International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Issue 3, 2022

Original Article

DONEPEZIL HYDROCHLORIDE LOADED SOLID LIPID NANOPARTICLES: FORMULATION, *IN VITRO–IN VIVO* PHARMACOKINETIC AND PHARMACODYNAMICS EVALUATION

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Received: 15 Jan 2022, Revised and Accepted: 25 Feb 2022

ABSTRACT

Objective: The aim of the present study was to design and *in vitro–in vivo* evaluation of Donepezil Hydrochloride Solid Lipid Nanoparticles (DHSLN).

Methods: A modified solvent injection method was used to produce Donepezil-loaded solid lipid nanoparticles. A Response Surface Method 3-factor, 2-level Box-Behnken design was applied to study the effect of independent variables on dependent variables. Then it was coated with tween 80 for ease of permeability through the blood-brain barrier due to intact absorption of solid lipid nanoparticles. The prepared SLN was evaluated for particle size, zeta potential analysis, Entrapment efficiency, *In vitro* drug release study, Field Emission-scanning electron microscopy, *In vivo* Pharmacokinetic and Pharmacodynamics studies.

Results: The results of coated optimized formulation showed an average particle size of 185.8 nm, entrapment efficiency of 78.52±2.54%, and *in vitro* drug release of 98.62±3.14% at 36h at pH 7.4. The pharmacokinetic data show higher C_{max} and improved bioavailability, which was also supported by behavioural changes observed in locomotor activity for surface-modified SLN formulation.

Conclusion: Thus, the current study successfully designed, developed an optimized SLN formulation. The surface-modified SLN proved to enhance the permeability of the drug through barrier, which led to the enhancement of Donepezil bioavailability and locomotor activity.

Keywords: Solid lipid nanoparticle, Optimization, Donepezil HCl, Particle size, Entrapment efficiency

DOI: https://dx.doi.org/10.22159/ijap.2022v14i3.44145. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Solid Lipid Nanoparticles are colloidal particles with a size between 50 to 1000 nm. They're made up of phospholipids in nanometric range with high loading efficiency and are designed to improve drug delivery for lowering drug toxicity and good stability in biological fluids. Surface modified Solid Lipid Nanoparticles are at the forefront of the rapidly emerging field of nanotechnology, with a wide range of potential uses in drug delivery, clinical treatment, research, and other domains. Lipid nanoparticles provide the opportunity for developing new therapies due to their unique size-dependent characteristics. The capacity to include pharmaceuticals into nanocarriers creates a new drug delivery concept that can be employed for secondary and tertiary drug targeting [1-4].

Poor drug loading capacity, drug expulsion following polymeric transition during storage, and relatively high water content of the dispersion (70-99.9%) have all been identified as potential drawbacks of SLN. The solubility of the drug in the lipid melt, the structure of the lipid matrix, and the polymeric state of the lipid matrix all limit the drug loading capacity of conventional SLN, If the lipid matrix contains molecules that are particularly similar (such as tristearin or tripalmitin), a perfect crystal with few defects forms. A highly ordered crystal lattice cannot accommodate large amounts of incorporated drugs because they are located between fatty acid chains, between lipid layers, and also in crystal imperfections. As a result, more complex lipids are more appropriate for increased drug loading. As a result, solid lipid nanoparticles are chosen for delivering Donepezil Hydrochloride for site-specific drug administration and also to enhance the permeability of drug [5, 6].

Donepezil is a reversible acetyl-cholinesterase inhibitor that acts centrally. Its primary therapeutic application is to treat Alzheimer's disease, where it is utilized to boost cortical acetylcholine levels. It is well absorbed in the gut, with a 100% oral bioavailability, and crosses the blood-brain barrier with ease. Donepezil comes in a variety of polymorphic forms, however, only polymorph 1 was used in this study. Since both active chemicals have a high solubility and are categorized as BCS class I compounds, particle size is not a factor in the production process or product functionality. The goal of this study was to improve Donepezil's permeability, bioavailability, and targeting efficiency by incorporating it into coated SLN and also to overcome the disadvantages of uncoated SLN like less loading efficiency, stability and drug expulsion due to polymeric transition during log storage [7, 8].

MATERIALS AND METHODS

Materials

Donepezil is a gift sample from Aurobindo Pharma Limited, Hyderabad. Tristearin (90%), Lecithin soya (30%) was purchased from Himedia Laboratories Pvt. Ltd., Mumbai. Double distilled water is used throughout the study. All materials and other chemicals were analytical reagent (AR) grade.

Methodology

Preparation of Donepezil SLNs

Various factors such as lecithin concentration (50, 60, 70 mg), drug concentration (5,7.5,10 mg), surfactant concentration (0.1, 0.2, 0.3 ml), stirring time (1 h), stirring speed (2000rpm), and sonication time (1 min) were fixed in optimization study, and their effect on particle size (nm), entrapment efficiency (%), and drug release (%) was determined by the Design-Expert software 7. A small modification of the previously reported modified solvent injection approach was used to make Donepezil SLNs. Lipids (soya lecithin, tristearin) was dissolved in ethanol with continuous stirring at 60 ° C., and the Donepezil HCl was added to the organic phase. An aqueous phase was prepared by dissolving surfactant in phosphate buffer solution (PBS) pH 7.4 and maintained at same temperature of organic phase (60°C). Hot organic phase was added to the aqueous phase with the aid of 24G needle syringe under continuous stirring (Remi Instruments, India) at a constant speed (2000rpm) for duration of 1 h and maintained at 60°C. This led to the formation of a dispersion, which was then filtered with a Whatmann filter paper in

order to remove excess lipid and ultra-sonicated using a probe sonicator (Lark, innovative technology, Chennai) and cooled to room temperature for SLNs formation [9-13].

