Physicochemical Characterization of Aceclofenac-Loaded Liposomes by Multimodal Analysis: Elucidating Structure, Stability and Drug-Lipid Interactions

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Abbreviations: ACE: Aceclofenac; ACN: Acetonitrile; AUC: Area under the curve; Chol: Cholesterol; $CDCl_3$: Deuterated chloroform; DLS: Dynamic light scattering; DMSO: Dimethyl sulfoxide; DMSO-d_6 (DMSO-d6): Deuterated dimethyl sulfoxide; DSC: Differential scanning calorimetry; FESEM: Field-emission scanning electron microscopy; FT-IR (FTIR): Fourier-transform infrared spectroscopy; He-Ne: Helium-neon (laser); HPLC: High-performance liquid chromatography; HR-TEM (HRTEM): High-resolution transmission electron microscopy; KBr: Potassium bromide; LOD: Limit of detection; LOQ: Limit of quantitation; MWCO: Molecular-weight cut-off; NMR: Nuclear magnetic resonance; PBS: Phosphate-buffered saline; PDI: Polydispersity index; PL (PL90G): Phospholipid (Phospholipon 90G); q.s. Quantity sufficient; RSD: Relative standard deviation; RT: Retention time; UV: Ultraviolet and XRD: X-ray diffraction.

***Abstract*—Liposomal drug delivery systems have emerged as a promising nanocarrier system to enhance treatment efficacy, stability and bioavailability of pharmaceutical agents. Liposomes reduce systemic toxicity through their unique bilayered vesicular structure, which enables them to encapsulate both hydrophilic and hydrophobic drugs, allowing for controlled and targeted release of the drugs. The precise formulation and detailed analytical characterization of these vesicular systems are crucial for understanding their performance and functionality. This study aims to evaluate the liposomal system through various advanced instrumentation tools systematically. Aceclofenac is a non-steroidal anti-inflammatory medication and it is extensively used for the treatment of pain and inflammation. In this research work, Liposomes were formulated using the thin film hydration technique, which is a widely accepted method for generating uniform and size-controlled vesicles. The key analytical parameters including particle size, polydispersity index and zeta potential were determined using Dynamic Light Scattering. Surface morphology, vesicle structure, and lamellarity were examined through High-Resolution Transmission Electron Microscopy (HRTEM), Field Emission Scanning Electron Microscopy and Optical Microscopy. Similarly, Molecular interactions and confirmation of drug encapsulation were analyzed using Fourier Transform Infrared Spectroscopy and Nuclear Magnetic Resonance. Furthermore, Thermal characteristics were evaluated using Differential Scanning Calorimetry, while crystallinity was assessed by X-ray Diffraction. Encapsulation efficiency was quantified through ultracentrifugation followed by UV-Visible spectrophotometry and High-Performance Liquid Chromatography. Additionally, *in-vitro* drug release studies were conducted to monitor release kinetics using validated analytical protocols. This study will primarily contribute to the instrumentation driven analytical assessment of liposomal drug delivery systems by applying advanced characterization techniques to evaluate nanocarriers.**

***Index Terms*—Aceclofenac, Controlled release, Drug-lipid interactions, DSC, Encapsulation efficiency, FESEM, FTIR, *In-vitro* release kinetics, Liposomal drug delivery, Nanocarriers, NMR, Physicochemical characterization, Thin film hydration, TEM and XRD.**

I. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) such as aceclofenac are widely prescribed for the

