IJMPR 2024, 8(7), 77-87

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article
SJIF Impact Factor: 6.669

LIPOSOMAL FORMULATIONS OF PRAZIQUANTEL: OPTIMIZATION THROUGH THIN FILM HYDRATION FOR IMPROVED DRUG DELIVERY

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Article Received on: 29/04/2024
Article Revised on: 19/05/2024
Article Accepted on: 09/06/2024

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ABSTRACT

The purpose of this study was to optimize the liposomal formulation of Praziquantel for administering large doses, utilizing the method of thin film hydration. Liposomal praziquantel was prepared by thin film hydration using the method of ultrasonication. The study systematically explored three formulations (F1: 1:6, F2: 1:8, F3: 1:10 by molar ratios) and was evaluated for quality of films, hydration, size reduction, purification, quantitation of free drug, and finally freeze-drying. Characterizations included DLS, FESEM, and ATR-FTIR. Thin film hydration yielded good quality films, ensured efficient hydration, and underwent downsizing to unilamellar vesicles, notably in F3 within 6 minutes. Purification resulted in a free drug precipitated in the order of F3 < F2 < F1. Free drug quantitation revealed F3 with the highest drug loading (98.7%), followed by F2 (82.5%) and F1 (65.6%). F3 exhibited a hydrodynamic radius of 99.4 nm ± 2.4 nm. FE-SEM confirmed liposomes to be spherical (158.14 ± 6.03 nm). FTIR suggested no interaction between SPC and praziquantel and also affirmed drug was incorporated into the bilayer. This preliminary study establishes reproducible parameters for uniform liposome formulations through thin film hydration, offering potential advancements in drug delivery systems. The results ensure a reproducible process and material-specific parameter settings for the preparation of uniform liposome formulations, addressing the inherent heterogeneity of thin-film hydration and opening avenues for the development of more complex formulations in the future.

KEYWORDS: Liposome, Praziquantel, Thin film hydration, Drug delivery, Characterization.

INTRODUCTION

Oral drug delivery has been recognized as an exceptionally attractive method among various drug delivery routes, mainly due to its ease of administration and also for its ability to deliver versatile formulations. However, the oral route encounters several challenges[1], one of which is the delivery of poorly soluble drugs. These drugs with low aqueous solubility (<100 µg/ml) face dissolution-limited absorption, requiring a high dose-to-solubility ratio, finally ending up with suboptimal oral bioavailability for the drug.[2] The diminished bioavailability ultimately can compromise the therapeutic efficacy, necessitating dose escalation[3], which contributes to the increased overall cost of the final formulation and, in some instances, to associated toxicity.[4]

Praziquantel, a well-known broad-spectrum antiparasitic drug used as the first choice for schistosomiasis and taeniasis in both humans and animals[5] is considered a poorly soluble drug belonging to the Class II of the Biopharmaceutical Classification System (BCS). The problem of low aqueous solubility, and the significant hepatic first pass, lowers the concentration of the drug, leading to less prolonged therapy and also less efficacy against immature worms in circulation, which leads to a mass treatment failure.[6]

Conventional formulation methods offer solutions under such situations by aiming to enhance the dissolution rate of medications by adhering to the principle of "larger the surface area, higher the dissolution rate".[7] However, traditional techniques come with limitations, including delayed onset of action, suboptimal oral bioavailability, challenges in achieving dosage proportionality, difficulty

in attaining steady-state plasma levels, and the potential for unwanted side effects.[8] To overcome this limitation of conventional formulations, nanomedicine emerges as a promising avenue, offering innovative medication delivery technologies with enhanced features.[9] Within nanocarriers, nanoparticulate drug delivery systems such as liposomes are garnering attention for their capability to solubilize water-insoluble drugs into nano-sized structures.[10]

Liposomes belong to the class of vesicular drug delivery systems, that constitute highly ordered assemblies of one or more concentric bilayers belonging to a broader classification of colloidal carriers systems.[11] Colloidal systems are nanoparticle dispersions typically in the size range of approximately 1-100 nm. Therefore, when a drug is dispersed or embedded within another molecular entity or continuous phase, the drug undergoes high-energy interactions with the carrier, thereby reducing the drug-drug interactions and facilitating improved dissolution. The amelioration in dissolution, in turn, contributes to enhanced drug absorption.[12]

Therefore, in this study, preparation of a conventional formulation of liposome was attempted for BCS Ⅱ drug praziquantel. Most of the conventional formulations of liposomal praziquantel are prepared in very small doses as outlined in studies.[6,13,14] In instances where specific studies necessitate higher doses, the production of formulations in small quantities can become intricate and often results in less homogeneity when scaled up. Hence in the current study, preparation of a conventional liposomal formulation of praziquantel was attempted using the technique of thin film hydration, to prepare a formulation having high drug loading and in optimal nano size range.

