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Original Article

FORMULATION AND OPTIMIZATION OF NIFEDIPINE LOADED NANOCARRIERS

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ABSTRACT

Objective: The main aim of this study to formulate a nifedipine-loaded nanocarrier for improving solubility and bioavailability.

Methods: To improve the solubility of drug, nifedipine-loaded nanocarrier (lipotomes) were prepared by using the film lipid hydration technique. lipotomes were prepared by using tween 80, which is used for increasing solubility and cetyl alcohol for lipophilic environment. Drug excipients interaction determined by FTIR. lipotomes were characterized for particle size, Entrapment efficiency and zeta potential. lipotomes were optimized by using Design-Expert 12 software. Optimized formula further lyophilized by using different cyroproyectant to improve the stability and oral administration of the drug.

Results: FTIR shows there was no interaction between formulation ingredients. Mean particle size, entrapment efficiency, zeta potential was determined and found to be 308.1 nm, 96.7%, 20.1mV, respectively. Surface morphology of lipotomes was observed by a scanning electron microscope (SEM). Optimized lipotomes was lyophilized with Mannitol (8% w/v) was the ideal cryoprotectant to retain the physicochemical characteristics of the OLT formulation after lyophilization.

Conclusion: Nifedipine loaded nanocarrier was successfully prepared, using film hydration method. Which have good particle size, EE% and zeta potential. After lyophilization no significant changes was observed in particle size with good physical stability, so it could be a good choice for conventional drug delivery system by doing further investigation as *in vitro* and *in vivo* study

Keywords: Lipotomes, Solubility, Nifedipine, Lyophilization, Nanocarriers

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INTRODUCTION

4-(2-Nitrophenyl)-2,6-dimethyl-3,5-dicarbomethoxy-1,4-dihydropyridine (Nifedipine) is under the class of calcium channel blocker,which is used for the treatment of angiocardiopathy. NI is poorly soluble in water (20 µg/ml). Although oral administration is the best convenient route and has better patient compliance; bioavailability of nifedipine has been limited by poor solubility, photo-instability, or short plasma half-life [1]. Improving solubility and membrane permeability could lead to enhance of oral bioavailability [2].

Many oral drugs like imipramine, morphine, lidocaine, etc. suffer from extensive first-pass effect, several methods were applied including changing the route of administration to rectal, injections, transdermal, intranasal, etc. to bypass the first-pass effect of the orally administered drugs [3-6]. Most preferred and favored route of administration by patients is oral route. To enhance the solubility of class II drugs in the GIT physiological conditions, several approaches have been developed including the formulation and development of nanocrystals, Micelles, Liposome's, Nanoparticles etc [7, 8].

Many limitations of different nanocarriers can be avoided through the selection of non-ionic surfactants with excellent biocompatibility profiles e. g. Tween® 80 and cetyl alcohol. Tween® 80 is safely used for oil-soluble vitamins as a solubilizer and its daily dose of administration is a 300 to 500 mg [9]. Cetyl alcohol is used as a food additive and listed by the FDA as GRAS [10]. Tween® 80 is used as a surfactant to enhance the solubility of poorly water-soluble drugs and cetyl alcohol is utilized to impart a lipophilic environment and enhance lymphatic uptake [11]. Comparing to conventional liposomes, lipotomes are superior over the due to replacing the main liposomal components, i.e., phosphatidylcholine and cholesterol with Tween®80 and cetyl alcohol. Due to ability to enzymatic degradation of liposome which contain Phosphatidylcholine shows low stability and high drug leakage in the GIT physiological conditions [12–14].

Drugs like budesonide, fluticasone propionate, chlorpromazine, verapamil, isradipine, felodipine, raloxifene, pentozocine base, dronedarone, lozartan and propafenone used as other candidates for lipotomes preparation [6]. Thin-film hydration technique. was used for the preparation of Lipotomes. Which is having an advantage over solid lipid nanoparticles which require more tedious and complicated methods of preparation [15].

MATERIALS AND METHODS

Materials

Nifedipine was gifted by Emcure pharmaceuticals,. Pune. Tween 80, Cetyl alcohol, (T80), mannitol, Aerosil, procured from Thermosil Fine Chem Pvt. Ltd. Pune.

