. Hence the plain entacapone has shown higher dissolution in pH 6.8 phosphate buffer whereas in other conditions due to the unionized form of entacapone, the dissolution was very low. However the lipid-solid formulation has shown improved dissolution irrespective of pH condition. The improvement in the dissolution can be due to the fact the excipients and process used in the preparation of the lipid-solid dispersions resulted in the solubility of the drug independent of the pH condition. This study indicated the efficiency of the formulation in improving the dissolution in all pH conditions thereby the absorption of the entacapone throughout the GI tract can be expected.

And 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 is suggested to be the main site for absorption for entacapone. Solid dispersion preparation with lipids increased the dissolution of entacapone even at pH 1.2, pH 4.5 and 6.8 indicating a pH independent drug release. This clearly demonstrates that the preparation of Lipid-solid dispersions for entacapone with lipid excipients helped to overcome the incomplete pH dependent dissolution as a rate limiting step in its absorption process.

In the recent years the utilization of lipid excipients in the enhancement of oral bioavailability of poorly soluble drugs has become prominent. The efficacy of lipid excipients and solid dispersion formulations in improving the aqueous dissolution was demonstrated by taking entacapone as candidate drug. The entacapone is very poorly soluble and poorly intestinal permeable and belongs to BCS class IV. In the present work the solid dispersion formulations with lipid excipients for entacapone were prepared by spray drying process. For the selection of suitable lipid composition various experiments were performed and the mixture of Gelucire 50/13 and Compritol at a ratio of 1:3 was selected. The drug dispersion in the lipid mixture and spray drying resulted in the fully dried particles. The prepared entacapone solid lipid dispersion particles were duly characterized for particle size and its distribution, DSC evaluation for entacapone morphological conversion, FTIR study for evaluating the chemical compatibility between the drug and lipids and *In vitro* dissolution. The particle size distribution study has shown the lower particle size of the drug and the size distribution is very narrow. The DSC thermograms revealed the morphological conversion of the entacapone to amorphous form after spray drying. And the FTIR spectras have proven the chemical compatibility between the entacapone and the lipid mixture excipients. Based on the *in vitro* drug release, it

was clearly evident that there was a significant improvement in the rate of dissolution as compared to the drug release profiles of plain drug. Moreover interestingly the dissolution of entacapone from the formulation is pH independent and in all the pH conditions studied the solubility of spray dried powder comprising drug substance along Gelucire 50/13 and carrier Mannitol was higher than the plain drug and spray dried drug substance alone. This kind of dissolution trend is very useful in improving the oral absorption of the poorly soluble drugs.

The enhancement of aqueous solubility can be attributed to the factors such as reduced particle size, amorphous form of drug, increased solubility of drug by lipids. Hence it is concluded that the aqueous solubility of poorly soluble drugs can be increased by preparation of lipid-solid dispersions by spray drying using lipid excipients.

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