Because the optimization method only takes a few runs with three or four variables, a 15-run, 3^2 Box-Behnken design was used to generate polynomial models for the optimization process. This model could be used to investigate the quadratic response surface and build a second-order polynomial model using Design-Expert software (Trial Version10.1.6, Stat-Ease Inc., MN). The design consisted of replicated center points and a set of points located at the midpoints of each edge of the multidimensional cube, which defined the region of interest for evaluating the main effects, interaction effects, and quadratic effects of the formulation ingredients, as well as optimizing the formulation [14-17]. Table 1 shows the experiment design matrix created by the software.

Table 1: Box-behnken optimization design

Std	Run	Factor 1	Factor 2	Factor 3
		A: Lipid	B: Surf	C: Drug
		level/mg	level/ml	level/mg
14	1	0/60	0/0.2	0/7.5
12	2	0/60	1/0.3	1/10
5	3	-1/50	0/0.2	-1/5
3	4	-1/50	1/0.3	0/7.5
7	5	-1/50	0/0.2	1/10
9	6	0/60	-1/0.1	-1/5
1	7	1/50	-1/0.1	0/7.5
6	8	1/70	0/0.2	-1/5
4	9	1/70	1/0.3	0/7.5
15	10	0/60	0/0.2	0/7.5
11	11	0/60	-1/0.1	1/10
10	12	0/60	1/0.3	-1/5
8	13	1/70	0/0.2	1/10
2	14	1/70	-1/0.1	0/7.5
13	15	0/60	0/0.2	0/7.5

Drug and excipient compatibility studies

Fourier-transform infrared spectroscopy (FTIR) studies

FTIR studies of Donepezil (pure drug) and Donepezil SLN were conducted to assess any changes in the drug's main functional groups and to establish that the drug was entrapped in lipid. The experiment was carried out using a direct sampling method on an IR spectrophotometer (Shimadzu, FTIR 8700) in the frequency range of 400 to 4000 cm^{-1} [18].

Differential scanning calorimetry (DSC) studies

Donepezil (pure drug) and Donepezil SLN were studied using DSC to see if there was any difference in the drug's melting enthalpy, glass transition temperature, or interactions with ingredients. The research was conducted using a DSC Q1000 TA equipment. Approximately 2-5 mg of sample was deposited in standard aluminium pans and scanned in the range of 5 °C to above its melting point with a temperature increment speed of 10 °C/min under dry nitrogen (flow rate 50 ml/min) as effluent gas [19].

Liquid X-ray diffraction (XRD) studies

XRD tests of Donepezil (pure drug) and Donepezil SLN were conducted to determine the crystallinity of the drug in both pure and SLN form. A Siemens DIFFRAC plus 5000 liquid diffractometer with CuK radiation was used for the experiment (1.54056A). The voltage and current of the tube were set to 40 kV and 40 mA, respectively. Each sample was scanned with a step size of 0.01° at 1 step between 10° and 40° [20].

Evaluation of SLN

Particle size and zeta-potential analysis

To obtain optimal particle counts, all samples were diluted in a 1:10 ratio with deionized water. Zetasizer was used to determine the particle size and zeta-potential of optimal SLN dispersions (Malvern Instruments Ltd., UK). Before measurement, all samples were diluted with double distilled water to attain an acceptable concentration [21-26].

Entrapment efficiency

The size exclusion approach (Sephadex G–50 mini-column) was used to determine it. To expel and remove void volume containing SLN into the centrifuged tubes, 2 ml of SLN dispersion was poured dropwise on the top of the column and then centrifuged at 2000rpm for 2 min at room temperature (Remi Instruments Pvt. Ltd, India). The amount of entrapped drug was measured spectrophotometrically at λ max 271 nm (Shimadzu 1800, Japan) and the amount of eluted dispersion was lysed by disrupting with 0.1 % Triton X-100 [27-29].

$$\% \text{ EE} = \frac{\text{Total drug} - \text{Unentrapped drug}}{\text{Total drug}} \text{X } 100$$

***In vitro* drug release study**

In vitro release tests were carried out on the SLN using 150 ml of phosphate buffer (PBS) pH 7.4 as the dissolving medium, which was maintained at 37±0.5 °C. The dialysis bag method (Hi-media, molecular weight cut-off 12,000 Daltons) was modified to maintain a sink state and achieve acceptable repeatability in *in vitro* release studies. Two milliliters of Donepezil-loaded SLN dispersion were poured into the dialysis bag and threaded together before being placed into the pre-heated dissolving media. A magnetic stirrer running at 50 rpm was used to stir the suspension at 37±0.5 °C. At fixed time intervals up to 24 h, 5 ml of the sample was taken and replaced with the same volume of the new medium. The samples were spectrophotometrically examined at a wavelength of max 454 nm. Sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride dihydrate 0.008 g, and purified water q. s. 100 Ml were used to make Donepezil Hydrochloride [22-26].

Coating of optimized formulation (COF)

A 1% (w/v) polysorbate 80 (Tween® 80) solution was used to modify the surface of optimized SLNs. It was mixed into a nanoparticle suspension for surface coating for 30 min with the help of a magnetic stirrer. The resulting coated nanoparticles were centrifuged, redispersed and stored in the refrigerator for subsequent study [30-32].