All reagents were of the highest grade commercially available and other chemicals and solvents were of analytical grade and de-ionized distilled water was used for the preparation of all solutions.

Construction of standard calibration curve for nifedipine

Standard calibration curve of Nifedipine was developed using 0.1N HCl and estimated by UV-Visible spectrophotometer at 239 nm.

A stock solution of (1 mg/ml) of standard drug was prepared; the 1 ml of stock solution was further diluted with 100 ml 0.1 N HCl to get 10 µg/ml (working standard). later required dilutions (1µg, 2µg, 3µg, 4µg, 5µg, 6µg, 7µg, 8µg, 9µg and 10µg drug per ml solution) were made with 0.1N HCl To a series of 10 ml volumetric flasks aliquots standard solutions were taken and the volume was made up using a 0.1N HCl. The absorbance of these solutions was measured at respective wave length of maximum absorbance (239 nm) in UV Visible spectrophotometer. Standard calibration curve was obtained by plotting Absorbance values against respective concentration.

Solubility study of drug

Solubility of nifedipine was determined in various solvents. Excess Nifedipine (i. e Saturated solubility) was dispersed and stirred in different solvents for 48h at room temperature. Filter the solutions, weighed accurate quantities of the filtered supernatants, dilute the filtrate for further analysis by using spectrophotometrically at

239 nm. from the obtained data the solubility of nifedipine was calculated in the respective liquid vehicle [16].

Compatibility studies

Drug and excipients compatibility studies by FTIR spectrophotometer

The infrared spectra of pure drug and mixture of polymers and excipients were studied by FT IR spectroscopy using the KBR. The KBr discs were prepared by compressing the powders at pressure of 5 tons for 5 min. in a hydraulic press. Scans were obtained at a resolution of 4 cm^{-1}; from 4000 to 600 cm-1 here spectral changes in the mixture are the basis for the determination of compatibility. The obtained spectrums of different formulation combinations were shown below. The spectral analysis of the pure drug and excipients mixture were done [17].

Method of preparation of nifedipine lipotome

Hydration of thin lipid film (Bangham method)

Nifedepine loaded Lipotomes was prepared using a thin film hydration technique. The drug (30 mg) Tween 80), Cetyl Alcohol were weighed and dissolved in 10 ml of (2:1 v/v) mixture of chloroform: methanol in a 250 ml round-bottom flask [11]. The organic solvent was slowly evaporated using a rotary evaporator revolving at 150 rpm for 15 min at 75 °C under reduced pressure. After the formation of a thin dry film, thin film expose in phosphate buffer PH 7.4 for hydration. Appeared suspension will be agitated for 30 min and then sonicated for 1 Hr for size reduction.

Initially nine bathes of Different concentration of CA: Tween 80 w/w (F1-F9) was prepared. Based in Particle size and entrapment efficiency F4, F5, F6 showed the least particle size and maximum entrapment efficiency as shown in table 5. For optimization of bath 3^2 factorial design was applied.

Statistical design study

To study the influence of the different variables used for preparation, as a characteristic of formulated lipotomes Central composite experimental design Design-Expert software was used. (version 12) In this design and within 9 runs, two factors were evaluated. The independent variables were lipid: surfactant w/w ratio (X1) and X2= Rotations per minute (RPM) (X2). The the dependent variables are particle size (Y1: PS), Entrapment efficiency (Y2: EE) and Zeta potential (Y3: ZP) were selected.

Table 1: Experimental designing by 3^2factorial

Formulation variables	Levels coded			Dependent variable
	-1	0	+1	
X1= lipid: surfactant w/w ratio	1:4	1:5	1:6	Y1=Particle size (nm) Y2=Entrapment Efficiency (%) Y3=Zeta potential
X2= Rotations per minute(RPM)	130	150	170	

Table 2: A 3^2 Full factorial experimental design layout

Formulation code	Coded factor levels	
	X1	X2
F1	-1	-1
F2	0	-1
F3	+1	-1
F4	-1	0
F5	0	0
F6	+1	0
F7	-1	+1
F8	0	+1
F9	+1	+1

Characterization of lipotomes

Particle size analysis

Determination of average particle size of lipotomes was very important characteristic. It was determined by using MALVERN INSTRUMENTS, DRSSK LABS PVT. LTD.

