IJPSR (2023), Volume 14, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 19 January 2022; received in revised form, 01 December 2022; accepted, 17 December 2022; published 01 January 2023

FORMULATION, OPTIMIZATION AND EVALUATION OF HEXADECANOIC ACID PHYTOSOMAL GEL FOR ANTI-FUNGAL ACTIVITY

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Keywords:

Hexadecanoic acid, Carbapol 934, Thin film hydration method, Entrapment efficiency

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ABSTRACT: Phytosomes is vesicular drug delivery system in which phytoconstituents of herb extract surround and are bound by lipid. Hexadecanoic acid is the phytoconstituents of *Nigella sativa* oil. Hexadecanoic acid is hydrophobic and possesses less solubility and permeability. The study aims to formulate, optimize, and evaluation of hexadecanoic acid phytosomal gel for anti-fungal activity hexadecanoic acid loaded phytosomes were prepared using Thin film hydration method and were optimized using factorial design (3^2) using Design-expert® software (Version 7.0.0, Stat-Ease Inc., Minneapolis, USA) different formulations were prepared. Two different independent variables were used, which include: Amount of Soya lecithin (X1), amount of cholesterol (X2) and the responses are particles size determination (Y1), and entrapment efficiency (Y2). The optimized formulation of hexadecanoic acid phytosomes was incorporated into a Carbapol 934 gel base and 1.5% of hexadecanoic acid phytosomal gel was prepared which was evaluated for drug content, pH, Spreadability, viscosity and *in-vitro* drug release. The prepared hexadecanoic acid phytosomes had a maximize EE% was found to be (72.23%), particle size was found to be (715 nm), the Spreadability value was (10.4cm). The prepared phytosomal gel was found to be (88.4%), which represents good content uniformity. The viscosity was found to 1900 cps. The Cumulative percentage of drug release for the phytosomal gel was found to be 91.54±0.27 %. The phytosomal gel was stable at 4°C for 90 days. R^2 Values for the optimized formulation were found to be highest for the Higuchi model. This indicated that the drug release from all the formulations followed diffusion controlled release mechanism.

INTRODUCTION: The transdermal drug delivery system (TDDS) includes all topically administered drug formulations intended to deliver the active ingredient into circulation [1]. The vesicular approach is among the foremost illustrious drug delivery methods for topical delivery [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(1).519-29

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.14(1).519-29

The active constituents of plants are usually polar or water-soluble. However, water-soluble phytoconstituents like flavonoids, glycosidal aglycones, tannins, *etc*., are poorly absorbed due to their poor lipid solubility or larger molecular size, a barrier in passive diffusion, resulting in their poor bioavailability [3].

The phytosomal drug delivery system is a newly introduced patented technology developed to incorporate the water-soluble phytoconstituents or standardized plant extracts into lipids to produce lipid-compatible molecular complexes [4]. A stoichiometric ratio of 1:1 is considered to be the

most efficient ratio for preparing phospholipid complexes [5]. These complexes enhance the bioavailability of the active constituents and protect the valuable component of herbal extract from being destructed by digestive secretion and gut bacteria. As a result, they showed better absorption and improved pharmacological and Pharmacokinetic parameters than conventional herbal extract [6].

Hexadecanoic acid is the phytoconstituent of *Nigella sativa*. It showed multiple health-beneficial activities like anti-fungal, antihistaminic, antibacterial, anti-hypertensive, hypoglycemic, anti-inflammatory, and anti-arthritic actions. It is a major component of the oil from the fruit of oil palms (palm oil), making up to 44% of total fats.

Meats, cheeses, butter, and other dairy products also contain palmitic acid, amounting to 50– 60% of total fats. Palmitates are the salts and esters of palmitic acid. The palmitate anion is the observed form of palmitic acid at physiologic pH (7.4-7.8).

MATERIALS AND METHODS: Hexadecanoic acid and Soya lecithin were obtained as gift samples from Central drug house (p) Ltd, Bombay, India. Cholesterol, sodium hydroxide, dichloro-methane and carbopol934 were purchased from SD Fine-Chem Limited, India. Methanol, triethanolamine and chloroform were purchased from Fischer Scientifics, Mumbai, India.

Preparation of Phytosomes of Hexadecanoic Acid using Thin Film Method: The phytosomes of Hexadecanoic acid were prepared using the thin-film method. An accurate amount of soya lecithin, cholesterol, and Hexadecanoic acid were dissolved in a mixture of dichloromethane: methanol (2:1v/v) inadry, round bottom flask.

The organic solvent mixture was allowed to evaporate in the rotary evaporator adjusted to 60 rpm, at 40°C, for 15 minutes under low pressure to prepare a thin lipid filmon the wall of the round-bottom flask.

The film was hydrated with phosphate buffer pH7.4 by rotating 60 rpm for 1 h at room temperature. The multilamellar lipid vesicles (MLVs) were then sonicated using the ultrasonic probe sonicator for 30 minutes to reduce the vesicle size and stored at 4°C for further investigation [7].

TABLE 1: FORMULATION DESIGN OF HEXADECANOIC ACID PHYTOSOMES

	Quantities w/w% (100)								
Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Hexadecanoic acid(mg)	100	100	100	100	100	100	100	100	100
Soyalecithin	100	100	100	200	200	200	300	300	300
Cholesterol	25	50	75	25	50	75	25	50	75
Chloroform or methanol(2:1ml)	30	30	30	30	30	30	30	30	30
Phosphate buffer (7.4)	20	20	20	20	20	20	20	20	20
Distilled water (v/v)	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Design of Experiment: A three-level factorial design was implemented using Design-expert® software (Version 7.0.0, Stat-Ease Inc., Minneapolis, USA) software which required an experiment to be carried out at all possible combinations of the three levels of each of the factors considered. The independent variables used were the amount of Cholesterol(X1) and the amount of soya lecithin (X2). The independent variables were screened using a multilevel factorial design (3^2), and nine formulations of Hexadecanoic acid pyrosomes were obtained. All the formulations were prepared using the thin film hydration method and then evaluated particle size (Y1) and entrapment efficiency (Y2), to determine the optimized formulation.

