Preparation and Characterization of Ajmaline-Loaded Liposome Formulation

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ABSTRACT
The present study aimed to develop and characterize an Ajmaline-loaded liposomal formulation for improving the physicochemical stability, encapsulation efficiency, and controlled drug release behavior of Ajmaline. Liposomes were prepared by the thin-film hydration method using soya lecithin and cholesterol in different ratios. The prepared formulations were evaluated for particle size, polydispersity index (PDI), zeta potential, drug loading, entrapment efficiency, Fourier Transform Infrared (FTIR) spectroscopy, surface morphology, and in vitro drug release profile. HPLC analysis confirmed the purity of Ajmaline with a retention time of 15.820 minutes, while UV spectrophotometric analysis showed maximum absorption at 210 nm. FTIR studies indicated successful encapsulation of Ajmaline within the liposomal bilayer without significant drug–excipient interaction. The optimized formulation (L3) exhibited a particle size of 153.5 nm, PDI of 0.201, zeta potential of −34.8 mV, drug loading efficiency of 9.69%, and entrapment efficiency of 96.90%. FESEM analysis revealed spherical vesicles with smooth morphology. In vitro drug release studies demonstrated a biphasic sustained-release pattern with approximately 96.1% drug release over 24 hours. The findings suggest that liposomal encapsulation significantly improves the stability and controlled-release characteristics of Ajmaline.

Keywords: Ajmaline, Liposome, HPLC, XRD, in-vitro release.
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.Introduction

Cardiovascular disorders remain one of the leading causes of morbidity and mortality worldwide, creating a continuous demand for effective antiarrhythmic therapies. Among the available agents, Ajmaline is an important class Ia antiarrhythmic drug widely used in the diagnosis and management of cardiac conduction abnormalities, particularly ventricular arrhythmias and Brugada syndrome. Originally isolated from the medicinal plant *Rauwolfia serpentina*, Ajmaline is an indole alkaloid possessing potent sodium channel blocking activity that prolongs the action potential duration and refractory period of cardiac tissues. Its pharmacological action mainly involves inhibition of rapid inward sodium currents in myocardial cells, thereby stabilizing cardiac membranes and suppressing abnormal electrical conduction pathways. Because of these properties, Ajmaline has gained significant clinical relevance in electrophysiological investigations and diagnostic provocation testing for inherited arrhythmogenic syndromes (Antzelevitch et al., 2005; Wilde et al., 2002).

Despite its promising pharmacological efficacy, the clinical application of Ajmaline is associated with several limitations. The drug exhibits poor aqueous solubility, limited bioavailability, rapid systemic clearance, and short biological half-life, which collectively reduce its therapeutic effectiveness. Furthermore, conventional formulations may produce fluctuations in plasma drug concentration, leading to adverse cardiovascular effects such as hypotension, excessive cardiac depression, and proarrhythmic

complications. The instability of Ajmaline under physiological conditions and nonspecific distribution within the body further necessitate the development of an advanced drug delivery system capable of improving its therapeutic index while minimizing side effects (Singh and Lillard, 2009).

In recent years, nanotechnology-based drug delivery systems have emerged as an effective strategy to overcome the limitations associated with conventional pharmaceutical formulations. Among various nanocarriers, liposomes have attracted substantial attention due to their unique structural and functional properties. Liposomes are spherical vesicular systems composed of one or more phospholipid bilayers surrounding an aqueous core. These carriers are biocompatible, biodegradable, non-toxic, and capable of encapsulating both hydrophilic and lipophilic drugs. The amphiphilic nature of liposomes allows them to enhance drug solubility, protect encapsulated drugs from degradation, improve pharmacokinetic profiles, and facilitate controlled or targeted drug delivery (Bangham et al., 1965; Bozzuto and Molinari, 2015).

The utilization of liposomal drug delivery systems has revolutionized pharmaceutical research because of their ability to improve therapeutic efficacy and reduce systemic toxicity. Liposomes can prolong circulation time, enhance tissue penetration, and provide sustained drug release characteristics. Additionally, the phospholipid bilayer structure of liposomes closely resembles biological membranes, enabling efficient interaction with cellular systems and promoting improved drug uptake. Encapsulation of antiarrhythmic agents within liposomes may reduce dose-dependent toxicity and enhance therapeutic performance through controlled drug release and better bioavailability (Allen and Cullis, 2013).