Entrapment efficiency

The entrapment efficiency of lipotomes will be determined by ultracentrifugation at 30,000-40,000 rpm and 4 °C for 1 hour using ultracentrifuge. Following centrifugation, the supernatant and vesicles will be separated. The supernatant will be removed and drug quantity will be analyzed by analytical method. The percentage of drug encapsulated was determined after lysis of the prepared lipotomes with absolute alcohol and sonication for 10 min. The concentration of Nifedipine in absolute alcohol was determined spectrophotometrically at 239 nm using a UV-visible spectrophotometer. The encapsulation efficiency expressed as entrapment percentage was calculated through the following relationship [18-21]**.**

$$\text{Entrapment efficiency \%} = \frac{\text{Total drug} - \text{free drug}}{\text{Total drug}} \text{X100}$$

Zeta potential

Zeta potential of the diluted samples was measured for each formula to evaluate its physical stability Measurements were done in triplicates for three independent samples from each formula and then the average values SD were calculated.

Lyophilization of lipotomes

Different cryoprotectants were mixed with optimized lipotomal batch. The selected cryoprotectants were aerosol and mannitol used in four different concentrations (2%,4%,6%,8%)The lyophilized formulae in presence of surfactants took the abbreviation from NL1 to NL8 where NL1–NL4 formulae were containing mannitol and NL4–NL8 formulae were containing Aerosil,

The saccharides or cryoprotectants were dissolved in PBS buffer at different concentrations of 2, 4, 6, and 8% of dry lipids. Lipotomal suspensions were diluted in an equal volume of each saccharide buffered solution in 50 ml tubes, at 10% of fill volume. As control, lipotomal suspension was diluted in equal volume of PBS buffer. A very low freezing temperature seems to avoid damage of nanoparticles. Considering these assumptions, all the liposomal suspensions were stored for 6 h in a deep freezer and then freeze-dried for 24 h in chamber with pressure 6 Pa.[3] [22, 23].

Characterization of the lyophilized nifedipine lipotomes

Determination of moisture content and reconstitution time

Moisture content of the lyophilized lipotomal formulations was analyzed using Karl Fischer titrator. The initial moisture content in 0.5 gm of the investigated lyophilized formulations was determined and expressed as %w/w.

Reconstitution time; accurately weighed amounts of the lyophilized formulations were reconstituted using equal volume of distilled water as that being removed during the lyophilization process. The samples were reconstituted using vortex. The time (reconstitution time) taken by the lyophilized lipotomes to form an aqueous dispersion without any aggregates was measured. Each experiment was conducted in triplicates for each formula and the mean values SD were calculated [24].

Particle size of lyophilized lipotomes

PS of the lyophilized Nifedipine lipotomes were analyzed after reconstitution. The same analyzing method, as in case of lipotomal dispersions was used.

Scanning electron microscopy

Surface morphology of the Lyophilized lipotomal formulations was investigated using SEM (JXA-840; JEOL, Japan), after being coated with gold under vacuum [25].

RESULTS AND DISCUSSION

Construction of standard calibration curve for nifedipine

Calibration curves of Nifedipine in 0.1N HCL is represented in fig. 1. The curves were linear at the concentration range of 1-10 µg/ml with regression values of 0.9985. The high regression values indicate that the calibration curves follow Beer's law.

Table 3: Standard readings of nifedipine in UV

Concentration (µg/ml)	Absorbance at 239 nm
0	0
1	0.166
2	0.336
3	0.493
4	0.643
5	0.780
6	0.9523
7	1.114
8	1.288
9	1.399
10	1.598

Solubility of nifedipine

The saturated solubility of Nifedipine in water was found to be equal to 0.003 mg/ml, this indicated that the drug is very slightly soluble in water and it shows good solubility in methanol and chloroform as 0.25 mg/ml and 1.6 mg/ml respectively. it indicates methanol and Chloroform is good choice of solvent in method of preparation of lipotomes.

Solubility data of drug nifedipine in various liquid vehicles is shown in table 4.

