

Design and Evaluation of Forskolin-Loaded Liposomes

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Article History:

Submitted: 02.05.2024

Accepted: 14.05.2024

Published: 25.05.2024

ABSTRACT

The goal of this study is to design liposome carrier system of forskolin for the treatment of muscle relaxant that is capable of delivering the encapsulated drug over a prolonged period of time by overcoming the limitations of conventional dosage forms. Forskolin is partially water-soluble drug but has low permeability. The main objective is to improve the permeability thereby improving the bioavailability. It is prepared by thin film hydration method, using materials like non-ionic surfactants (cholesterol, soylécithin) and solvents such as chloroform and ethanol. The Fourier Transform Infrared Spectroscopy (FTIR) results revealed that there is no interaction between forsko-

lin and excipients; all the formulations showed better encapsulation efficacy. Scanning Electron Microscopy (SEM) analysis revealed the surface morphology of forskolin-loaded liposome while dissolution studies showed prolonged drug release. On comprising all formulations, F3 showed sustained release of 98.44% up to 12 h. This may be due to the longest saturated alkyl chain and shows the highest entrapment.

Keywords: Bioavailability, Forskolin, Liposomes, Sustained release, Encapsulation

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INTRODUCTION

Liposomes are known as non-ionic surfactant vesicles which are microscopic lamellar structures formed on admixture of a non-ionic surfactant, cholesterol and dicetyl phosphate with subsequent hydration with aqueous media (Chen S, *et al.*, 2019; Sankhyan A and Pawar PK, 2013). Liposomes are capable of entrapping a variety of drugs and found as an alternative delivery system, with similar physical properties and are comparatively inexpensive with other delivery systems.

In the current years, transferring drug molecules to the desired site in the biological systems has become a very precise and sophisticated area of pharmaceutical research. The role of the drug delivery system is not only limited to a drug package just meant for administration and convenience but also to bring a required improvement in pharmacological efficacy and safety by carrying the drug molecules to the required site in the most convenient manner. Drug delivery system using colloidal particulate carriers like liposome has distinct merits over conventional dosage form as the colloidal particulate can act as drug reservoirs. Among different nanovesicular carriers, liposomes are selected as a carrier of choice because of its dominance over others carrier with regard to stability and cost effectiveness (Stulzer HK, *et al.*, 2008; Shalini M, *et al.*, 2016). Conventional drug delivery systems face some significant challenges, such as unfavorable pharmacokinetics and undesirable side effects. Nanocarriers have been extensively investigated in the past decades to overcome the challenges associated with conventional drug delivery systems, due to the advantages such as-

- Facilitate targeted drug delivery to the diseased site
- Enhance absorption as surface area increases and hence increase bioavailability
- Improve pharmacokinetics and bio distribution of active agents
- Increase retention in biological systems and extend the efficacy of drugs

Forskolin exerts 75% bioavailability but in the presence of food the bioavailability reduces to 30%-50% due to its relative short half-life. The main aim and objective of this study was to design

a prolonged release Forskolin formulation which would benefit the patients by reducing dose frequency, increased patient compliance, effective treatment, reduction in plasma concentration fluctuations, and reduced side effects.

MATERIALS AND METHODS

Materials

Forskolin was obtained as a gift sample from Emami Pvt. Ltd., Kolkata. Soyabean and cholesterol purchased from lobe chemical, Mumbai. Chloroform and methanol of analytical reagent were used.

Preparation of forskolin-loaded liposomes

Forskolin-loaded liposomes were developed by thin film hydration method in a rotary evaporator. Accurately weighed amounts of non-ionic surfactants (span 20, 40, 60 and 80) and cholesterol were transferred in a round bottom flask of the rotary evaporator. The chloroform and methanol (4:1) solution was added to the flask and evaporated at 50°C for 15-20 min under vacuum. The formed film is then hydrated with phosphate buffer pH 7.4 containing drug and vortexed at room temperature for 20 min which forms milky white suspension. The resultant dispersion was then cooled in an ice bath and sonicated for 3-5 min. Then, the resultant liposomes were stored at refrigerator (4°C-6°C). Various formulations were prepared (Table 1).

Evaluation of liposomes

%practical yield: Percentage practical yield is calculated to know efficiency of any method, thus its help in selection of appropriate method of formulation. Liposome were collected and weighed to determine Practical Yield (PY) from the following equation. The results are shown in Table 2.