For a molecule like Ajmaline, liposomal encapsulation presents several potential advantages. Since Ajmaline exhibits poor stability and rapid elimination, incorporation into liposomal vesicles may improve drug retention, enhance circulation time, and protect the active molecule from premature degradation. Liposomes may also reduce the direct exposure of cardiac tissues to high concentrations of free drug, thereby minimizing toxic manifestations. Furthermore, liposomal systems can potentially facilitate sustained release behavior, enabling maintenance of therapeutic plasma concentrations for longer durations and reducing the frequency of administration. Such improvements may significantly enhance patient compliance and clinical outcomes in antiarrhythmic therapy.

The preparation and characterization of liposomal formulations are critical steps in the development of an effective drug delivery system. Various preparation methods including thin-film hydration, reverse-phase evaporation, ethanol injection, and sonication techniques have been employed for liposome fabrication depending on the physicochemical properties of the drug and intended therapeutic application. Among these methods, the thin-film hydration technique is one of the most commonly used approaches because of its simplicity, reproducibility, and ability to produce vesicles with high encapsulation efficiency. Characterization parameters such as vesicle size, polydispersity index, zeta potential, entrapment efficiency, morphology, drug loading capacity, and in vitro drug release behavior are essential for evaluating the stability and performance of liposomal formulations (Mozafari et al., 2008).

Particle size plays a crucial role in determining the biodistribution and cellular uptake of liposomes, whereas zeta potential reflects colloidal stability. Entrapment efficiency indicates the ability of liposomes to retain the drug within the vesicular structure, and in vitro release studies help predict drug release kinetics under physiological conditions. Morphological evaluation using electron microscopy

techniques provides insights into vesicle architecture and surface characteristics. Collectively, these characterization studies are necessary to optimize formulation parameters and ensure the development of a stable and therapeutically efficient liposomal system.

Considering the therapeutic importance of Ajmaline and the advantages offered by liposomal drug delivery, the present study aims to prepare and characterize an Ajmaline-loaded liposome formulation. The study focuses on improving the physicochemical stability, encapsulation efficiency, and controlled release properties of Ajmaline through liposomal encapsulation. The developed formulation may provide a promising approach for enhancing the pharmacological performance and therapeutic safety of Ajmaline in cardiovascular applications.

Materials and Methods

Chemicals and Reagents

The chemicals and reagents used for the preparation of Ajmaline-loaded liposomes included Soya-L-α-lecithin (phosphatidylcholine), cholesterol, butylated hydroxyanisole (BHA), chloroform, methanol, potassium dihydrogen phosphate, sodium chloride, acetonitrile, and orthophosphoric acid.

Preformulation Studies

Determination of Absorption Maxima (λmax) of Ajmaline

Ajmaline was dissolved in a phosphate buffer saline (PBS, pH 7.4) and methanol mixture in the ratio of 4:6. The prepared solution was scanned in the wavelength range of 200–400 nm using a UV-visible spectrophotometer to determine the absorption maxima (λmax). The PBS:methanol mixture was used as the blank solution.

Preparation of Phosphate Buffered Saline (PBS), pH 7.4

Phosphate buffered saline was prepared by dissolving 1.7 g of sodium dihydrogen phosphate and 0.391 g of sodium hydroxide in double-distilled water, and the final volume was adjusted to 250 ml. The pH was adjusted to 7.4 using a calibrated pH meter.

Preparation of Calibration Curve of Ajmaline

A stock solution of Ajmaline (10 mg/ml) was prepared in PBS:methanol (4:6). Serial dilutions were prepared to obtain concentrations ranging from 1–7 mg/ml. The absorbance of each dilution was measured spectrophotometrically against PBS:methanol (4:6) as blank, and a calibration curve was plotted between concentration and absorbance.