MATERIALS AND METHODS

Materials

L-α-Phosphatidylcholine (P5638), Type Ⅱ-S, and praziquantel was purchased from Sigma-Aldrich Chemical Pvt. Ltd., Bengaluru, India, and Merck India Ltd., Bangalore, India, Solvent: Chloroform was purchased from M/s Merck India Ltd., Bangalore, India, Phosphate buffered saline (PBS) of pH 7.4: PBS of pH 7.4 was prepared by dissolution of 1 PBS tablet (Sigma-Aldrich (P4417) in 200 ml of deionized water. The solution was stirred in a magnetic stirrer until no precipitate was observed. Sucrose pure (84973) was purchased from Sisco Research Laboratories (SRL) Pvt. Ltd. India.

Synthesis of liposomal praziquantel by thin film hydration method

Liposomal formulations of praziquantel were created by utilizing the technique of thin film hydration with minor modifications.[13] The study examined three molar ratios of praziquantel (PRZ) to L-α phosphatidylcholine (Soy phosphatidylcholine/SPC) to optimize praziquantel loading into liposomes. The study began with praziquantel to soy-phosphatidylcholine (SPC) ratio of 1:6 (F1) followed by the preparation of formulations at ratios of 1:8 (F2) and 1:10 (F3).

Preparation of Phosphatidylcholine-Chloroform-Drug mixture

To the prepared SPC-chloroform solution, accurately weighed and added 100 mg of praziquantel powder. The mixture was stirred uniformly using a hot plate magnetic stirrer until complete dissolution of the drug was achieved. The quantities of each component for the three distinct formulations (F1, F2, F3) investigated were composed according to the values specified in Table 1. The solubility of SPC in chloroform was taken as 100 mg/ml. The molecular weights of L-α phosphatidylcholine and praziquantel were taken as 776 g/mol (Da) and 312.4 g/mol, respectively.

Subsequently, the drug-SPC mixture was transferred to a 1-liter round-bottom flask, of a rotary vacuum evaporator (M/s Buchi, Switzerland), to facilitate the solvent evaporation process. The parameters of the vacuum evaporator were configured at 105 rpm, 284 bar of pressure, and 35 °C temperature for the bath. The flask was subjected to vacuum conditions to extract the majority of chloroform over 45 minutes. Afterward, any residual chloroform was removed by leaving the flask in a vacuum desiccator overnight.

Table 1: Composition of thin film mixture containing soy-phosphatidylcholine, chloroform and praziquantel.

Ratio (SPC: PRZ)	Weight of SPC (g)	Volume of Chloroform (ml)	Molarity of SPC (mM)	Amount of praziquantel (mg)	Molarity of PRZ in mixture (mM)
1:6 (F1)	1.5	15	128.8	100	21.3
1:8 (F2)	2	20			16
1:10 (F3)	2.5	25			12.8

Hydration of lipid film

The lipid film was hydrated using sucrose in phosphate-buffered saline (sucrose: lipid ratio of 2:1) pH 7.4, following the method outlined elsewhere.[15] For the hydration step, 20 ml of the prepared solution was transferred into rotary flasks containing thin films, each with varying lipid-drug molar ratios. The precise composition of the hydration mixture is detailed in Table 2.

The hydration process was executed in a rotary flask connected to a rotary apparatus without the application of a vacuum. The rotation speed was set at 250 rpm, and a water bath was maintained at a temperature of 45°C,

taking into consideration the phase transition of SPC within the range of -20 to -30 °C.[16] Periodically, the flask underwent a brief removal, followed by 5 minutes of bath sonication, and then resumed rotation until the thorough detachment of the lipid film from the flask surface was confirmed. The hydration process was sustained for 1 hour. After hydration, the dispersion was transferred to a beaker and stored at 4°C for aging before further downsizing.

Table 2: Composition of phospholipid dispersions (F1, F2, F3) after hydration with 20 ml of sucrose in PBS of pH 7.4.

SPC: PRZ	SPC (g)	Volume of hydration (ml)	Concentration of lipid in hydration fluid (mg/ml)
1:6 (F1)	1.5	20	75
1:8 (F2)	2	20	100
1:10 (F3)	2.5	20	125

Downsizing of liposome

The size reduction process was accomplished utilizing the probe sonication method, employing a UP200Ht - 200W, 26kHz Handheld Ultrasonic Homogenizer (M/s Hielscher Ultrasonics, Germany). Liposomal praziquantel, with varying molar ratios, was positioned in a 50 ml beaker and subjected to sonication using a 6 mm titanium sonotrode (S26d7, with a processing capacity of 20-500 ml) for 6 minutes in two cycles of 3 minutes each (3 minutes ON, 3 minutes OFF, 3 minutes ON). The sonication process was conducted at 40% amplitude and 120 watts of power, with the setup maintained under an ice water bath to prevent overheating of the probe. After sonication, the liposomes were securely sealed and stored in a refrigerator at 4°C for subsequent processing.

Purification of liposomes

The liposomal drug preparation underwent purification by removing unencapsulated drugs from the liposomes, employing high-speed centrifugation (Eppendorf 5430R, M/s Eppendorf, Germany).[17] Specifically, 1 ml of liposomal praziquantel from the 20 ml prepared dispersion was transferred into 2 ml Eppendorf tubes (1 ml volume) and subjected to high-speed centrifugation at 12,500 g for 15 minutes. The resulting precipitated-free drug was utilized for quantifying the drug loading in liposomes.[13] The supernatant, which contained the encapsulated drug, was separated and utilized for dosage formulation.