Table 4: Solubility of drug in different solvents

Solvent	Solubility (mg/ml)
Methanol	0.25±0.26
Chloroform	1.6±0.90
Ethanol	0.18±0.85
Water	0.003±0.48
Tween 80	0.21±0.32

Values represent mean±SD (n =3)

Compatibility studies

Drug and excipients compatibility studies by FTIR spectrophotometer

Drug-excipients interaction was studied using Fourier transform infrared (FTIR) spectroscopy, and the results are presented in fig. 2, 3, 4. Nifedipine demonstrates characteristic peaks in FTIR at 1225 cm−1 (due to its CH deformation) and 1682 cm−1 (due to C-C stretching) [26]. In this study both Drug Nifedipine and Mixture of Drug with Excipients (Cetyl alcohol) showed the characteristic peak about at same wavelength.

From this study and the graphs based on peaks and wave numbers that specific functional group, no additional peaks were obtained which indicates that there is no significant interaction between drug and excipients.

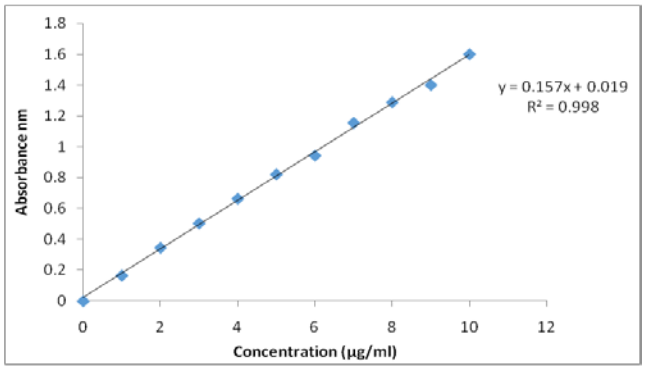

Fig. 1: Standard graph of nifedipine in 0.1N HCl

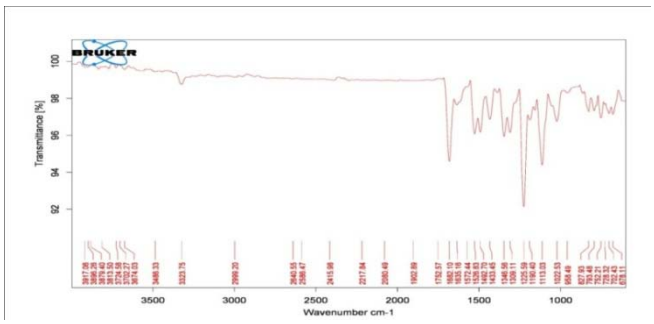

Fig. 2: FTIR of pure nifedipine

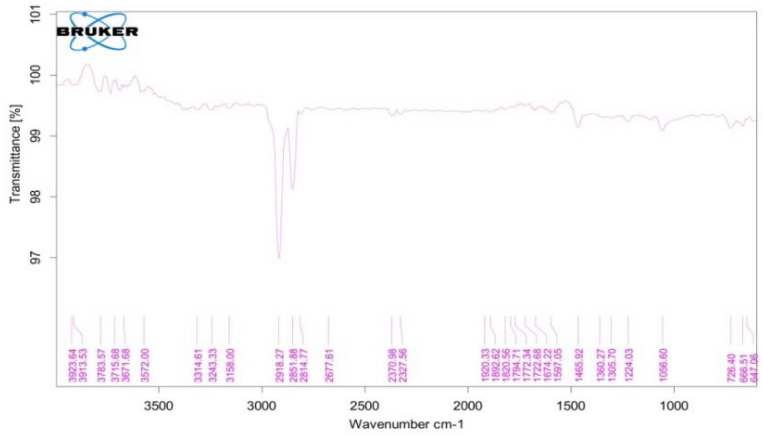

Fig. 3: FTIR of cetyl alcohol

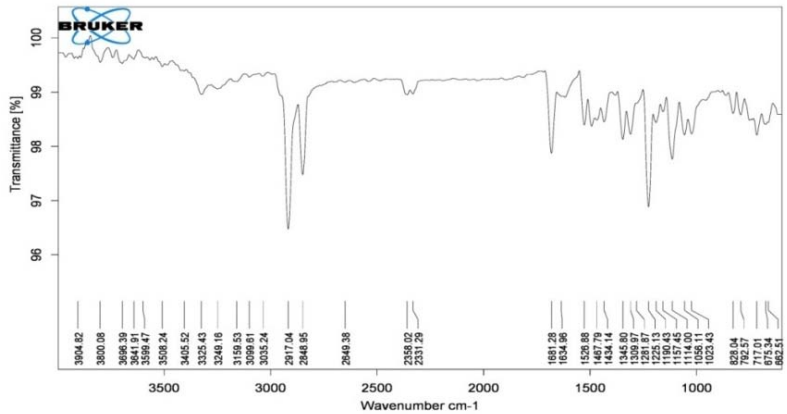

Fig. 4: FTIR of mixture of drug and excipient

Characterization of prepared lipotomes

Analysis of particle size, entrapment efficiency and zeta potential

Table 5: Particle size and entrapment efficiency of lipotomes prepared using film hydration method

Formulations	CA: Tween 80 w/w	Drug (mg)	Chloroform: methanol (v/v)	Particle size (nm)	Entrapment efficiency (%)
F1	1:1	30	2:1	485.3±2.7	79.1±3.2
