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DEVELOPMENT AND EVALUATION OF EPLERENONE LIPOSOMAL DRUG DELIVERY SYSTEM

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Abstract: The present study was aimed at the development and evaluation of an Eplerenone liposomal drug delivery system using the thin-film hydration method to enhance drug encapsulation and achieve sustained drug release. Eplerenone liposomes were prepared by the thin-film hydration technique using phosphatidylcholine and cholesterol in different ratios. Eight formulations (F1–F8) were developed by varying the cholesterol concentration while maintaining a constant amount of phosphatidylcholine. The prepared liposomes were evaluated for particle size, zeta potential, surface morphology by scanning electron microscopy (SEM), drug entrapment efficiency, in vitro drug release using a Franz diffusion cell, drug release kinetics, and stability according to ICH guidelines. The prepared liposomes showed vesicle sizes ranging from 284 to 389 nm. SEM analysis confirmed the formation of spherical vesicles with smooth surfaces and minimal aggregation. Drug entrapment efficiency ranged from 86.98% to 95.68%, with formulation F4 exhibiting the highest entrapment efficiency (95.68%). The optimized formulation also demonstrated the highest cumulative drug release (98.35%) over 12 hours, indicating sustained release behavior. Stability studies performed under ICH storage conditions for three months showed no significant changes in drug release or physical characteristics, confirming good formulation stability. The study successfully developed a stable Eplerenone liposomal formulation with high drug entrapment efficiency, nanosized vesicles, and sustained drug release characteristics. The optimized formulation (F4) demonstrated superior physicochemical properties and controlled drug release, indicating that liposomal encapsulation is a promising approach for enhancing the delivery and therapeutic performance of Eplerenone.

Keywords: Eplerenone, Liposomes, Thin-film hydration, Phosphatidylcholine, Cholesterol, Drug entrapment efficiency, Sustained drug release, Franz diffusion cell, Higuchi model, Stability studies.

INTRODUCTION

Vesicular drug delivery systems are carrier systems in which drug molecules are enclosed within lipid-based vesicles.1 Liposomal drug delivery systems have emerged as promising vesicular carriers for enhancing the delivery of therapeutic agents.2 Liposomes are spherical vesicles composed primarily of phospholipid bilayers surrounding an aqueous core and can accommodate drugs with different physicochemical properties.3 The development of an eplerenone liposomal drug delivery system may consequently provide an alternative approach for improving the performance of conventional eplerenone therapy.4 Eplerenone is a selective aldosterone receptor antagonist primarily used in the management of hypertension and heart failure, particularly in patients with left ventricular dysfunction following myocardial infarction.5 The present study was therefore undertaken with the objective of developing and evaluating eplerenone-loaded liposomes using suitable phospholipids and cholesterol as structural components. 6 The prepared liposomes were evaluated for particle size, polydispersity index, zeta potential, entrapment

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efficiency, morphology, drug content, and in-vitro drug-release characteristics.7

MATERIALS

Eplerenone was procured from Hetero Labs, HYD. Phosphatidylcholine and Cholesterol were obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

METHODOLOGY

Formulation development

Table-1: Composition of lipids for preparation of liposomes

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Phosphatidylcholine (mg)	100	100	100	100	100	100	100	100
Cholesterol(mg)	100	200	300	400	500	600	700	800
Chloroform (ml)	10	10	10	10	10	10	10	10
Methanol	5	5	5	5	5	5	5	5
Drug(mg)	25	25	25	25	25	25	25	25
Phosphate buffer pH 7.4(ml)	10	10	10	10	10	10	10	10