HPLC Method for Drug Estimation

The chromatographic analysis of Ajmaline was performed according to the British Pharmacopoeia (2010) using a stainless-steel column packed with octadecylsilyl silica gel maintained at 40°C. The mobile phase consisted of acetonitrile and 0.001 M potassium dihydrogen orthophosphate solution in the ratio of 400:600, adjusted to pH 2.0 with orthophosphoric acid. The flow rate was maintained at 1.5 ml/min and detection was carried out at 210 nm. The sample solution was prepared by dissolving 50 mg of Ajmaline in methanol with sonication for 10 minutes followed by dilution with mobile phase. A 10 µl sample volume was injected into the HPLC system.

Preparation of Ajmaline-Loaded Liposomes

Ajmaline-loaded liposomes were prepared using the thin-film hydration method. Different ratios of phosphatidylcholine and cholesterol were used for formulation development. Precisely weighed quantities of Soya-L-α-lecithin, cholesterol, BHA (2% w/w of total lipid), and Ajmaline were transferred into a clean and dry round-bottom flask containing chloroform.

The organic solvent was evaporated using a rotary vacuum evaporator operated at 120 rpm and 40°C, resulting in the formation of a thin lipid film on the inner wall of the flask. The flask was subsequently placed in a vacuum desiccator overnight for complete removal of residual solvent.

Hydration of the dry lipid film was performed using isotonic phosphate buffer

saline (pH 7.4) at 60°C, which was above the phase transition temperature of phospholipids. The flask was rotated at 100 rpm until complete dispersion of the lipid film occurred. The resulting liposomal suspension was sonicated for 30–40 minutes using a bath sonicator to reduce vesicle size.

The preparation was allowed to stand at room temperature for 1 hour for vesicle formation and subsequently stored at 4°C for 24 hours under inert atmospheric conditions. The dispersion was then centrifuged at 15,000 rpm for 1 hour at 4°C. The separated liposomal vesicles were stored at −40°C overnight followed by lyophilization until complete drying of the formulation was achieved.

Table 1. Composition of Ajmaline-Loaded Liposome Formulations

Formulation Code	Phosphatidylcholine : Cholesterol Ratio	Drug (mg)
L1	1:1	10
L2	2:1	10
L3	2.5:1	10
L4	3:1	10

Physicochemical Characterization of Ajmaline Liposomes

FTIR Spectroscopy

Fourier-transform infrared (FTIR) spectroscopy was performed to investigate possible drug–excipient interactions. Pure Ajmaline, phosphatidylcholine, cholesterol, physical mixtures, and lyophilized formulations were individually mixed with potassium bromide in a ratio of 1:100 and compressed into pellets using a hydraulic press under 5.5 metric tons pressure. The pellets were scanned over the range of 400–4000 cm^{-1} using an FTIR spectrophotometer.

Surface Morphology by FESEM

The surface morphology of the lyophilized liposomal formulations was evaluated using field emission scanning electron microscopy (FESEM). Freeze-dried samples were mounted on metal stubs, coated with platinum using an ion sputtering device, vacuum dried, and examined under FESEM.

Particle Size Analysis

The particle size distribution of reconstituted liposomes was determined using dynamic light scattering (DLS) technique with a Zetasizer Nano ZS 90 instrument. The lyophilized formulations were dispersed in PBS (pH 7.4) and analyzed for mean particle diameter and size distribution.

Polydispersity Index (PDI)

The polydispersity index of liposomal formulations was measured using the Zetasizer Nano ZS 90 instrument to evaluate homogeneity and distribution uniformity of vesicles.

Zeta Potential Measurement

Zeta potential analysis was carried out using the Zetasizer Nano ZS instrument to determine the surface charge and colloidal stability of liposomal vesicles.

Determination of Drug Loading and Loading Efficiency

Lyophilized liposomal formulations equivalent to 1 mg/ml were dispersed in PBS:methanol (4:6), sonicated, and centrifuged at 10,000 rpm for 15 minutes. The absorbance of the supernatant was measured at 210 nm using UV-visible spectrophotometry. Drug content was determined using the calibration curve of Ajmaline.