$\%practical\ yield = \frac{practical\ yield}{theoretical\ yield} \times 100$

%drug content: Liposome equivalent to 50 mg of forskolin was taken and dissolved in distilled water for the extraction of encapsulated drug with regularly shaking and kept undisturbed for 24 h for complete extraction. The extract was filtered and diluted serially with phosphate buffer pH 7.4 and the absorbance was measured at 210 nm and the drug content was calculated from the calibration curve, as shown in Table 3.

Table 1: Composition of liposome containing forskolin

Composition	Quantities									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Forskolin	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Soy lecithin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Span 20	1	-	-	-	0.5	0.5	0.5	-	-	-
Span 40	-	1	-	-	0.5	-	-	0.5	0.5	-
Span 60	-	-	1	-	-	0.5	-	0.5	-	0.5
Span 80	-	-	-	1	-	-	0.5	-	0.5	0.5
Ethanol (ml)	5	5	5	5	5	5	5	5	5	5
Chloroform (ml)	20	20	20	20	20	20	20	20	20	20
Phosphate buffer (pH 7.4)	25	25	25	25	25	25	25	25	25	25

Table 2: %practical yield of liposomes

Formulation code	%yield
F1	77.6
F2	79.2
F3	94.2
F4	83.6
F5	84.4
F6	87.2
F7	85.2
F8	92.4
F9	86.8
F10	87.6

Table 3: %drug content of liposome (n=3)

Formulation code	%drug content \pm SD
F1	86.84 \pm 0.14
F2	88.62 \pm 0.24
F3	97.18 \pm 0.37
F4	89.99 \pm 0.28
F5	91.93 \pm 0.37
F6	92.25 \pm 0.48
F7	95.96 \pm 0.14
F8	96.37 \pm 0.24
F9	93.46 \pm 0.24
F10	94.59 \pm 0.14

%drug content=test absorbance/standard absorbance × 100

In vitro drug release: The *in vitro* drug release was studied using United States Pharmacopeia (USP) dissolution apparatus (Karthick K and Kumaran KS, 2016; Gupta A, *et al.*, 2007). Liposome equivalent to 50 mg of forskolin were placed in the dialysis membrane tubes. The tubes were immersed in dissolution vessel containing phosphate buffer pH 7.4 maintained at 37°C ± 0.5°C. Samples were withdrawn at periodic time intervals and replaced with equal amount of buffer to maintain sink condition. The samples were analyzed by Ultraviolet (UV)/visible spectrophotometer (Table 4).

Drug release kinetics: The release data obtained from various formulations were studied further for fitness of data in different kinetic models such as zero order, first order, Higuchi equation, Korsmeyer-Peppas and Hixson-Crowell release models as presented in Tables 5 and 6.

Scanning electron microscopy

Scanning: Electron microscopy of the liposome was performed to exam-

ine the particle size and surface morphology. Liposomes were mounted on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the equipment. The pictures were taken using a scanning electron microscope (Figure 1).

Fourier Transform Infrared Spectroscopy (FTIR) studies: The chance of drug-excipients (cholesterol and non-ionic surfactants) interactions was investigated by FTIR spectrum studies. The FTIR spectrum of pure drug (forskolin) and combination of drug with excipients were recorded using FTIR spectrophotometer. The spectrum was scanned in the wavelength region of (4000-400) cm⁻¹. The results are shown in Table 7.

Stability studies: Stability studies for the optimized formulation were carried out at elevated temperature 45°C ± 2°C with Relative Humidity (RH) 75% for a period of 3 months according to the International Council for Harmonization (ICH). At definite time periods, the samples from each batch were taken and evaluated for drug release (Table 8).

Table 4: %comparison of dissolution data of liposomes containing forskolin (n=3)

