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Potential of microemulsified entacapone drug delivery systems in the management of acute Parkinson's disease

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ABSTRACT

Objective: To design solid self-microemulsifying drug delivery system (S-SMEDDS) of entacapone and evaluate for its anti-Parkinson's potentials.

Methods: Solubility studies were performed in various vehicles *i.e.*, oils, surfactants and co-surfactants and pseudo-ternary phase diagrams were plotted to understand the microemulsion formation region. Liquid self-microemulsifying drug delivery systems (SMEDDS) were developed using gingelly and rice bran oil as lipid vehicles, Tween 80 and Span 20 as surfactants and glycerin, propylene glycol as co-surfactants. They were characterized by Fourier transform infrared spectroscopy, pH, viscosity, zeta potential, polydispersibility index and droplet size analysis and evaluated for drug content, *in-vitro* release, *in-vitro* diffusion and *ex-vivo* permeation. Optimized liquid SMEDDS were converted into S-SMEDDS by adsorption and melt granulation procedures. Characterization by differential scanning calorimetry, SEM, micrometrics, reconstitution property, moisture content and evaluation by drug content, drug release kinetics and shelf-life were performed for S-SMEDDS. Parkinsonism was induced and pharmacodynamic potentials of S-SMEDDS were evaluated.

Results: S-SMEDDS formulation AG8 had shown the highest drug release of 90.92% within 60 min. Pharmacodynamic studies also proved the efficiency of entacapone S-SMEDDS against Parkinsonism.

Conclusions: Entacapone S-SMEDDS is an effective drug delivery system that offers more predictable and extensive drug release with enhanced shelf-life in the treatment of acute Parkinsonism.

1. Introduction

Parkinson's disease (PD) occurs at a prevalence of 52.85 per 100 000 with progressive disorder in the movements. The annual mortality rate was 2.89/100 000 approximately with an average risk rate of 8.98 deaths per year[1]. PD is due to the damage of dopaminergic neurons in nigro-striatal pathway resulted with dementia, depression and autonomic dysfunction. In the later stages of the disease, the non-motor symptoms often predominate. The chief symptoms of PD involve suppression of voluntary movements (hypokinesia), tremor at risk, usually starting in the hands that tend to reduce in voluntary action and muscle rigidness[2]. The first-line drugs used to treat PD are levodopa (dopamine precursor), selegiline (monoamine oxidase inhibitor), tolcapone and entacapone (catecholamine-o-methyl transferase-inhibitors), amantadine (drugs that release dopamine), bromocriptine, lysuride and ropinirole (dopamine receptor agonists).

Entacapone is a catecholamine-o-methyl transferase inhibitor belongs to Biopharmaceutics Classification System (BCS) class

IV with high lipophilicity[3]. It is an analogue of Tolcapone, claimed to be less hepatotoxic. It shows 98% protein binding, 0.4–0.7 h half-life, log p of 2.8, 35% bioavailability and exhibits hepatic metabolism. Its action is primarily peripheral with shorter duration of action compared to tolcapone. The main indication for this drug is to treat early 'end-of-dose' deterioration as it does not cause dyskinesia[4]. Apart from this, diarrhea, gastrointestinal problems like nausea and abdominal pains, dry mouth were frequently occurring side effects[5].

The solubility, dissolution and bioavailability of poorly soluble BCS class II and IV drugs can be improved by complexation with cyclodextrins[6], micronization, solid dispersions[7], solid mixtures and nanosuspension[8]. Development of lipid based formulations was one among them which lured a phenomenal attention of the researchers[9]. Enhanced permeation, versatility of lipidic excipients, low risk profile, high market potential pulls up the lipid based formulations, an alternative technique to administer the poorly soluble drugs effectively[10]. Self micro-emulsifying drug delivery systems (SMEDDS) is an efficient lipid based formulation[11] that enhances the bioavailability of hydrophobic drugs[12]. SMEDDS are isotropic, thermodynamically stable anhydrous mixture of drug, lipids, surfactants, co-surfactants which leads to the formation of fine o/w microemulsion upon gentle agitation when it intacts gastrointestinal fluids. SMEDDS are generally formulated as liquid dosage forms or solid dosage forms by incorporating into soft gelatin capsules. Encapsulation of SMEDDS in soft gelatin capsules leads to the economical burden in the manufacturing process due to increased fabrication expenditure[13].

Oral administration of SMEDDS enables the microemulsion formation spontaneously in gastrointestinal tract and thereby the formed small sized droplets present large interfacial area for the absorption of drug at the desired site of action. In these systems, dissolution phase can be eliminated due to presence of hydrophobic moiety in solution form or small lipid droplets which maintains the drug in the dissolved state throughout its transport to the intestinal membrane's aqueous unstirred layer[14]. When these SMEDDS are diluted with water, they can form the thermodynamically stable, transparent microemulsion droplets of size <50 nm[15]. The major advantage of SMEDDS is that the drug is maintained in dissolved form in gastrointestinal tract throughout its period[16]. Upon oral administration, peristaltic movements of stomach and small intestine provide the gentle agitation needed for self-emulsification *in-vivo*[17]. Despite of the advantages, SMEDDS also pose some limitations in terms of stability aspects, formulation methodology, possible interaction of formulation when filled in the capsule and storage aspects like temperature and humidity[18,19]. To overcome those potential problems, liquid SMEDDS were incorporated into solid carrier to convert them into solid SMEDDS (S-SMEDDS)[20]. The rationale selection of self-emulsifying formulation is based on solubility ability in vehicles, prediction of self emulsifying region from phase diagram and microemulsion droplet size.

S-SMEDDS offer many advantages such as low economic burden, high process control, better stability and good patient acquiescence when compared to liquid SMEDDS[21]. The major limitation of entacapone is its poor solubility and its minimum dose of 200 mg administered up to 8 times a day. To circumvent this limitation, SMEDDS becomes an effective method to reduce the dose and frequency of entacapone administration by enhancing its solubility and bioavailability. The present study was aimed to develop and characterize the entacapone S-SMEDDS and also to carry out preclinical evaluation.

2. Materials and methods

2.1. Materials

Entacapone was the gratuitous of Ms. Hetero Drugs Ltd., Hyderabad. Double refined gingelly, olive, coconut, palm, soybean and rice bran oils were procured from market. Isopropyl myristate, oleic acid, polyethylene glycol (PEG) 400, PEG 600, Tween 20, Tween 80, Span 80 and Span 20 were purchased from S.D. Fine Chemicals Ltd., Mumbai. Glycerin and propylene glycol were obtained from Merck Specialties Ltd., Mumbai. The other chemicals of analytical grade were used in the study.

2.2. Solubility study

To the 5 mL of different vehicles like oils, surfactants and co-surfactants, an excess quantity of entacapone was added. This blend was equilibrated at (37 ± 1) °C for 72 h. After equilibration, centrifugation was carried out for 20 min at 3 000 r/min and undissolved drug was removed by filtration using 0.45 μm membrane filter. Further quantification was done by diluting the supernatant with methanol and analyzed by UV–spectrophotometer (Shimadzu UV 1700).