Estimation of drug loading in liposome

UV-Visible spectroscopy

The determination of drug loading in liposomal praziquantel formulations (F1, F2, F3) was conducted through an indirect method, involving the measurement of the amount of free drug formed as a pellet after high-speed centrifugation.[13] The pellet, which contained an unencapsulated drug, was dissolved in 1 ml of methanol through vortexing using a vortex mixer (M/s Remi, India) for 5 minutes. Subsequently, from this stock solution, 10 µl of the sample was extracted, to which 2990 µl of methanol was added (total volume of 3 ml). The quantification of drug loading in liposomes was then conducted using a UV-visible spectrophotometer (BioMate 3S, M/s Thermo Scientific, Germany).

$$\text{Drug loading \%} = \frac{Total\ amount\ of\ added\ drug - free\ unencapsulated\ drug}{Total\ amount\ of\ added\ drug} \text{ X } 100$$

A calibration curve was established for praziquantel in methanol at 206 nm, utilizing concentrations ranging from 1 µg/ml to 10 µg/ml. This curve was employed for the determination of the unknown amount of the drug in liposomal formulations F1, F2, and F3.

Freeze drying of liposomes

The formulation with the highest drug loading underwent freeze-drying using a high-capacity upright freeze dryer (M/s Operon, Korea). The liposome dispersion was first placed in the cold trap for 1 hour and subsequently transferred to the freeze dryer operating at -80°C for comprehensive drying. After a 5-day freeze-drying process, samples were collected, securely packed, and stored in a refrigerator at 2-8°C for subsequent characterization and dosage formulation.

Characterization of liposomes

Formulation with an optimal drug-to-lipid ratio that maximized the drug loading and had good stability with storage was selected for characterization.

Particle size analysis using dynamic light scattering spectroscopy (DLS)

The hydrodynamic radius of the liposome dispersion post-hydration and the drug-loaded liposomes were evaluated using a Nanoparticle Analyzer (Horiba SZ-100 series, M/s Horiba Ltd, United States). Distilled water served as the dispersion medium for analyzing the size of the liposomes.[18] All the parameters were selected through sequential trails of sample analysis which provided a value of minimum standard deviation for the measured particle size. Samples were prepared by extracting 50 µl from the 20 ml colloidal dispersion. This extract was then mixed with 20 ml of distilled water until a solution ranging from clear to slightly hazy was

achieved. From this infinitely diluted solution, 3 ml was transferred to a quartz cuvette for dynamic light scattering (DLS) analysis to ascertain the particle size. The determination of the particle size involved multiple iterations through trial and error.

Morphology of liposomes- Field emission Scanning electron microscopy

The morphology of Liposomal praziquantel was examined through Field Emission Scanning Electron Microscopy (Carl Zeiss Sigma, M/s Carl Zeiss, Germany). In summary, 1 mg of the sample was suspended in 1 ml of distilled water, and 2 µl of this suspension was applied to a sample stub. After drying the stub in an oven at 45°C for 10 minutes, the samples were gold-coated using an Ion Sputter and analysed at an accelerating voltage of 5 Kv.[19] Image analysis was conducted using SmartSEM software v5.07.

Characterization of liposomes using Attenuated total reflectance – Fourier transform infrared spectroscopy (ATR-FTIR)

The interaction among various components in the liposomal formulation was evaluated by analysing the FTIR spectra of free liposomes, drug-loaded liposomes in PBS, and drug-loaded liposomes with sucrose in PBS. Spectra were acquired using an FTIR spectrometer (Perkin Elmer Spectrum Two M/s Perkin Elmer, UK) with a sampling station equipped with an ATR accessory (M/s Perkin Elmer, UK). The samples were scanned at room temperature within the range of 400–4,000 cm^{-1}, with a scanning speed of 2 mm/s and a resolution of 4 cm^{-1}.

RESULTS AND DISCUSSION

Preparation of drug-loaded liposome

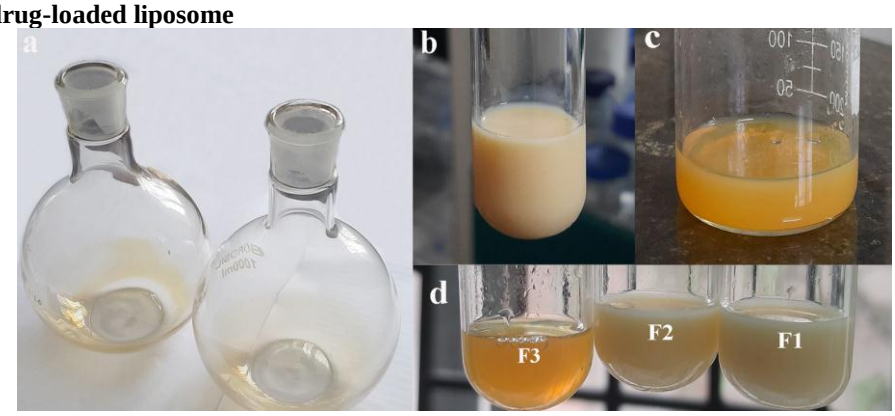

Figure 1: a) Thin film formation after complete evaporation of chloroform, b) Drug loaded liposomes formed after hydration of 1 hr c) Free liposome formed after hydration of 1 hr d) Colloidal nature of formulations (F3, F2, F1) after probe sonication for period of 6 minutes under ice bath.