F2	1:2	30	2:1	567.2±3.9	74.7±1.6
F3	1:3	30	2:1	646.6±6.5	69.6±2.7
F4	1:4	30	2:1	218.1±7.1	89.7±3.9
F5	1:5	30	2:1	269.2±12.4	96.3±1.1
F6	1:6	30	2:1	293.2±10.4	92.1±2.7
F7	1:7	30	2:1	141.3±1.2	88.8±2.1
F8	1:8	30	2:1	159.3±11.2	87.6±1.1
F9	1:9	30	2:1	194.3±0.9	82.8±4.2

Values represent mean±SD (n =3)

Particle size

By using different ratio of Tween 80 and cetyl alcohol nifedipine lipotomes was prepared and particle size of lipotomes varies from 308.1 nm to 404.3 nm

Fig. 5A presents the effect of different factors on the mean PS. These investigated factors were X1= lipid: surfactant w/w ratio and X2= Rotations per minute (RPM) from the presented figure, it is evident that both factors showed significant effects on the mean PS (p-values<0.001). It was manifest that increasing the Tween 80 in case of factor (X1) and RPM in case of factor (X2) led to a significant decrease in the mean PS produced lipotomes with smaller particle size [19].

Entrapment efficiency

EE of all Nifedipine loaded lipotomes prepared formulations were within the range of 79.1% to 96.3%, as shown in table 6 and fig. 5B. X1= lipid: surfactant w/w ratio and X2= Rotations per minute (RPM) showed the significant effect on entrapment efficiency. Increase in lipid: surfactant w/w ratio and RPM decrease in entrapment efficiency. Formulation (F4) which has 1:4 lipid: surfactant ratio and 150 RPM showed the maximum entrapment efficiency [27].

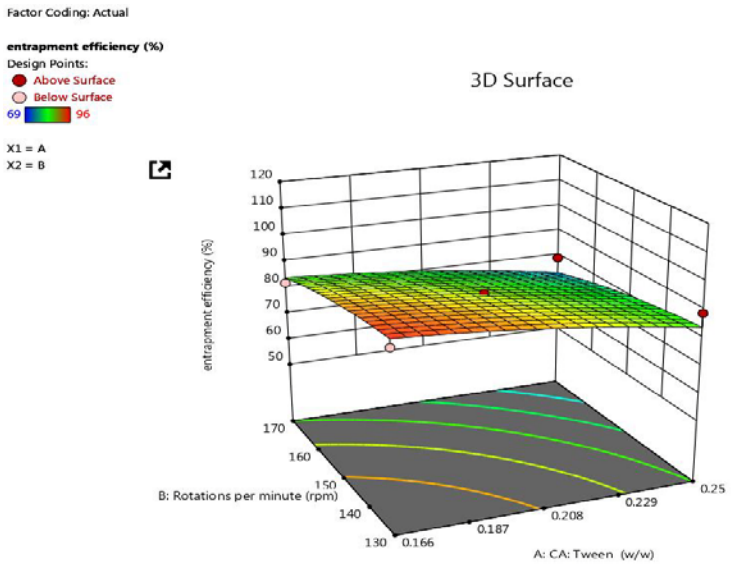

Fig. 5A: Response surface plots for the effects of lipid: surfactant w/w ratio (X1) and rotations per minute (RPM) (X2) on the mean particle size of liposomal formulations