TABLE 2: INDEPENDENT VARIABLES

Coded values level	**Independent variables**	
	X_1, Cholesterol	**X_2, Soy lecithin**
-1	25	100
0	50	200
+1	75	300

TABLE 3: FORMULATION OF HEXADECONIC ACID PHYTOSOMES SUSPENSION

Formulation Code	Drug	Cholesterol	Soy lecithin	Chloroform
F1	100	25	100	30
F2	100	25	200	30
F3	100	25	300	30
F4	100	50	100	30
F5	100	50	200	30
F6	100	50	300	30
F7	100	75	100	30
F8	100	75	200	30
F9	100	75	300	30

Evaluations of Phytosomes:

Entrapment Efficiency: 100mg of Hexadecanoic acid phytosomal complex was added to phosphate buffer pH 7.4 and were centrifuged at 2000 rpm for 30 min 4˚C to allow the separation of the entrapped drug from the un-entrapped drug using a Remi ultracentrifuge which results in the formation of sediment and supernatant. This unentrapped drug was separated by removing the supernant then the sediment was lysed with methanol and analyzed at 279 nm using a UV-visible spectrophotometer. The percentage of drug entrapment was calculated by using the formula [8].

$$EE\% = \text{Amount of Entrapped Hexadecanoic acid} / \text{Total Amount of Hexadecanoic acid} \times 100$$

SEM Analysis: Then, the particle size of the formulation was viewed and photographed using Scanning Electron Microscope [8].

Determination of Particle size and Zeta potential: The average diameter and surface charge property of Hexadecanoic acid were estimated by dynamic light scattering (DLS) using a particle size analyzer at 25°C.

***In-vitro* Diffusion Studies:** A diffusion study of formulations was carried out using Franz diffusion cells through a dialysis membrane. The dialysis membrane was soaked in distilled water for 24 hours. Franz diffusion cells contain two compartments upper donor and a lower receptor compartment. The receptor compartment was filled with 7.4 phosphate buffer. The donor compartment contained 100 ml of pyrosomes on the dialysis membrane with an exposure area of 2 cm2 to the receptor medium. The whole assembly was kept on a magnetic stirrer at 600 rpm for 600 minutes, and samples were withdrawn at an interval of 1 hour for 10 hours and replaced with an equal volume of buffer. Samples were appropriately diluted with buffer and analyzed using a UV spectrophotometer at 279 nm.

Preparation of Phytosomal Gel: The phytosomes were formulated into a gel for ease in application. Carbopol 934 was dispersed in water to prepare 1% w/w dispersion. The dispersion was mechanically stirred and then neutralized with 0.5% v/v triethanolamine solution. The neutralized dispersion was kept overnight to remove any entrapped air. Finally, Phytosomes were then added to the dispersion [9].

Homogeneity: The consistency was determined by using homogenizer [10].

Spreadability: Two glass slides of 20 cm × 20 cm were selected. The phytosomal gel was placed between the slides.

A 100 g was placed on the upper slide to press the gel uniformly to format the inlayer. The time taken for the separation was noted using a top clock. The following equation was used for this purpose [11]:

$$S = m \times L / T$$

Where, S – Spreadability, m-Weight tied to the upper slide, l-Length of the glass t - Time taken in seconds.

Viscosity: The viscosity of phytosomal gel was measured using a Brookfield viscometer using spindle number S64 rotated at a speed of 12 rpm for an a10-run time at 37°C [10].

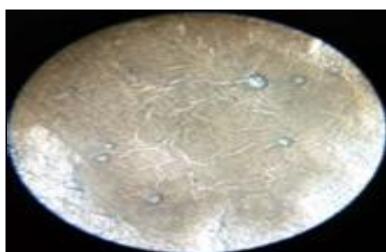
Measurement of pH: One gram of gel was dispersed in 20 mL of distilled water, and a digital pH meter was used to determine the pH value.

Drug Content: 1 gm of gel was dissolved in a 100mL of phosphate buffer pH 7.4. The resultant

solution was filtered, and drug content was analyzed spectrophotometrically [12].

Stability Study: The stability study of Hexadecanoic acid Phytosomalgel (phospholipid and soya lecithin) was conducted at refrigerated temperature (4°C) and room temperature (30°C) as per Guidelines ICH. Samples were analyzed for physical appearance, drug content and *in-vitro* diffusion study after 30, 60, and 90 days.

Vesicle Shape and Morphology: The preliminary characterization of Hexadecanoic acid phytosomes (prior to sonication) was done using an optical microscope. The optical microscopic images of hexadecanoic acid-loaded vesicles are shown in **Fig. 1.**



OPTICAL PHOTOMICROGRAPH OF HEXA-DECANOIC ACID LOADED PHYTOSOMES FORMULATION

TABLE 4: MEAN VESICLE SIZE OF HEXA-DECANOIC ACID PHYTOSOMES

Formulation code	Size of Vesicle (nm)
F1	5117.6
F2	5612.2
F3	4314.4
F4	6107.3
F5	3712.8
F6	4417.5
F7	3612.4
F8	5213.8
F9	6018.5

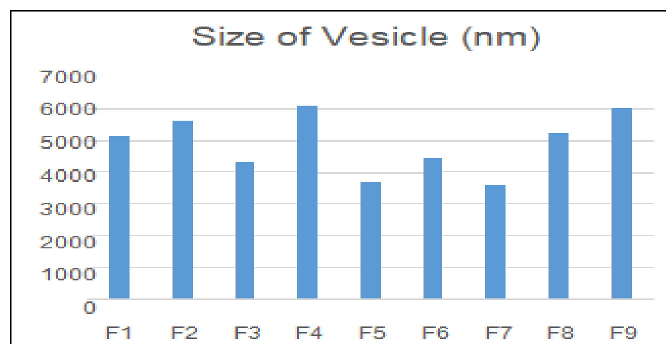


FIG. 1: VESICLE SIZE OF HEXADECANOIC ACID PHYTOSOME

As shown in Table, it was found that the prepared Hexadecanoic acid phytosomes exhibited a good EE%, with values ranging from (50.32) to (72.23).

