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DEVELOPMENT OF ALOE VERA EXTRACT LOADED LIPOSOMES FOR SKIN HYDRATION AND WOUND HEALING

Payal Jain*[1], M. Sindhuja [2], B. Venu Gopal [2], S. Rohini[2], Shaik Majid[2]

[1]Associate Professor, Department of Pharmacognosy, St. Mary's College of pharmacy, Hyderabad, Telangana.

[2]Students, Department of Pharmacognocy, St. Mary's College of pharmacy, Hyderabad, Telangana.

Abstract:

The present study aimed to formulate and evaluate Aloe vera extract-loaded liposomes to enhance the therapeutic efficacy and controlled delivery of Aloe vera for topical applications. FTIR analysis confirmed the compatibility of Aloe vera extract with the selected lipids and excipients, as no significant changes in characteristic peaks were observed. The prepared liposomal formulations were evaluated for particle size, morphology, entrapment efficiency, and drug release behavior. SEM analysis revealed spherical vesicles with uniform morphology. Among the formulations, F2 exhibited the smallest particle size of 179 nm, while formulation F3 showed the highest drug entrapment efficiency of 85.19%. In vitro drug release studies demonstrated sustained drug release over a period of 8 hours, with formulation F3 exhibiting the highest cumulative drug release of 98.51%. The optimized formulation (F3) was further subjected to stability studies under various storage conditions for three months. The formulation showed no significant changes in its physical characteristics or drug release profile, retaining more than 95% drug release throughout the study period, indicating good stability. The results suggest that Aloe vera extract-loaded liposomes are a promising vesicular drug delivery system capable of enhancing drug encapsulation, providing sustained release, and maintaining formulation stability. Therefore, the optimized liposomal formulation may serve as an effective carrier for the topical delivery of Aloe vera extract.

***Keywords**: Aloe vera, Liposomes, Vesicular Drug Delivery System, Entrapment Efficiency, In Vitro Drug Release, Stability Studies, Topical Delivery.*

Corresponding author:

Payal Jain,

Associate Professor, Department of Pharmacognosy, St. Mary's College of Pharmacy, Hyderabad, Telanagana.

Mobile:

Email Id:

QR CODE

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INTRODUCTION:

Liposomes are simple microscopic vesicles in which an aqueous volume is entirely enclosed by membrane composed of lipid molecules. Various amphipathic molecules have been used to form liposomes. The drug molecules can either be encapsulated in aqueous space or intercalated in to the lipid bilayers. The exact location of drug will depend upon its physico-chemical characteristics and the composition of the lipids.

Phospholipids are the major structural components of the biological membranes in the human body, where two types of phospholipids exist i.e. phosphodiglycerides and sphingolipids, together with their corresponding hydrolysis products. The way they work and form membranes are elegant and miraculous. Each phospholipid molecule has three major parts, one head and two tails.4 The head is made from three molecular components: choline, phosphate, and glycerol. The head is hydrophilic in other words, it is attracted to water. Each tail is a long, essential fatty acid chain. These fatty acids are hydrophobic that is, they are repelled by water. When phospholipids are put in an aqueous (water-based) solution, the hydrophilic heads of the phospholipids form a line side by side with their tails behind much like swimmers at a starting gate. Then because the tails are hydrophobic, another phospholipid layer will line itself up tail-to-tail in response to the same aqueous environment. This natural alignment creates two-rows of tightly fitted phospholipid molecules, called a phospholipid bilayer. It is these phospholipid bilayers that form the membranes around and within every cell in our bodies.

MATERIALS

Aloe vera extract was procured from Synpharma Research Labs, Hyd, Cholesterol, Lecithin, Phosphatidylcholine, Chloroform and Methanol were obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

METHODOLOGY:

FTIR Spectroscopic Analysis

For FTIR spectroscopy KBr powder was dried out at 60°C for one hour. The dried KBr powder was uniformly mixed with drug and IR spectra was taken for this mixture using ATR-FTIR 4000 (Jasco, Japan) IR Spectrophotometer.

Extraction process:

Fresh leaves of Aloe vera were collected and washed thoroughly with distilled water to remove dirt and other foreign materials. The base portion of the leaves was cut and the leaves were kept in a vertical position for about 15–20 minutes to allow the yellow latex to drain completely. This step was carried out to remove the anthraquinone-containing latex, which may cause irritation.The outer green rind of the leaves was carefully removed using a sterile knife, and the inner transparent mucilaginous gel was collected aseptically. The collected gel was homogenized using a blender to obtain a uniform gel mass.The homogenized gel was filtered through muslin cloth followed by Whatman filter paper to remove fibrous materials and impurities. The obtained filtrate was considered as fresh Aloe vera gel extract.The prepared extract was stored in an airtight container under refrigerated conditions until further use for formulation studies.

