

Development, Characterization and In vitro Evaluation of Donepezil solid Lipid Nanoparticles

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ABSTRACT

Solid lipid nanoparticles (SLNs) loaded with donepezil were prepared by hot homogenization followed by probe ultrasonication technique. Donepezil SLNs were composed of lipids (1 to 5 %w/v) such as trimyristin, tristearin, glycerol monostearate and Compritol which were stabilized by soya lecithin (0.5 to 2.5 %w/v) and poloxamer 188 (0.5 to 2.5 %w/v). Developed donepezil SLNs were characterized for size, zeta potential, entrapment efficiency and content uniformity. Selected method of preparation was able to produce nanoparticles of size range 102.5 to 179.3 nm possessing zeta potential -23.5 to -34.9 mV with entrapment efficiency 86.65 to 89.64%. FTIR and DSC studies showed that there was no interaction between donepezil and selected lipids. Sterilization by autoclave increased size of SLNs from 1.5 to 1.9 times, however, there was no decrease in the zeta potential of the formulations. Short term stability studies showed that size of SLNs maximum increased by 38 nm whereas their entrapment efficiency was lowered by 1.3 % only. In vitro release studies revealed that 61.58 to 74.82 % of donepezil released from developed SLNs after 24 hours and found that drug release followed non-Fickian transport (0.556 to 0.658).

Keywords: Solid lipid nanoparticles (SLNs), Donepezil, FTIR, DSC, Sterilization, Stability study, In vitro release study.

INTRODUCTION:

Solid lipid nanoparticles were first introduced as an alternative drug delivery system to liposomes and polymeric nanoparticles. Much of the research work focused on solid lipid nanoparticles as drug carriers by many research groups across the globe to exploit the benefits of SLNs and to avoid disadvantages of other novel carrier systems such as nanoemulsions, nanosuspensions, liposomes and polymeric micro and nanoparticles¹⁻³. SLNs can be stated as less toxic because of the biological origin of lipid component of the SLNs while compared against polymeric NPs⁴. Because of their proved advantages such as precise controlled drug release⁵, drug targeting, enhanced bioavailability high drug protection, biocompatibility, high loading efficiency of lipophilic drugs and ease of preparation, SLNs were investigated against many chronic diseases⁶⁻⁹.

To develop SLNs as alternative novel drug delivery systems, many techniques have been approached in the literature such as microemulsion¹⁰⁻¹³, modified high shear homogenization and ultrasound techniques, emulsification-diffusion method¹⁴, emulsification and low-temperature solidification¹⁵, solvent injection method¹⁶ and solvent diffusion method¹⁷. Manufacturing process of linagliptin solid lipid nanoparticles was optimized using Quality by Design approach (QbD) by identifying the impact of critical process parameters on critical quality attributes and stated that less errors in the production of solid lipid nanoparticles¹⁸.

In case of neurodegenerative disorders such as Alzheimer's disease (AD) death of brain cells causes memory loss and cognitive decline. The cholinesterase inhibitors (ChEIs) block the breakdown of acetylcholine. As a result, more acetylcholine as neurotransmitter is available in the brain, and it may become easier to form new memories. Donepezil hydrochloride is one among the four ChEIs approved by the FDA is used by most

physicians because the fourth drug tacrine has more undesirable side effects. Donepezil also known as Aricept is a new anti-Alzheimer drug increases the levels of acetylcholine in the brain involved in memory function¹⁹⁻²². It is used to treat symptoms of dementia²³. Physico chemical properties of donepezil²⁴ such as low molecular weight 379.5 g/mol and acceptable log P value 4.14 permits its loading into solid lipid nanoparticles. Concentration of anticancer drugs such as idarubicin, etoposide, doxorubicin and camptothecin increased in brain when administered them as SLNs. The low intrinsic toxicity and biodegradability of lipids used in SLN are valuable features in terms of brain disorder management²⁵. Hence, donepezil further to exhibit better therapeutic action, its high concentration in the brain region is essential, therefore, in the present study, we tried to develop donepezil SLNs as initial attempt.

In our research trials, we have employed hot homogenization followed by probe ultrasonication technique to develop donepezil SLNs. This approach does not require removal any organic solvents from the final preparation like in other methods. The present research aims to develop, characterize and in vitro evaluate the donepezil loaded solid lipid nanoparticles.