Field emission-scanning electron microscopy (FE-SEM)

At 25±2 °C, the surface morphology of COF was investigated using a FE-SEM (BRUKER, x-Flash 6130, USA). The SLN dispersion was spread out on a silicon wafer and allowed to dry for 24 h. It was also examined at 50.000 times magnification with a 10 kV accelerating voltage [36, 37].

***In vivo* studies**

Albino wistar male rats were used in this study to illustrate the effect of the improved formulation on psychosis-induced animals and to determine the bioavailability of Donepezil. The Institutional Animal Ethical Committee approved the animal study protocol. The animals were housed in propylene cages that were filled with rice husk. The temperature and humidity levels in the room were kept constant. Throughout the process, ethical rules were followed. The animals were rehabilitated. After the experimental procedure was completed, IAEC, Sri Padmavathi School of Pharmacy, Tiruchanoor, authorized all animal experiments (IAEC No: SPSP: 1016/PO/Re/S/06/CPCSEA/2017/014.

Pharmacokinetic study

The animals were placed into three groups, each of which had six animals. The first group received simply drug oral suspension (0.5 mg/kg); the second group received drug-loaded SLN oral suspension (0.5 mg/kg); and the third group received surface-modified SLN. The oral feeding tube was used to give the entire medication solution. At predetermined intervals, blood samples were taken from the tail vein (0, 0.5, 1, 1.5,3, 6, 16, 22 and 24 h). To avoid blood clotting, an EDTA solution was added. Each blood sample received 1.5 ml of acetonitrile before being centrifuged at 3000 rpm for 10 min. The supernatant liquid was collected and evaluate the plasma drug concentration in HPLC. These studies were carried out using 4.6×250 mm "Phenomenex" C18 HPLC column with 5 µ particles. Each time, 20 µl of sample was injected. Acetonitrile: potassium dihydrogen phosphate buffer pH3.2 (5:95 % v/v) was utilized as the mobile phase. The flow rate was kept constant at 1 ml/min. Donepezil hydrochloride were spectrophotometrically analyzed at λ max 271 nm for Donepezil hydrochloride [23-37]. All studies on animals were approved by IAEC, Sri Padmavathi School of Pharmacy, Tiruchanoor (IAEC No: SPSP: 1016/PO/Re/S/06/CPCSEA/2017/014)

Pharmacodynamics study

The animals were placed into four groups, each of which had six animals. Group I was a normal control group with no induction or treatment; Group II was a negative control group that was induced with Ketamine (30 mg/kg for 5 d); Group III was induced with Ketamine (30 mg/kg for 5 d) along with treatment with Donepezil SLN (0.5 mg/kg); Group IV was induced with Ketamine (30 mg/kg for 5 d) together with treatment with Donepezil surface-modified SLN (0.5 mg For 5 d, animals in groups II-IV were injected with 30 mg/kg of Ketamine solution. The induction of psychosis was confirmed by measuring locomotor activity. After 1.5 h of dosing as per the above groups the locomotor activity was checked for 5 min using actophotometer [29-37]. All studies on animals were approved by IAEC, Sri Padmavathi School of Pharmacy, Tiruchanoor (IAEC No: SPSP: 1016/PO/Re/ S/06/CPCSEA/2017/014).

RESULTS AND DISCUSSION

Optimization of formulation and process variables

Box-behnken Design (3^2 Factorial Design in Design expert programme) with Polynomial Quadratic Model and Multiple Linear Regression approach was used to optimize formulation and process parameters for SLN formulation, as shown in table 2-5. The quadratic model suggests a P value of 0.0083 in the sum of squares. Selected the highest order polynomial with significant additional terms and no aliasing. The chosen model has a minor lack of fit, as evidenced by the P-value of 0.3099 obtained from the Lack of Fit test. The Model F-value of 66.61 indicates that the model is statistically significant. An F-value of this magnitude has a 0.01 % chance of occurring due to noise. Model terms with P-values less than 0.0500 are significant. A, B, AC, B^2, C^2 are important model terms in this situation. The model terms are not important if the value is bigger than 0.1000. Model reduction may improve our model if there are many inconsequential model terms (not including those required to support hierarchy). The F-value of 2.38 for the Lack of Fit indicates that it is not significant in comparison to the pure error. Due to noise, a significant Lack of Fit F-value has a 30.99 % chance of occurring. A minor lack of fit is acceptable, as it is required to fit the model. The Adjusted R^2 of 0.9768 is reasonably close to the Predicted R^2 of 0.8926; that is, the difference is less than 0.2. The signal-to-noise ratio is measured by enough precision. It is preferable to have a ratio of more than four. The signal-to-noise ratio of 24.639 suggests that the signal is adequate. As a result, we can use this model to navigate the design space. When all other factors are maintained constant, the coefficient estimate provides the expected change in response per unit change in factor value. In an orthogonal design, the intercept is the overall average response of all the runs. The coefficients are modifications based on the factor settings around that average. The variance inflation factor (VIF) is 1 when the factors are orthogonal; VIFs more than 1 imply multi-colinearity; the higher the VIF, the more severe the factor correlation. VIFs of fewer than ten are considered tolerable. For given levels of each element, the equation in terms of coded factors can be used to make predictions about the response. The high levels of the factors are coded as +1 and the low levels of the factors are coded as -1 by default. By comparing the factor coefficients, the coded equation can be used to determine the relative impact of the components. It was discovered from the data that there was a good association between lipid concentration and particle size (r^2= 0.9917). It determines whether there is an increase in lipid concentration as particle size increases (when using ANOVA). It exhibits P<0.0001, indicating that there is a substantial difference in PS when Lipid concentration rises, indicating positive linear regression. Entrapment Efficiency was shown to increase as surfactant concentration was increased (EE). However, one of the particles' maximal conductivity abilities was obtained. There is no change in entrapment efficiency when surfactant levels rise above 0 (r^2 = 0.9866 linear regression). It could be because of the influence of higher lipid and surfactant concentrations. P-value = 0.0001 was calculated using ANOVA, indicating that increasing surfactant and lipid concentrations resulted in a significant change in EE. The relation between surfactant and lipid concentration on Drug Release shows regression correlation values as r^2= 0.9850. Increase in lipid and surfactant concentration leads to an increase in entrapment efficiency, and further shows a good control and increase in drug release. The results are shown in fig. 1. From the checkpoint analysis data, solution-1 (table 6) SLN formulation was selected for further studies [18-20].