management of pain and inflammation. Despite their therapeutic efficacy, the conventional formulations of aceclofenac are often associated with poor aqueous solubility, limited bioavailability and gastrointestinal side effects. These limitations necessitate the development of advanced drug delivery systems that can enhance therapeutic performance while minimizing systemic toxicity. However, Liposomal drug delivery systems have emerged as a promising nanocarrier platform due to their unique bilayered vesicular architecture which enables the encapsulation of both hydrophilic and hydrophobic drugs (1). Liposomes offer controlled and targeted drug release, improved pharmacokinetics and reduced adverse effects which makes them ideal candidates for reformulating NSAIDs. However, the success of liposomal formulations depends heavily on precise fabrication techniques and robust physicochemical characterization to ensure stability, uniformity and effective drug-lipid interactions. In this research, aceclofenac-loaded liposomes were prepared using the thin-film hydration method which is a well-established technique for producing size-controlled vesicles with high encapsulation efficiency. To comprehensively evaluate the formulation, a multimodal analytical approach was employed. Particle size, polydispersity index (PDI) and zeta potential were measured using dynamic light scattering (DLS). At the same time, vesicle morphology and lamellarity were assessed via high-resolution transmission electron microscopy (HRTEM), field emission scanning electron microscopy (FESEM) and optical microscopy. Drug encapsulation and molecular interactions were confirmed using Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR). Thermal behavior and crystallinity were analyzed through differential scanning calorimetry (DSC) and X-ray diffraction (XRD), respectively. Encapsulation efficiency was quantified using ultracentrifugation followed by UV-visible spectrophotometry and high-performance liquid chromatography (HPLC). *In-vitro* drug release studies were conducted to assess release kinetics under physiologically relevant conditions.

This study presents a comprehensive physicochemical characterization of aceclofenac-loaded liposomes, offering insights into their structural integrity, stability and drug-lipid interactions. The findings aim to contribute to the instrumentation driven evaluation of liposomal drug delivery systems and support their potential application in enhancing NSAID therapy.

2. MATERIALS

Aceclofenac (ACE; M/s Ipca Laboratories Pvt. Ltd., Mumbai, India) was obtained ex-gratis by the respective company. Cholesterol (Chol; M/s Loba Chemie, Mumbai, India) and phosphatidylcholine (Phospholipon 90G; M/s Lipoid GmbH, Nattermannallee, Germany) were procured from their respective suppliers. Chloroform, methanol, ethanol, acetonitrile and other solvents used in formulation and analytical work were of HPLC or analytical grade and sourced from Merck (Germany). Buffer salts (potassium dihydrogen phosphate, sodium dihydrogen phosphate, sodium chloride) and other chemicals were of analytical grade and used without further purification. Dialysis membranes with appropriate molecular-weight cut-off for *in vitro* release studies were obtained from HiMedia Laboratories (Mumbai, India). Ultrapure water from a Milli-Q® Integral system (M/s Merck Millipore, Billerica, USA) was used throughout the study.

3. METHODS

3.1. Analytical method development and validation

An ultraviolet (UV) spectrophotometric method and a high-performance liquid chromatography (HPLC) method were established to quantify aceclofenac (ACE). For UV analysis, 10 µg mL^{-1} ACE solutions were prepared in various solvents and scanned from 200–400 nm to identify the absorption maximum. A consistent λ_{max} of 276–278 nm was observed across solvents and this wavelength was used for subsequent quantitation. The HPLC method employed a Waters Alliance e2695 system fitted with an Inertsil ODS-3 C18 column (250 × 4.6 mm, 5 µm). The mobile phase consisted of acetonitrile:methanol: 0.035 M phosphate buffer (pH 7.2) in a ratio of 30:3:67 (v/v), delivered at 1.5 mL min^{-1}. Detection was performed at 276 nm. Standard solutions (10–100 µg mL^{-1}) were injected (10 µL) to construct a calibration curve (2).

3.2. Solubility studies

To identify suitable solvents for formulation and release studies, solubility of aceclofenac was evaluated in various organic solvents (methanol, ethanol, acetonitrile, chloroform, DMSO and isopropanol) and phosphate buffers of pH 6.0–7.4. Excess drug was added to 5 mL of each solvent and the suspensions were shaken at 37±1°C for 24 h. After equilibration, samples were centrifuged, filtered through 0.22 µm membranes and analysed by HPLC at 276 nm to determine the dissolved drug concentration. Each measurement was performed in triplicate (3).

3.3. Selection of *in vitro* release medium

Because ACE exhibits low aqueous solubility in aqueous solutions, mixed solvent systems were evaluated to maintain sink conditions during in vitro release studies. A series of phosphate buffered saline (PBS, pH 7.4) and ethanol mixtures containing 10, 20, 30 and 40 % ethanol (v/v) was prepared. The solubility of ACE in each mixture was determined using the method described above. Taking into account solvent effects on solubility and pH, suitable PBS/ethanol mixture (v/v) was selected as the release medium. This medium was used throughout the release experiments (4).