Method Preparation of liposomes

Aloe vera extract -loaded liposomes can be prepared by a modified thin-film hydration technique. First, the required lipids such as Phosphotidyl choline and cholesterol are accurately weighed and dissolved in a volatile organic solvent such as chloroform–methanol (2:1 v/v). The solvent mixture is evaporated under reduced pressure in a rotary evaporator at about 40 °C to form a uniform dry lipid film on the inner wall of the flask, and any traces of solvent are removed by flushing with nitrogen or argon. An aqueous phosphate buffer (pH ≈ 7.4) containing the desired concentration of aloe vera extract is then added to the dried lipid film, and the flask is gently rotated or vortexed so that the film hydrates and detaches, entrapping the Aloe vera extract solution within multilamellar vesicles. The size of the vesicles is reduced and a more uniform population obtained by probe sonication, high-pressure homogenisation or extrusion through polycarbonate membranes of decreasing pore size. Unencapsulated drug is removed by centrifugation or by passing the suspension through a size-exclusion (gel-filtration) column. Finally, the liposomal suspension is sterilised—preferably by aseptic processing or, if particle size permits, by filtration through a 0.22 µm sterile filter—and stored under refrigeration at 2–8 °C to maintain stability.

Table-1: Composition of lipids for preparation of liposomes

Ingredients	F1	F2	F3	F4
Phosphatidylcholine (mg)	100	150	200	250
Cholesterol(mg)	50	50	50	50
Chloroform (ml)	10	10	10	10
Methanol	5	5	5	5
Extract (mg)	100	100	100	100
Phosphate buffer pH 7.4(ml)	10	10	10	10

Evaluations of liposomes

Encapsulation efficiency determination

The encapsulation efficiency of Aloe vera extract liposomes is determined by first separating the free (unencapsulated) drug from the liposome-entrapped drug and then measuring the amount of each fraction. A known volume of the liposomal suspension is subjected to ultracentrifugation at about 100,000 RPM for roughly half an hour at 4 °C, or alternatively passed through a size-exclusion (gel filtration) column such as Sephadex G-50 or G-75. This step allows the liposomes, which elute in the void volume or form a pellet, to be separated from the free Aloe vera extract that remains in the supernatant or later fractions. The entrapment efficiency of the liposomes was estimated using the following equation.

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

First, an accurately measured aliquot of the liposomal suspension is diluted with filtered deionized water or isotonic buffer to an appropriate concentration so that the sample is optically clear enough for light-scattering measurements but still gives a strong scattering signal. The diluted dispersion is transferred to a clean, dust-free cuvette, taking care to avoid formation of air bubbles. The cuvette is then placed in the sample holder of the DLS instrument, which is typically maintained at a controlled temperature (for example 25 °C). A laser beam of fixed wavelength is directed through the suspension, and the instrument detects fluctuations in the intensity of scattered light arising from the Brownian motion of the liposomes.

SEM analysis

First, a small volume of the liposomal suspension is diluted with deionised water and a drop is placed on a clean, conductive specimen holder or on an aluminium SEM stub pre-covered with a thin layer of conductive adhesive such as carbon tape. The sample is allowed to air-dry at room temperature so that the vesicles form a dry film; sometimes a mild vacuum desiccator is used to ensure complete removal of surface moisture without collapsing the structure. To prevent charging of the non-conductive lipid material under the electron beam, the dried sample is then coated with a very thin (≈10 nm) conductive layer—commonly gold using a sputter coater. The prepared stub is mounted in the SEM chamber, the instrument is evacuated to the required vacuum and operated at an accelerating voltage typically between 5 and 20 kV. Images are recorded at different magnifications to examine the overall morphology, surface texture and approximate size of the liposomes. The micrographs obtained provide qualitative confirmation of the spherical shape and surface characteristics and can be complemented with particle size measurements from other techniques such as dynamic light scattering.