$$PS = +161.53 + 31.69\,A - 7.44\,B + 3.07\,C + 1.63AB - 8.40AC - 4.35\,BC + 2.42\,A^2 - 10.33B^2 - 7.80C^2$$

$$EE = +67.90 + 6.74A - 1.60B - 0.3050C - 1.74AB - 1.38AC - 0.7225BC + 2.32A^2 - 2.63B^2 - 2.21C^2$$

$$DR = +91.87 + 7.24A - 1.61B - 0.1213C + 0.0925AB - 0.8025AC - 1.11BC - 0.9275A^2 - 4.09B^2 - 2.84C^2$$

FT-IR studies

FT-IR tests were carried out on the following samples, such as Donepezil and Donepezil lipid mixture, in order to explore the structural composition of the drug and excipients in the form of functional group frequencies and their reproducibility in excipient mixtures and formulations. Fig. 2 shows typical FT-IR spectra of the material mentioned above. When compared to Donepezil Lipid mixture, which shows infrared absorption at 1601.00; 1473.69, 717.55 cm^{-1}, pure Donepezil showed high infrared absorption at 1605, 1589, 1500, 749, 702 cm^{-1}. Another finding was that the IR spectra of Donepezil lipid did not contain any new peak, indicating that there was no strong interaction and no incompatibility between the excipients in the formulation [13, 14].

DSC studies

Due to drug entrapment in the lipid, the melting point of Donepezil (pure drug) was marginally reduced in Coated SLN, from 224.5 °C (fig. 3) to 222.2 °C (fig. 3A), possibly due to the presence of excipients. Because the drug was encased in lipid, the melting point of lipid peaked at 222.2 °C. According to studies, the majority of SLNs have a less organized crystal organization, resulting in an amorphous dissolved state within the lipid, confirming the drug loading [15].

Table 2: Optimization design showing the effect of independent variable on dependent variable

Std	Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
		A: Lipid	B: Surf	C: Drug	PS (Minimize)*	EE (Maximize)*	DR (Maximize)*
		level/mg	level/ml	level/mg	Nm	%	%
14	1	0/60	0/0.2	0/7.5	158.5±1.42	67.12±2.17	92.61±3.84
12	2	0/60	1/0.3	1/10	135.3±2.12	61.51±1.64	81.28±3.42
5	3	-1/50	0/0.2	-1/5	112.4±2.20	60.81±2.26	80.63±2.40
3	4	-1/50	1/0.3	0/7.5	109.8±1.32	60.16±2.30	77.75±3.84
7	5	-1/50	0/0.2	1/10	138.4±2.30	62.13±3.28	82.53±2.66
9	6	0/60	-1/0.1	-1/5	142.8±3.22	63.18±2.02	86.37±3.84
1	7	1/50	-1/0.1	0/7.5	132.2±2.12	61.19±1.45	80.05±2.80
6	8	1/70	0/0.2	-1/5	190.7±1.34	76.67±2.36	95.29±3.84
4	9	1/70	1/0.3	0/7.5	178.3±2.42	70.53±2.54	93.83±2.46
15	10	0/60	0/0.2	0/7.5	161.7±2.40	67.84±3.60	91.14±3.84
11	11	0/60	-1/0.1	1/10	154.6±1.20	64.85±2.42	87.82±2.46
10	12	0/60	1/0.3	-1/5	140.9±2.14	62.73±2.82	84.29±3.84
8	13	1/70	0/0.2	1/10	183.1±2.46	72.46±2.13	93.98±2.60
2	14	1/70	-1/0.1	0/7.5	194.2±2.20	78.52±2.54	95.76±3.84
13	15	0/60	0/0.2	0/7.5	164.4±2.16	68.75±2.81	91.86±2.44

*Data represents in mean±SD (n=3)

Table 3: ANOVA for quadratic model, which shows the response of independent variable on PS

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	9547.44	9	1060.83	66.61	0.0001	Significant
A-LIPID	8032.78	1	8032.78	504.36	<0.0001	
B-SURF	442.53	1	442.53	27.79	0.0033	
C-DRUG	75.65	1	75.65	4.75	0.0812	
AB	10.56	1	10.56	0.6632	0.4525	
AC	282.24	1	282.24	17.72	0.0084	
BC	75.69	1	75.69	4.75	0.0811	
A^2	21.64	1	21.64	1.36	0.2964	
B^2	393.94	1	393.94	24.73	0.0042	
C^2	224.88	1	224.88	14.12	0.0132	
Residual	79.63	5	15.93			
Lack of Fit	62.19	3	20.73	2.38	0.3099	not significant
Pure Error	17.45	2	8.72			
Cor total	9627.08	14				