Time (h)	Pure drug	%cumulative drug release (meant ± SD)									
		F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
0.5	27.99 ± 0.14	16.13 ± 0.10	15.16 ± 0.06	10.47 ± 0.05	14.16 ± 0.10	17.03 ± 0.05	17.41 ± 0.05	10.53 ± 0.05	10.63 ± 0.22	12.75 ± 0.09	13.16 ± 0.05
1	48.74 ± 0.14	27.97 ± 0.05	26.22 ± 0.05	20.72 ± 0.09	25.34 ± 0.05	25.34 ± 0.05	28.84 ± 0.05	21.06 ± 0.14	20.81 ± 0.25	23.06 ± 0.10	24.88 ± 0.11
2	70.86 ± 0.16	70.86 ± 0.16	44.31 ± 0.05	37.16 ± 0.05	41.78 ± 0.05	47.59 ± 0.05	48.53 ± 0.05	37.16 ± 0.05	37.34 ± 0.30	39.63 ± 0.05	10.63 ± 0.22
3	97.40 ± 0.94	72.91 ± 0.10	57.16 ± 0.06	44.72 ± 0.09	54.97 ± 0.11	60.72 ± 0.05	62.69 ± 0.11	45.81 ± 0.11	51.13 ± 0.05	47.69 ± 0.11	49.87 ± 0.19
4	-	90.06 ± 0.05	70.72 ± 0.05	55.34 ± 0.05	72.72 ± 0.05	70.94 ± 0.14	73.81 ± 0.05	55.91 ± 0.11	61.53 ± 0.05	61.53 ± 0.05	60.69 ± 0.11
5	-	96.49 ± 0.03	89.41 ± 0.11	64.06 ± 0.22	82.66 ± 0.05	85.09 ± 0.05	89.88 ± 0.11	66.06 ± 0.05	72.72 ± 0.20	69.69 ± 0.05	72.75 ± 0.19
6	-	-	72.75 ± 0.19	72.75 ± 0.19	72.75 ± 0.19	72.75 ± 0.19	98.31 ± 0.19	76.16 ± 0.05	82.91 ± 0.05	80.50 ± 0.11	80.50 ± 0.11
7	-	-	-	-	-	82.81 ± 0.06	94.78 ± 0.10	94.69 ± 0.16	91.75 ± 0.11	96.75 ± 0.49	97.32 ± 0.82
8	-	-	-	-	-	-	-	-	-	91.81 ± 0.05	97.53 ± 0.05
9	-	-	-	-	-	-	-	-	-	-	98.44 ± 0.10

Table 5: Correlation coefficient (r) and release rate constant (k) values of forskolin-loaded liposomes

Kinetic models	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Zero order										
r	0.998	0.994	0.997	0.993	0.995	0.994	0.997	0.997	0.995	0.993
k	23.87	22.15	18.58	20.89	23.79	24.26	18.58	18.67	19.81	20.87
First order										
r	1	0.999	1	0.999	1	0.999	0.999	1	0.999	0.999
k	0.667	0.557	0.307	0.382	0.531	0.6	0.326	0.358	0.379	0.415
Higuchi model										
r	0.99	0.991	0.995	0.993	0.993	0.993	0.991	0.99	0.994	0.995
k	45.39	40.52	30.5	38.06	39.68	40.86	33.32	34.57	34.35	35.4
Korsmeyer-Peppas model										
r	0.998	0.999	0.996	0.999	0.998	0.998	0.998	0.999	0.997	0.996
k	0.857	0.74	0.711	0.745	0.71	0.707	0.715	0.816	0.68	0.668
Hixson Crowell model										
r	0.999	0.998	0.999	0.997	0.999	0.998	0.999	0.999	0.998	0.998
k	0.639	0.52	0.333	0.41	0.498	0.538	0.334	0.334	0.365	0.389
T ₅₀	2.09	2.44	3.5	2.62	2.18	2.1	3.41	2.92	3.23	3.01
T ₉₀	4	5.07	9.6	6.13	5.39	5.01	7.49	7.6	7.17	6.6

Table 6: Kinetic data for the optimized formulation

Time (h)	Zero order %cumulative drug release	First order time	First order log %cumulative drug remaining	Higuchi square root of time	Higuchi %cumulative drug release	Korsemeyer-Peppas log time	Korsemeyer-Peppas log %cumulative drug release	Hixson-Crowell time	Hixson-Crowell cube root of %drug remaining
1	20.72	1	1.899	1	20.72	0	1.316	1	4.295
2	37.16	2	1.798	1.414	37.16	0.301	1.57	2	3.975
4	44.72	3	1.742	1.7325	44.72	0.477	1.65	3	3.809
4	55.34	4	1.649	2	55.34	0.602	1.743	4	3.547
5	64.06	5	1.555	2.236	64.06	0.698	1.806	5	3.3
6	73.63	6	1.421	2.449	73.63	0.778	1.867	6	2.976
8	82.81	8	1.235	2.828	82.81	0.903	1.918	8	2.58
10	91.81	10	0.913	3.162	91.81	1	1.962	10	2.015
12	98.44	12	0.193	3.464	98.44	1	1.993	-	-