Drug loading and loading efficiency were calculated using the following equations:

$$\%\ Drug\ Loading = \frac{Weight\ of\ Drug\ in\ Formulation \times 100}{Total\ Weight\ of\ Formulation}$$

$$\%\ Loading\ Efficiency = \frac{Weight\ of\ Drug\ in\ Formulation \times 100}{Total\ Amount\ of\ Drug\ Added}$$

Entrapment Efficiency

Entrapment efficiency was determined by centrifugation method. Liposomal

dispersions were centrifuged at 15,000 rpm for 30 minutes at 4°C to separate unentrapped drug. The supernatant containing free drug was analyzed spectrophotometrically at 210 nm. The amount of entrapped drug was calculated by subtracting the amount of free drug from the total amount of drug used during formulation preparation.

The percentage entrapment efficiency was calculated using the following equation:

$$\%EE = \frac{Total\ Drug - Free\ Drug}{Total\ Drug} \times 100$$

In-vitro Drug Release Study

The in vitro drug release study was performed using the dialysis bag diffusion technique. Dialysis membranes were soaked overnight in PBS (pH 7.4). Liposomal formulation equivalent to 10 mg of Ajmaline was placed inside the dialysis bag and immersed in 100 ml PBS maintained at 37 ± 0.5°C with continuous stirring at 100 rpm.

At predetermined time intervals, 5 ml samples were withdrawn and replaced with equal volumes of fresh dissolution medium to maintain sink conditions. The samples were analyzed at 210 nm using UV-visible spectrophotometry, and cumulative percentage drug release was calculated using the standard calibration curve.

Results and Discussion

HPLC Assessment of Ajmaline

The purity and identity of Ajmaline were evaluated using High-Performance Liquid Chromatography (HPLC) according to the British Pharmacopoeia method. The chromatogram demonstrated a prominent and sharp peak at a retention time of 15.820 minutes, confirming the presence of Ajmaline as the major component in the sample.

The major peak accounted for 63.07% of the total chromatographic area, while the remaining minor peaks represented trace impurities or degradation products present within acceptable analytical limits. The sharpness and symmetry of the major peak indicated good purity and suitability of the drug for formulation development. The chromatographic profile confirmed that the obtained Ajmaline sample was chemically stable and appropriate for liposomal encapsulation studies.

Table 2. HPLC Retention Time of Ajmaline

Retention Time (min)	Area %	Compound
15.820	63.07	Ajmaline

Preparation of Calibration Curve of Ajmaline

Determination of Absorption Maxima (λmax)

The UV spectrophotometric scanning of Ajmaline in phosphate buffer saline (PBS, pH 7.4) and methanol mixture (4:6) revealed a maximum absorption peak at 210 nm. This wavelength was selected for all subsequent spectrophotometric analyses including drug loading, entrapment efficiency, and in vitro drug release studies.

Calibration Curve Analysis

The calibration curve of Ajmaline was prepared within the concentration range of 1–6 mg/ml. The absorbance values increased proportionally with increasing drug concentration, demonstrating compliance with Beer-Lambert's law.

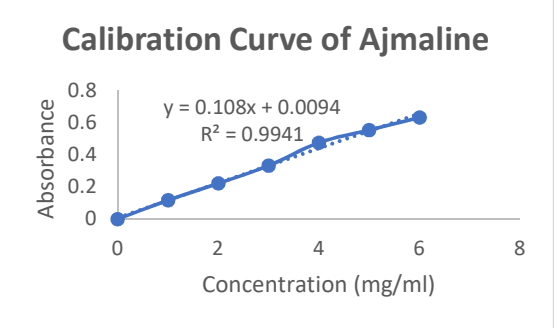

Figure 1. Calibration curve of Ajmaline

The obtained calibration plot showed excellent linearity between concentration and absorbance, indicating the reliability and accuracy of the analytical method for quantification of Ajmaline in liposomal formulations. The developed calibration model was therefore considered suitable for further formulation evaluation studies.

FTIR Study of Drug–Excipient Interaction

Fourier Transform Infrared (FTIR) spectroscopy was performed to investigate the compatibility of Ajmaline with formulation excipients such as phosphatidylcholine, cholesterol, and BHT. The FTIR spectrum of pure Ajmaline exhibited characteristic peaks at 2937 cm^{-1}, 2867 cm^{-1}, and 1714 cm^{-1} corresponding to stretching vibrations of functional groups present in the drug molecule. However, these peaks were absent or significantly reduced in the FTIR spectrum of the liposomal formulation. Additionally, a characteristic peak at 1737 cm^{-1} was observed, indicating the presence of lecithin in the liposomal membrane.