ANOVA-analysis of variance

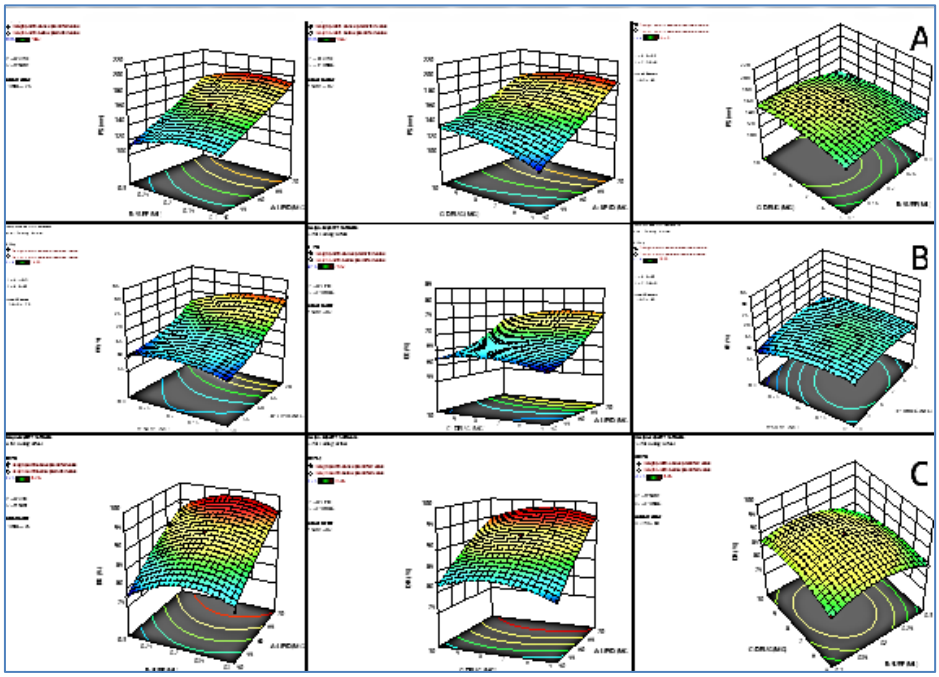

Fig. 1: 3D Contour plot showing Independent variable effect on (A) Particle size (nm); (B) Entrapment efficiency (%); (C) Percentage amount of drug release (24h)

Table 4: ANOVA for quadratic model, which shows the response of independent variable on EE

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	473.14	9	52.57	40.86	0.0004	significant
A-LIPID	363.02	1	363.02	282.15	<0.0001	
B-SURF	20.51	1	20.51	15.94	0.0104	
C-DRUG	0.7442	1	0.7442	0.5784	0.4812	
AB	12.11	1	12.11	9.41	0.0278	
AC	7.65	1	7.65	5.94	0.0588	
BC	2.09	1	2.09	1.62	0.2587	
A^2	19.93	1	19.93	15.49	0.0110	
B^2	25.47	1	25.47	19.80	0.0067	
C^2	18.02	1	18.02	14.01	0.0134	
Residual	6.43	5	1.29			
Lack of Fit	5.10	3	1.70	2.55	0.2944	not significant
Pure Error	1.33	2	0.6672			
Cor Total	479.58	14				

Table 5: ANOVA for quadratic model which shows the response of independent variable on DR

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	533.59	9	59.29	36.60	0.0005	significant
A-LIPID	419.05	1	419.05	258.67	<0.0001	
B-SURF	20.64	1	20.64	12.74	0.0161	
C-DRUG	0.1176	1	0.1176	0.0726	0.7984	
AB	0.0342	1	0.0342	0.0211	0.8901	
AC	2.58	1	2.58	1.59	0.2629	
BC	4.97	1	4.97	3.07	0.1402	
A^2	3.18	1	3.18	1.96	0.2203	
B^2	61.92	1	61.92	38.22	0.0016	
C^2	29.68	1	29.68	18.32	0.0079	
Residual	8.10	5	1.62			
Lack of Fit	7.02	3	2.34	4.33	0.1933	not significant
Pure Error	1.08	2	0.5403			
Cor Total	541.69	14				

Table 6: Point prediction table

Response	Predicted mean	Predicted median	Observed	Std dev	SE mean	95% CI low for mean	95% CI high for mean	95% TI low for 99% Pop	95% TI high for 99% Pop
PS	167.808	167.808	163.31	3.998	2.1293	162.335	173.282	142.04	193.576
EE	69.7804	69.7804	70.64	1.134	0.6053	68.2247	71.3362	62.4566	77.1042
DR	93.0942	93.0942	94.85	1.272	0.6798	91.3485	94.8399	84.876	101.312

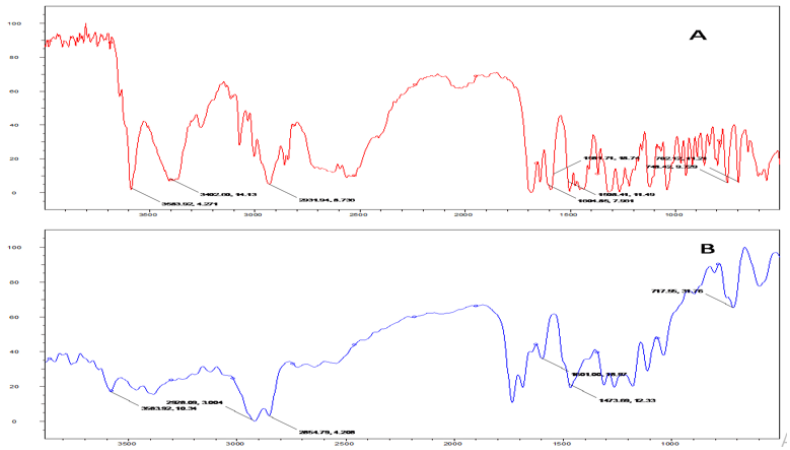

Fig. 2: FTIR spectrum of (A) Donepezil hydrochloride; (B) Donepezil hydrochloride solid lipid nanoparticle