MATERIAL AND METHODS:

Materials:

Donepezil was purchased from Yarrow Chem Products (Mumbai, India). Trimyristin (Dynasan 114) was generously supplied by Sasol Germany GmbH (Hamburg, Germany). Tristearin, Soya Lecithin 30% and Poloxamer 188 (Pluronic F 68) were purchased from HiMedia (Mumbai, India). Glycerol monostearate was purchased from Research-Lab Fine Chem. Industries, (Mumbai, India). Compritol 888 (glyceryl behenate) was generously gifted by Gattefosse India Pvt. Ltd. (Mumbai, India). Centrisart-1 filters (molecular weight cutoff 20,000) were purchased from Sartorius Stedim Lab Ltd. (Stonehouse, England). Dialysis membrane-70 was purchased from HiMedia (Mumbai, India). Other chemicals and reagents used were of analytical grade. Partitioning Behaviour of Donepezil in Various Lipids:

To determine partition co-efficient of donepezil, 10 mg of donepezil was accurately weighed, dispersed in a mixture of

melted lipid (1 gm) and 1 ml of hot distilled water and shaken for 30 minutes in a hot water bath. The mixture was cooled to room temperature, further aqueous phase was separated by ultracentrifugation with the help of Centrisart and analyzed for drug concentration by HPLC.

Preparation of Donepezil Solid Lipid Nanoparticles: SLNs were prepared by hot homogenization followed by probe ultrasonication technique²⁶. Donepezil (10 mg), lipids such as trimyristin, tristearin, glycerol

monostearate and compritol (100 to 500 mg) and soya lecithin (50 to 250 mg) as shown in Table 1, were dissolved in 10 ml of mixture of chloroform and methanol (1:1). Organic solvents were completely removed by placing round bottom flask under rotation in water bath and to get drug dispersed lipid layer. Thus drug embedded lipid layer obtained was melted by heating at 5 °C above melting point of the lipid. In another beaker, hot aqueous solution of poloxamer 188 was prepared by dissolving of poloxamer 188 (50 to 250 mg) in distilled water (10 ml). Hot aqueous phase was added to molten lipid phase slowly during homogenization which was carried out at 20,000 rpm for 5 minutes by ultrahomogenizer (Heidolph, Germany). Coarse emulsion so obtained was immediately ultrasonicated using probe sonicator (Sonics, USA) for 25 min. So obtained hot nanoemulsion was slowly cooled to room temperature to initiate the recrystallization of the lipids to obtain solid lipid nanoparticles. Blank SLNs were prepared in a similar manner by omitting drug. Blank SLNs prepared with trimyristin, tristearin, glycerol monostearate and compritol are abbreviated as B-TM, B-TS, B-GMS and B-CP, respectively. In a similar way, donepezil loaded SLNs are abbreviated as D-TM, D-TS, D-GMS and D-CP.

Table 1: Composition of donepezil SLNs prepared using various lipids at different percentages.

Donepezil (mg)	Lipids*		Soya lecithin (mg)	Poloxamer (mg)	Distilled water q.s. to
	% w/v	Taken (mg)			
10	1	100	50	50	10 ml
10	2	200	100	100	10 ml
10	3	300	150	150	10 ml
10	4	400	200	200	10 ml
10	5	500	250	250	10 ml

*Lipids taken were trimyristin, tristearin, glycerol monostearate and compritol

Measurement of Size and Zeta Potential of SLNs: Mean particle size and zeta potential of prepared SLNs were analyzed by Dynamic Light Scattering technique using Malvern

Zetasizer Nano ZS. Samples were diluted appropriately with the aqueous phase of the formulation for the measurements. Size of blank and drug loaded SLNs was compared using the Student's t-test.

Drug Excipients Compatibility Studies:

Compatibility of pure drug donepezil with selected lipids was checked by Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC). The samples used for these two studies were donepezil, lipid (compritol) and physical mixture of donepezil with compritol (1:1).

FTIR spectra were recorded in the region of 4000 to 400 cm⁻¹ using FTIR spectrophotometer (Shimadzu Corporation, Japan). The samples were mixed with potassium bromide and compressed using hydraulic press to make the pellet.

DSC analysis was performed using DSC-60 Plus (Shimadzu, Japan). The samples were sealed in an aluminum pan and heated under nitrogen (30 ml/min). Heating range employed was 10-300 °C with a heating rate of 5 °C / min. Reference sample used was an empty aluminum pan.

Effect of Sterilization by Autoclave:

Dispersions of donepezil SLNs of different lipids were filled into the vials and subjected for sterilization by autoclave at 121 °C and 15 psi of pressure for 30 min.

Short Term Stability Study:

Donepezil SLNs of different lipids were stored at 25 °C for three months to observe the influence of storage conditions on size and entrapment efficiency of SLNs.