In Vitro Drug release study

In-vitro drug release of Aloe vera extract-loaded liposomes was evaluated by the dialysis bag diffusion method under sink conditions. A known quantity of liposomal suspension containing a measured amount of Aloe vera extract was placed in a pre-soaked dialysis membrane having a molecular weight cut-off of 10–12 kDa, which retained the liposomes while allowing the free drug to diffuse

through the membrane.The dialysis bag was sealed properly and immersed in phosphate-buffered saline (PBS, pH 7.4) maintained at 37 ± 0.5 °C with continuous stirring using a magnetic stirrer or shaking water bath to simulate physiological conditions.At predetermined time intervals, aliquots of the release medium were withdrawn and replaced with an equal volume of fresh PBS pH 7.4 to maintain sink conditions and constant volume throughout the study.The collected samples were analyzed for Aloe vera extract content using a UV–Visible spectrophotometer at 278 nm. The cumulative percentage drug release was calculated and plotted against time to obtain the drug release profile of the liposomal formulation.

Determination of pH :

The pH of the prepared Aloe vera extract-loaded liposomal formulation was determined using a calibrated digital pH meter. Before measurement, the pH meter was calibrated using standard buffer solutions of pH 4.0, 7.0, and 9.2.An adequate quantity of the liposomal suspension was taken in a clean beaker and the electrode of the pH meter was immersed completely into the formulation. The pH was measured at room temperature and the readings were recorded. All measurements were carried out in triplicate and the average value was calculated.The pH of the formulation was maintained within the acceptable range suitable for topical application to avoid skin irritation.

Stability studies:

The stability of optimized liposomes formulation was assessed for a period of 3 Months by storing the liposomes at 3 specified temperature conditions, i.e. 25±20 /60% RH (Room temperature; RT), 40±2°C/75% RH (Accelerated conditions) and 2-80 C (Refrigerator; control). The liposomal formulation was kept in sealed laminated aluminum tubes (10 ml capacity) which were previously flushed with nitrogen. Samples were periodically taken at specified time period of 0, 1, 2and 3months. These were tested for drug release, in the manner described.

RESULTS& DISSUSSION:

Drug-excipient compatibility studies (FT-IR)

The compatibility between the extract and the selected lipid and other excipients was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the extract-lipid mixture, which confirmed the absence of any chemical interaction between the Drug, lipid and other chemicals.

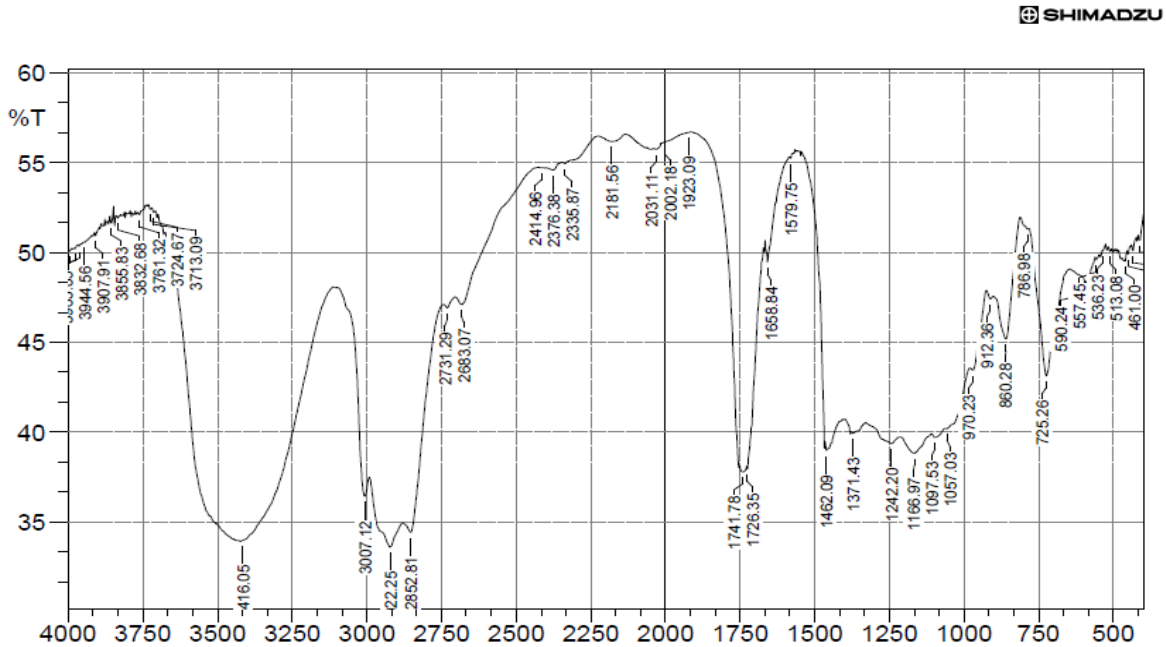

Fig-1: FTIR Studies of Aloe vera extract

Table-2: Characteristic Peaks for Aloe vera extract

S.No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	C=O stretching	1000-1500	1166.97
2	N–H stretching	3000-2500	2852.81
3	C–H stretching	2000-1500	1741.78