2.3. Pseudo-ternary phase diagram construction

Pseudo-ternary phase diagrams were constructed by water titration method using oil, surfactant, co-surfactant and water at different possible combinations. Different ratios of oil to surfactant/cosurfactant were chosen in the series of 1:9 to 9:1. Then, water was added drop wise to the mixtures of oil and surfactant/co-surfactant of different ratios and agitation was provided using magnetic stirring until a homogenous mixture was obtained. The transparent mixture thus obtained were identified and considered as microemulsions[22].

2.4. Drug loaded liquid SMEDDS formulation

Liquid SMEDDS were formulated by using various ratios of oil (gingelly oil, rice bran oil), surfactant (Tween 80, Span 20), co-surfactant (propylene glycol, glycerin) and entacapone (50 mg). Initially, drug was dissolved in oil which was further added to surfactant and co-surfactants taken in glass vials. Gentle stirring and vortex mixing of the components at 37 °C was done till entacapone was completely dissolved. The mixture was then maintained at room temperature till its further usage for characterization[22]. The liquid SMEDDS composition was given in Table 1.

2.5. Selection of optimized liquid SMEDDS

The liquid SMEDD formulations (G1-G16 and R1-R16) were evaluated to estimate the drug content and drug release. The S-SMEDDS were prepared using liquid SMEDD formulations of promising drug release profiles. Liquid SMEDDS that had shown promised release profiles were subjected to characterization studies *viz.* pH, viscosity, globule size, zeta potential and polydispersity index.

Table 1
The composition of liquid SMEDDS.

Formulation	% Weight of oil	Surfactant: cosurfactant (Smix)	Smix ratio	% Weight of Smix
G1	9	Tween 80: propylene glycol	1:1	79
G2	8		1:2	65
G3	9		2:1	68
G4	6		2:3	69
G5	15	Tween 80: glycerin	1:1	60
G6	8		1:2	55
G7	15		2:1	58
G8	18		2:3	72
G9	5	Span 20: glycerin	1:1	48
G10	12		1:2	58
G11	11		2:1	48
G12	20		2:3	51
G13	9	Span 20: propylene glycol	1:1	79
G14	9		1:2	78
G15	18		2:1	68
G16	10		2:3	80
R1	7	Tween 80: propylene glycol	1:1	55
R2	5		1:2	50
R3	8		2:1	68
R4	8		2:3	65
R5	17	Tween 80: glycerin	1:1	68
R6	9		1:2	71
R7	11		2:1	75
R8	9		2:3	76
R9	7	Span 20: glycerin	1:1	61
R10	8		1:2	60
R11	9		2:1	71
R12	7		2:3	65
R13	8	Span 20: propylene glycol	1:1	68
R14	5		1:2	50
R15	4		2:1	45
R16	10		2:3	80

G: Gingelly oil; R: Rice bran oil.

2.6. Evaluation of liquid SMEDDS

2.6.1. Drug content analysis

One milliliter sample of liquid SMEDD formulations were diluted with methanol. Entacapone in the samples was quantified by spectrophotometrically at 310 nm after suitable dilutions.

2.6.2. In-vitro drug release studies of liquid SMEDDS

In-vitro drug release estimation of liquid SMEDDS were carried out by United States Pharmacopoeia dissolution apparatus type I at 50 r/min. A total of 900 mL of 0.1 mol/L HCl which was maintained at (37 ± 0.5) °C was used as the dissolution medium. An appropriate volume of liquid SMEDDS was filled into hard gelatin capsules of "size 0". Under the sink conditions, 5 mL of aliquot was withdrawn from the dissolution medium at predetermined regular intervals of time, and filtered through 0.45 µm nylon filter. The quantity of drug released from SMEDDS was quantified from drug absorbance analysis at 307.5 nm.

2.6.3. Diffusion studies

Diffusion studies were executed in a Franz diffusion cell using cellophane membrane as a barrier. The formulation was applied on the membrane placed between donor and acceptor

compartment. Constant stirring at (37 ± 1) °C was maintained by keeping the whole set up on a thermostatic magnetic stirrer. The aliquots were collected at periodical intervals of time up to 6 h. The receptor compartment was set for sink condition using phosphate buffer solution pH 7.4[23]. *Ex-vivo* studies were carried out by replacing cellophane membrane with goat intestine sac. The results were compared with that of pure drug.

2.6.4. Determination of flux

The cumulative amount of drug permeated was plotted against time and the angular coefficient of that curve provides the flux (J) value. The following equation was used to calculate the permeability coefficient (K_p)[24]:

$$K_p = J/C$$

where, C being the initial concentrations of drug in the SMEDDS formulation.

2.7. Characterization of liquid SMEDDS

2.7.1. Fourier transform infra-red spectroscopy (FTIR)

Drug and excipients compatibility was analyzed by using attenuated total resonance (ATR) FTIR spectrophotometer (Agilent CARY 630 ATR-FTIR). The spectra of entacapone, gingelly oil formulations (G8, G16), rice bran oil formulations (R8, R16) of liquid SMEDDS were recorded. A sample of material was placed on the diamond ATR crystal and analyzed by using Agilent resolutions pro software. Each spectrum of sample was collected from 32 single average scans at 4 cm^{-1} resolution in the absorption area of 600–4000 cm^{-1}.

2.7.2. pH and viscosity

A 10 mL liquid SMEDDS formulation was used to determine the pH by glass membrane electrode (ELICO LI 200, Hyderabad, India). Brookfield viscometer (spindle #63) of Middleboro, MA-LDLV-E model maintained at 10 r/min was used to determine the viscosity of SMEDDS.

2.7.3. Zeta potential and droplet size analysis

Excess amount of water was added to SMEDDS taken in a volumetric flask and the flask was inverted regularly for gentle mixing that results in fine emulsion formation. Zeta potential and droplet size, polydispersity index of formed microemulsions were determined by using zeta sizer (HSA 3000, Horiba Scientific, Singapore). The formula used to calculate the polydispersity index was:

$$Đ_M = M_w/M_n$$

where, M_w being the weight-average molar mass and M_n being the number-average molar mass.

2.8. Preparation of S-SMEDDS

S-SMEDDS was prepared by executing the techniques *i.e.*, adsorption and melt granulation. The compositions of S-SMEDDS are presented in Table 2.

2.8.1. Adsorption technique

Liquid SMEDDS containing entacapone was mixed with aerosil 200 that acts as adsorbent carrier to prepare S-SMEDDS.

Table 2
The composition of S-SMEDDS by adsorption and melt granulation methods.

Method	Formulation code	Liquid SMEDDS equivalent to 50 mg of drug	Aerosil 200 (mg)	PEG 2000 (mg)	Talc (mg)
Adsorption	AG8	G8	300	–	20
	AG16	G16	300	–	20
	AR8	R6	300	–	20
	AR16	R12	300	–	20
Melt granulation	MG8	G8	–	280	20
	MG16	G16	–	280	20
	MR8	R6	–	280	20
	MR16	R12	–	280	20

In this method, aerosil 200 was taken in a porcelain dish and liquid SMEDDS was added drop-wise onto the carrier. After that, for uniform distribution of individual formulation, homogenization was done using glass-pestle. Then the mixture was passed through sieve # 80 and then little quantity of talc was added to it and air-dried finally.

2.8.2. Melt granulation technique

Hydrophilic carrier, PEG 2000 was melted and to that molten mass liquid SMEDDS and stir it continuously so that solidification of the mixture takes place. Then the mixture was passed through sieve # 80 and talc was added. Both samples were stored in a vacuum desiccator.