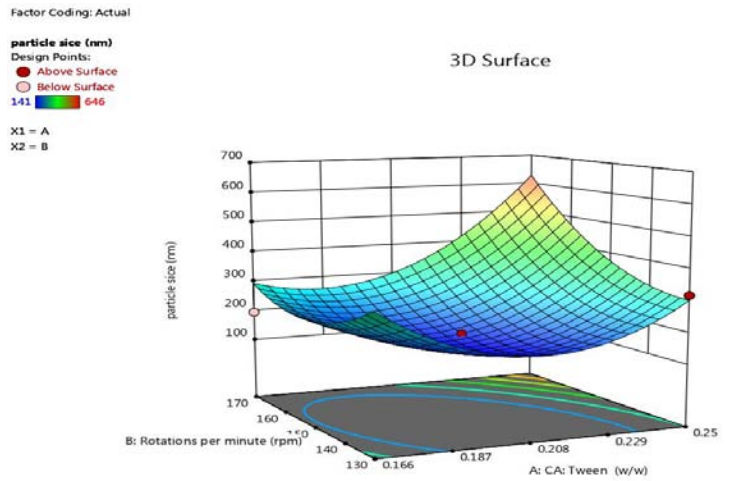

Fig. 5B: Response surface plots for the effects of lipid: surfactant w/w ratio (X1) and rotations per minute (RPM) (X2) on entrapment efficiency of lipotomal formulations

Zeta potential

ZP values of the prepared formulations between–20.1and–26.7 mV, as shown in table 6 the higher the zeta potential higher the repulsive force between particle which prevent aggregation of the nanovesicles. The zeta potential is a good indicator for the stability of the nanoparticles. Nanoparticles carrying high electric charge will protect the nanoparticles in from aggregation due to the high repellent forces between the particles. It was previously mentioned that high absolute zeta potential values provide excellent stability for the nanoparticles [28]. From the zeta potential results, it is observed that obtained values will Shows a good degree of stability for all the prepared lipotomal formulations.

Table 6: Experimental runs, independent variables and measured responses of the 3^2 full factorial experimental designs

Formulae	X1= lipid: surfactant w/w ratio	X2= Rotations per minute (RPM)	Y1= Particle size (nm)	Y2= Entrapment efficiency (%)	Y3= Zeta potential (mV)
F1	1:4	130	385.3±2.69	92.48±3.19	-24.4±1.1
F2	1:5	130	367.2±4.14	94.2±5.17	-22.1±1.0
F3	1:6	130	346.6±3.81	89.4±4.09	-23.0±0.5
F4	1:4	150	308.1±2.79	96.7±5.31	-20.1±0.2
F5	1:5	150	389.2±4.29	95.3±3.31	-24.2±0.6
F6	1:6	150	393.2±4.71	90.1±5.44	-26.7±0.8
F7	1:4	170	341.3±2.16	84.7±3.39	-25.4±0.6
F8	1:5	170	359.3±3.41	92.6±4.61	-26.1±1.0
F9	1:6	170	404.3±5.52	91.8±4.21	-26.2±0.6

Values represent mean±SD (n =3)

Optimization of lipotomes

Nifedipine loaded lipotomes were prepared by film hydration method. The study showed that particle size and entrapment efficiency of drug was significantly affected by lipid: surfactant ratio and stirring Rate (RPM). so it was demonstrate that optimal formulation (F4) having lipid to surfactant ratio (1:4),chloroform to methanol ratio(2:1) and stirring rate (150RPM) was chosen best formula showing least particle size (308.1 nm) and maximum entrapment efficiency(96.7%).

Lyophilization of lipotomes

Optimized lipotomal formula (F4) was used for lyophilization, to improve the drug stability and drug retention in lipotomes lyophilization was carried out of optimized batch by using different cyroprotectant as aerosol and mannitol used in four different concentrations (2%,4%,6%,8%). lyophilized formula containing 8% mannitol (w/v)showed minimum moisture content, good reconstitution time, proved to be suitable for further studies as invite and *in vivo* study. after lyophilization no significant changes occur in lipotomes particle size.