HPLC Analysis of Donepezil:

Mobile phase was prepared by mixing acetonitrile and freshly prepared 0.02M sodium phosphate in the ratio of 40:60 v/v, respectively. Mobile phase was degassed with the help of bath sonicator. The chromatographic system consisted of a Shimadzu LC-10AT solvent delivery pump equipped with a 20 µl loop and rheodyne sample injector. Analytical column used was C18RS (25 cm X 4.6 mm ID). Detector used was SPD-10A VP dual wavelength UV-Visible detector (Shimadzu) and the eluate was monitored at 270 nm. The sensitivity was set at 0.001 AUFS. Flow rate was kept at 1ml / min. Column temperature was maintained at 25 °C.

Standard Graph of Donepezil:

10 mg of donepezil was accurately weighed and dissolved in mobile phase using 10 ml standard volumetric flask to get a stock solution (1 mg/ml). Working standards (5, 10, 15, 20, 25 and 30 µg/ml) of donepezil were prepared by diluting stock solution with mobile phase, 20 µl of each sample was injected into the HPLC column.

Drug Content and Entrapment Efficiency:

Drug content: To 1 ml of formulation, 4 ml of methanol was added and vortexed for 15 min. Drug concentration was determined by HPLC.

$$\text{Drug Content (\%)} = \frac{\text{Practical amount of drug (mg)}}{\text{Theoretical amount of drug (mg)}} \times 100$$

Entrapment efficiency:

The entrapment efficiency of the SLN for donepezil was determined by measuring the concentration of free drug in the aqueous phase. To separate aqueous phase from SLN dispersion, ultracentrifugation was carried out using Centrisart, (consisting of filter membrane, molecular weight cut-off 20,000 Dalton). The unit was centrifuged at 5000 rpm for 15 minutes. The solid lipid nanopartilces along with encapsulated drug remained in the outer chamber and aqueous phase moved into the sample recovery chamber through filter membrane. The concentration of the donepezil in the aqueous phase was estimated by HPLC. Entrapment efficiency is calculated based upon the formula as mentioned.

$$\text{Entrapment efficiency} = \frac{\text{Drug content (mg)} - \text{Amount of drug in aqueous phase (mg)}}{\text{Drug content (mg)}} \times 100$$

In vitro Release Studies of Donepezil SLN:

In vitro release studies were performed using modified Franz diffusion cell²⁷. Dialysis membrane having pore size 2.4 nm, was soaked in double distilled water for 12 hours before mounting in a Franz diffusion cell. Donepezil SLN dispersions (2 ml) was placed in the donor compartment and the receptor compartment was filled with 22 ml of release medium i.e. phosphate buffer, pH 7.2. At fixed time intervals (0.5, 1, 2, 3, 6, 12 and 24 hrs), 0.2 ml of the sample was withdrawn from

receiver compartment through side tube. Fresh medium was replaced to maintain constant volume of release medium. Samples were analyzed by HPLC method as described previously at 270 nm.

RESULTS AND DISCUSSION:

Partitioning Behaviour of Donepezil:

Partitioning of a drug towards lipid carrier during the preparation is essential to get high drug entrapment efficiency and loading capacity of drug delivery system. Partition coefficient of a drug can be obtained by measuring its relative solubility in a mixture of lipid and water. Partition co-efficient is calculated by taking the ratio of the amount of donepezil in lipid phase to aqueous phase. In order to calculate concentration of drug in aqueous phase, calibration equation i.e. $y = 26970x + 3472$, with the regression co-efficient $R^2 = 0.999$ obtained by HPLC was used. The results obtained are shown in the Table 2. Partition co-efficient of donepezil in the selected lipids ranged from 10.53 to 17.71, there was no much variation in the obtained values in case of different lipids. However, relatively high partition coefficient (17.71) and log P values (1.25) of donepezil are observed in case of tristearin lipid. Thus, donepezil possess adequate log P values (1.02 to 1.25) to be entrapped in the selected lipids.

Table 2: Partition co-efficient and log P values of donepezil in selected lipids (mean \pm SD, n=3)

Lipid	Amount of donepezil (mg)		Partition co-efficient	Average partition coefficient	Average Log P Value
	In aqueous Phase	In lipid phase			
Glycerol monostearate	0.945	9.055	09.58	10.53 \pm 1.16	1.02
	0.895	9.107	10.19		
	0.779	9.221	11.83		
Compritol	0.725	9.277	12.83	12.74 \pm 0.51	1.11
	0.758	9.242	12.19		
	0.704	9.296	13.20		
Trimyristin	0.657	9.345	14.22	14.01 \pm 0.19	1.15
	0.673	9.327	13.85		
	0.668	9.332	13.97		
Tristearin	0.559	9.441	16.88	17.71 \pm 0.98	1.25
	0.505	9.495	18.80		
	0.542	9.458	17.45		