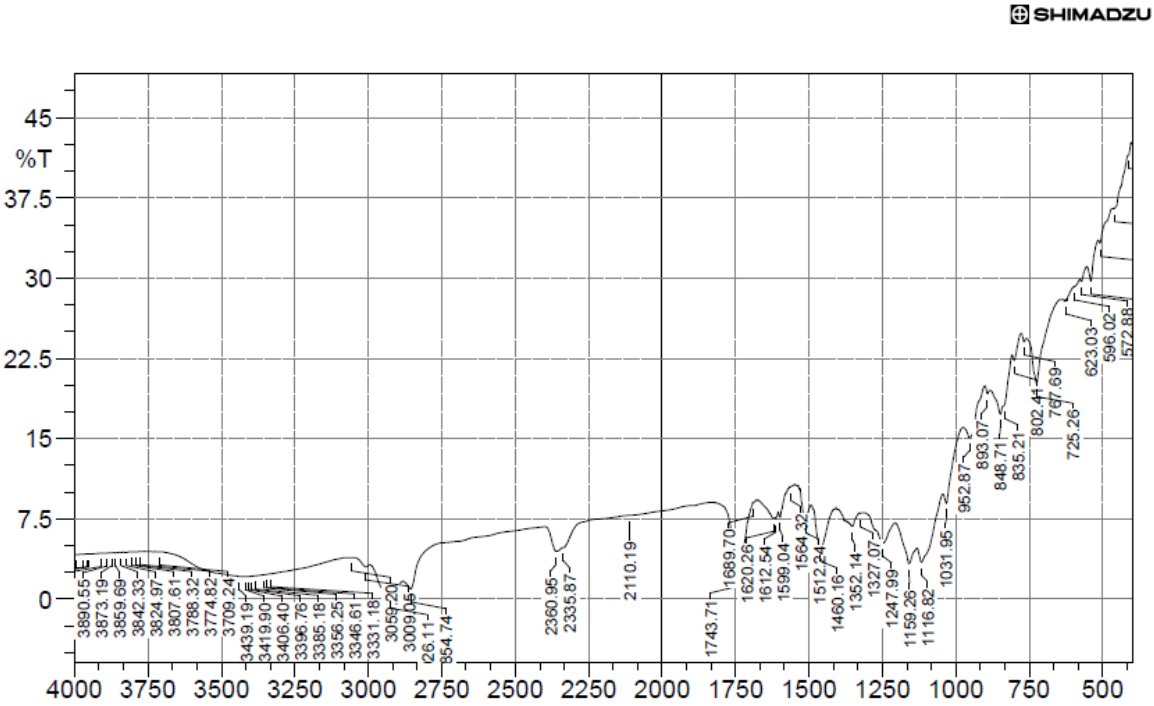

Fig-2: FTIR Studies of optimized formulation

Table-3: Characteristic Peaks for optimized formulation

S.No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	C=O stretching	4000-3500	3842.33
2	N–H stretching	2500-2000	2110.19
3	C–H stretching	2000-1500	1620.26

Particle size

Vesicle shape: Vesicle shape of the prepared formulation was found to be spherical from the SEM (scanning electron microscope) analysis at 15.00kV.

SEM Analysis

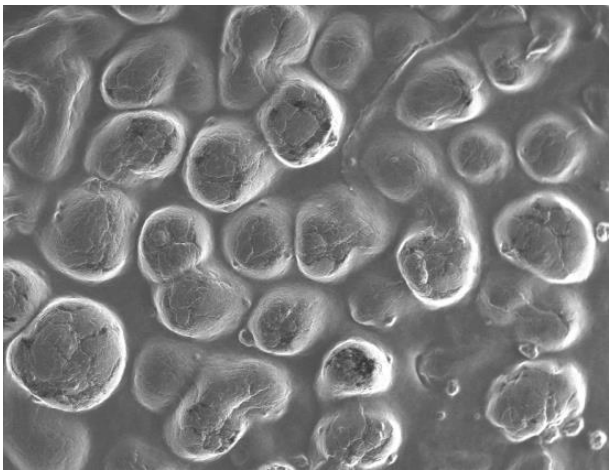

Fig-3: SEM Analysis of liposomes

Vesicle size:

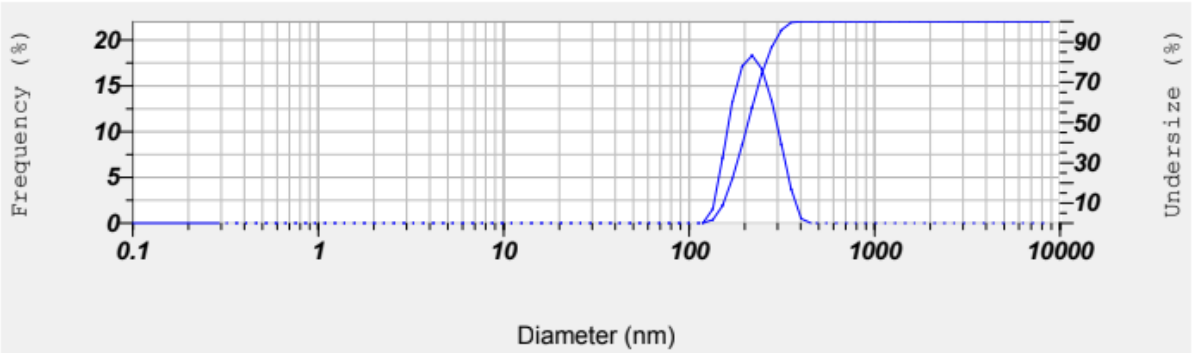

Fig-4: particle size liposomes

Table-4: Mean particle size of different formulation of liposomes

Formulation No.	Particle size
F1	258
F2	179
F3	243
F4	222

Drug entrapment efficiency

Table-5: Different batches of liposome made by using different ratio of lipids

Formulation no.	% DEE
F1	82.39
F2	82.10
F3	85.19
F4	78.93

Drug release studies

Table-6: Cumulative percentage drug release from various formulation of liposomes

Time	F1	F2	F3	F4
0	0	0	0	0
1	14.89	16.13	18.10	17.58
2	26.65	28.19	28.93	27.82
3	34.52	36.82	38.59	37.17
4	46.98	48.19	48.53	47.35
5	58.49	57.58	59.28	58.10
6	65.55	68.55	71.25	69.85
7	79.88	81.29	83.65	82.35
8	92.20	96.30	98.51	95.58

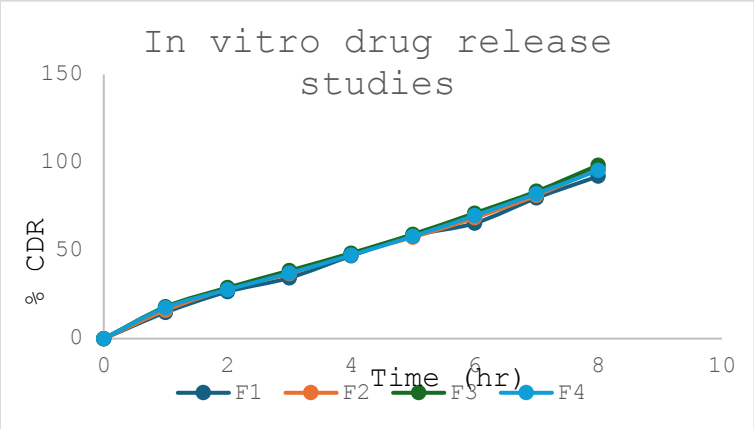

Fig-5: In vitro drug release of (F1-F4) formulations

Formulation F3 were found to release the drug in 8 h. The cumulative percentage release was found to be 98.51 %.

Stability studies

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

Table- 7: Results of stability studies of optimized formulation F-3

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-3	**25^{0}C/60%RH % Release**	98.51	97.82	96.37	95.80	**Not less than 85 %**
F-3	**30^{0}C/75% RH % Release**	98.51	97.43	96.22	95.32	**Not less than 85 %**
F-3	**40^{0}C/75% RH % Release**	98.51	97.25	96.15	95.20	**Not less than 85 %**

CONCLUSION:

The present study successfully developed and evaluated Aloe vera extract-loaded liposomes for enhanced drug delivery. FTIR studies confirmed the compatibility of the extract with the selected excipients without any significant interaction. Among all formulations, F3 was identified as the optimized formulation based on its high entrapment efficiency (85.19%) and maximum cumulative drug release (98.51%) with sustained release over 8 hours. SEM analysis showed spherical vesicles, indicating successful liposome formation. Stability studies demonstrated that the optimized formulation remained stable for three months with no significant changes in drug release, confirming its suitability as a stable and effective liposomal drug delivery system.

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