Preparation of liposomes

Accurately weighed quantities of Eplerenone (25 mg), phosphatidylcholine (100 mg), and cholesterol (100–800 mg according to formulation F1–F8) were transferred into a clean, dry 250 mL round-bottom flask. A solvent mixture of chloroform (10 mL) and methanol (5 mL) in the ratio of 2:1 (v/v) was added to dissolve the lipid components and the drug completely, producing a clear homogeneous solution. The organic solvents were then removed using a rotary vacuum evaporator maintained at 40 ± 2°C under reduced pressure with a rotation speed of 80–100 rpm. Evaporation continued until a thin, uniform lipid film was formed on the inner wall of the flask. The flask was further kept under vacuum for 2–4 hours (or overnight in a vacuum desiccator) to ensure complete removal of residual organic solvents. The dried lipid film was hydrated with 10 mL of phosphate buffer (pH 7.4) preheated to 50–60°C, which is above the phase transition temperature of phosphatidylcholine. Hydration was carried out by rotating the flask at 60 rpm for 30–60 minutes, allowing the lipid film to swell and detach from the flask wall, resulting in the formation of multilamellar liposomes. The resulting liposomal dispersion was subjected to probe sonication or bath sonication for 5–10 minutes to reduce the vesicle size and obtain small unilamellar vesicles with a narrow size distribution. Alternatively, the dispersion may be extruded through polycarbonate membrane filters of appropriate pore size to obtain uniform-sized liposomes. The prepared liposomes were allowed to equilibrate at 4°C for 12–24 hours to improve vesicle stability and drug entrapment. The liposomal suspension was then stored in airtight amber-colored glass containers under refrigerated conditions (2–8°C) until further evaluation.8

Evaluations of liposomes

Encapsulation efficiency determination

The encapsulation efficiency (EE%) of the prepared Eplerenone liposomes was determined by separating the unentrapped (free) drug from the liposome-entrapped drug using the ultracentrifugation method. A 2 mL sample of the freshly prepared liposomal dispersion was transferred into centrifuge tubes and centrifuged at 15,000–20,000 rpm for 30–45 minutes at 4°C using a refrigerated ultracentrifuge. After centrifugation, the supernatant, containing the unentrapped (free) Eplerenone, was carefully collected and appropriately diluted with methanol or phosphate buffer (pH 7.4). The amount of free drug present in the supernatant was determined using a UV–Visible spectrophotometer at 240 nm against a suitable blank. The pellet containing the liposomes was washed with phosphate buffer (pH 7.4) to remove any loosely adsorbed drug. The washed pellet was then lysed by adding methanol to disrupt the lipid bilayer and release the entrapped drug. The solution was mixed thoroughly and analyzed spectrophotometrically at 246 nm to determine the amount of entrapped Eplerenone.9

Entrapment efficiency (%) = [(TD – FD)/TD] × 100 (1)

Where

FD is the quantity of drug estimated in the filtrate and

TD is the theoretical amount of tacrolimus that is present in liposomal preparation.

Particle Size Analysis

The average particle size and particle size distribution of the prepared Eplerenone liposomes were determined using Dynamic Light Scattering (DLS) with a particle size analyzer (e.g., Malvern Zetasizer). Before analysis, the liposomal suspension was diluted 100-fold with filtered distilled water or phosphate buffer (pH 7.4) to avoid multiple scattering effects. The diluted sample was transferred into a disposable cuvette, and measurements were performed at 25 ± 1°C with a scattering angle of 90°. Each formulation was analyzed in

triplicate, and the average particle size (nm) and polydispersity index (PDI) were recorded.10

Scanning Electron Microscopy (SEM) Analysis

The surface morphology of the prepared Eplerenone liposomes was examined using Scanning Electron Microscopy (SEM). A small amount of the lyophilized liposomal sample was mounted on a double-sided adhesive carbon tape attached to an aluminum stub. The sample was sputter-coated with a thin layer of gold under vacuum to improve electrical conductivity. The coated sample was observed under the scanning electron microscope at suitable accelerating voltages (10–20 kV), and micrographs were recorded at different magnifications to evaluate the shape, surface characteristics, and aggregation of liposomes.11