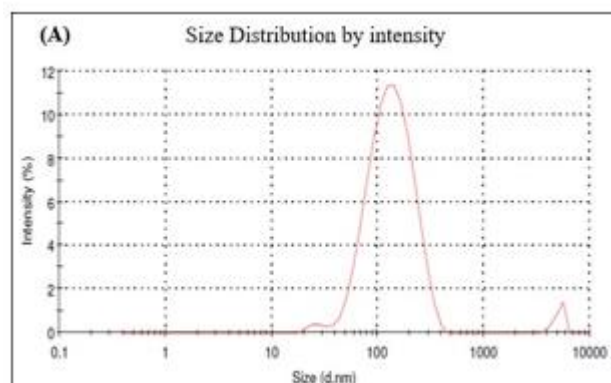
Optimization of Amount of Lipids:

To develop donepezil SLNs, four lipids such as trimyristin, tristearin, glycerol monostearate and compritol were tried at different percentages from 1 – 5 %w/v as mentioned in the Table 1. Soya lecithin and poloxamer 188 were selected as stabilizers and lipid to sum of stabilizers ratio (1:1) was kept constant in all the formulations. Soya lecithin and poloxamer were chosen in equal quantities. SLNs were prepared by hot homogenization followed by ultra sonication technique. All

five blank SLNs prepared with trimyristin / tristearin (1 – 5 %w/v) were translucent and stable. However, in case of SLNs prepared with GMS 1- 4 %w/v were translucent and stable whereas preparation of GMS 5 %w/v was viscous to creamy consistency. Blank SLNs prepared with compritol were stable only in case of 1- 3 %w/v lipid, other two preparations (4- 5 %w/v compritol) were viscous to creamy consistency. In a similar way, drug loaded SLNs prepared with four different lipids at 1 – 5 %w/v, these donepezil SLNs exhibited similar stability behavior while compared to their corresponding blank SLNs. These observations indicated that while selecting percentage of lipids in the formulation, there is need to consider the chemical nature of the lipids. Hence, type of lipid and its percentage in the formulation is important and to be considered to optimize the final formulation.

Average Particle Size and Zeta Potential of Donepezil SLNs:

A pattern of size distribution by intensity of SLNs measured by Malvern Zetasizer is shown in Fig. 1(A). PDI of all the formulations was less than 0.4 indicating narrow size distribution of the obtained nanoparticles. As the percentage of trimyristin in the formulation increased from 1 to 5 %w/v the particle size of blank SLNs increased from 102.5 \pm 4.6 to 130.7 \pm 3.5 nm, whereas donepezil SLNs of trimyristin increased from 115.9 \pm 6.1 to 147.9 \pm 6.4 nm. The similar trend observed in case of SLNs prepared with other three lipids tristearin, glycerol monostearate and compritol. While the lipid percentage increased from 1 to 5 %w/v, the viscosity of dispersed phase increases and produced comparatively larger sized nanoparticles. Average size of SLNs increases with increase in viscosity of dispersed phase has been reported²⁸.



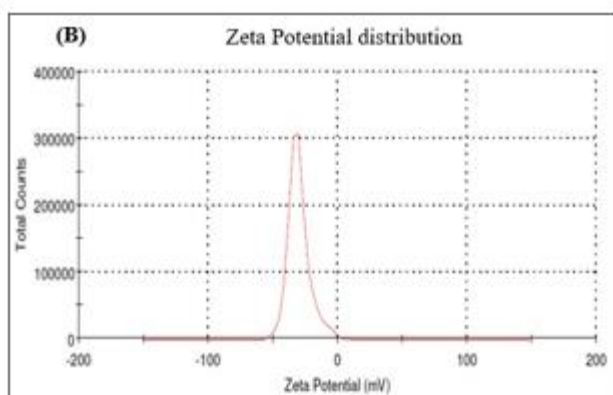


Fig.1: (A) Size distribution and (B) zeta potential distribution of donepezil SLNs by Malvern Zetasizer.

In the literature, it is found that donepezil SLNs were prepared by solvent emulsification diffusion technique using glyceryl behenate as lipid and blend of two water soluble surfactants tween 80 and poloxamer 188. They achieved the mean particle size of SLNs ranged from 99.42 to 217.19 nm²⁹. In our research work, we prepared the SLN by hot oil in water nano-emulsification method using soya lecithin (oil soluble) and poloxamer 188 (water soluble) stabilizers. Our method of preparation able to produce compritol based donepezil SLNs in a size range 156.4 to 179.3 nm. However, it has been stated that the combination of emulsifiers might prevent particle agglomeration more efficiently³⁰. Hence, compritol (glyceryl behenate) could be successfully used for the development of drug loaded SLNs. In other studies, compritol has been proven as capable lipid to produce valsartan SLNs of size 224 nm³¹ and loteprednol etabonate SLNs of size 141 nm³².