XRD studies

The characteristics of matrix SLNs can be changed by lecithin. As a result, only the hard lipid component influences melting and crystallization. The pure medication Donepezil showed high peaks at 2θ of 10.4, 24.8, 25.6, 32.4, and 34.6, indicating that it is crystalline. The curved peak of Donepezil-loaded SLNs demonstrates that SLNs are amorphous. The results are shown in fig. 4(A) and 4(B).

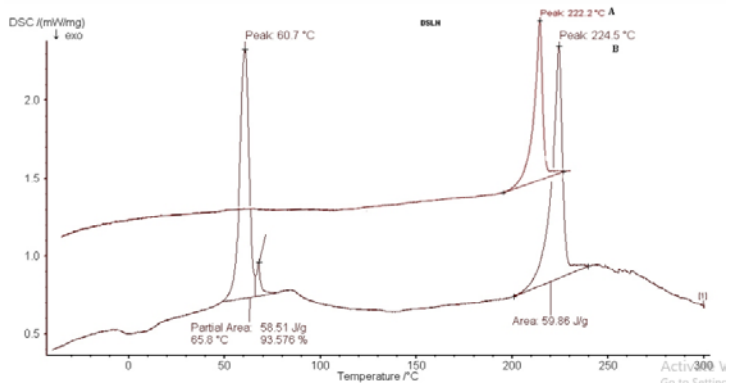

Fig. 3: DSC images of (A) Donepezil coated optimized SLNs and (B) Donepezil (pure drug)

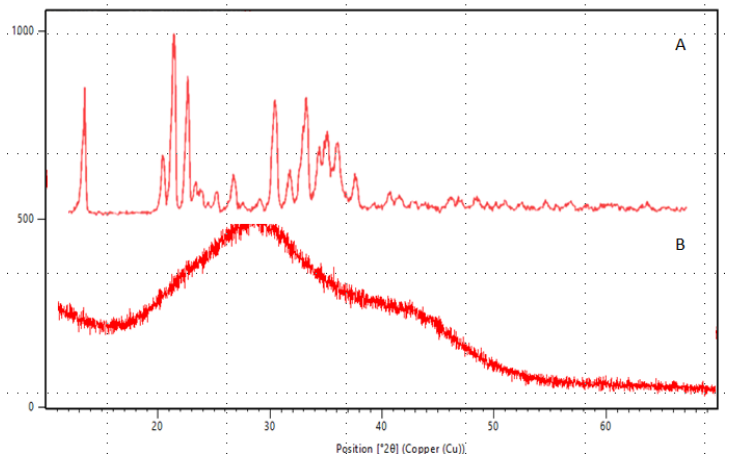

Fig. 4: XRD pattern of (A) Pure donepezil drug; (B) Coated optimized donepezil SLNs