in vitro drug release study

The in vitro drug release of the prepared Eplerenone liposomal formulation was evaluated using a Franz diffusion cell apparatus. A dialysis membrane (molecular weight cut-off 12,000–14,000 Da) was soaked overnight in phosphate buffer (pH 7.4) before use to ensure complete hydration. The hydrated membrane was mounted carefully between the donor and receptor compartments of the Franz diffusion cell, with the smooth surface facing the donor compartment. The receptor compartment was filled with 25 mL of phosphate buffer (pH 7.4), which served as the diffusion medium. The receptor medium was maintained at 37 ± 0.5°C using a circulating water bath, and continuously stirred with a magnetic stirrer at 300–500 rpm to maintain uniform mixing and sink conditions. An accurately measured quantity of the Eplerenone liposomal suspension equivalent to 25 mg of drug was placed into the donor compartment over the dialysis membrane. The donor compartment was covered with parafilm or an appropriate cap to prevent evaporation during the study. At predetermined time intervals 1 mL of the receptor medium was withdrawn and immediately replaced with an equal volume of fresh phosphate buffer (pH 7.4) maintained at the same temperature to maintain sink conditions. The collected samples were filtered through a 0.45 µm membrane filter and analyzed using a UV–Visible spectrophotometer at 246 nm to determine the amount of Eplerenone released.12

Drug Release Kinetics[13]

The in vitro drug release data obtained from the optimized liposomal formulations were fitted into various kinetic models to determine the mechanism of drug release.

The mathematical models used were:

- Zero-order kinetics
- First-order kinetics
- Higuchi diffusion model
- Korsmeyer–Peppas model

The regression coefficient (R^2) was calculated for each model. The model exhibiting the highest R^2 value was considered the best fit for describing the drug release behavior. The release exponent (n) obtained from the Korsmeyer–Peppas equation was used to determine the mechanism of drug release.

Stability Studies[14]

Stability studies of the optimized Eplerenone liposomal formulation were carried out according to ICH Q1A(R2) guidelines.

RESULTS AND DISCUSSION

Vesicle size:

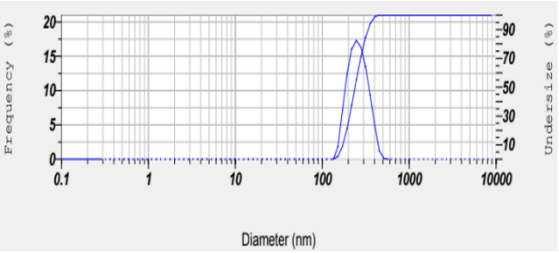


Fig-1: particle size of Eplerenone liposomes

SEM Analysis

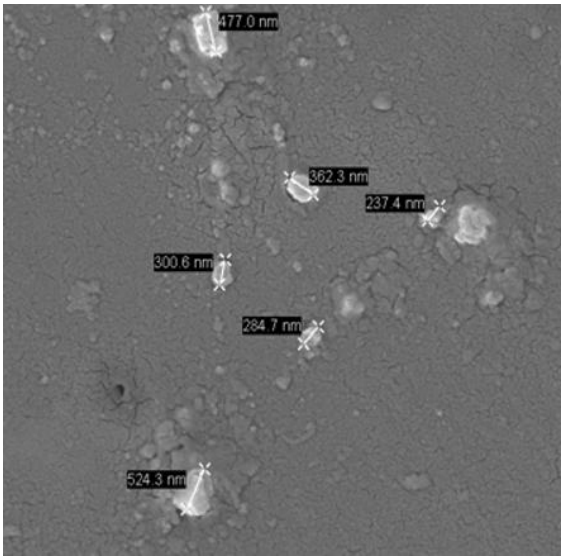


Fig-2: SEM Analysis of Eplerenone liposomes

Table-2: Mean particle size of different formulation of liposomes

Formulation No.	Particle size
F1	356
F2	300
F3	297
F4	284
F5	363
F6	371
F7	389
F8	324