The above dried products containing 50 mg entacapone equivalent were filled into "size 0" capsules. Before filling into capsules, the powders were subjected to micromeritic properties studies like bulk density, Hausner ratio and angle of repose[23,25].

2.9. Evaluation of S-SMEDDS

2.9.1. Reconstitution ability of S-SMEDDS

Dilution study was performed to determine the reconstitution ability of S-SMEDDS upon diluting with water that mimics the stomach environment of oral administration. Then 50 mg of S-SMEDDS was added to 50 mL of double distilled water maintained at 37 °C taken in a beaker and stirred continuously using magnetic stirrer. The propensity of emulsification and emulsion droplets formation were noted regularly with respect to time. When clear emulsion was formed after stopping of stirring, it was judged quantitatively "good" and when turbid or milky white emulsion was formed indicating drug precipitation upon dilution and it was judged as "not good emulsion"[26].

2.9.2. Drug content

The S-SMEDDS formulations were dissolved in methanol for 20 min and then filtered using 0.45 μm nylon filter. The filtrate upon suitable dilution was used for quantification by spectrophotometric analysis.

2.9.3. In-vitro drug release of S-SMEDDS

Dissolution studies of S-SMEDDS were carried out United States Pharmacopoeia type I dissolution apparatus to determine the *in-vitro* drug release. A total of 900 mL of 0.1 mol/L HCl maintained at (37.0 ± 0.5) °C was used as the dissolution medium and study was performed at 50 r/min. A 50 mg equivalent of S-SMEDDS encapsulated in hard gelatin capsules of size "0" was placed into the dissolution medium. Aliquot of 5 mL was collected at regular intervals of time and filtered. Amount of drug released was estimated by measuring absorbance at 307.5 nm.

2.9.4. Moisture content determination

The moisture content in the S-SMEDDS was estimated by accurately weighing 1 g of S-SMEDDS and dried at 100 °C for 4 h. Then the sample was allowed to cool and weight of dried sample was measured. The moisture content of sample is calculated using the following equation.

% Moisture content = (Weight of wet sample − Weight of dry sample)/Weight of dry sample × 100.

2.9.5. SEM of S-SMEDDS

The external morphology of entacapone and S-SMEDDS formulations (AG8 and MR8) was analyzed by a scanning electron microscope (Thermoscientific SU 1510). The formulations were adhered to the brass specimen club using double sided platinum coated electrically conductive adhesive tape for 300 s at 15 Ma.

2.9.6. Differential scanning calorimetry (DSC)

DSC analysis of entacapone and S-SMEDDS (AG8 and MR8) was performed using Perkin Elmer PYRIS 6. The S-SMEDDS samples were placed in the aluminum pan which was heated over a range of 35–350 °C at the rate of 10 °C/min. Nitrogen purging at a rate of 100 mL/min was done to maintain the inert atmosphere.

2.9.7. Stability of entacapone SMEDDS

Entacapone S-SMEDDS were sealed in "0" size capsules and kept in stability chambers at 75%, 85% and 96% relative humidity (RH) for about 3 months. Physical and chemical stabilities were evaluated using the samples withdrawn at 0, 1, 2 and 3 months. Phase separation, drug precipitation upon dilution with water was evaluated to determine the physical stability. Drug content estimation represents the chemical stability. Shelf life of the formulations were determined using the formula $t_{90} = 0.1052/K$ in which K means first-order rate constant.

2.10. Pharmacodynamic evaluation

Male Albino Wistar rats (150–200 g) were housed and acclimatized under standard laboratory conditions. They were divided into 6 groups containing 6 rats per group. Group 1 animals which received water only were considered as positive control. Animals in Group 2 were served as negative control and administered with chlorpromazine (CPZ) (3 mg/kg, *i.p.*). Animals of Group 3 were administered with CPZ (3 mg/kg, *i.p.*), L-dopa and carbidopa combination (LDC) (10 mg/kg, *i.p.*); Group 4 animals received CPZ (3 mg/kg, *i.p.*), LDC (10 mg/kg, *i.p.*) and entacapone (20 mg/kg, *p.o.*), Group 5 animals received CPZ (3 mg/kg, *i.p.*), LDC (10 mg/kg, *i.p.*), AG8 (20 mg/kg, *p.o.*) and Group 6 animals received

CPZ (3 mg/kg, *i.p.*), LDC (10 mg/kg, *i.p.*) and MR8 (20 mg/kg, *p.o.*).

2.10.1. Anti-Parkinson 's activity

Pharmacodynamic evaluation was carried-out by following CPZ induced Parkinson's model for 21 days. Behavioral (catalepsy score) and biochemical parameters estimations (lipid peroxidation, nitrite levels, reduced glutathione, catalase) were done to support the study. All experimental procedures involving animals were conducted in accordance to Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines and approved by Institute Animal Ethics Committee of the Sree Vidyanikethan College of Pharmacy, Tirupati, India with reference No.: 930/a/06/CPCSEA.

Catalepsy score was determined by block method. Score of 0.5 is assigned when the rats fail to move about by touching or gently pushing after placing upon the table. Total score of 1 is allotted in the 2nd stage, for each paw (0.5) if it failed to restore the position of front paws within 15 s after placing on 3 cm block. In the 3rd stage a score of 2 is given, for each paw (1) if posture retention is not attained within 15 s after placing the front paws on 9 cm block. Cut off score for catalepsy is 3.5[27].

Lipid peroxidation, reduced glutathione, nitrite and catalase were estimated by following Wills, Ellman, Griess reagent assay, Beers and Sizer method, respectively. Lipid peroxidation was analyzed from thiobarbituric acid reactive substances (TBARS) levels. TBARS was estimated by treating the rat brain tissue homogenate with thiobarbituric acid–trichloro acetic acid (TBA–TCA) reagent. The homogenate mixture was heated for 15 min, then cooled and centrifuged for 10 min. The colored supernatant was analyzed spectrophotometrically at 532 nm against blank[28].

Reduced glutathione was estimated by following method. In this method, the brain tissue homogenate (1 mL) was precipitated by addition of 1 mL of 10% TCA and centrifuged to collect supernatant portion. The supernatant portion was added with 4 mL of phosphate solution and 0.5 mL of 5,5′-dithiobis-(2-nitrobenzoic acid) reagent and analyzed at 412 nm[29].

Increased oxidative-stress in brain can lead to brain tissue damage and in turn produces the nitric oxide. Whereas produced nitric oxide in turn oxidized spontaneously to yield nitrite and nitrate. Thus nitrite levels were estimated from nitric oxide production in this work. Brain tissue homogenate and Griess reagent of equal volumes were incubated for 10 min and analyzed at 548 nm to determine the nitrite levels[30].

Catalase levels were estimated based on the principle of hydrogen peroxide decomposition by the catalase enzyme evidenced with the reduction of absorbance with time. In this method, a homogeneous mixture was prepared by combining brain tissue homogenate (0.1 mL), 50 mmol/L phosphate buffer (1.9 mL) and 30 mmol/L hydrogen peroxide (1 mL) and its absorbance was noted down at 240 nm initially and after 3 min. The variation in the absorbance values was used to estimate the catalase levels[31].