3.4. Optimization of Formulation Components

A 3 × 3 factorial design was applied to study the effects of formulation variables. Liposomal formulations were prepared using a fixed drug load of ACE, while the lipid composition and solvent volume were systematically varied. Phospholipid and cholesterol were combined at w/w ratios of 70:30, 80:20 and 90:10, representing increasing phospholipid content relative to total lipid mass. Each lipid ratio was evaluated across three levels of organic solvent volume (10, 15, and 20 mL) using a chloroform: methanol mixture (2:1 v/v) as the drug-lipid dissolving phase. This resulted in a total of nine formulations, enabling assessment of the combined effects of lipid ratio and solvent volume on liposome formation and stability. Phosphate-buffered saline (PBS, pH 7.4) was used as the hydration medium for all formulations. Nine formulations (F1–F9) were prepared according to this design. Each batch was evaluated for physical stability, mean vesicle size, polydispersity index (PDI), vesicle count, in vitro drug release and entrapment efficiency. Table 4.6 summarizes the different combinations evaluated (5).

3.5. Preparation of Ace-Loaded Liposomes

Thin film hydration method was used to prepare the ACE–loaded liposomes (**Figure 3.1).** Accurately weighed amounts of phospholipid, cholesterol and ACE were dissolved in chloroform: methanol (2:1) in a round bottom flask. The solvent was evaporated at 60 ± 2 °C under reduced pressure using rotary evaporator at 120 rpm. When the solvents were completely evaporated, a thin lipid film was formed which was then hydrated at 35 ± 2 °C with PBS (pH 7.4) to obtain a homogenous suspension of ACE-loaded liposomes. Then, the suspension was kept for 24 h at room temperature for complete hydration process (6).

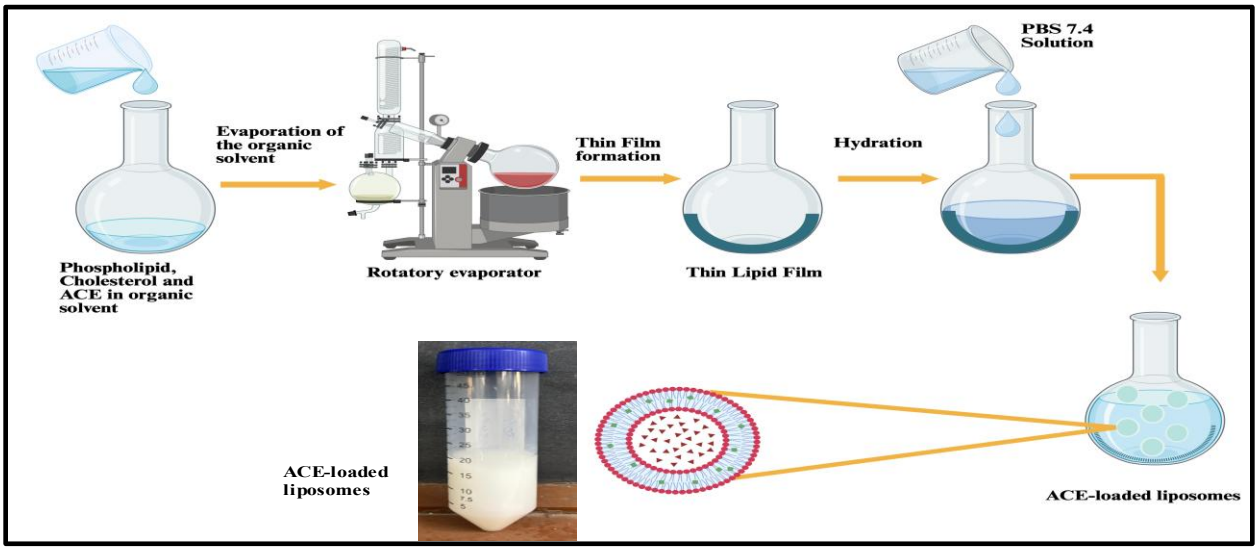

Figure 3.1: Thin Film Hydration method for liposome preparation.

3.6. Physicochemical and structural characterisation.

3.6.1. Physical stability

After preparation, each liposomal formulation was stored and visually examined for signs of instability. Formulations were inspected periodically for aggregation (flocculation), sedimentation or phase separation. Only samples showing no visible aggregation or separation were considered physically stable and were taken forward for further characterization (7).