Entrapment Efficiency:

TABLE 5: ENTRAPMENT EFFICIENCY OF HEXADECANOIC ACID PHYTOSOMES

S. no.	Formulation	Entrapment Efficiency (%) Y2
1	PF1	50.32
2	PF2	54.12
3	PF3	56.56
4	PF4	54.23
5	PF5	58.11
6	PF6	60.23
7	PF7	64.35
8	PF8	68.11
9	PF9	72.23

The values are expressed as mean, ± SD, (n=3).

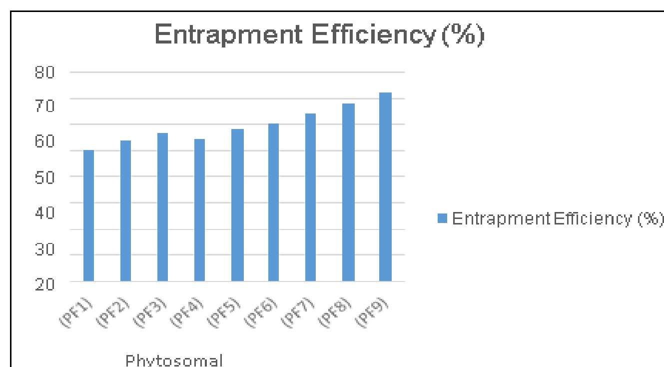


FIG. 2: ENTRAPMENT EFFICIENCY OF HEXADECANOIC ACID PHYTOSOME

TABLE 6: COMPOSITION AND CHARACTERISTICS OF FORMULATIONS

Formulation Code	*X1*, Cholesterol	*X2* Soy lecithin	*Y1*, Particle size (nm)	*Y2*, Entrapment Efficiency (%)
F1	25	100	605	50.32
F2	25	200	630	54.12
F3	25	300	640	56.56
F4	50	100	650	54.23
F5	50	200	680	58.11
F6	50	300	688	60.23
F7	75	100	692	64.35
F8	75	200	710	68.11
F9	75	300	715	72.23

***In-vitro* Diffusion Studies:** Diffusion studies of all formulations were carried out using dialysis membrane for 10 hrs and samples were analyzed using a double-beam UV Visible Spectrophotometer.

TABLE 7: *IN-VITRO* DIFFUSION STUDIES

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Time (min)	CDR %	CDR %	CDR %	CDR %	CDR %	CDR %	CDR %	CDR %	CDR %
0	0	0	0	0	0	0	0	0	0
1	21.97 ±0.2	16.86 ±0.71	18.21 ±0.3	22.93 ±0.6	28.16 ±0.34	21.14 ±0.51	19.16 ±0.35	20.9 ±0.6	21.22 ±0.37
2	33.27 ±0.8	30.69 ±0.3	27.56 ±0.4	32.71 ±0.23	30.81 ±0.27	24.45 ±0.36	29.55 ±0.72	25.76 ±0.26	32.66 ±0.46
3	45.26 ±0.54	47.72 ±0.73	33.73 ±0.28	44.12 ±0.49	33.24 ±0.49	33.21 ±0.43	35.34 ±0.75	33.45 ±0.31	36.55 ±0.21
4	57.23 ±0.7	50.73 ±0.12	44.93 ±0.58	56.77 ±0.57	45.64 ±0.36	39.67 ±0.21	43.65 ±0.25	46.68 ±0.37	48.69 ±0.64
5	66.64 ±0.2	51.27 ±0.3	49.81 ±0.22	67.10 ±0.42	52.19 ±0.49	45.15 ±0.20	63.17 ±0.21	55.25 ±0.27	54.27 ±0.38
6	70.25 ±0.84	58.14 ±0.80	52.91 ±0.8	70.45 ±0.34	66.75 ±0.34	52.16 ±0.19	72.15 ±0.58	64.16 ±0.39	68.64 ±0.38
7	76.54 ±0.91	62.56 ±0.23	63.10 ±0.8	73.74 ±0.21	77.12 ±0.68	66.18 ±0.29	78.37 ±0.69	68.14 ±0.82	75.74 ±0.81
8	78.25 ±0.5	72.71 ±0.89	79.98 ±0.4	75.26 ±0.61	79.19 ±0.43	72.85 ±0.37	80.88 ±0.67	76.14 ±0.76	79.78 ±0.69
9	81.54 ±0.52	82.18 ±0.2	84.99 ±0.21	86.33 ±0.37	82.79 ±0.26	84.27 ±0.61	82.23 ±0.34	84.13 ±0.21	86.33 ±0.37
10	86.64 ±0.62	88.46 ±0.7	90.45 ±0.5	89.55 ±0.63	88.36 ±0.43	90.11 ±0.49	86.45 ±0.59	88.73 ±0.34	92.98 ±0.92

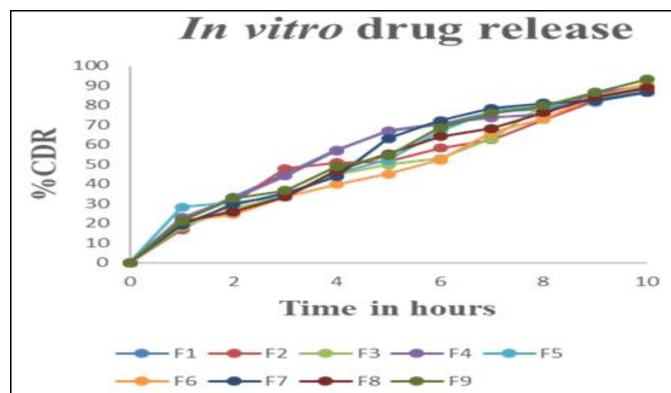


FIG. 3: *IN-VITRO* DIFFUSION STUDIES OF PHYTOSOMAL FORMULATIONS

Particle Size (Y3) and Zeta Potential of Hexadecanoic Acid Phytosomes: From the results, all the hexadecanoic acid phytosomes have particle size less than 279nm and as such are effective for transdermal application.