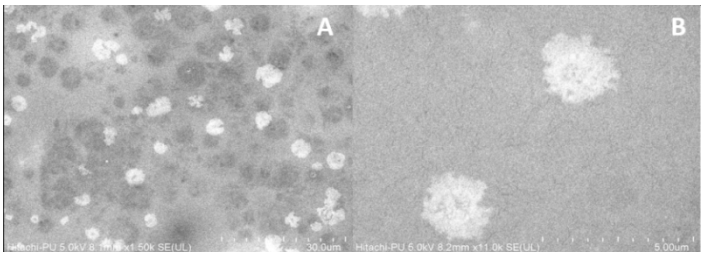

Fig. 5: FESEM images of donepezil hydrochloride loaded coated SLNs

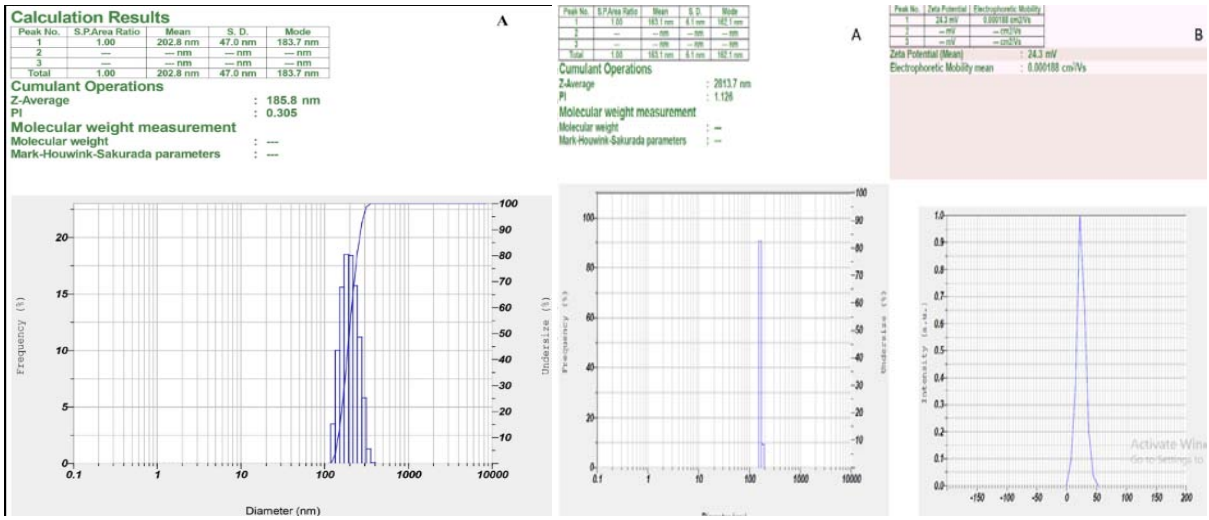

Fig. 6: (a) Particle size distribution curve, (b) Zeta potential curve images of coated optimized donepezil SLNs

FESEM studies

The surface topography of optimized coated SLN was clearly explained by FESEM analysis of the Coated Optimized formulation, as illustrated in fig. 5. The particle was rough, nearly spherical, and monodisperse in nature, with Tween 80 surface modification of the SLN obvious in the FESEM image [20, 21].

Physical characterization of coated SLN

Particle size, size distribution and zeta potential curve of coated SLN are shown in fig. 6, respectively. The average particle size, PDI and zeta potential were found to be 185.8 nm, which has brain permeability ≤(20 0 nm), 0.305 showed an broader particle distribution and -24.3 mV indicates that prepared SLNs were stable respectively due to high surface charge (fig. 6a, fig. 6b). The % entrapment efficiency and % drug release at 36 h in pH 7.4 PBS of coated SLN was found to be 78.52±2.54% and 98.62±3.14 %, respectively, this may be due to the strong layer coated on the SLNs by Tween 80. The particle shape is almost spherical as shown in fig. 5. The characteristic peaks of the drug disappeared and were replaced by the peak of tristearin. Remaining peaks also either shifted or replaced in the IR spectrum of Coated SLN are shown in fig. 2. This established the drug entrapment in a lipid matrix and the compatibility between the drug and excipients used in the formulation [23-25].

***In vitro* drug release kinetics**

The drug release of the F14 was observed up to 24h (93.64±3.24), whereas the drug release from coated formulation was 36h (98.62±3.14), indicating that due to surface modification, the drug release is sustained as shown in fig. 7. The release kinetics of F14, Coated SLN was applied for various kinetic models by using PCP Disso V3.01 software. The best fit model for both is Korsmeyer-Peppas. n=0.624 (F14), 0.524 (Coated Formulation), and k=15.426, 15.024 for F14 and Coated formulation respectively. Where n = 0.5, is indicative of release mechanism (slow diffusion) of Donepezil from SLN matrix, which depicts that this fits in time-dependent Fickian diffusion drug release from insoluble lipid matrix. The best linearity was followed by the matrix kinetics (R^2-0.9924, 0.9902) as shown in table 7 [26-28].

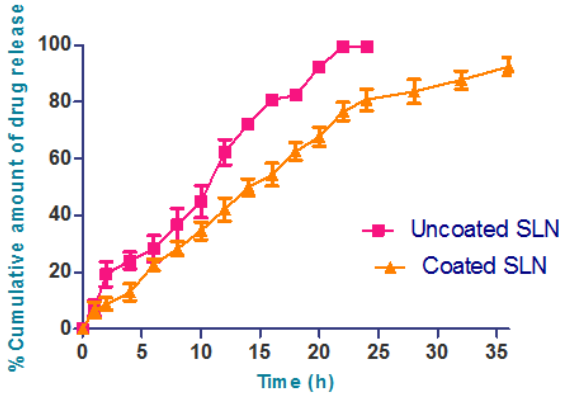

Fig. 7: *In vitro* drug release of (F14) before coating, after coating of SLNs (Data represents in mean±SD (n=3))

Table 7: Drug release kinetics values for optimized formulation

Kinetics model	Model fitting (Average) values for F14		Model fitting (Average) values for coated formulation	
	R	K	R	K
Zero order	0.742	4.344	0.798	3.344
T-test	4.562	(Passes)	5.864	(Passes)
1st order	0.984	-0.082	0.978	-0.094
T-test	11.42	(Passes)	17.62	(Passes)
Matrix	0.9924	17.542	0.9902	14.344
T-test	14.824	(Passes)	18.424	(Passes)
Peppas	0.986	15.426	0.954	15.024
T-test	14.520	(Passes)	15.464	(Passes)
Hix. Crow.	0.954	-0.021	0.984	-0.013
T-test	8.924	(Passes)	12.062	(Passes)

***In vivo* pharmacokinetic studies**

Pharmacokinetic study as shown table 8; fig. 8, was performed to understand the extent of bioavailability enhancement of Donepezil modified SLN in animals. Donepezil surface modified SLN showed improved bioavailability orally. Donepezil surface modified SLN shows maximum plasma drug concentration in oral route with slower drug elimination from body. Though T_{max} for all formulations remained same the onset of action was found to be faster. The surface modified SLN shows good C_{max} and AUC when compared to other two treatment groups. This data infers that there was a significant improvement of bioavailability of Donepezil Hydrochloride when it converted to surface modified SLN. Higher C_{max} and improved bioavailability was also supported by behavioural changes observed in locomotor activity for surface modified SLN when compared to other treatment groups [26-28].