Drug entrapment efficiency

Table-3: Different batches of liposome made by using different ratio of lipids

Formulation no.	% DEE
F1	86.98
F2	89.17
F3	90.25
F4	95.68
F5	94.53
F6	88.12
F7	87.58
F8	91.23

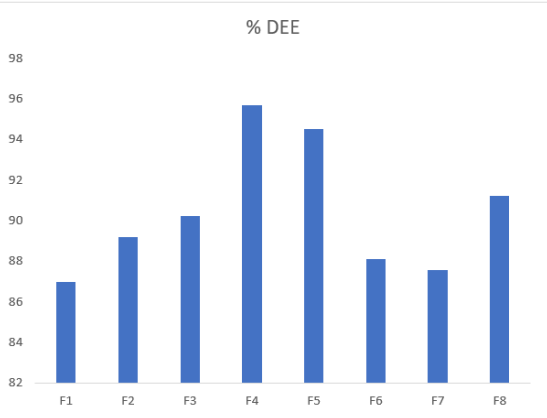


Fig-3: Drug entrapment efficiency of all formulation

Discussion

Vesicle Size Analysis

The results showed that the mean particle size of the formulations ranged from 284 nm to 389 nm, indicating successful formation of nanosized liposomal vesicles. Among all the formulations, F4 exhibited the smallest mean particle size (284 nm), whereas F7 showed the largest particle size (389 nm).

SEM Analysis

Scanning Electron Microscopy (SEM) was performed to examine the morphology and surface characteristics of the optimized Eplerenone liposomes. The SEM micrographs demonstrated that the prepared liposomes were predominantly spherical in shape with a smooth and uniform surface. The vesicles appeared discrete, well defined, and showed minimal aggregation, indicating successful liposome formation by the thin-film hydration method. The observed morphology confirms that the selected phosphatidylcholine and cholesterol composition produced stable vesicular structures capable of efficiently encapsulating Eplerenone. The absence of surface cracks or collapsed vesicles further indicates the structural integrity of the liposomes, which is essential for maintaining drug entrapment and providing sustained drug release.

Drug Entrapment Efficiency

Overall, formulation F4 was identified as the optimized formulation because it exhibited the highest drug entrapment efficiency (95.68%), indicating superior drug-loading capacity and better vesicle stability. The high encapsulation efficiency is expected to contribute to sustained drug release, reduced drug leakage during storage, and improved therapeutic performance of the liposomal formulation.

Drug release studies

Table-4: Cumulative percentage drug release from various formulation of liposomes

Time (hr.)	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	17.86	18.96	16.53	19.83	15.69	16.37	19.12	16.38
2	25.92	26.39	28.49	29.46	26.72	25.49	26.37	25.43
3	35.89	36.82	38.46	38.47	37.42	38.12	37.46	38.42
4	48.12	49.58	46.35	48.58	47.51	46.32	45.82	48.79
6	57.83	58.43	59.82	62.35	63.26	65.97	63.38	65.28
8	73.59	75.82	76.38	79.58	75.69	76.28	77.83	74.58
10	82.16	83.55	85.29	88.43	84.51	85.58	86.91	87.42
12	93.57	94.71	95.36	98.35	96.85	97.46	95.36	96.81