In the present study, a conventional liposomal formulation of praziquantel was undertaken and was prepared in larger doses to mitigate the inhomogeneity and repetition errors associated with small dosage preparation. Vacuum evaporation of chloroform from formulations F1, F2, and F3 in a rotary vacuum apparatus yielded uniform thin films within the rotary flask, as depicted in Figure 1a. The applied parameters of 105 rpm, 35°C, and 284 bar pressure guaranteed the consistency of the thin film formation across iterations, without evident creases or gaps in the film. This is of paramount importance, as the quality of the dry film significantly impacts the final size and size distribution of liposomes.[20]

The hydration process of drug-loaded lipid films resulted in a homogeneous dispersion with a visibly milky-white appearance, as illustrated in Figure 1b. The successful hydration of the lipid film was confirmed by the complete detachment of the film from the flask surface with a minimal amount of hydration fluid. In contrast, the hydration of the film without drug content led to a translucent yellow dispersion, devoid of the milky appearance, as depicted in Figure 1c. It was also observed that, the thinner the film, the less the volume of hydration fluid required for complete and successful hydration. This could be due to the increased surface area available for the hydration fluid to act.

The size reduction of praziquantel formulations (F1, F2, F3) through probe sonication after overnight aging produced final formulations with distinctive colloidal properties, as shown in Figure 1d. Among these formulations, only the F3 formulation exhibited a uniformly dispersed colloidal solution, characterized by a translucent nature, in contrast to F2 and F1. This translucent colloidal state, especially favouring F3 can be assumed to be due to the occurrence of a size change, resembling the formation of small unilamellar vesicles (SUVs) from multilamellar vesicles (MLVs).[21] This sudden change in size was also reflected in the DLS analysis, with a reduction of the mean hydrodynamic radius of formulation (F3) from 257.5 ± 4.9 nm to 99.4 ± 2.4 nm after sonication. The turbidity can be assigned to light scattering induced by residual large particles that persist in the suspension.[22]

Under similar conditions of sonication, F3 formulation showing spontaneous vesicle formation into a lower vesicle size compared to F2 or F1 may be due to liposome size in F3 being comparable to the ultrasound wavelength of the sonicator. If the liposome dimensions were less than the US wavelength, the pressure field acting on the liposome would remain relatively uniform, and no deformation would occur. However, when the size matches the wavelength, the liposome experiences a pressure gradient that leads to a shear force which has the potential to disrupt the bilayer structure.[23] Also due to the high lipid dose in F3, sonication might have produced a large number of bilayer phospholipid fragments (BPF)[24], and when it exerts the hydrophobic effect in the presence of hydration volume.[25] available for F3, it can be assumed to produce a small sized vesicle when compared to F2 and F1 (more phospholipid fragments, less aqueous volume encapsulated).

Assessment of drug loading efficacy of liposome

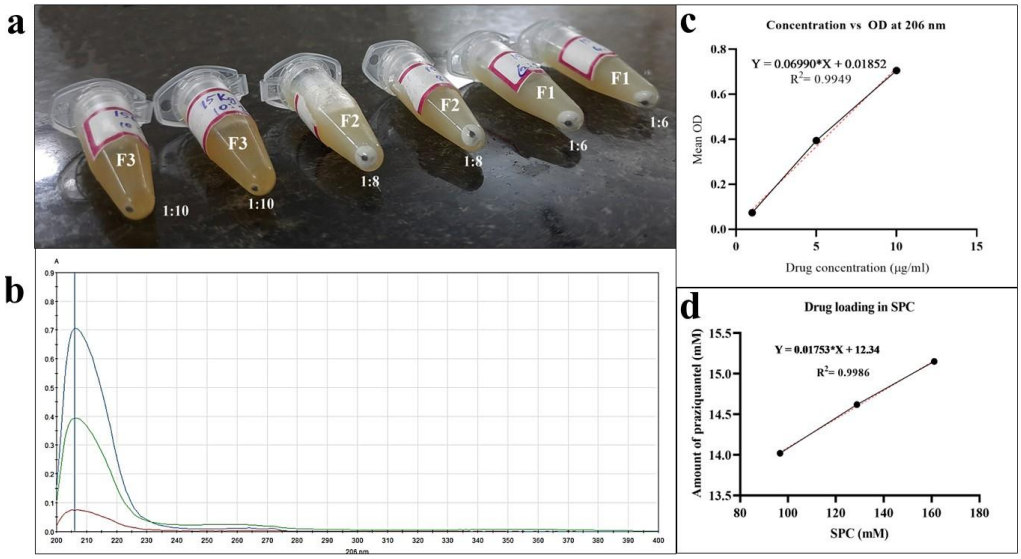

Figure 2: a) Precipitate formation from all formulations: F3(1:10) < F2(1:8) < F1(1:6) after purification at 12,500g for 15 minutes, b) Response linearity of praziquantel at 206 nm at concentrations of 1, 5 and 10 µg/ml in methanol c) Calibration curve for praziquantel (Mean OD vs Drug concentration) in methanol at 206 nm at concentrations 1, 5 and 10 µg/ml d) Linearity in drug loading between soy-phosphatidylcholine (96.64, 128.8, 161.08 mM) and praziquantel (16 mM).