Optimize Formulation: The central composite design (CCD) was used to find the suitable variables. Total 9 experimental runs were executed, and the recorded results are represented in Table

ANOVA for Quadratic Model:

Response 1: Particle Size: The Model F-value of 201.70 implies the model is significant. P-values less than 0.0500 indicate model terms are significant. In this case A, B, B^2 are significant model terms.

The Predicted R^2 of 0.9668 is in reasonable agreement with the Adjusted R^2 of 0.9921; i.e., the difference is less than 0.2. Adeq Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. Here ratio of 40.723 indicates an adequate signal.

Response 2: Entrapment Efficiency: The Model F-value of 308.60 implies the model is significant. P-values less than 0.0500 indicate model terms are significant. In this case, A, B, A^2 are significant model terms. The Predicted R^2 of 0.9784 is in reasonable agreement with the Adjusted R^2 of 0.9948; i.e. the difference is less than 0.2. Adeq Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable.

Response Analysis through Polynomial Equations:

Effect of Variables on Particle Size: Data was analyzed to fit full second-order quadratic or cubic polynomial equation(s) with added interaction terms to correlate the various studied responses with the examined variables. As depicted in 2D and 3D-plots **Fig. 1A** and **2A**, it is indicated that at lower levels of cholesterol, an increase in the levels of soy lecithin concentration showed a positive influence on particle size. Similarly, by increasing cholesterol levels at constant soy lecithin concentration, increase in particle size was observed. Thus, lowest levels of cholesterol and soy lecithin concentrations resulted in minimum particle size. The final mathematical model in terms of coded factors as determined by the Design Expert software is shown below in Eq. (1) for particle size.

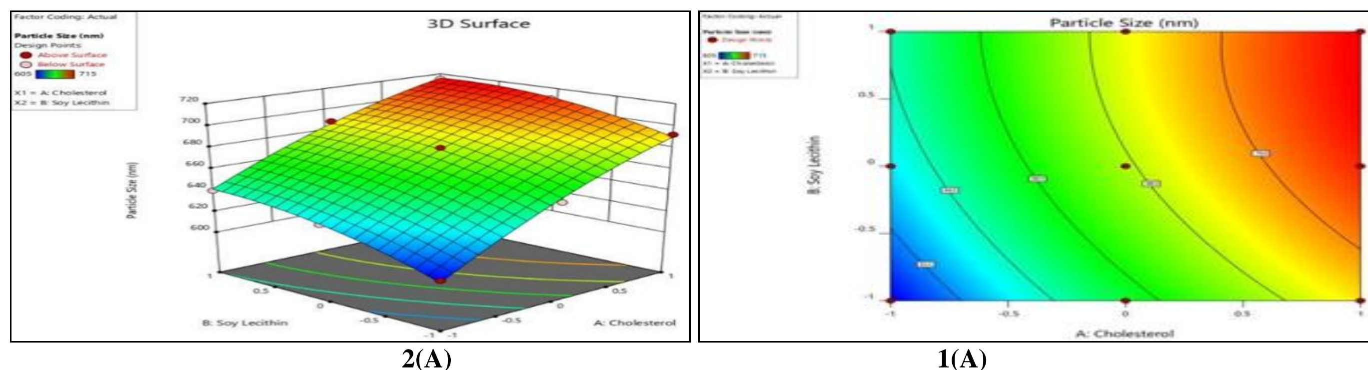
Response 1: Particle size, *Y1*

$Y1 = 678.22 + 40.33\ X1 + 16.00\ X2 - 3.00\ X1X2 - 7.33\ X^{2} - 8.33X^{2}$ …. Eq.(1)

Effect of Variables on Entrapment Efficiency: As depicted by 2D contour plot **Fig. 2B** and 3D response surface plot **Fig. 2B**, the percent entrapment efficiency of drug is positively correlated with *X1,* cholesterol concentration and *X2,* soy lecithin concentrations. The final mathematical model in terms of coded factors as determined by the Design-Expert software is shown below in Eq. (2) for entrapment efficiency

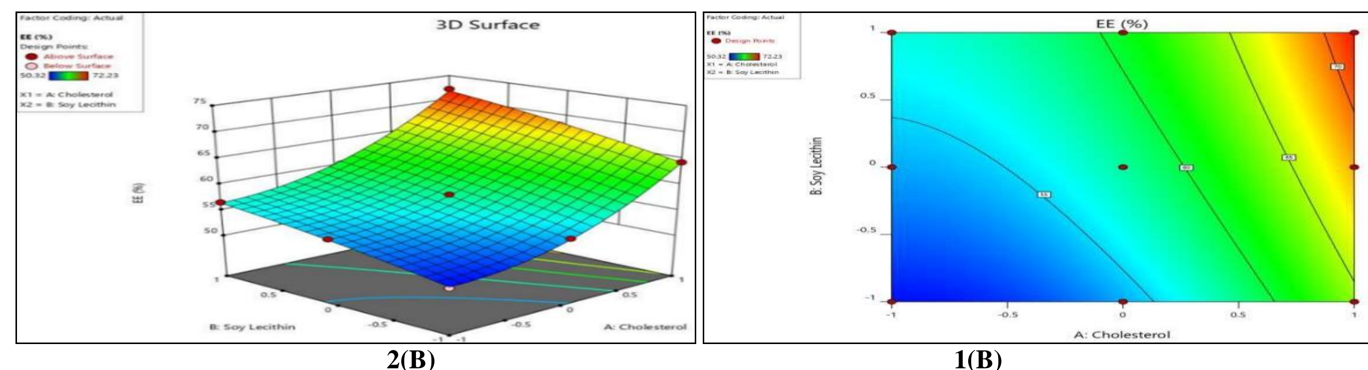
Response 2: Entrapment Efficiency, *Y2*

$Y1 = 57.83 + 7.28\ X1 + 3.35\ X2 + 0.4100\ X1X2 + 3.42X12 - 0.4600\ X22$…. Eq. (2)