Average particle size of blank and donepezil SLNs prepared with various lipids at different percentages (1 – 3 % w/v) are shown in the Fig. 2. Average particle size of B-TM-1% is 102.5 ± 4.6 nm whereas respective drug loaded SLN i.e. D-TM-1% is 115.9±6.1 nm. Compared to blank SLNs drug loaded SLNs showed the slight higher size in all the formulations.

To compare the size and zeta potential of SLNs of various lipids, SLNs prepared with various lipids at 3%w/v are shown in the Table 3. Average particle size of blank SLNs of different lipids are in the following order B-TM (112.2±5.4 nm) < B-TS (118.0±5.5 nm) < B-GMS (135.6±6.2 nm) < B-CP (152.2±6.6 nm). Similar trend was observed in case of donepezil SLNs.

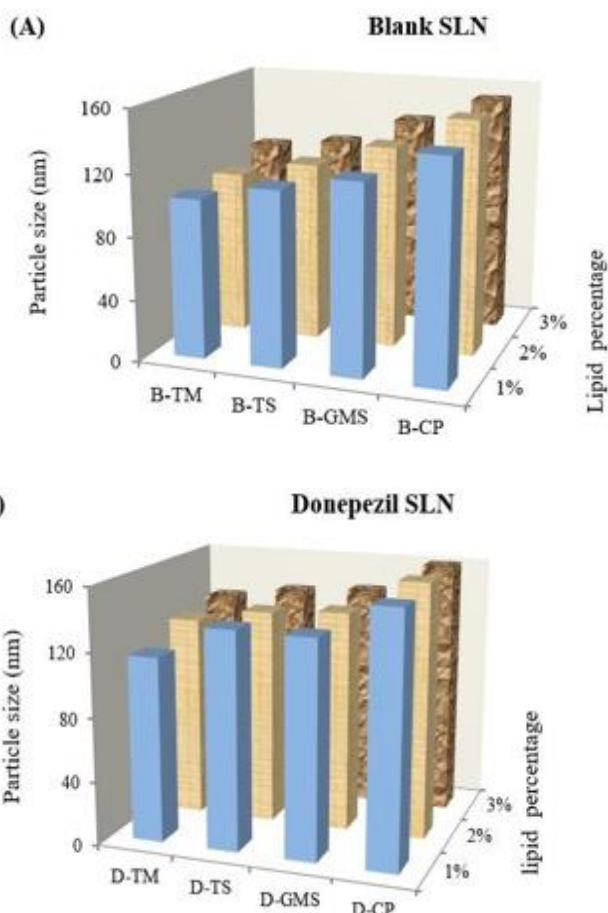


Fig. 2: Average particle size of SLNs (A) without drug and (B) with donepezil prepared using four different lipids at 1 to 3 % w/v.

Table 3: Particle size and zeta potential of SLNs prepared with various lipids at 3 %w/v (mean ± SD, n = 3).

Blank SLNs				Donepezil SLNs			
Formulation Code	Size (nm)	PDI	Zeta potential (mV)	Formulation code	Size [†] (nm)	PDI	Zeta potential (mV)
B-TM-3%	112.2±5.4	0.276	-23.5	D-TM-3%	131.7±9.6	0.349	-31.7
B-TS-3%	118.0±5.5	0.317	-26.8	D-TS-3%	138.2±3.0	0.348	-33.8
B-GMS-3%	135.6±6.2	0.394	-32.2	D-GMS-3%	141.2±8.4	0.389	-34.9
B-CP-3%	152.2±6.6	0.398	-27.7	D-CP-3%	179.3±8.4	0.359	-31.8

[†]Statistical significance with drug versus without drug is p < 0.05.

An illustration of zeta potential distribution of SLNs measured by Malvern Zetasizer is shown in Fig. 1(B). Zeta potential of various blank SLNs prepared with different lipids (1-5 % w/v) ranged from -21.7 to -32.2 mV whereas drug loaded SLNs exhibited from -28.9 to -34.9 mV. Drug loaded SLNs showed slightly higher zeta potential than their corresponding blank SLNs (Table 3).

Compatibility of Lipids:

FTIR spectra of donepezil, compritol and physical mixture of donepezil and compritol are shown in the Fig.

3. Spectra of donepezil exhibited strong intense peaks at

1693.5 cm⁻¹ (due to C = O carbonyl stretching vibrations), 1593.2 cm⁻¹ (due to C = C aromatic ring stretching) and 1313.5 and 1261.4 cm⁻¹ (due to aromatic amine C-N stretching). These peaks represent main functional groups in the chemical structure of donepezil. These functional groups retained in the spectra of physical mixture of donepezil and compritol at 1697.3 cm⁻¹ (C = O), 1595.1 cm⁻¹ (C = C) and 1305.8 and 1261.4 cm⁻¹ (C-N). Hence, there was no interaction between the drug and lipid compritol.