After subjecting formulations (F1, F2, and F3) to high-speed centrifugation at 12,500g for 15 minutes to eliminate undissolved free drug, white precipitates were observed in varying amounts at the bottom of Eppendorf tubes for each formulation as in Figure 2a. This satisfactory separation of free drug from all formulations, achieved at high centrifugal force could be due to the increased density of the suspending media i.e. PBS in the study, which could have prevented either liposome or free drug from precipitating out at a low centrifugal force.[26]

Among the formulations, F3 exhibited the least undissolved drug precipitate, followed by F2, and then F1. Analysing the relationship between the amount of soy-phosphatidylcholine (SPC) in each formulation and the from the drug precipitated, revealed a linear relationship inversely proportional to one another. This observation can be simply accorded to the property of liposomes to solubilize hydrophobic drugs to their bilayer structure[27], with the sense that an increase in phospholipid concentration accommodates more hydrophobic drugs. In the study, to solubilize 16 mM of praziquantel required 161 mM of SPC. This observation of a higher drug-to-lipid ratio in contrast to the 1:5 ratio as the minimum ratio for complete solubilization of praziquantel without precipitation[13] may be due to a higher centrifugal force applied in the study.

The response linearity of the signal area for praziquantel in methanol was established at 206 nm by analysing samples with concentrations of 1, 5, and 10 µg/ml in scan mode within the UV region (200 to 300 nm) using UV-visible spectroscopy, as illustrated in Figure 2b. The standard curve, generated by plotting the concentrations of 1, 5, and 10 µg/ml of praziquantel in methanol against their corresponding OD values at 206 nm, yielded a linear equation: $y = 0.0699x + 0.0185$, with an R^2 value of 0.9949, as depicted in Figure 2c.

Table 3: Drug loading % in formulations F1, F2 and F3 analysed using technique of UV spectroscopy.

Drug: SPC	Molarity of SPC in a formulation (mM) of 20 ml	Molarity of praziquantel (mM)		Loading efficiency (w/w%)
		Before purification (mM)	After purification (mM)	
F1 (1:6)	96.64	16	10.5	65.6
F2 (1:8)	128.8		13.2	82.5
F3 (1:10)	161.08		15.8	98.7

The quantification of undissolved free drugs in formulations using UV-visible spectroscopy revealed liposomal formulations with varying drug loading percentages, as summarized in Table 3. Among the formulations investigated, F3 exhibited the highest drug loading percentage at 98.7 ± 0.48 %, representing the sample with the least drug precipitate. This was followed by F2 with a drug loading percentage of 82.3 ± 1.64 %, and F1 with a drug loading percentage of 65.5 ± 4.74%. A linear correlation was also noted between the varying lipid concentrations used in the study and the total input drug being incorporated into the lipids, as depicted in Figure 2d. The F3 formulation, with a concentration of 161.08 mM of SPC was able to incorporate the highest amount of drug ie., 15.8 mM, followed by F2 with 13.2 and then F1 with 10.5 mM. The formulation F3 with a 1:10 drug-to-lipid ratio was selected as the best formulation due to its high drug loading capacity and stability observed on storage.

Observing a greater difference in the loading efficiency (% fraction of total input drug loaded) between formulations, even without a larger variation in the phospholipid concentration (75mg/ml, 100 mg/ml, and 125 mg/ml), for the same amount of drug may be because of the favourable drug to lipid interaction achieved with the 1:10 drug to lipid ratio. This may be similar to the "pocket theory" postulated in a study incorporating SOD into soy-phosphatidylcholine to study protein-lipid interaction. The study proposed that SODs are inserted vertically into pockets formed between the cholesterols in the bilayer, which allows favourable interaction of lipids with proteins.[28] But for this interaction to occur, optimal bilayer thickness is also important which possibly requires a higher lipid concentration. Another study on doxorubicin loaded liposomes also reported a loading efficiency greater than 97%, prepared with DSPC, Cholesterol, and DOTAP in a 1:10 drug-to-lipid ratio.[29]