The disappearance of major drug peaks in the formulation spectrum suggested successful encapsulation of Ajmaline within the lipid bilayer rather than simple surface adsorption. Furthermore, no appearance of additional peaks or significant peak shifting was observed, indicating absence of chemical incompatibility between the drug and excipients. Thus, FTIR analysis confirmed successful liposome formation and compatibility of formulation components.

Particle Size Distribution Study

Particle size analysis was carried out using dynamic light scattering (DLS) to evaluate the average vesicle diameter of the prepared liposomal formulations. The mean particle size of the formulations ranged between 96.46 nm and 153.5 nm.

Table 3. Particle Size of Liposomal Formulations

Formulation	Particle Size (nm)
L1	105.5
L2	124.0
L3	153.5
L4	96.46

All formulations were found within the nanometer range, confirming successful preparation of nanoliposomes. Nanosized vesicles are advantageous because they enhance surface area, improve cellular uptake, and facilitate sustained drug delivery. Smaller vesicles also contribute to enhanced bioavailability and improved pharmacokinetic performance. Among the formulations, L4 exhibited the smallest particle size, which may facilitate better penetration and improved therapeutic performance.

Polydispersity Index (PDI)

The polydispersity index (PDI) was evaluated to determine the homogeneity and uniformity of vesicle size distribution.

Table 4. Polydispersity Index of Liposomal Formulations

Formulation	PDI
L1	0.364
L2	0.384
L3	0.201
L4	0.276

The obtained PDI values indicated narrow particle size distribution for all formulations. Generally, PDI values below 0.3 represent uniform vesicle distribution and good formulation homogeneity. Formulation L3 exhibited the lowest PDI value (0.201), indicating the narrowest distribution range and highest uniformity among all formulations. Such homogeneity is essential for formulation stability and reproducible drug delivery behavior.

Zeta Potential Study

Zeta potential measurements were performed to evaluate the colloidal stability of liposomal dispersions.

Table 5. Zeta Potential of Liposomal Formulations

Formulation	Zeta Potential (mV)
L1	−29.6
L2	−28.3
L3	−34.8

L4	−31.5

All formulations exhibited negative zeta potential values, indicating electrostatic repulsion between vesicles and reduced aggregation tendency. Formulations having zeta potential values greater than ±30 mV are generally considered physically stable. Among all formulations, L3 exhibited the highest negative zeta potential (−34.8 mV), indicating superior colloidal stability and reduced probability of particle aggregation during storage.

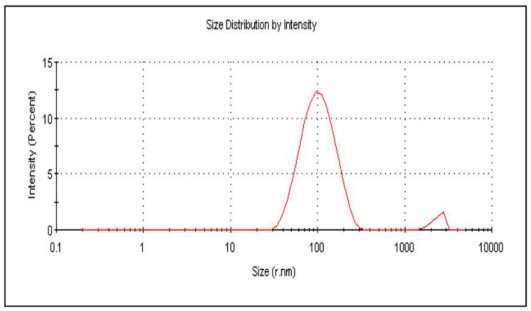

Figure 2. Particle size distribution pattern of lyophilized formulation F3

Drug Loading and Loading Efficiency

Drug loading studies were performed to evaluate the amount of Ajmaline successfully incorporated within the liposomal vesicles.

Table 6. Drug Loading Efficiency of Liposomal Formulations

Formulation	PC:CH Ratio	Drug Loading Efficiency (%)
F1	1:1	8.122
F2	2:1	9.57
F3	2.5:1	9.69
F4	3:1	9.43

The results demonstrated that increasing phosphatidylcholine concentration improved drug loading up to an optimum ratio. Formulation F3 exhibited the highest loading efficiency (9.69%), suggesting optimum lipid bilayer formation and efficient accommodation of Ajmaline within the vesicular system.