2(A) 1(A)

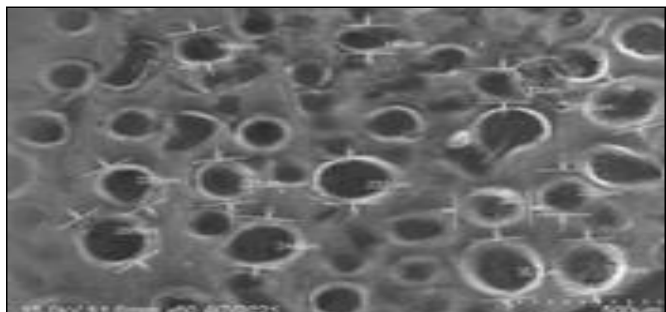
FIG. 4: 3D RESPONSE SURFACE PLOT AND 2D CONTOUR PLOT AND FOR EVALUATING INFLUENCE OF CHOLESTEROL (X1) AND SOY LECITHIN (X2) ON PARTICLE SIZE NM (Y1)



2(B) 1(B)

FIG. 5: 3D RESPONSE SURFACE PLOTS AND 2D CONTOUR PLOT FOR EVALUATING INFLUENCE OF CHOLESTEROL (X1) AND SOY LECITHIN (X2) ON % ENTRAPMENT EFFICIENCY (Y2)

Characterization of the Optimized Formulation: Surface Morphology of Phytosomes: The Hexadecanoic acid phytosomes are found to be spherical in shape and vesicle size is found to be in the range of 279 nm.



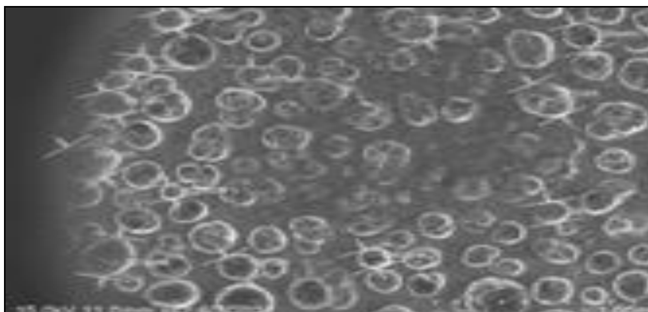


FIG. 6: SEM OF PHYTOSOMES AT 500µM **FIG. 7: SEM OF PHYTOSOMES AT 1.00µM**

FIG. 8: SEM IMAGES OF HEXADECANOIC ACID PHYTOSOMES

Particle size Distribution and Zeta Potential Determination: Zeta potential of hexadcanoic acid phytosomes of formulation showed good stability.

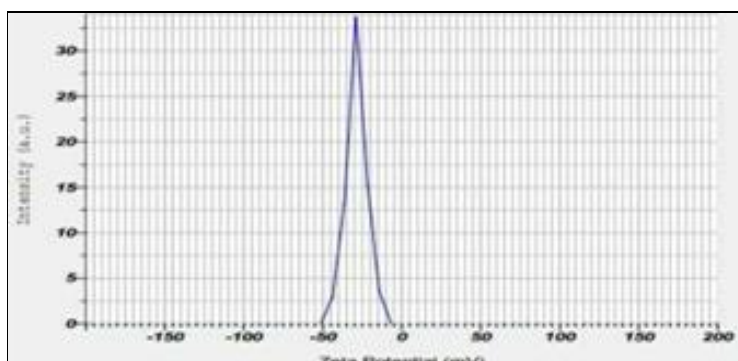


FIG. 9: ZETA POTENTIAL OF F9 FORMULATION

202103100439026.nsz
Measurement Results
Date : 10 March 2021 04:39:26
Measurement Type : Particle Size
Sample Name : HEXADECANOIC ACID PHY GEL-Size
Scattering Angle : 90
Temperature of the holder : 25.0 deg. C
T% before meas. : 33280
Viscosity of the dispersion medium : 2.035 mPa.s
Form Of Distribution : [Standard]
Representation of result : Scattering Light Intensity
Count rate : 1175 kCPS
Calculation Results

Peak No.	S.P.Area Ratio	Mean	S.D.	Mode
1	1.00	[illegible]	[illegible]	[illegible]
2	---	[illegible]	[illegible]	[illegible]
3	---	[illegible]	[illegible]	[illegible]
Total	1.00	[illegible]	[illegible]	[illegible]

Histogram Operations
Size (Median) : 6059.0 nm
Mode : 6684.7 nm
% Cumulative (1) : 10.0 (%) - 4774.9 (nm)
% Cumulative (2) : 50.0 (%) - 6059.0 (nm)
% Cumulative (3) : 90.0 (%) - 7217.7 (nm)
% Cumulative (4) : 30.0 (%) - 5474.2 (nm)
% Cumulative (5) : 40.0 (%) - 5774.0 (nm)
% Cumulative (6) : 50.0 (%) - 6059.0 (nm)
% Cumulative (7) : 20.0 (%) - 5173.5 (nm)
% Cumulative (8) : 70.0 (%) - 6624.9 (nm)
% Cumulative (9) : 95.0 (%) - 7373.5 (nm)
% Cumulative (10) : 100.0 (%) - 8510.6 (nm)
Cumulant Operations
Z-Average : 6747.4 nm
PI : 0.677