3.6.2. In vitro drug release

The release profile of aceclofenac from liposomes was assessed using a dialysis diffusion method. A weighed quantity of liposomal dispersion (0.5 g) was sealed in a dialysis membrane bag and suspended in 50 mL of release medium (PBS pH 7.4/ethanol 30 % v/v) maintained at 37 ± 1 °C with constant stirring. At predetermined intervals, aliquots of the external medium were collected and replaced by fresh medium to maintain sink conditions. Drug content in each sample was quantified by the validated HPLC method at 276 nm (8).

3.6.3. Drug entrapment efficiency (EE)

Entrapment efficiency was determined by ultracentrifugation method. A known amount of liposomal suspension was centrifuged at 40 000 rpm for 1 h at 4 °C to pellet the liposomes. The supernatant containing free drug was collected and analysed by HPLC (9). The pelleted liposomes were dissolved in a chloroform: methanol (2:1 v/v) mixture, made up to volume with methanol and the entrapped drug content was quantified by HPLC. Entrapment efficiency (%) was calculated using formula (Eqn. 3.1).

$$\%\, Entrapment = \frac{Entrapped\ Drug\ (mg)}{Total\ Drug\ Added\ (mg)} \times 100 \quad \ldots(3.1)$$

3.6.4. Vesicle count

Vesicle concentration was estimated using an improved Neubauer hemocytometer. The liposomal suspension was diluted 10 times with 0.9 % saline and loaded into the hemocytometer grid (10). Vesicles in multiple squares were counted under the microscope and the number of vesicles per millilitre was calculated using the standard hemocytometer formula as per Eqn 3.2.

$$No.of\ vesicles\ per\ cubic\ mm = \frac{No.of\ vesicles\ in\ small\ squares \times Dilution\ factor \times 4000}{Total\ no.of\ small\ squared\ counted} \ldots. (3.2)$$

3.6.5. Particle size and zeta potential

A small aliquot of the liposomal suspension was diluted with filtered deionized water to avoid multiple scattering. Measurements were made at 25 °C on a Malvern Zetasizer Nano ZS90 equipped with a 4 mW He–Ne laser (λ = 633 nm) and a backscatter angle of 173°. Zeta potential was determined using disposable folded capillary cells, and each measurement was performed in triplicate (11).

3.6.6. Field-emission scanning electron microscopy (FESEM)

Diluted liposome samples were deposited onto aluminium stubs and dried in a vacuum desiccator. The dried films were sputter-coated with about 5 nm of gold before being examined using a Hitachi SU8010 FE-SEM operating at 5–10 kV (12).

3.6.7. High-resolution transmission electron microscopy (HR-TEM)

A drop of diluted liposomal suspension was placed on a carbon-coated copper grid, allowed to air-dry without staining, and examined on a JEOL JEM-2100 transmission electron microscope at 200 kV (13).

3.6.8. Differential scanning calorimetry (DSC)

Samples (2–5 mg) of pure ACE, PL, Chol and ACE-Loaded liposomal formulation were sealed in aluminium pans with an empty pan as reference. Thermal scans were recorded from 30 °C to 350 °C at 10 °C min^{-1} under a nitrogen purge (50 mL min^{-1}) using a Setaram Setline DSC (14).

3.6.9. Fourier-transform infrared (FT-IR) spectroscopy

Each sample was finely ground with spectroscopic-grade KBr and pressed into a pellet. Spectra were acquired on a PerkinElmer Spectrum Two over 4000–400 cm^{-1} at 4 cm^{-1} resolution (typically 32 scans) (15).

3.6.10. X-ray diffraction (XRD)

Powdered samples of ACE and ACE-Loaded liposome were analysed on a Malvern Panalytical Empyrean diffractometer having a Cu Kα source (λ = 1.5406 Å)

operating at 40 kV and 30 mA. Patterns were collected over 2θ range of 5–60° with a step size of 0.02° and a scan speed of 2° min^{-1} (16).

3.6.11. Nuclear Magnetic Resonance (NMR)

Samples of ACE, PL and ACE-Loaded liposomal were dissolved in appropriate deuterated solvents (DMSO-d_6, $CDCl_3$ and D_2O). ^1H NMR spectra were collected at 298 K on a Bruker Avance Neo 500 MHz spectrometer and processed with TopSpin software (17).