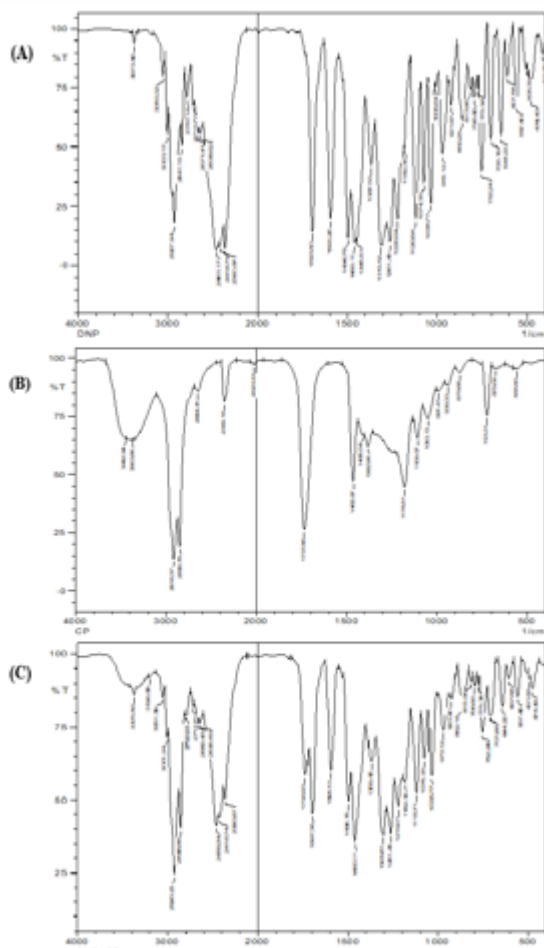


Fig. 3: FTIR spectra of (A) donepezil, (B) compritol and (C) physical mixture of donepezil and compritol.

DSC thermograms of donepezil, compritol and physical mixture of donepezil and compritol is shown in the Fig. 4.

Thermogram of donepezil exhibited sharp melting peak at 232.15 °C indicating crystalline nature of the drug. Whereas compritol exhibited melting peak at 73.02 °C. Thermogram of physical mixture showed the drug melting peak at 227.45 °C. Melting peak of drug in physical mixture is sharp and not much shifted indicating compatibility of the drug and lipid compritol. The similar trend was observed in case of FTIR and DSC reports of other three lipids such as trimyristin, tristearin and

GMS. Therefore, lipids can be used for formulation of lipophilic drug such as donepezil without any interaction.

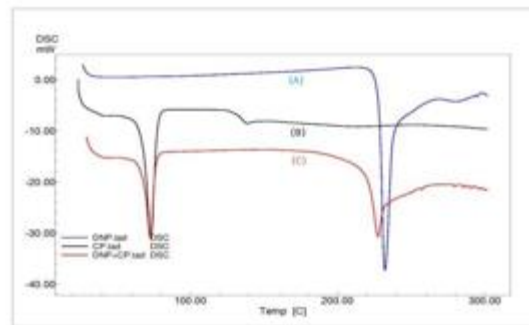


Fig. 4: Overlaid thermograms of (A) donepezil, (B) compritol and (C) physical mixture of donepezil and compritol.

Drug Content and Entrapment Efficiency:

Standard graph of donepezil was plotted by HPLC using six standard concentrations 5-30 µg/ml (Fig. 5), the average standard equation and the regression co-efficient obtained were $y = 26970x + 3472$, $R^2 = 0.999$ used for the calculation. Drug content and entrapment efficiency of various donepezil SLNs are shown in Table 4. For optimized formulations drug content was found to be in the range of 99.20 to 99.73 % whereas entrapment efficiency obtained was in the range of 86.65 to 89.64 %..

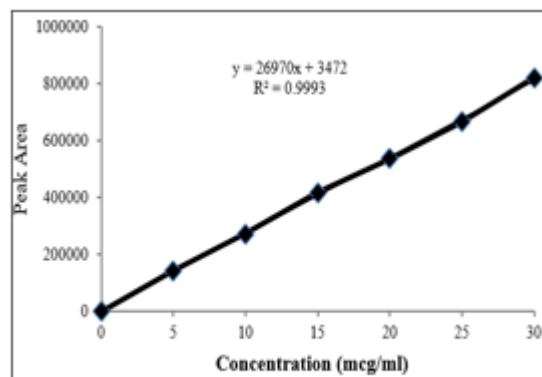


Fig. 5: Standard graph of donepezil in mobile phase at 270 nm by HPLC.