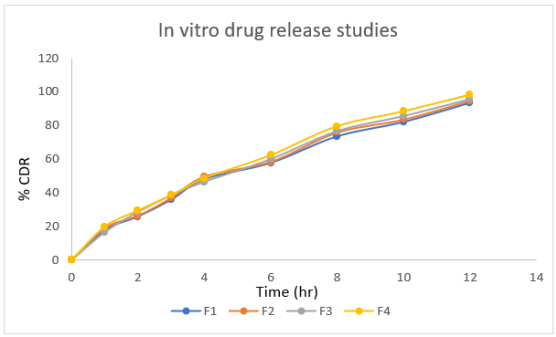


Fig-4: In vitro drug release of (F1-F4) formulations

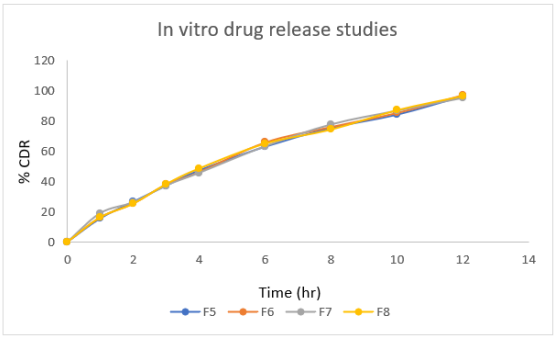


Fig-5: In vitro drug release of (F5-F8) formulations

Discussion

Overall, the results demonstrate that formulation F4 produced the most desirable release profile, delivering nearly complete drug release over 12 hours while maintaining excellent vesicle characteristics and high drug entrapment efficiency. The sustained release behavior observed is expected to improve therapeutic efficacy by maintaining prolonged drug availability and reducing dosing frequency.

Drug release kinetics:

The results obtaining in vitro release studies were plotted in different model of data treatment as follows:

- Cumulative percent drug released vs. time (zero order rate kinetics)
- Log cumulative percent drug retained vs. time (First Order rate Kinetics)
- Cumulative percent drug released vs. square root of time (Higuchi's
- Classical Diffusion Equation)
- Log of cumulative % release Vs log time (Peppas Exponential Equation