Surface Morphology by FESEM

Field Emission Scanning Electron Microscopy (FESEM) was performed to study the morphology and surface characteristics of the lyophilized liposomes. The micrographs revealed that the liposomal vesicles were predominantly spherical with smooth surface morphology. Slight aggregation was observed, which may be attributed to the lyophilization process. The observed morphology confirmed successful formation of liposomal vesicles with intact bilayer structure. The FESEM results were in agreement with particle size and PDI studies, further supporting the nanoscale nature of the optimized formulation. Based on particle size, PDI, zeta potential, and drug loading efficiency, formulation L3/F3 was selected as the optimized liposomal formulation.

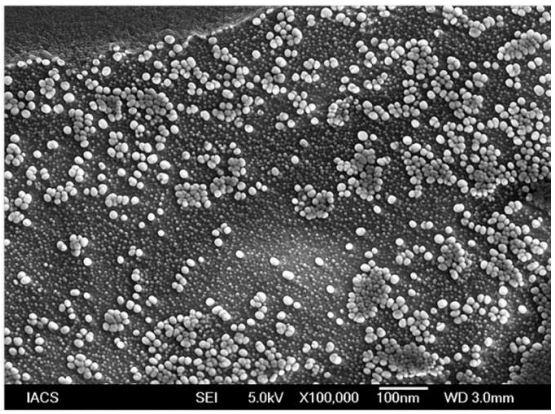

Figure 3. Scanning electron micrograph of freshly prepared lyophilized liposomes L3

Entrapment Efficiency Study

Entrapment efficiency studies were performed to determine the ability of liposomes to retain Ajmaline within the phospholipid bilayer system.

Table 7. Entrapment Efficiency of Liposomal Formulations

Formulation	Entrapped Drug (mg)	Entrapment Efficiency (%)
L1	8.12	81.22

L2	9.57	95.70
L3	9.69	96.90
L4	9.43	94.30

The entrapment efficiency increased with increasing phospholipid concentration up to formulation L3. The highest entrapment efficiency was observed for L3 (96.90%), indicating optimum vesicle formation and efficient drug incorporation. The enhanced entrapment may be attributed to increased lipid bilayer domains available for Ajmaline accommodation. A slight reduction in L4 may be due to excessive lipid concentration causing vesicle instability or leakage. These findings confirmed successful encapsulation of Ajmaline within the liposomal system.

In-vitro Drug Release Study

The in vitro drug release profile of optimized formulation L3 was evaluated using the dialysis bag diffusion method in PBS (pH 7.4).

Table 8. In-vitro Drug Release Profile of Optimized Formulation L3

Time (h)	Cumulative Drug Release (%)
1	18.4
2	29.7
4	45.3
6	58.6
8	69.8
10	78.2
12	85.6
24	96.1

The optimized formulation exhibited a biphasic drug release pattern consisting of an initial burst release followed by sustained release over 24 hours. The initial burst effect may be attributed to drug molecules adsorbed on the liposomal surface, whereas the sustained phase resulted from gradual diffusion of entrapped Ajmaline through the phospholipid bilayer.

Approximately 96.1% cumulative drug release was observed after 24 hours, indicating efficient controlled-release behavior. Sustained drug release from liposomal vesicles may help maintain therapeutic plasma concentrations for prolonged durations while reducing dosing frequency and minimizing adverse effects associated with conventional formulations. These results demonstrate the potential of liposomal encapsulation for improving the therapeutic performance of Ajmaline.

Conclusion

The present investigation successfully developed and characterized Ajmaline-loaded liposomal formulations using the thin-film hydration technique. The prepared liposomes exhibited nanosized vesicles with satisfactory physicochemical properties, including suitable particle size distribution, narrow polydispersity index, high zeta potential, and excellent drug entrapment efficiency. FTIR studies confirmed compatibility between Ajmaline and formulation excipients, while FESEM analysis demonstrated spherical and uniform vesicle morphology. Among all formulations, L3 was identified as the optimized formulation due to its superior stability, highest entrapment efficiency (96.90%), and desirable sustained-release characteristics. The in vitro drug release study revealed prolonged and controlled release of Ajmaline over 24 hours, indicating the potential of liposomes to improve therapeutic efficacy and reduce dosing frequency. Overall, the study demonstrated that liposomal encapsulation is an effective strategy for enhancing the stability, bioavailability, and controlled delivery of Ajmaline, thereby offering promising potential for improved antiarrhythmic therapy.

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