2.11. Statistical analysis

Statistical analysis of the obtained data was performed by Kruskal Wallis ANOVA followed by Dunnet's test and *P* < 0.05 was set as a level of significance.

3. Results

3.1. Solubility study

As drug solubilization capacity of individual vehicle is a key determinant for the formulation of liquid SMEDDS, solubility of entacapone in different vehicles were tested to select the suitable vehicles and the results are shown in Figure 1. Entacapone exhibited maximum solubility in gingelly oil, rice bran oil (oily phases), Tween 80, Span 20 (surfactants) and propylene glycol, glycerin (co-surfactants) which were further selected as components for SMEDDS formulation.
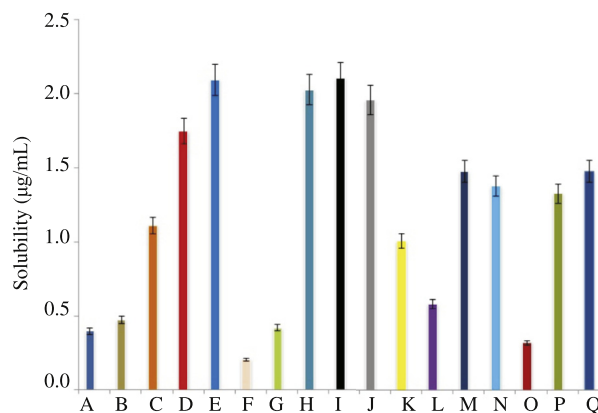

Figure 1. Solubility of entacapone in various vehicles.
A: Isopropylmyristate; B: Olive oil; C: Coconut oil; D: Palm oil; E: Rice bran oil; F: Oleic acid; G: Sunflower oil; H: Soybean oil; I: Gingelly oil; J: Propylene glycol; K: PEG 400; L: PEG 600; M: Glycerin; N: Span 20; O: Span 80; P: Tween 20; Q: Tween 80.

3.2. Pseudo-ternary phase diagrams

The pseudo-ternary diagrams were plotted as per the compositions given in Table 1. Increase in the concentrations of surfactant/co-surfactant results in the increase of microemulsion region and their phase diagrams were represented in Figure 2. Liquid SMEDDS were prepared by adding 50 mg of entacapone to the oil and surfactant mixture ratio that were selected from the pseudo-ternary phase diagram.

3.3. Evaluation of liquid SMEDDS

3.3.1. FTIR
FTIR studies of entacapone and selected liquid SMEDDS (G8, G16, R8 and R16) were shown in Figure 3.

3.3.2. Drug content
The amount of drug present in liquid SMEDDS was assessed by methanol dilution. The drug content of entacapone SMEDDS was between 82% and 98% for both gingelly oil and rice bran oil formulations. The drug content values were given in Table 3.

3.3.3. In-vitro drug release
From the formulations 85%–95% of drug release was observed by the end of 60 min. G8 formulation showed higher
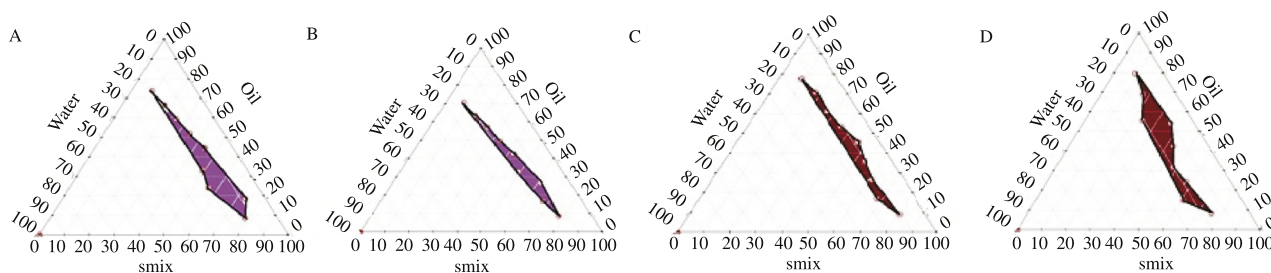
Figure 2. Triplots of optimized liquid SMEDDS formulation.
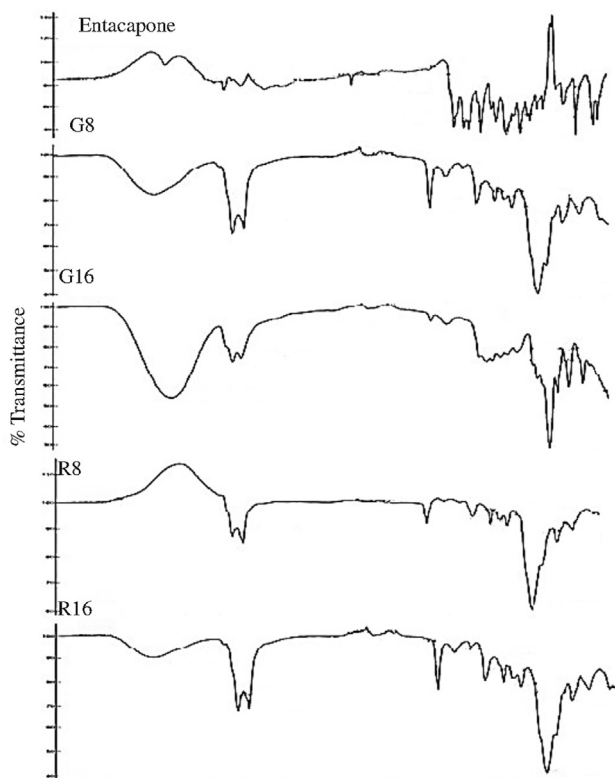A: G8; B: G16; C: R8; D: R16.

Figure 3. Comparative FTIR spectra of entacapone and SMEDDS.

Table 3

Percent drug content of the liquid SMEDDS.

Formulation code	% Drug content	Formulation code	% Drug content
G1	82.03 ± 0.64	R1	86.16 ± 0.62
G2	86.12 ± 0.32	R2	88.09 ± 0.32
G3	86.06 ± 0.48	R3	86.31 ± 0.57
G4	90.25 ± 0.59	R4	95.42 ± 0.68
G5	88.12 ± 0.46	R5	82.18 ± 0.36
G6	82.31 ± 0.43	R6	94.53 ± 0.52
G7	83.50 ± 0.58	R7	89.25 ± 0.47
G8	98.19 ± 0.26	R8	94.05 ± 0.49
G9	83.12 ± 0.43	R9	89.12 ± 0.54
G10	84.08 ± 0.32	R10	91.35 ± 0.32
G11	87.32 ± 0.48	R11	86.24 ± 0.38
G12	89.52 ± 0.36	R12	95.48 ± 0.59
G13	91.16 ± 0.27	R13	85.16 ± 0.46
G14	84.09 ± 0.62	R14	87.13 ± 0.38
G15	89.18 ± 0.57	R15	88.51 ± 0.46
G16	95.23 ± 0.32	R16	95.23 ± 0.29

Results were expressed as mean ± SD ($n = 6$).

drug release of 96.42% than other gingelly oil formulations. R8 formulation showed higher drug release of 88.24% than other rice bran oil formulations. G8, G16, R8 and R16 had shown maximum release of 96.63%, 94.42%, 88.24% and 86.32%, respectively than the other formulations in 60 min. The drug release patterns from G1-G16 and R1-R16 formulations were illustrated in Figure 4. At the end of 30 min of dissolution study, all liquid SMEDDS have shown 50% drug release, which indicates that the microemulsions had been formed with good globule size in range of 5–100 nm.