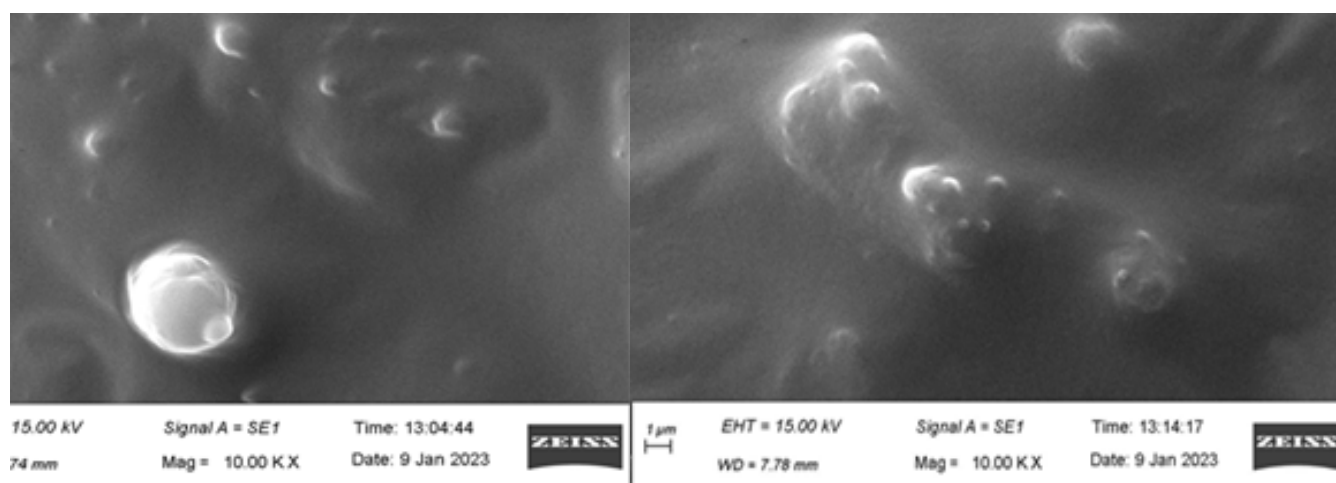


Figure 1: SEM image of forskolin

Table 7: FT-IR interpretation of pure drug and optimized formulation

Functional group	Characteristic peaks (cm ⁻¹)	Observed peaks of forskolin (cm ⁻¹)	Observed peaks (optimized formulation) (cm ⁻¹)
C-S (stretching)	600-800	672.35	670.65
C-N (stretching)	1020-1250	1193.64	1193.33
C-H (bending)	1450-1470	1647.12	1467.45
N H (bending)	1580-1650	1581.9	1582.41
C=O (stretching)	1690-1760	1740.9	1740.86
S-H (stretching)	≈2550	2561.28	2561.61

Table 8: Dissolution data of optimized formulation

Time (h)	%cumulative drug release (mean \pm SD)			
	Initial	1 st month	2 nd month	3 rd month
0.5	10.47 \pm 0.05	10.47 \pm 0.14	10.34 \pm 0.24	10.28 \pm 0.24
1	20.72 \pm 0.09	20.72 \pm 0.16	20.66 \pm 0.24	20.53 \pm 0.16
2	44.72 \pm 0.09	37.13 \pm 0.28	36.94 \pm 0.43	36.84 \pm 0.28
3	44.72 \pm 0.09	44.31 \pm 0.27	44.03 \pm 0.30	43.88 \pm 0.41
4	55.34 \pm 0.05	55.06 \pm 0.87	54.75 \pm 0.90	54.34 \pm 0.87
5	64.06 \pm 0.22	63.78 \pm 1.09	63.47 \pm 1.15	62.75 \pm 1.13
6	73.63 \pm 0.05	73.56 \pm 1.55	73.25 \pm 1.63	72.22 \pm 1.28
7	82.81 \pm 0.06	82.53 \pm 0.87	82.06 \pm 1.03	81.78 \pm 0.91
8	91 \pm 0.05	91.78 \pm 0.84	91.50 \pm 0.50	91.16 \pm 0.55
9	98.44 \pm 0.10	98.06 \pm 1.44	97.75 \pm 1.11	97.13 \pm 1.14

RESULTS AND DISCUSSION

%practical yield

From the results, it was observed that there was no significant loss of the drug and excipients during the preparation of liposomes by thin film hydration method.

%drug content

The solutions were analyzed for drug content spectrophotometrically at 210 nm. The drug content was calculated by estimating the amount of drug in liposomes. Results shown in Table 3 revealed that there was no significant loss of the drug during the preparation and all the formulations exhibited fairly uniform drug content.