Table 8: Comparative *in vivo* pharmacokinetic studies data between donepezil treatment groups

Group	Ke (h^{-1})	T_{max} (h)	C_{max} (µg/ml)	$AUC_{0-\alpha}$(µg/ml/h)
Group I: Donepezil drug solution (0.5 mg/kg)	0.0824±0.002	2±0.02	0.94±0.02	26.84±2.2
Group II: Donepezil SLN (F14) (0.5 mg/kg)	0.0562±0.004	2±0.04	2.38±0.02	53.22±3.8
Group III: Donepezil surface modified SLN (COF) (0.5 mg/kg)	0.0428±0.002	2±0.02	4.24±0.08	112.42±4.8

Note: K_e elimination rate constant, $T_{1/2}$ Elimination half-life, C_{max} maximum plasma concentration at time T_{max}, AUC area under the curve. Data represents mean±SD (n=3)

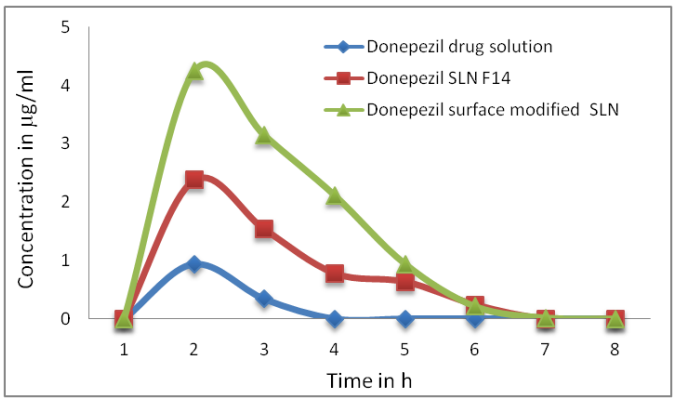

Fig. 8: *In vivo* pharmacokinetic graph between donepezil treatment groups. Data represents in mean, (n=3)

In vivo pharmacodynamics studies

Psychosis was induced in animals by injecting Ketamine for 5 d. From the decreased locomotor activity and distorted movements it was confirmed that psychosis was induced. The locomotors activity was checked after 1.5 h of administration. It was found that, the locomotor effect of Donepezil surface modified SLN oral solution was more as compared to Donepezil SLN (F14) and Donepezil plain drug solution. Change in Locomotor activity (rat muscle coordination activity) by each group was statistically significant with a probability<0.001. From the statistically significant results it was concluded that effect of Donepezil surface-modified SLN was more when compared to Donepezil SLN (F14) administered by oral route. Muscle coordination assessed by the Rota rod apparatus were significantly deteriorated ($p<0.05$) in the ketamine induced group compared to the control as shown in fig. 8. When compared to the ketamine induced group, Donepezil SLN (F14) treatment group and Donepezil surface modified SLN showed better significant retention time ($p<0.05$) attributed to improvement in muscle coordination. Both treatment groups showed significant good muscle coordination when compared to ketamine induced group. Similarly, the locomotor score evaluated by Actophotometer was significantly diminished ($p<0.05$) in the ketamine group in comparison to the control group. This indicates the induction of oxidative stress and depletion in the neurons. Treatment with Donepezil surface modified SLN ($p<.05$) improved the locomotor score as compared to the diseased group as shown in fig. 9 [29-32].

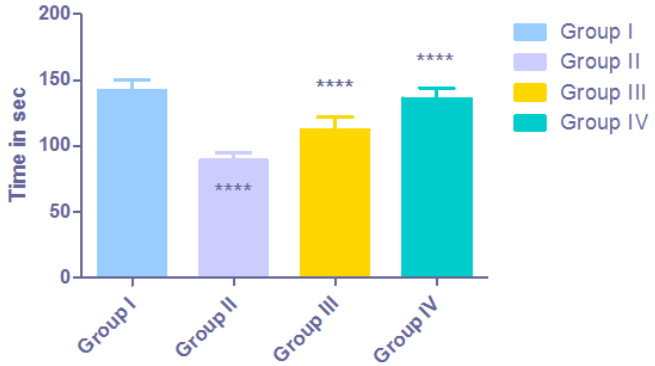

Fig. 9: *In vivo* pharmacokinetic graph between donepezil treatment groups. Data represents in mean±SD (n=3)

CONCLUSION

The SLN was formulated, optimized and coated with Tween 80 and the parameters like Particle Size (PS in nm); % Entrapment Efficiency and *in vitro* drug release for 24 h were evaluated for before coating (PS=163.31±2.41; EE=70.64±3.62; %DR=94.85±2.87) and after coating 185.8 nm, 78.52±2.54%EE, 98.62±3.14 DR 36h. These parameters showed significant changes while formulating SLN along with various formulation and process variables. From the above-discussed data, it was concluded that the solvent injection method was an optimized technique for the preparation of Donepezil SLN containing with selected formulation variables like Lecithin as lipid, Tween 80 as Surfactant with ultrasonication process. This developed technique will be an effective and reproducible for the formulation of SLN. Thus, the current study successfully designed, developed and optimized SLN formulation of Donepezil using a 3^2 Box-behnken design with enhanced membrane permeability. Thus, the current study successfully designed, developed an optimized SLN formulation. The surface modified SLN proved to enhance the permeability of drug through the barrier, which leads to the enhancement of Donepezil's bioavailability and locomotor activity.

ACKNOWLEDGEMENT

DST-FIST, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati. Sophisticated Analytical Instruments Facility (SAIF), Punjab University, Chandigarh. SAIF, IITM, Chennai.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

All authors have none to declare.

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