3.3.4. In-vitro diffusion studies

The *in-vitro* diffusion process revealed the amount of drug perfusion through the membrane passively and the availability of drug at the site of application. Study was conducted for 6 h and the samples were analyzed at 307.5 nm. The results were depicted in Figure 5 and Table 4.

Table 4

In-vitro and *ex-vivo* diffusion studies for liquid SMEDDS.

Formulation	*In-vitro* studies		*Ex-vivo* studies	
	Flux ($\mu g/cm^2/h$)	Diffusion coefficient (cm/h)	Flux ($\mu g/cm^2/h$)	Permeability coefficient (cm/h)
Entacapone	1.400	2.8×10^{-2}	1.000	2.0×10^{-2}
G8	2.407	4.8×10^{-2}	1.881	3.7×10^{-2}
G16	2.152	4.3×10^{-2}	2.050	4.1×10^{-2}
R8	1.964	3.9×10^{-2}	1.673	3.3×10^{-2}
R16	1.923	3.8×10^{-2}	1.700	3.4×10^{-2}

3.3.5. Ex-vivo studies

Permeation studies were performed to estimate the formulations' capacity to cross bio-membrane. The permeation study was conducted for 6 h and the results are shown in Figure 6. In this study the amount of drug permeated through intestinal membrane was more in SMEDDS formulation than that of pure drug as per the flux and permeability coefficient values demonstrated in Table 4.

3.4. Characterization of optimized liquid SMEDDS for physical properties

3.4.1. pH and viscosity

The pH of the formulations was almost neutral *i.e.* 7 owing to the exploitation of neutral and/or non-ionic excipients in the formulation which made them suitable for oral use. The
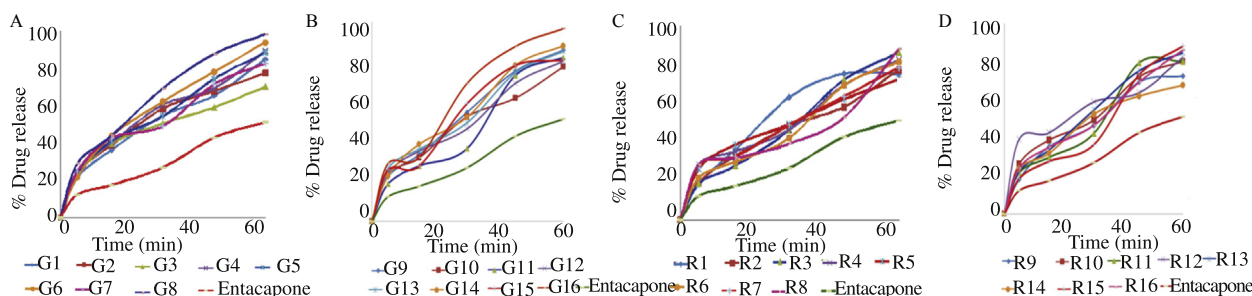
Figure 4. Percent of drug release of liquid SMEDDS.

A: % Drug release of liquid SMEDDS from G1-G8; B: % Drug release of liquid SMEDDS from G9-G16; C: % Drug release of liquid SMEDDS from R1-R8; D: % Drug release of liquid SMEDDS from R9-R16.
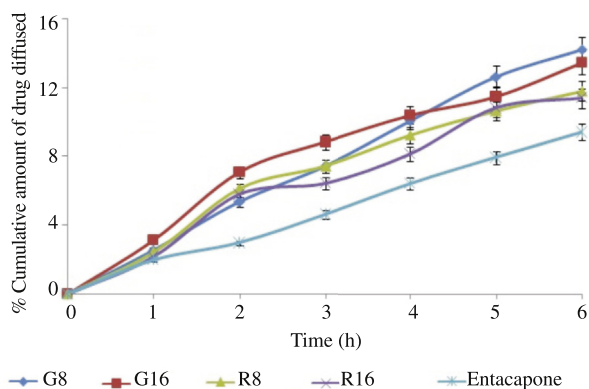

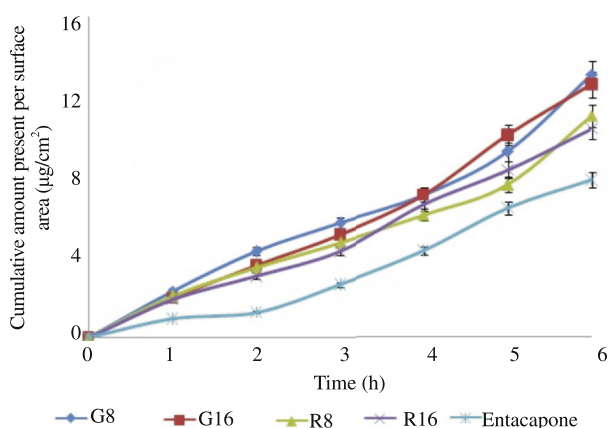**Figure 5.** Drug diffusion profile of liquid SEEDDS formulations compared with entacapone.

Figure 6. Cumulative amount of drug present surface area of SMEDDS formulation compared with entacapone.

viscosity of the liquid SMEDDS was in the range of 218–225 cps. The pH and viscosity values were tabulated in Table 5.

Table 5

Physicochemical properties of selected liquid SMEDDS.

Formulation	pH	Viscosity (cps)	Droplet size (nm)	Polydispersity index	Zeta potential (mV)
G8	7.1	223.20	59.7	3.274	−27.0
G16	7.2	225.03	5.3	10.000	−45.6
R8	7.0	220.12	103.0	1.963	−43.4
R16	7.1	218.15	144.2	2.963	−29.9

3.4.2. Droplet size and zeta potential

The droplet size, zeta potential and polydispersity index were given in Table 5. Droplet size of the formulations exhibited a

versatile range from 5.3 to 144.2 nm. Polydispersity index was in the range of 1.963–10.000. The particle size analysis results specified that the emulsion droplets were not distributed uniformly. The R16 formulation of S:CoS (2:3) ratio containing propylene glycol as co-surfactant exhibited a large size due to its high oil content. Zeta potential values were in the range of −27.0 to −45.6 mV in diluted form.

3.5. Evaluation of S-SMEDDS

3.5.1. Micromeritic properties

Micromeritic properties of S-SMEDDS of entacapone like bulk, tapped density and Hausner ratio were assessed. The adsorption technique powder had bulk density of 0.76–0.86 g/cm^3 and Hausner ratio was in the range of 1.11–1.16. The bulk density of melt granulation S-SMEDDS was observed to be in between 0.82 and 0.86 g/cm^3 and Hausner ratio in between 1.10 and 1.21 as shown in Table 6. The angle of repose was in the range of 28° to 34° by adsorption technique and 32° to 41° by melt granulation techniques as shown in Figure 7.

Table 6

Micromeritics and reconstitution time of S-SMEDDS.