In vitro drug release

The formulated niosomes were subjected to *in vitro* drug release studies using phosphate buffer pH 7.4 as a dissolution medium (Ruckmani K and Sankar V, 2010; Jadon PS, *et al.*, 2009; Bansal, *et al.*, 2013; Patel KK, *et al.*, 2012; Qumbar M, *et al.*, 2017). The amount of forskolin released was estimated spectrophotometrically at 210 nm. The free drug was released 97.40% within 3 h. F1 showed release of 96.49% within 5 h, F2 showed release of 97.50% within 6 h, F3 showed release of 98.44% within 12 h, F4 showed release of 94.78% within 8 h, F5 showed release of 97.56% within 6 h, F6 showed release of 98.31% within 6 h, F7 showed release of 94.69% within 8 h, F8 showed release of 97.53% within 10 h, F9 showed release of 96.75% within 8 h and F10 showed release of 97.32% within 8 h. These results showed that forskolin-loaded liposomes have shown sustained release when compared to pure drug. This is because the drug is released slowly for a prolonged period of time in liposomal formulation (Kumar YA and Setty CM, 2017; Sunilkumar MR, *et al.*, 2016; Meenakshi M and Elango K, 2016). The difference in the drug release was attributed to the structure of surfactant. As known, non-ionic surfactants such as span 20, 40 and 60, have the same head group and different alkyl chain. Among these

surfactants, only span 80 have an unsaturated alkyl chain. The introduction of double bonds made the chains bend. This means that the adjacent molecular cannot be tight when they form the membrane of liposomes. These cause the membrane to be more permeable, which possibly made the lowest entrapment efficiency of the span 80 formulation. However, of the other three kinds of non-ionic surfactants, span 60 has the longest saturated alkyl chain and show the highest entrapment. The encapsulation efficiency shows the trend C16 (span 60) > C14 (span 40) > C12 (span 20). It proposes that the length of alkyl chain is a crucial factor of permeability and high entrapment. Out of all 10 formulation, F3 consists of span 60 was considered as optimized formulation.

The time point to dissolve 50% of drug is t_{50} which was found to be 2.09 h for F12, 44 h for F2, 3.50 h for F3, 2.62 h for F4, 5.39 h for F5, 5.01 h for F6, 7.49 h for F7, 7.60 h for F8, 7.17 h for F9 and 6.60 h for F10 formulations. The drug release data were fitted in different kinetic equations and “r” values are shown table. The drug release patterns from the liposome have found to be followed the first-order kinetic model predominantly followed by Hixson-Crowell’s cube root model as well. This release patterns are evident with the correlation coefficient “r” values. Zero order, first order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell data were drawn for the optimized formulation to depict the release kinetics of the drug.

Scanning Electron Microscopy (SEM) analysis

Scanning: Electron microscopy was used to characterize the surface morphology. The particle size of forskolin was found to be 74 nm whereas particle size of forskolin-loaded liposome was found to be 7.78 μ m. The particle size of forskolin was size reduced 10 times on liposomal formulation.

Pure forskolin showed principal absorption peaks at 672.35 cm^{-1} (C-S stretching), 1193.64 cm^{-1} (C-N stretching), 1467.12 cm^{-1} (C-H bending), 1581.90 cm^{-1} (N-H bending), 1740.90 cm^{-1} (C=O stretching), and 2561.28 cm^{-1} (S-H stretching). The identical peaks of C-S stretching, C-N stretch-

ing, C-H bending, N-H bending, C=O stretching, and S-H stretching vibrations were also noticed in the spectra of drug-loaded liposome. FT-IR spectra revealed that there was no interaction between the drug and the polymers used for liposome preparation.

Stability studies: A stability study for the optimized formulation was carried out at elevated temperature ($45 \pm 2^\circ\text{C}$) and RH of $75\% \pm 5\%$ for a period of 3 months as per ICH guidelines. Samples were withdrawn at definite time periods (1st month, 2nd month and 3rd month) and dissolution studies were performed. The results from dissolution studies indicated that there was no significant difference of drug release. Stability studies indicated that optimized formulation F3 was stable.

CONCLUSION

The forskolin-loaded liposomes were prepared by thin film hydration method. Cholesterol and non-ionic surfactants were used for the preparation of liposome. Span 20, 40, 60 and 80 were used as non-ionic surfactants. The prepared forskolin-loaded liposomes were evaluated for %yield, %drug content, FTIR, SEM analysis, and stability studies. The *in vitro* release studies were performed. Good results were obtained for %yield, %drug content, and *in vitro* studies. FT-IR spectra revealed that there was no interaction between the drug and the polymers used for liposome preparation. F3 formulation containing cholesterol and span 60 exhibited the release of 98.44% up to 12 h. On comprising all formulations, F3 showed sustained release up to 12 h. This may be due to longest saturated alkyl chain of span 60 and shows the highest entrapment. From this study, it is concluded that forskolin-loaded liposome have shown sustained release when compared to pure drug.

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