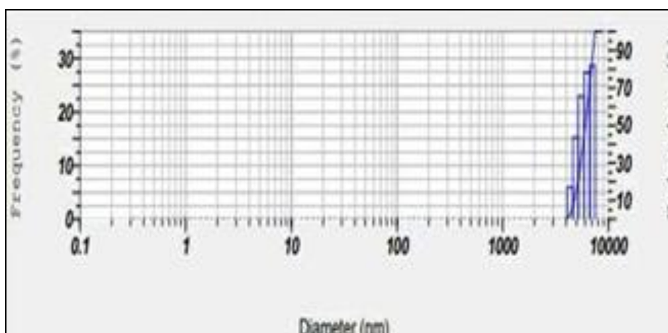


FIG. 10: PARTICLE SIZE DISTRIBUTION OF F9 FORMULATION

Formulation of Hexadecanoic Acid Phytosomal Gel: The hexadecanoic acid phytosomal gel was prepared using soya lecithin with 1% Carbopol 934 as agelling agent. The concentration of HA in the prepared phytosomalgel was 1.5% w/w.

Evaluations of Hexadecanoic Acid Phytosomal Gel:

1.5% Hexadecanoic Acid Soya Lecithin Phytosomal Gel: Hexadecanoic acid phytosomal gel was smooth and homogenous. The spreadability value was 10.4cm, which indicates that the gel can be spared easily on optimization of soya lecithin phytosomal gel of hexadecanoic acid the skin surface with little stress.

The viscosity of 1.5% hexadecanoic acid soya lecithin phytosomal gel was found to be 1900 cps. The pH value was 7.4 ± 0.24, which is considered within the normal pH range for topical preparations. The actual drug content of the hexadecanoic acid phytosomalgel was found to be 88.4± 1.51% %, which represents good content

uniformity. The *in-vitro* drug release was found to be 91.54±0.27 **Table 8.**

TABLE 8: EVALUATION OF HEXADECANOIC ACID GEL

Evaluation	Results
Homogenicity	Good
Spreadability (cm)	10.4cm
Viscosity (cps)	1900
pH measurements	7.4 ± 0.24
Drug content (%)	88.4± 1.51%
In-vitro drug release (%)	91.54±0.27

TABLE 9: DRUG CONTENT AND CONTENT UNIFORMITY

Formulation code	Drug content (%)			MEAN±SD
1.5 % Hexadecanoic acid gel	88.2	88.8	88.6	88.4±1.51%

In-vitro Drug Release:

TABLE 10: CDR, JSS AND KP OF 1.5% HEXADECANOIC ACID PHYTOSOMAL GEL

Formulations	CDR (%)	Flux (Jss) (µg/cm^2/h)	ty Coefficient (kPa) (cm/h)
Hexadecanoic acid gel	91.54 ± 0.27	4.86	1.071 ± 0.94

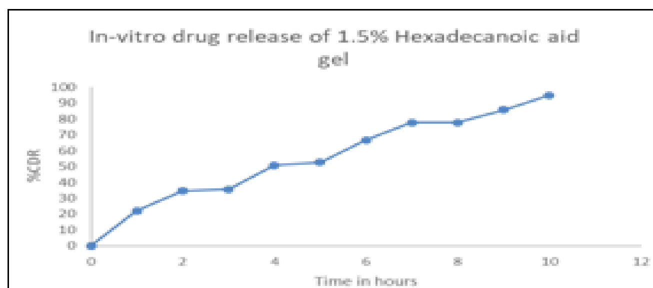


FIG. 11: *IN-VITRO* DRUG RELEASE OF HEXADECANOIC ACIDPHYTOSOMAL GEL

Pharmacokinetic Profiles for the Phytosomal Gel: The drug release kinetic studies were estimated to determine the type of release mechanism followed. Release kinetic study of soya lecithin phytosomal gel of Hexadecanoic acid optimized was performed for different kinetic equations. R^2 value for the optimized formulation of Hexadecanoic acid phytosomal gel was found to be highest for the Higuchi model. This indicated that the drug release from all the formulations followed diffusion controlled release mechanism. 'n' value was estimated from line a regression of log (Mt/M) vs log t, and it was found that drug release follows Quasifickian mechanism.

TABLE 11: RELEASE KINETICS FOR OPTIMIZED FORMULATION

	ZERO	FIRST	HIGUCHI	PEPPAS
	% CDR Vs T	Log % Remain Vs T	%CDR Vs √T	Log C Vs Log T
Slope	9.116.773	-0.142354303	30.47232164	1.300281031
Intercept	10.76.395	2.18634792	-7.981872699	0.815035248
Correlation	0.986801926	-0.847603452	0.986228597	0.800090803
R 2	0.973778041	0.970131612	0.971546845	0.640145293