Formulation	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Hausner ratio	Reconstitution time (min)
AG8	0.83 ± 0.54	0.95 ± 0.08	1.14	2.5
AG16	0.84 ± 0.48	0.94 ± 0.11	1.11	2.0
AR8	0.76 ± 0.19	0.86 ± 0.43	1.13	2.0
AR16	0.86 ± 0.22	0.89 ± 0.18	1.16	1.8
MG8	0.80 ± 0.09	0.97 ± 0.23	1.21	2.2
MG16	0.84 ± 0.12	0.94 ± 0.17	1.10	2.3
MR8	0.86 ± 0.31	0.87 ± 0.32	1.12	2.0
MR16	0.82 ± 0.45	0.96 ± 0.26	1.17	2.1

Values were expressed as mean ± SD (n = 3).

3.5.2. Reconstitution properties of S-SMEDDS

Reconstitution time for S-SMEDDS formulations ranges from 1.8 to 2.5 min as represented in Table 6 indicating spontaneous microemulsion formation. Phase separation or phase inversion of microemulsion was not evidenced after 2 h.

3.5.3. Drug content of S-SMEDDS

The drug content in AG8, AG16, AR8, AR16, MG8, MG16, MR8 and MR16 formulations was varied between 81% and 95%. More entacapone was present in S-SMEDDS of adsorption than S-SMEDDS of melt granulation due to higher specific surface of aerosol 200. The AG8 formulation had the highest

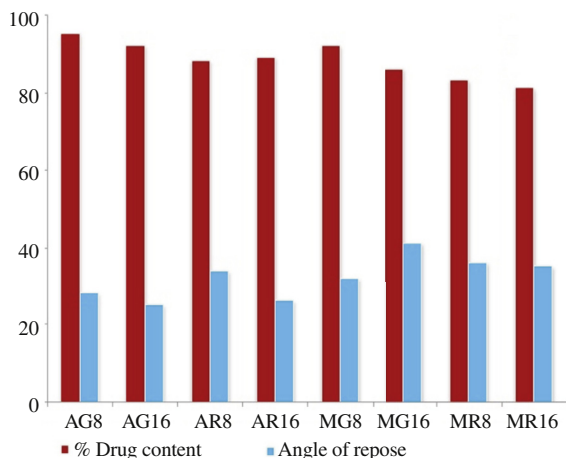drug content in S-SMEDDS prepared by both methods as represented in Figure 7.

Figure 7. Angle of repose and percent drug content of S-SMEDDS.

3.5.4. In-vitro drug release from S-SMEDDS

Dissolution study was performed for AG8, AG16, AR8, AR16, MG8, MG16, MR8 and MR16 S-SMEDDS formulations. A highest release of 90.56% was obtained in AG8 formulation by adsorption method which in-turn composed of glycerin as a co-surfactant followed by AG16 (86.63%), AR8 (82.48%), AR16 (80.62%). The highest release of 86.32% was obtained with MG8 formulation of melt granulation followed by MR8 (82.48%), MR16 (78.62%), MG16 (74.34%) and entacapone (51.21%) by 60th min as depicted in Figure 8. The drug release profiles from the S-SMEDDS were fitted to various kinetic models to know their release mechanism. Correlation coefficient value (r) was found predominant for first-order and Hixson–Crowell model as tabulated in Table 7, representing the
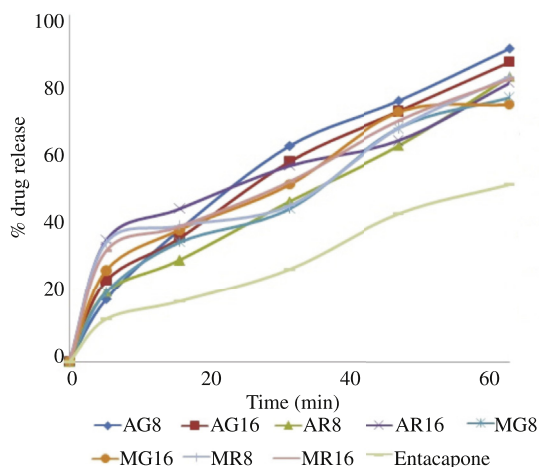

Figure 8. Percent of drug release from S-SMEDDS.

release of entacapone from S-SMEDDS followed varied release mechanisms.

3.5.5. Moisture content

This test was performed to find out the moisture content of S-SMEDDS. AG8 S-SMEDDS showed moisture content as 42.0% and MR8 formulation showed 66.6%. The moisture content determination was done to assess the moisture content in prepared S-SMEDDS.

3.5.6. SEM analysis

SEM study of entacapone, S-SMEDDS formulations AG8 and MR8 were carried out and results were evidenced that the crystalline nature of entacapone; AG8 and AR8 were existed as loose aggregates with smooth texture as depicted in Figure 9.
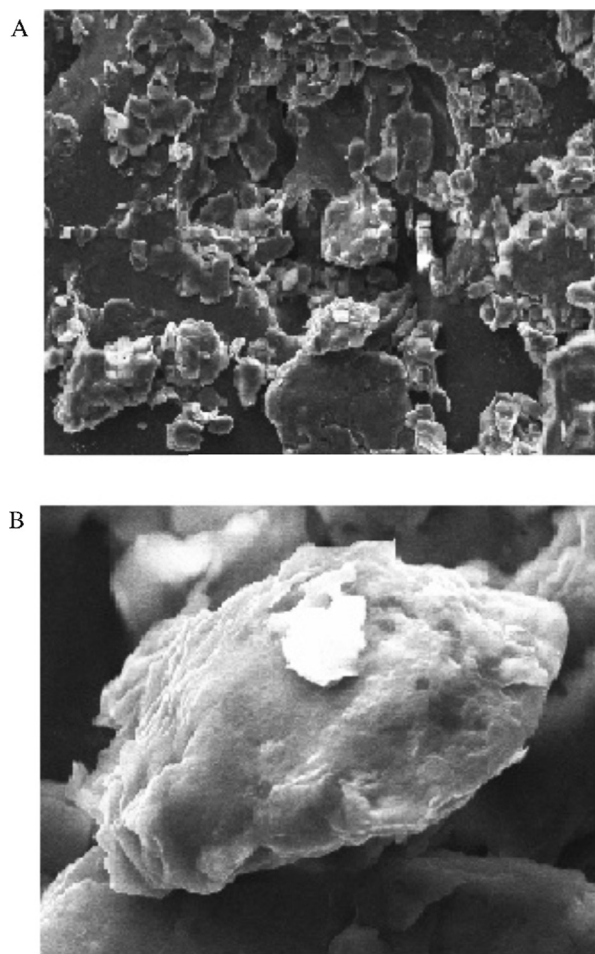
Figure 9. SEM images of SMEDDS.
A: AG8 formulation; B: MR8 formulation.

Table 7
Release kinetics of optimized S-SMEDDS.