Table 4: Drug content and entrapment efficiency of various donepezil SLNs (mean ± SD, n = 3).

Formulation Code	Drug Content			Entrapment Efficiency		
	Peak Area	Concentration (mg/ml)	Drug Content (%)	Peak Area	Aqueous Concentration (mg/ml)	Entrapment Efficiency (%)
D-TM-3%	3367422	0.11939	99.44±0.34	3072233	0.114	88.06±0.78
D-TS-3%	3373023	0.11992	99.58±0.61	2883839	0.1099	89.64±0.53
D-GMS-3%	3383109	0.11995	99.73±0.57	3330988	0.131	86.65±0.39
D-CP-3%	3354522	0.11984	99.20±0.46	3100869	0.115	87.72±0.41

Entrapment efficiency of drug is depended upon its solubility in the lipid melt which is generally more than in the solidified lipid. Long chain triglycerides with higher melting points exhibit higher entrapment efficiency due to their lipophilic nature. Furthermore, the presence of mono and diglycerides in the lipid matrix promotes drug solubilisation and entrapment efficiency³³. Thus the selected lipids trimyristin and tristearin being long chain triglycerides, whereas GMS being monoglyceride and compritol being mixture of mono and diglycerides exhibited good entrapment efficiency of donepezil SLNs.

Effect of Sterilization by Autoclave:

In order to check the feasibility of administering SLNs by intravenous route, effect of sterilization on particles size of SLNs was studied. Effect of sterilization on characteristics of donepezil SLNs is shown in the Table 5.

Table 5: Effect of sterilization on characteristics of donepezil SLNs (mean \pm SD, n = 3).

SLN	Size (nm)		Zeta Potential (mV)		Entrapment Efficiency (%)	
	Before	After	Before	After	Before	After
D-TM-3%	131.7 \pm 4.6	254.3 \pm 5.2	-51.7	-53.4	88.08 \pm 0.78	87.68 \pm 0.47
D-TS-3%	138.2 \pm 3.0	257.9 \pm 6.3	-55.8	-56.1	89.04 \pm 0.53	88.72 \pm 0.39
D-GMS-3%	141.2 \pm 8.4	271.0 \pm 6.8	-54.9	-55.8	88.65 \pm 0.39	88.98 \pm 0.64
D-CO-3%	179.5 \pm 8.4	277.5 \pm 5.9	-51.8	-54.6	87.72 \pm 0.41	86.49 \pm 0.74

Size of donepezil SLNs, after sterilization increased to 1.5 to 1.9 times compared to the original size. The high temperature maintained during autoclave causes SLNs to convert into a hot o/w nano-emulsion, consequently modifies the size of the hot nano-emulsion droplets. On further slow cooling, the SLNs reformed, with small coalesce, producing larger SLN than the initial ones. However, still the size of SLNs were in nanorange. It was observed that after sterilization there was no decrease in the zeta potential of the formulations. After sterilization by autoclave, entrapment efficiency of SLNs was decreased slightly (at most 3 %), it shows that drug donepezil is favourably partitioned into melted lipids rather than aqueous medium even at high temperature. Hence, SLNs prepared with lipids, stabilized by soya lecithin and poloxamer could be subjected to autoclave for sterilization purpose. Therefore, SLNs possess another advantage of allowing autoclave sterilization, an essential step towards formulation of intravenous and ocular preparations³⁴.

Stability of SLNs:

Stability data of donepezil SLNs storing at room temperature (25 °C) is shown in the Table 6. After 3 months of storage, size of SLNs maximum increased by 38 nm. This increase in size may be due to aggregation of nanoparticles. Entrapment efficiency was lowered by 1.3 % only, which indicated no leakage of drug from the nanoparticles into dispersion medium. Whereas the drug donepezil was found to be stable under photolytic and dry heat conditions determined by HPLC³⁵⁻³⁶.

Table 6: Influence of storage conditions on size and entrapment efficiency of donepezil SLNs (mean \pm SD, n = 3).

SLN	Size (nm)			Entrapment Efficiency (%)	
	Initial	1 month	3 months	Initial	3 months
D-TM-3%	131.7 \pm 4.6	147.5 \pm 6.2	166.5 \pm 1.7	88.08 \pm 0.78	87.68 \pm 0.47
D-TS-3%	138.2 \pm 3.0	148.9 \pm 8.1	171.5 \pm 6.3	89.04 \pm 0.53	88.72 \pm 0.39
D-GMS-3%	141.2 \pm 8.4	158.5 \pm 4.9	179.2 \pm 7.7	88.65 \pm 0.39	88.98 \pm 0.64
D-CO-3%	179.5 \pm 8.4	191.5 \pm 3.8	215.6 \pm 5.8	87.72 \pm 0.41	86.49 \pm 0.74