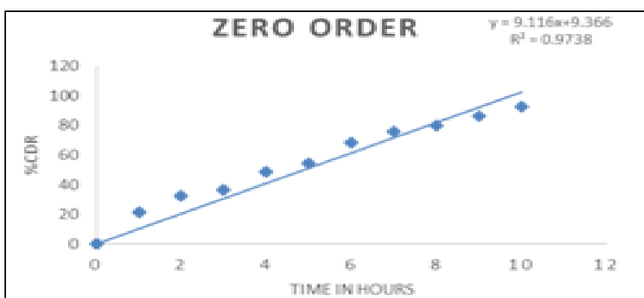


FIG. 12: ZERO ORDER KINETICS FOR THE PHYTOSOMAL GEL

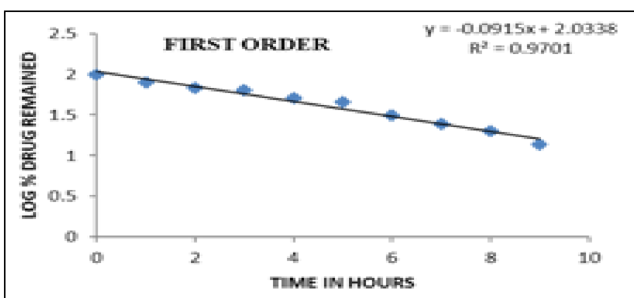


FIG. 13: FIRST ORDER KINETICS FOR THE PHYTOSOMAL GEL

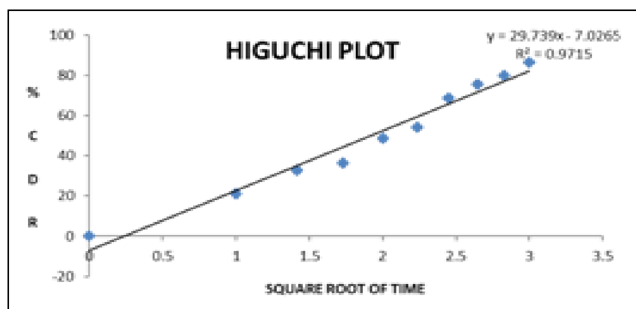


FIG. 14: HIGUCHI MODEL FOR THE PHYTOSOMAL GEL

Stability Studies: Stability studies were performed as per the conditions of ICH guidelines for climatic zone IV. Table shows stability studies of the phytosomal gel. The studies showed that the phytosomal gel was found to be more stable at 4°C when compared to other temperatures.

TABLE 12: STABILITY STUDIES OF HEXADECANOIC ACID PHYTOSOMAL GEL AT REFRIGERATED AND ROOM TEMPERATURE

Parameters/ Temperature	0th day		30th day		60th day		90th day	
	4˚C±2˚C	30˚C± 2˚C	4˚C±2˚C	30˚C± 2˚C	4˚C±2˚C	30˚C±2˚C	4˚C±2˚C	30˚C±2˚C
DC (%)	88.4 ±1.51%	88.2 ±1.51%	86.77 ±0.32	82.4 ±0.88	85.3 ±0.88	77.8 ±0.46	84.5 ±0.56	73.9 ±0.74
CDR (%)	91.54 ±0.27	91.05 ±0.27	91.7 ±0.36	93.2 ±0.85	96.3 ±0.77	90.6 ±0.38	96.9 ±0.65	87.5 ±0.36

The values are expressed as mean, ± SD (n=3) DC- Drug Content, C.D.R- Cumulative Drug Release.

Anti-Microbial Activity:

Preliminary Screening for Anti-fungal Activity [13-22]:

Microorganism used: Standard cultures *of Candida albicans* (NCIM-2708), *Aspergillus niger* (2079), were obtained from Owaisi Hospital and Research centre, Hyderabad, Telangana. The organisms were maintained by sub-culturing at regular intervals with 24 hr in nutrient agar medium.

Preparation of Test and Standard Solutions: The test solutions of Phytosomes were dissolved in DMSO, as Amphotericin- B standard was dissolved in sterilized water to get a concentration of 200 µg / 1 ml DMSO (0.1 ml) was used as solvent control.

Preparation of Standard Inoculum: The above-mentioned quantities of different ingredients were accurately weighed and dissolved in the appropriate amount of distilled water. The prepared media was sterilized by autoclaving at 121°C for 15 minutes.

TABLE 13: COMPOSITION OF NUTRIENT AGAR MEDIUM

Sl. no.	Ingredients	Weight (g)
1	Beef extract	4.0
2	Peptone	5.0
3	Agar	20.0
4	Distilled water	q.s.1000ml
5	pH	7.4

Anti-fungal Screening by Cup Plate Method: This method is based on the diffusion of anti-fungal components from reservoir hole to the surrounding inoculated nutrient agar medium, so that the growth of fungal is inhibited as a zone around the hole.

Procedure: A sterile borer was used to prepare cups of 10 mm diameter in the agar media spread with the microorganisms. 0.1 ml of inoculums (of 10^4 to 10^6 CFU / ml population prepared from standardized culture, adjusted with peptone water) was spread on an agar plate by spread plate technique. Accurately measured (0.1 ml) solution of each sample and standard were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8°C for two hours for effective diffusion of test compounds and standards. Later, they were incubated at 37 ºC for 24 h. The presence of definite zones of inhibition around the cup indicated fungal activity.

RESULTS:

Activity of Phytosomes against *Candida albicans*: The antifungal effect of different phytosomes such as F4, F6, and optimized formulation (OF) is shown in Table no, Figure. F4 showed antifungal activity of 9.5mm, F6 11.3mm and 12.4 zone of inhibition compared with standard Amphotericin- B at 200µg/ml, which showed 14.9mm zone of inhibition.

Activity of Phytosomes against *Aspergillus niger*: The antifungal effect of different phytosomes such as F4, F6 and optimized formulation (OF) is shown in Table no, Figure. F4 showed antifungal activity of 9.1mm, F6 10.8 mmand OF 13.4mmzone of inhibition, when compared with standard Amphotericin- B at 200µg/ml, which 13.7mm showed zone of inhibition.