Characterization of liposomal praziquantel

Particle size analysis using the DLS technique

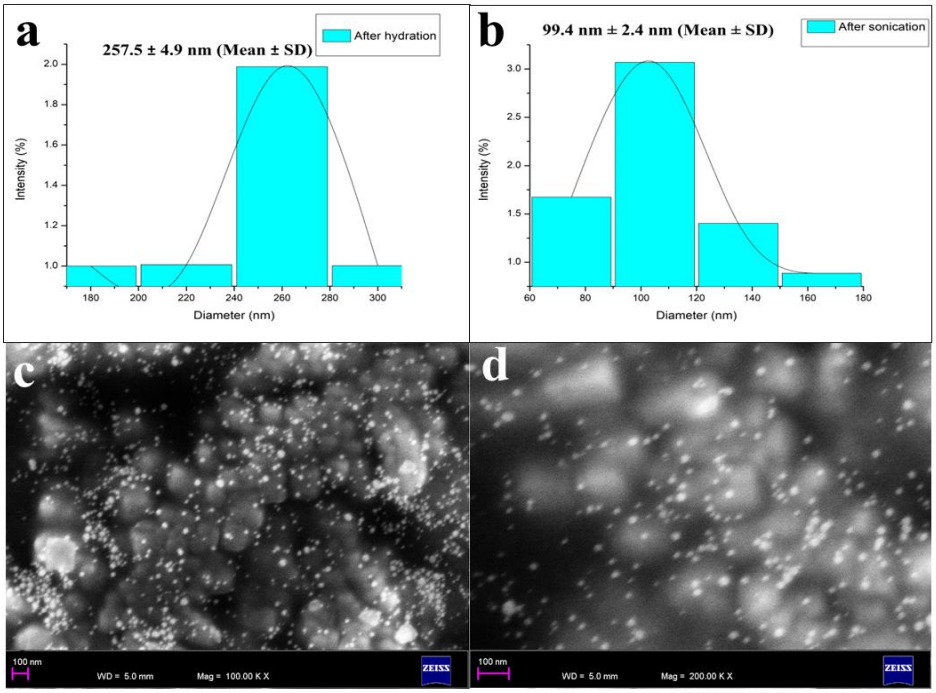

Figure 3: a) DLS analysis of particle size for F3 formulation after hydration, b) DLS analysis of particle size for F3 formulation after sonication, c) FE-SEM analysis of F3 formulation at 100 KX, d) FE-SEM analysis of F3 formulation at 200 KX.

The size and size distribution of F3 formulation analysed using the technique of dynamic light scattering obtained a mean hydrodynamic radius (n=3) of 257.5 ± 4.9 nm (Mean ± SD) for liposomes after hydration, monodisperse with a narrow distribution form as in figure 3a. A decrease in mean hydrodynamic radius (n=3)

was obtained after sonication to 99.4 nm ± 2.4 nm (Mean ± SD) with the same mode as in Figure 3b. This size change to nanometre range was similar to the earlier reported studies. A study on praziquantel-loaded conventional liposomes report a similar size change to 49.65 nm for drug-loaded and 70.87 nm for empty liposomes when sonicated using an Ultrasonic processor after keeping in ice bath for 45 minutes.[13] Another study for capsaicin loaded liposome composed of choles- terol, sodium cholate and isopropyl myristate also report a mean size range in nanometer range, between 50 to 60 nm.[30]

Generally, liposomes intended for drug delivery applications are desirable to fall within the size range of 50-200 nm, particularly for inhalation and parenteral use. Additionally, smaller liposomes exhibit increased circulation within the organism, whereas larger ones are swiftly removed from the bloodstream.[31]

Liposome morphology using Field emission scanning electron microscopy

The morphology of liposomes analyzed through FE-SEM obtained less uniform spherical-shaped liposomes with smooth surfaces, appearing as fused vesicles having a mean particle size of 158.14 ± 6.03 nm (Mean ± SD). It is to be appreciated that the spherical shape offers certain advantages to liposomal drug delivery, mainly because the geometric characteristics of nanoparticles significantly influence their rheological behavior in systemic circulation. Isotropic nanoparticles (spherical) exhibit a more consistent flow and margination property when compared to anisotropic particles.[32]

The mean particle size obtained through FESEM as in Figure 3c was slightly higher than that obtained from DLS as in Figure 3b. This change could be from the vesicle fusion that occurs during the sample drying process[33] or can be due to the effect of freeze drying of liposomes.[34] When analysed for particle size before and after freeze drying (Sa/Sb), the ratio was obtained to be 1.6. This being away from the optimal ratio of 1, it can be assumed that the application of cryoprotectant externally could not prevent vesicular retention, and possibly a method of external and internal addition of sucrose as reported for curcumin loaded liposome[35] could be more beneficial for cryoprotection. However, it is to be acknowledged that the effect of freeze drying on loading efficiency is more significant for drugs that are incorporated into an aqueous compartment rather than the bilayer.[36]

Characterization by ATR-FTIR

Table 4: ATR-FTIR spectral data of various functional groups in free, drug loaded sucrose free and sucrose incorporated liposome.