In vitro Drug Release Kinetics:

Drug release studies of donepezil SLNs was carried out using dialysis membrane having pore size 2.4 nm. Drug release profiles obtained for donepezil SLNs of various lipids are shown in the Fig. 6. Release studies showed that there was burst release of drug for initial three hours and followed by prolonged release until 24 hours. A burst release might be due to the higher temperature used while homogenization and ultrasonication during the preparation of SLNs and presence of surfactants in the formulation. At high temperature and presence of surfactants the solubility of the drug in the water phase increases, further while cooling, drug enrichment happen on outer surface of the SLNs. Drug present in outer layer of SLNs releases in burst manner. Similar type of release i.e. initial burst release followed by a prolonged release was observed in case of prednisolone loaded SLNs due to increase in temperature and increase in surfactant concentration³⁷. Further, continuous slow release of donepezil happened by diffusion of the drug to the surface of the SLNs and later by partitioning into the aqueous phase. In vitro release studies revealed that percentage of donepezil released from developed

SLNs formulations was in the following order D-TM-3% (74.82%) > D-GMS-3% (68.90%) > D-TS-3% (66.02%) > D-CP-3% (61.58%).

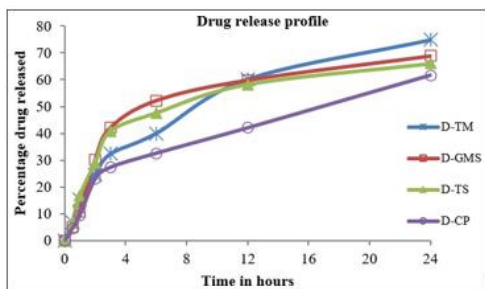


Fig. 6: *In vitro* drug release profiles of Donepezil SLNs of various lipids in phosphate buffer (pH 7.2).

potential and entrapment efficiency of SLNs was not much altered by autoclave sterilization. *In vitro* release studies showed that optimum percentage of drug released in a sustained manner. Drug release from SLNs followed Higuchi and Korsmeyer-Peppas models and drug release was anomalous diffusion. Thus developed donepezil solid lipid nanoparticles shall be utilized for better treatment of Alzheimer's disease.

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Table 7: Drug release kinetics of donepezil SLNs

Formulation code	Model dependent approaches, equations and Regression co-efficient (R ²)			
	Zero order (%CDR $\frac{1}{\sqrt{t}}$)	First order (Log %CDR $\frac{1}{\sqrt{t}}$)	Higuchi (%CDR $\frac{1}{\sqrt{t}}$)	Korsmeyer-Peppas (Log %CDR $\frac{1}{\sqrt{t}}$ Log T)
D-TM-3%	y = 2.673x + 17.77 R ² = 0.882	y = 0.032x + 1.226 R ² = 0.621	y = 16.01x + 0.485 R ² = 0.977	y = 0.384x + 1.144 R ² = 0.952
D-GMS-3%	y = 2.347x + 22.22 R ² = 0.678	y = 0.033x + 1.225 R ² = 0.444	y = 14.90x + 3.136 R ² = 0.845	y = 0.658x + 1.106 R ² = 0.853
D-TS-3%	y = 2.146x + 23.03 R ² = 0.712	y = 0.028x + 1.285 R ² = 0.466	y = 13.51x + 7.667 R ² = 0.873	y = 0.556x + 1.187 R ² = 0.864
D-CP-3%	y = 2.135x + 13.86 R ² = 0.877	y = 0.034x + 1.099 R ² = 0.566	y = 12.70x + 0.239 R ² = 0.960	y = 0.626x + 1.005 R ² = 0.912

In order to understand the drug release patterns, obtained release data was processed into different model dependent approaches such as zero order, first order, Higuchi and Korsmeyer-Peppas. After processing release data, so obtained kinetic equations and regression values (R²) of donepezil SLNs are shown in the Table 7. Drug release from SLNs followed Higuchi and Korsmeyer- Peppas models rather than zero and first order. Release exponent value ‘n’ of Korsmeyer-Peppas models characterizes the release mechanism of drug. For four donepezil SLNs ‘n’ values obtained were ranged from 0.556 to 0.658 (i.e. 0.45 < n < 0.89), hence, the drug release from SLNs followed anomalous diffusion or non- Fickian transport.

CONCLUSION:

Stable donepezil SLNs with good entrapment efficiency were produced successfully by homogenization followed by probe ultrasonication technique employing hot oil in water nano-emulsion method. Biocompatible lipids such as trimyristin, tristearin, GMS, and compritol are better stabilized by soya lecithin and poloxamer 188 in the nanorange. Size, zeta

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