Model		Adsorption				Melt granulation			
		AG8	AG16	AR8	AR16	MG8	MG16	MR8	MR16
Zero order	r	0.8526	0.8628	0.8556	0.8603	0.8612	0.8432	0.8413	0.8406
First order	r	0.9962	0.9954	0.9982	0.9842	0.9886	0.9823	0.9745	0.9912
Higuchi	r	0.9878	0.9899	0.9942	0.9876	0.9885	0.9924	0.9918	0.9826
Peppas	r	0.9228	0.9611	0.9222	0.9568	0.9226	0.9752	0.9825	0.9868
Baker–Lonsdale	r	0.9665	0.9732	0.9782	0.9716	0.9632	0.9428	0.9724	0.9684
Erosion	r	0.9816	0.9924	0.9821	0.9818	0.9762	0.9808	0.9728	0.9624
Hixson–Crowell	r	0.9826	0.9948	0.9912	0.9918	0.9823	0.9826	0.9845	0.9910

3.5.7. DSC studies

The thermographs of entacapone, AG8 and MR8 formulations had shown peaks at 139 °C to 293 °C indicating the variance in solid state characteristics, supporting the solubility enhancement in SMEDDS formulations. The thermographs depicted in Figure 10 exhibited a change in melting point of the drug showing the effect of surfactant mixture.

3.5.8. Stability study

The stability aspects of optimized S-SMEDDS (AG8 and MR8) were carried out at various storage conditions for 90 days at 75%, 85% and 96% RH according to International Conference on Harmonization guidelines. The capsules were investigated for physical and chemical stability by observing physical integrity, drug content and *in-vitro* drug release at the end of 15, 30, 60 and 90 days and the data were presented in Table 8.

3.5.9. Pharmacodynamic evaluation

Chlorpromazine induced Parkinson's model was followed and anti-Parkinson potentials of entacapone in S-SMEDDS were evaluated by observing the catalepsy score, lipid peroxidation, reduced glutathione, nitrite and catalase levels and the results were reported in Table 9.

Increase in catalepsy score was observed in Group 2 animals that received CPZ. Significant reduction in the catalepsy score was noticed in all the groups (3, 4, 5 and 6) at a level of
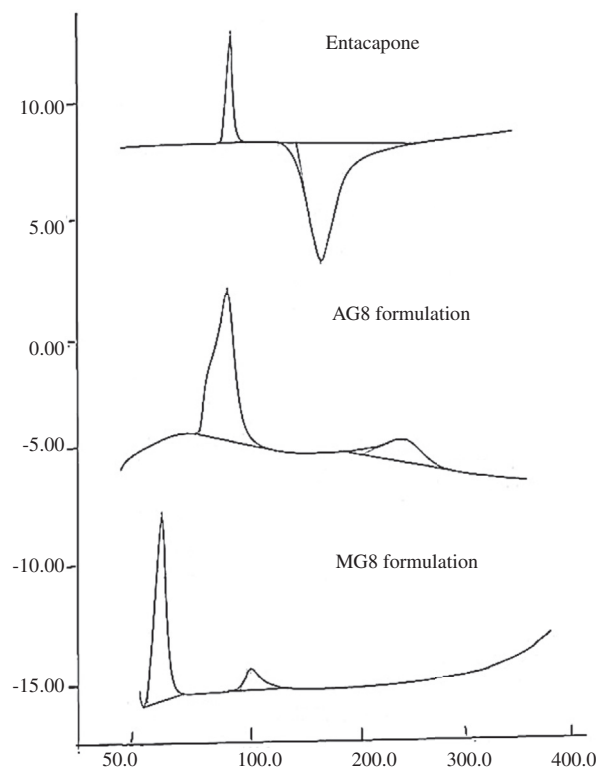

Figure 10. DSC thermograms of entacapone, AG8 and MR8 formulations.

Table 8

Stability study data of S-SMEDDS.

Conditions			Formulations	15 days	30 days	60 days	90 days
Physical appearance		75% RH	AG8	Intact	Intact	Intact	Slight elongation
			MR8	Intact	Intact	Intact	Slight elongation
		85% RH	AG8	Intact	Intact	Slight elongation	Elongated
			MR8	Intact	Intact	Slight elongation	Elongated
		96% RH	AG8	Intact	Slight elongation	Elongated	Elongated
			MR8	Intact	Slight elongation	Elongated	Elongated
Chemical stability	Drug content (%) mean ± SD ($n = 3$)	75% RH	AG8	87.12 ± 0.59	87.22 ± 0.33	84.14 ± 0.51	83.24 ± 0.45
			MR8	83.04 ± 0.37	83.17 ± 0.18	84.12 ± 0.69	83.15 ± 0.31
		85% RH	AG8	87.17 ± 0.56	87.11 ± 0.65	79.09 ± 0.33	75.13 ± 0.94
			MR8	83.23 ± 0.54	81.07 ± 0.72	76.16 ± 0.48	75.36 ± 0.54
		96% RH	AG8	87.42 ± 0.37	85.16 ± 1.23	69.47 ± 0.56	64.24 ± 0.64
			MR8	83.29 ± 0.15	79.22 ± 0.21	70.48 ± 0.45	63.42 ± 0.48
	Drug release (%) mean ± SD ($n = 3$)	75% RH	AG8	90.52 ± 0.58	90.02 ± 0.08	90.42 ± 0.28	89.62 ± 0.28
			MR8	80.22 ± 0.45	81.22 ± 0.11	80.12 ± 0.42	80.12 ± 0.42
		85% RH	AG8	90.52 ± 0.68	90.52 ± 0.78	89.27 ± 0.68	89.27 ± 0.68
			MR8	80.25 ± 0.56	80.23 ± 0.12	80.10 ± 0.20	80.10 ± 0.20
		96% RH	AG8	90.62 ± 0.80	90.62 ± 0.08	89.24 ± 0.18	88.24 ± 0.68
			MR8	81.22 ± 0.31	81.62 ± 0.16	81.08 ± 0.26	81.06 ± 0.21

Table 9

Behavioral and biochemical assessment.

Groups	Catalepsy score	TBARS (nmol/L/mg of protein)	GSH (nmol/L/mg of protein)	Nitrite (nmol/L/mg of protein)	Catalase (µmol/L/mg of H_2O_2 degraded/min)
Group 1	0.0000 ± 0.0000	27.7700 ± 0.7583	18.1500 ± 0.6489	4.5200 ± 1.0478	103.0500 ± 0.5889
Group 2	2.5800 ± 0.5147[*]	44.1500 ± 0.7189[*]	6.2400 ± 0.8946[*]	16.2500 ± 0.3475[*]	74.8700 ± 0.4821[*]
Group 3	1.2500 ± 0.3942[*,a]	37.9000 ± 0.7965[*,a]	10.520 0 ± 1.2470[*,a]	12.4800 ± 0.9473[*,a]	97.6100 ± 1.0415[*,a]
Group 4	1.0800 ± 0.9731[*,a]	34.2500 ± 0.6245[*,a]	14.9800 ± 0.5874[*,a]	10.530 0 ± 1.1023[*,a]	114.940 0 ± 0.2532[*,a]
Group 5	0.5800 ± 0.8327[*,a,b]	27.8200 ± 1.3001[*,a,b]	20.7400 ± 0.9127[*,a,b]	6.2800 ± 0.6471[*,a,b]	126.8500 ± 1.2133[*,a,b]
Group 6	0.6600 ± 0.7983[*,a,b]	31.5300 ± 0.8112[*,a,b]	16.2900 ± 0.6382[*,a,b]	7.0600 ± 0.8419[*,a,b]	121.1100 ± 0.0001

[*]: $P < 0.05$ statistically significant compared to Group 1 (positive control); [a]: $P < 0.05$ statistically significant compared to Group 2 (CPZ treated-negative control); [b]: $P < 0.05$ statistically significant compared to Group 4 (treated with pure drug-entacapone).