Functional groups	Free liposome (cm^{-1})	Transmittance %	Praziquantel liposome without sucrose (cm^{-1})	Transmittance %	Praziquantel liposome with sucrose (cm^{-1})	Transmittance %
-OH	3382	71.47	3393.13	83.81	3333.07	64.84
-CH2 asymmetric	2923.23	75.85	2923.10	70.10	2923.28	76.30
-CH2 symmetric	2853.29	83.40	2853.09	78.20	2853.29	82.35
-Carbonyl	1732.25	83.97	1736.84	77.81	1737.39	84.84
-C=C-	1643.68	81.27	-	-	1641.97	80.29
-N-C=O (Praziquantel)	-	-	1650.82	80.60	-	-
-PO2- (antisymmetric)	1217.85	78.14	1214.36	73.30	1214.10	75.94
-P-O-C (Symmetric)	1064.10	65.47	1063.18	61.73	1048.02	47.99
-C-O (Sucrose)					993.38	50.48
-C-C (Sucrose)					925.91	67.37

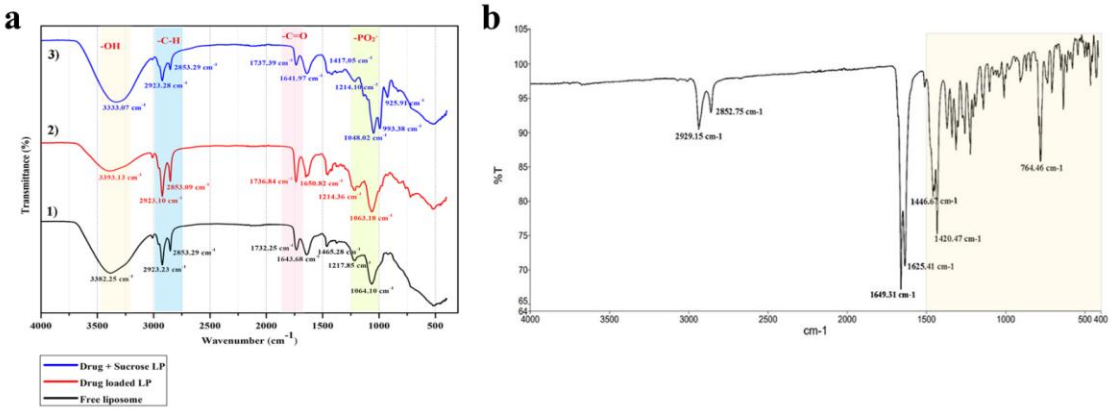

Figure 4: a) ATR-FTIR spectra of F3 formulation: 1) Free liposomes, 2) Praziquantel loaded liposome without sucrose, 3) Drug loaded liposome with sucrose, b) ATR- FTIR spectra of praziquantel.

Analysis of FTIR spectra of free liposomes without sucrose revealed characteristic bands of soy phosphatidylcholine (PC). A broad band for the intermolecular bonded OH group was obtained at 3382 cm^{-1}. Peaks for asymmetric and symmetric vibration of -CH_2 groups in the acyl chains were obtained at wavenumber 2923.23 cm^{-1} and 2853.29 cm^{-1}. A weak band for the carbonyl stretching was obtained at 1732.25 cm^{-1}. Unsaturation in PC for the C=C double bond was obtained at 1643.68 cm^{-1} and a weak band near 1465.28 cm^{-1} for the angular bending vibration of the -CH group. Typical bands for the antisymmetric vibration of PO_2^- and P-O-C vibration/PO_2^- symmetric vibration were obtained at 1217.85 and 1064.10 cm^{-1} respectively as in Figure 4a:1. These obtained bands for soy-phosphatidylcholine was in accordance with the previous other studies, one such is in a study evaluating the effect of phytosterol butyrate ester on soy-phosphatidylcholine.[37]

FTIR analysis of praziquantel was per the FTIR analysis reported elsewhere.[38,39] The FTIR spectra of praziquantel revealed specific peaks associated with the drug as in Figure 4b. Notably, the peaks at 2929.15 and 2852.75 cm^{-1} were discerned, corresponding to the C-H asymmetric and symmetric CH_2 vibrations. Additionally, the band at 1625.41 cm^{-1} indicated carbonyl stretching, while the peaks at 1446.67 and 1420.47 cm^{-1} were attributed to the C=C axial deformation of the aromatic ring. Overlapping bands of C-N axial deformation were observed between 1350 to 1000 cm^{-1}. The spectrum also displayed angular deformation at 764 cm^{-1}, suggestive of C-H outside the plane to the aromatic ring as in Figure 4b.