TABLE 14: ZONE OF INHIBITION ON HEXADECANOIC ACID FORMULATION AGAINST *C. ALBICANS* AND *A. NIGER*

Formulation	*C. albicans* (Mm)	*A. niger* (Mm)
PNA-F4	9.5	9.1
PNA-F6	11.3	10.8
PNA-OF	12.4	13.4
Amphotericin- B	14.9	13.7

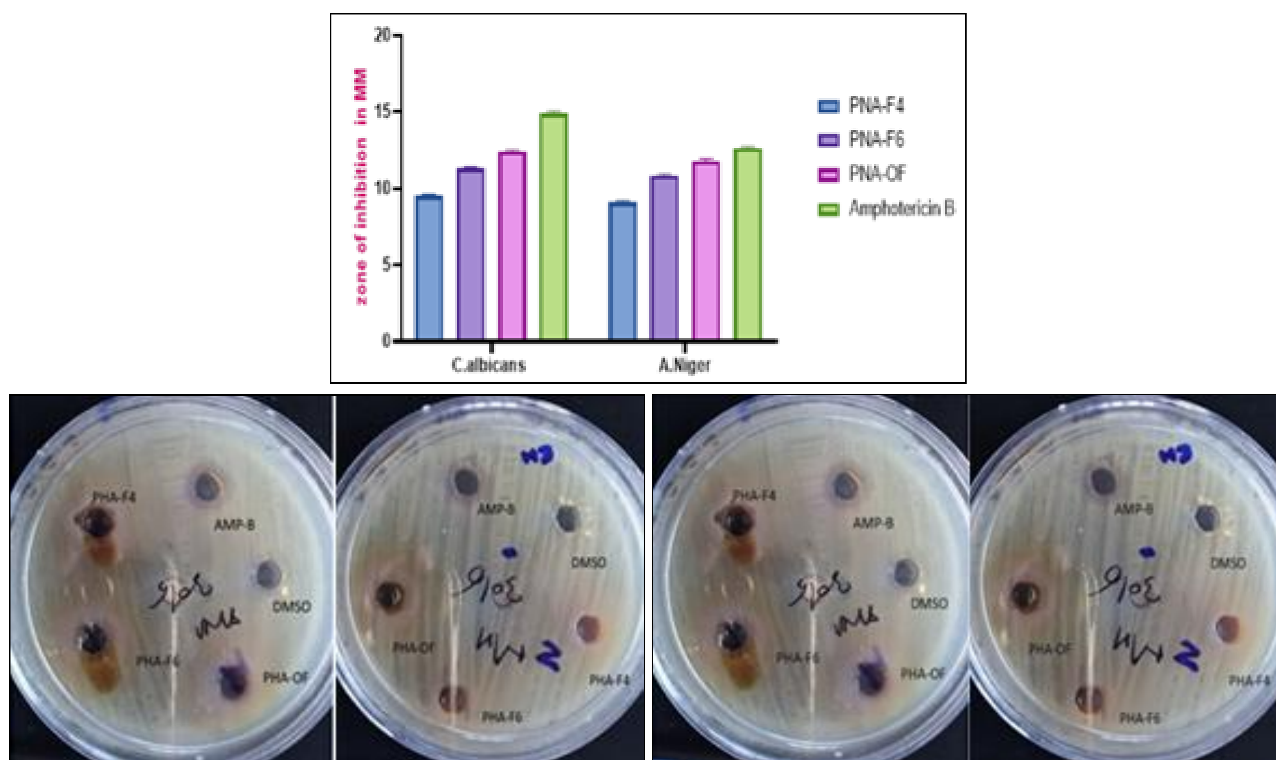


FIG. 15: ZONE OF INHIBITION ON HEXADECANOIC ACID FORMULATION AGAINST *C. ALBICANS*

CONCLUSION: The study aims to formulate, optimize and evaluate hexadecanoic acid phytosomal gel for anti-fungal activity. Hexadecanoic acid has numerous therapeutic effects, including the anti-fungal effect. Preformulation studies show high solubility of Hexadecanoic acid in Chloroform, and FTIR shows no interaction between drug and excipients. The absorption maxima of Hexadecanoic acid in chloroform was found to be 279 nm. The software was for the Design of an Experiment used with 3^2 factorial design. After optimization of formulation variables, it was found that the optimized formulation was found to contain (75mg, 300mg) of X1 and X2, respectively, which gave high %EE (72.23), %CDR (92.98%) and small particle size (715nm). SEM of optimized Hexadecanoic acid phytosomes appeared as spherical, well-identified, unilamellar nanovesicles. The optimized formulation of phytosomes was further formulated to gel with a concentration of 1.5% w/w of Hexadecanoic acid. The Spreadability value is 10.4±0.26 cm, which indicates that they can be spared easily on the skin surface with little stress. The pH value is 7.4±0.24, which is considered within the normal pH range for topical preparations. The actual drug content of the Hexadecanoic acid phytosomal gel was found to be 88.4±1.51 %, which represents good content uniformity. The viscosity of Hexadecanoic acid phytosomal gel is found to 1900cps. The percentage of drug release for Hexadecanoic acid phytosomal gel is 91.54±0.27%, indicating that Hexadecanoic acid phytosomal gel has high release and permeability. When release kinetics is applied it follows Higuchi model, it was found that drug release follows quasi fickian mechanism. Finally, stability studies showed that Phytosomal gel prepared using soya lecithin is more stable at $4^{o}C$ when compared to room temperature. Hexadecanoic acid phytosomal gel made up of soya lecithin showed good release kinetics along with good stability.

Future Scope: Future study of hexadecanoic acid phytosomes for various *in-vivo* studies in animals like colon cancer, Anti-inflammatory i.e, ulcerative colitis and productive effect of neurotoxicity.

ACKNOWLEDGEMENTS: The Authors acknowledge the Deccan School of Pharmacy and University College of Technology (Osmania University). Hyd for providing sources to completethe research work and thank Dr. Roshan S for guiding.

CONFLICTS OF INTEREST: NIL

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How to cite this article:
Fatima Z and Shahidulla SM: Formulation, optimization and evaluation of hexadecanoic acid phytosomal gel for anti-fungal activity. Int J Pharm Sci & Res 2023; 14(1): 519-29. doi: 10.13040/IJPSR.0975-8232.14(11).519-29.

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