Characterization of the lyophilized nifedipine lipotomes

Determination of moisture content and reconstitution time

Prepared lyophilized lipotomes shows low moisture content ranging from 1.49±0.11 to 2.59±0.01 (w/w) lipotomal formulations containing mannitol showed lower initial moisture content when compared to lipotomal formulations containing the same concentration of arosil where, formula NL4 containing 8% (w/v) mannitol possessed the lowest moisture content, 1.49% w/w This difference was accredited to the ability of mannitol to resist moisture uptake in comparison to arosil. Lyophilized samples pre-treated with cryoprotectants like mannitol or arosil showed better re-dispersion upon reconstitution. Moisture content and reconstitution time determined for lyophilized lipotomal formulations is represented in table 7. From the table, it is evident that the lyophilized lipotomes containing mannitol as cyroprotectant show the less moisture uptake and good redespersibility.

Particle size

Cyroprotectant with different concentrations was used for lyophilization, it was observed that 8% mannitol preserve the partical size of lipotomes no significant changes in particle size after lyophilizationthe. Mean particle size of lyophilized nifedipine lipotomes was shown in table 7.

Scanning electron microscopy

SEM was utilized to examine the morphology of the optimized lyophilized lipotomal formulations (NL4). Showed that the air-dried NL4 formula were regularly spherical in shape with smooth surface as shown in fig. 6.

Table 7: Moisture content, reconstitution time and particle size of lyophilized lipotomes

Formula	Moisturecontent (%w/w)	Reconstitution time (min)	Particle size (nm)
NL1	2.19±0.15	1.91±0.10	1019.13±3.07
NL2	1.93±0.11	1.62±0.09	929.17±6.65
NL3	1.73±0.20	0.92±0.12	817.12±9.56
NL4	1.49±0.11	0.63±0.05	318.6±2.97
NL5	2.59±0.01	2.80±0.14	998.52±7.18
NL6	2.31±0.08	2.49±0.17	813.19±9.56
NL7	1.84±0.17	1.84±0.10	654.50±8.54
NL8	1.69±0.05	1.39±0.08	519.28±5.51

Values represent mean±SD (n =3), NL: Nifedipine lipotomes

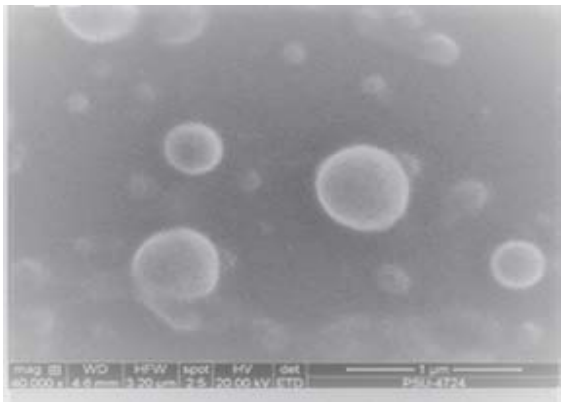

Fig. 6: SEM of lyophilized nifedipine lipotomes (NL4)

Selection of the lyophilized lipotomal formula

From the above results, it is observed that the lyophilized lipototoes (NL-4) formula pre-treated with 8% mannitol (w/v) showed good result compared to other formulations. As lowest reconstitution time, PS and moisture content. Hence, formula NL-4 was selected for further investigations.

CONCLUSION

Lipotomes was prepared by using cetely alcohol and tween 80 which provide combine the lipophilic environment and solubilizing power respectively. That is, lipotomes offer dual action for enhancing drug's oral bioavailability. lipotomes is a stable and easily prepared platform for oral administration. The prepared lipotomes are mainly composed of lipophilic fatty alcohol and a hydrophilic surfactant, which create or provide microenvironment for the entrapment of a wide range of active ingredients. In this study, we designed nifedipine lipotomes by using film hydration method Hence, optimized lyophilized lipotomes was prepared at the chosen optimal factors composition, and its evaluation showed development of a successful formulation with very good compatibility, convenient particle size and high drug entrapment efficiency. This study concludes. However in further studies lyophilized lipotomes formulation to be used as better option of the conventional drug delivery system in the treatment of hypertension. further investigations need to be conducted *in vitro* and *in vivo* study.

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Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

The author has no conflict of interest to declare.

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