Zero order kinetics

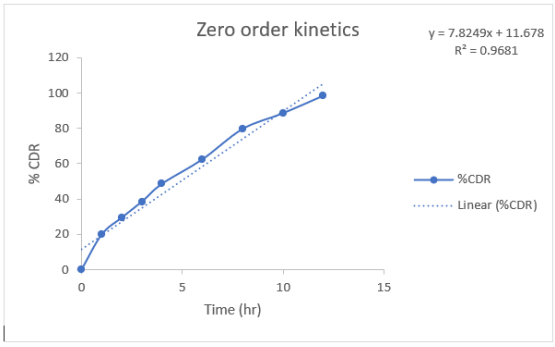


Fig-6: Zero order kinetics of optimized formulation

First order kinetics

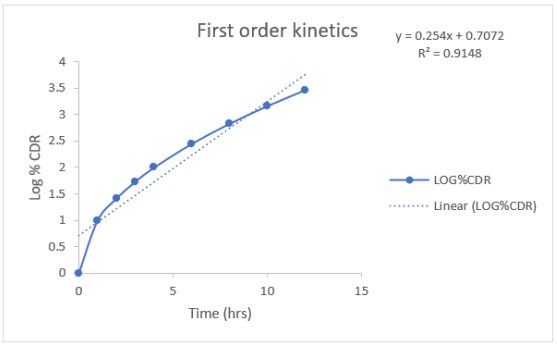


Fig-7: First order kinetics optimized formulation

Higuchi model

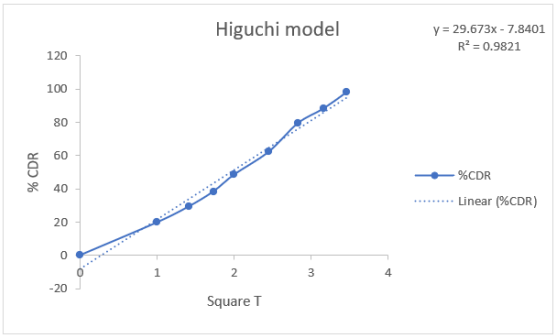


Fig-8: Higuchi model optimized formulation

Korsmeyer peppas

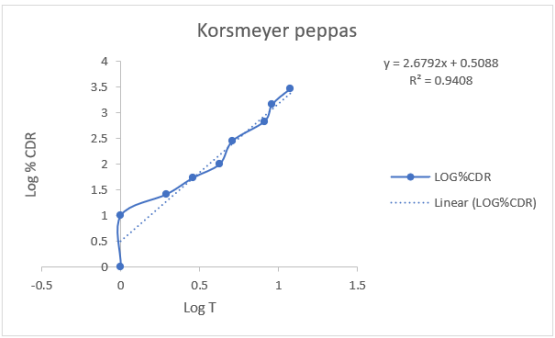


Fig-9: Korsmeyer peppas optimized formulation

The values of in vitro release were attempted to fit into various mathematical models. Plots of zero order, first order, Higuchi matrix, Peppas were respectively. The table indicates that r2

values are higher for Higuchi's model compared for all the formulation.

The in vitro drug release data of optimized formulation F4 were fitted into various kinetic models. This suggests that drug release from liposomes is primarily governed by diffusion through the lipid matrix, confirming controlled drug release behavior.

Stability studies

There was no significant change in physical and chemical properties of the tablets of formulation F-4 after 3 months. Parameters quantified at various time intervals were shown;

Table- 5: Results of stability studies of optimized formulation F-4

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-4	250C/60%RH % Release	98.35	97.53	96.37	95.80	Not less than 85 %
F-4	300C/75% RH % Release	98.35	97.12	96.25	95.38	Not less than 85 %
F-4	400C/75% RH % Release	98.35	97.06	96.21	95.25	Not less than 85 %

The stability of the optimized liposomal formulation F4 was evaluated under different storage conditions according to ICH guidelines for a period of three months. The formulation was stored at 25 ± 2°C/60% RH, 30 ± 2°C/75% RH, and 40 ± 2°C/75% RH, and the percentage drug release was determined at regular intervals. The results indicated only a slight decrease in drug release during storage. At 25°C/60% RH, the cumulative drug release decreased from 98.35% initially to 95.80% after three months. Similarly, at 30°C/75% RH and 40°C/75% RH, the percentage drug release decreased marginally to 95.38% and 95.25%, respectively. The observed reduction in drug release was minimal and remained well within the acceptable pharmacopoeial specification of not less than 85% drug release. No significant changes were observed in the physical appearance of the formulation, indicating that the liposomal vesicles remained stable throughout the storage period. The minor decrease in drug release may be attributed to slight lipid rearrangement or gradual oxidation of phospholipids during storage; however, these changes were not sufficient to affect the overall performance of the formulation. These findings confirm that the optimized formulation F4 possesses good physical and chemical stability under both room temperature and accelerated storage conditions. The retention of high drug release throughout the stability study demonstrates that the liposomal formulation is suitable for long-term storage and has the potential to maintain its therapeutic performance during its shelf life.

CONCLUSION

Among all the formulations, F4 was identified as the optimized formulation based on its smallest vesicle size (284 nm), highest drug entrapment efficiency (95.68%), and maximum cumulative drug release (98.35%). The optimized lipid composition provided an appropriate balance between membrane stability and permeability, resulting in efficient drug encapsulation and controlled drug release. Drug release kinetic studies demonstrated that the optimized formulation followed the Higuchi diffusion model, confirming that the release of Eplerenone was primarily governed by diffusion through the phospholipid bilayer. Stability studies further demonstrated that the formulation remained physically and chemically stable under both long-term and accelerated storage conditions without any significant deterioration. The findings of this study indicate that liposomal drug delivery is a promising approach for improving the therapeutic performance of Eplerenone by enhancing drug encapsulation, providing sustained drug release, and potentially improving patient compliance. Therefore, the optimized liposomal formulation has significant potential for further in vivo pharmacokinetic, pharmacodynamic, and clinical investigations, which may ultimately contribute to the development of an effective commercial liposomal formulation of Eplerenone.

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