FTIR analysis for praziquantel-incorporated liposomes obtained peaks of various characteristics as in Figure 4a: 2 and Table 4. A broad band with a low dip was obtained for intermolecular bonded OH stretch. Peaks stronger in intensity than free liposomes were obtained for -CH_2 vibrations in the acyl chain as observed by a decrease in transmittance to 70.10% and 78.02 % respectively. Band for the carbonyl stretching was obtained at a higher wavenumber of 1736.84 cm^{-1} with a decrease in transmittance to 77.81%. A new band at 1650.82 cm^{-1} was obtained. A slight decrease in wavenumber to 1214.36 cm^{-1} and 1063.18 cm^{-1} was also observed for the PO2- antisymmetric and symmetric vibration. Primarily, minimal changes in peaks between the spectra of free liposomes and drug-loaded liposomes indicated an absence of any significant chemical interaction between praziquantel and soy-phosphatidylcholine. A similar finding was also reported for praziquantel in solid lipid nanoparticles.[40] The study assumes no modification of the drug is induced by soy-lecithin. In the spectrum of drug-loaded liposomes, a decrease in transmittance observed in bands of acyl chains (70.10%, 78.20%) without a change in wavenumber may be attributed to the alignment of CH bands of praziquantel near acyl chains, suggesting a potential hydrophobic interaction (van der Waal's forces) rather than a hydrogen bond formation. A slightly higher wavenumber for the carbonyl stretches in drug-loaded liposomes (1736.84 cm^{-1}) was also observed which was assumed to originate from the two amide carbonyl groups of praziquantel.[39] Also, not much change in wavenumber was observed when comparing the polar regions of SPC in free (1217.85 cm^{-1}, 1064.10 cm^{-1}) and drug-loaded liposome (1214.36 cm^{-1}, 1063.18 cm^{-1}). From these findings, it can be assumed that the drug in liposomes is fully positioned in the hydrophobic part of the phospholipid. Complete disappearance of fingerprint region (< 1500 cm^{-1}) of praziquantel spectrum without any overlap with peaks of SPC as in 4a also indicates a complete inclusion of drug into the liposome without formation of any physical mixtures as reported for praziquantel in beta cyclodextrins[41], or between Mitomycin C and Soy-phosphatidylcholine in a phytosome preparation.[42]

FTIR spectra for praziquantel-loaded liposomes in sucrose obtained a spectrum similar to that of hydrated phosphatidylcholine, but with some changes as illustrated in figure 4a: 3 and table 4. Compared to the other two spectra a broader and stronger bend was obtained for the intermolecular bonded OH group. The intensity of symmetric and antisymmetric vibration of

CH_2 was reduced as observed as an increase in transmittance to 76.30 and 82.35 respectively when compared to drug-loaded liposome without sucrose. Peak for carbonyl stretching was less intense (increase in transmittance to 84.84) and obtained at 1737.39 cm^{-1}. Compared to the other two preparations a stronger bond with a reduction in wavenumber was obtained for antisymmetric and symmetric stretching of PO2-. New peaks were obtained at 993.38 and 925.91 cm^{-1} as in figure 4 a)3. From the broadband obtained at 3333.07 cm^{-1} (Intermolecular bonded OH region), reduction in the wavenumber of -P-O-C symmetric stretching from 1063.18 cm^{-1} (Liposomal praziquantel without sucrose) to 1048.02 cm^{-1} in drug-loaded liposomes with sucrose (very low transmittance of 47.99%, as indicated in table 4), and a general increased transmittance for all the other functional groups indicate the possible positioning of sucrose, assumed to be in the interface of bilayer and external water.

The protective effect of sucrose (polysaccharide) and its mechanism of cryoprotection have been mentioned in many studies.[43,44] Of the many theories, the "Water Replacement Theory" posits the stabilizing impact of protectors to replace bound water surrounding bilayers through specific interactions with the polar region of the lipid head group under conditions of low hydration. This was very evident from the FTIR spectrum of praziquantel-loaded liposome with sucrose. The emergence of new bands at 993.38 cm^{-1} and 925.91 cm^{-1} can be ascribed to the C-O and C-C stretching, considered the skeletal modes of sucrose vibration.[45]

CONCLUSION

Keeping in mind the already published research on the conventional liposomal formulation of praziquantel, a one-pot synthesis method to prepare a high-dose liposome formulation of "Praziquantel", an anti-schistosomal drug was successfully attempted in the present study. The establishment of this method involved careful consideration and evaluation of many material and process factors in detail, mostly lacking in earlier studies to produce an optimal liposome formulation using minimal components. These efforts aimed at achieving a consistent, repeatable procedure for a high drug loading preparation reduced the inherent heterogeneity associated with the thin film hydration method. Physical characterization of liposomes performed with DLS technique obtained final formulation in nano-size range and further confirmation using FESEM obtained liposomal praziquantel exhibiting spherical morphology. Finally, chemical characterization by FTIR analysis understood the interactions among the components in the formulation. Drug-loaded liposome demonstrated the complete inclusion of the drug within the liposome possibly located within the bilayer and sucrose between the bilayer-external water interface. This arrangement extrapolated from the FTIR spectrum ensures the drug is dispersed in a lipid environment with additional membrane protection from sucrose during the freeze-drying process. Overall, a detailed bulk synthesis preparation process for liposomal praziquantel using the natural l-α phosphatidylcholine to alleviate the low oral bioavailability of praziquantel was attempted with scope for many further characterization and material inclusion.

ACKNOWLEDGEMENT

The authors report that there are no financial or non-financial interests that are directly or indirectly related to the work submitted for publication.

Funding

The authors are thankful to the Kerala Veterinary and Animal Sciences University, Pookode, Wayanad for the financial aid vide No Acad(3)/6270/2020(i).

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