$P < 0.05$. However, the AG8 had shown a remarkable decrease in the levels of catalepsy score. Significantly reduced TBARS and nitrate levels and augmented glutathione and catalase levels were observed in the groups treated with AG8 and MR8 compared to group received pure entacapone at a level of $P < 0.05$. S-SMEDDS of entacapone exhibited high free radical scavenging activity against nitric oxide and reduced the levels of TBARS compared to pure entacapone.

4. Discussion

The preferred oils *i.e.* rice bran oil and gingelly oil has similar hydrophile-lipophile balance values of 7, due to which the drug exhibited higher solubility in them. Compared with entacapone, all the physical mixtures showed slight change in their wave numbers of characteristic drug peaks which are considered as insignificant. Upon FTIR results obtained, it was confirmed that drug has not undergone any chemical interaction with excipients.

The drug content results were evident that there was no significant drug loss in formulation of SMEDDS. Small amounts of drug loss might be lost due to higher side viscosities of SMEDDS and also due to transfer of the formulations. Due to their submicron droplet size they could diffuse more intensively through biomembrane. After 1 h, entire drug was expected to be available in systemic circulation, since the drug is in the mo-lecular form and therefore, solubility was not a criterion.

The formulations at 2:3 S:coS ratio had shown more drug release than the other ratios of S:coS formulations due to more concentration of surfactant which consequences significant interfacial tension reduction. The enhanced drug release was referred to their higher co-surfactant concentration owing to the liquefaction of the interfacial film and increasing the film flex-ibility to form globule of low size than higher surfactant for-mulations[32]. The drug release had shown variations due to the solubilization of surfactants and co-surfactants used. Glycerin being a short chain alcohol, when used as a co-surfactant had shown greater particle size reduction. Less hydrophilic glycerin sets the hydrocarbon chains of Tween 80 to align towards oil phase between their moieties ensuing the effective van der Waal attraction, which forms strong and stable film opposing coales-cence. Propylene glycol with less –OH groups, has a constraint in decreasing interfacial tension to remarkable low level along with Tween 80. At higher concentrations, co-surfactant provides more flexibility to the formulations.

Diffusion study demonstrated that the amount of drug diffused/permeated along the membrane was high in formulated SMEDDS than that of pure drug and the results of flux and permeability coefficient were tabulated as Table 4. The G8 formulation showed sustained release when compared with other formulation and entacapone. According to results obtained, G8 had shown the enhanced permeability when compared with other formulations and even with pure entacapone. The improvement in diffusion and permeation was because of the microemulsion droplets formation, which reduced the interfacial tension owing to the presence of Tween 80 (hydrophile–lip-ophile balance-15).

Zeta potential was found to be less as liquid SMEDDS contain non-ionic surfactants like Tween 80 and Span 20 that could diminish the interactions among globules by steric forces of repulsions rather than electrokinetic approach. Negative zeta

potential values of SMEDDS are due to presence of lipid phases attributed to the stability maintenance of SMEDDS.

The outcome of micromeritic analysis indicated that they simulate good flow properties. Compared to melt granulation technique, adsorption technique S-SMEDDS showed best results due to the adsorbent carrier Aerosil 200. The carriers used in melt granulation being waxy in nature consequences poor flow. Manufacturing of S-SMEDDS by melt granulation required the addition of glidant to enhance the flow overcoming the hurdles in production arena.

The faster drug release of entacapone from S-SMEDDS might be due to spontaneous production of microemulsion. Higher drug release was observed for adsorption based S-SMEDDS than melt granulation S-SMEDDS. The major reason for this was the behavior of the carrier while contacting water. In adsorption method based S-SMEDDS, liquid SMEDDS gets adsorbed onto the carrier surface and lies in the voids of surface until it contacts the water. Once liquid SMEDDS contacts with water it forms microemulsions immediately. Whereas in melt granulation based S-SMEDDS, the drug gets mixed up with the molten hydrophilic carrier and solubilization of carrier is required prior to the drug release which in turn depends on the thickness of polymer matrix. Neither methodology nor the types of carriers used in S-SMEDDS had significant effect on the globule size.

The higher moisture content in MR8 formulation was due to the hydrophilic carrier *i.e.* PEG 4000. The low moisture content in AG8 formulation was due to adsorbent carrier *i.e.* Aerosil 200 which is in inert nature.

The S-SMEDDS' SEM images represented that they possess smooth texture with uniform surface supporting the adsorption of entacapone onto the carrier properly.

In the stability studies at 96% RH conditions, due to moisture absorption by the capsule shell, they lost their integrity which consequences a negligible drug loss that results in insignificant weight change of capsules. Shelf-life of AG8 and MR8 was found to be 1.75 and 1.05 years respectively. Thus S-SMEDDS formulations proffer greater stability with enhanced shelf-life.

Entacapone is a selective reversible catecholamine-o-methyl transferase inhibitor used in combination with levodopa. The neuropathophysiology of Parkinsonism is related to the gener-ation of free radicals and oxidative stress. Induction of Parkin-sonism by CPZ led to increase in catalepsy score, lipid peroxidation (represented as TBARS), nitrite levels and decreased levels of glutathione and catalase. Test group animals were treated with LDC, LDC/entacapone, LDC/AG8 and LDC/MR8 to explore the anti-Parkinson's potentials of S-SMEDDS.

It was found from the results that S-SMEDDS formulation of AG8 had exhibited significant anti-Parkinson potentials when compared to MR8 formulation and entacapone which is due an immediate release of entacapone from S-SMEDDS prepared by adsorption method.

Entacapone being BCS class IV category poses very poor oral bioavailability. Liquid SMEDDS of entacapone were designed to overcome these hurdles based on its solubility in oil, surfactant and co-surfactant. The highest drug release was observed from the formulations having higher co-surfactant concentration due to the less particle size. The optimized liquid SMEDDS based on drug release were formulated into S-SMEDDS by employing methodologies *i.e.*, adsorption and melt granulation. Drug release from S-SMEDDS prepared by adsorption method was effective compared to melt granulation

method. Hence, Aerosil 200 as a solid adsorbent carrier is efficient in the formulation of S-SMEDDS to promote the rate of dissolution and intestinal permeability. The drug release from the liquid formulations is more than that of solid forms. This could be due to the existence of drug in dispersed form at molecular state in liquid SMEDDS whereas it was not in S-SMEDDS. The solid forms have to undergo the dissolution process and hence it was not instantaneous. The disadvantages of the liquid SMEDDS, such as the phase separation and leaching could be overcome by selecting and designing them into the solids. The solid form of SMEDDS provided better stability in shelf. The results were well supported by the pharmacodynamic studies in which S-SMEDDS had proven to be effective against Parkinsonism. In general, S-SMEDDS present advantages with accuracy of dose, shelf-life of formulation and more patient compliance when compared to liquid SMEDDS. Further, S-SMEDDS formulation technologies can be forwarded for pilot scale process and be fortified with clinical research.

Conflict of interest statement

The authors report no conflict of interest.

Acknowledgments

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