3.6.12. UV–visible spectroscopy

For λ_{max} determination pure Aceclofenac was carried out in different solvents (e.g., methanol, ethanol acetonitrile and aqueous solutions) using a Lambda 35 UV-Visible Spectrophotometer (PerkinElmer Analytical Sciences, Singapore). Measurements were performed using quartz cuvettes of 10 mm path length over the wavelength range of 200–400 nm. The obtained λ_{max} values were used for analytical method development and further HPLC quantification studies (18).

4. RESULTS AND DISCUSSIONS

4.1. Analytical method development and validation

4.1.1. UV Spectrophotometric Method

The UV scan of ACE (200–400 nm) in different solvent systems showed well-defined absorption maxima with excellent Beer–Lambert linearity. The observed λ_{max} with calibration r^2 were shown in Table 4.1. This data confirm solvent dependent spectral positions around 276–277 nm with high linearity suitable for quantitative analysis in the tested media.

Table 4.1: λ_{max} values (and calibration r^2) of ACE in different solvent systems.

Solvent system	λ_{max} (nm)	r^2
Non-aqueous solvents		
ACN	276.17	0.9951
Methanol	277.65	0.9999
Ethanol	276.44	0.9981
Aqueous solvents		
ACN: methanol: Potassium Dihydrogen orthophosphate buffer (0.035M) of pH 7.2 in the ratio of (30:3:67)	276.27	0.9901
Phosphate Buffer of pH 7.4: Ethanol (70:30)	277.60	0.9913

4.1.2. HPLC Method Validation and Estimation of ACE

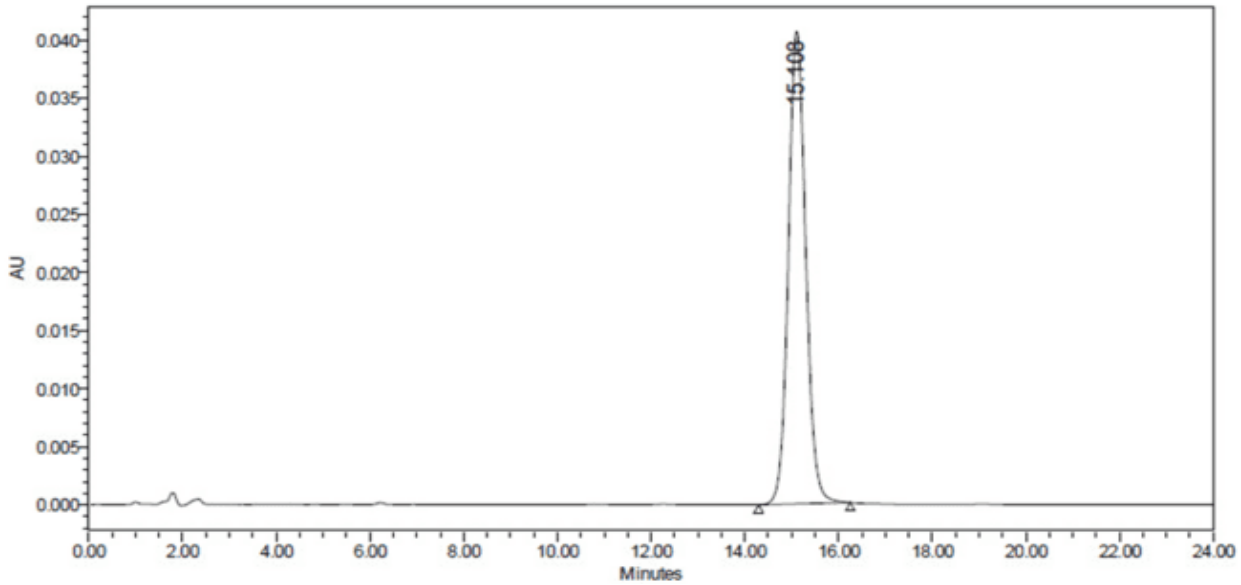

Figure 4.1: HPLC chromatogram of ACE.

Linearity: The retention time of ACE was 15.1 ± 0.09 min. Linear regression analysis was used to evaluate the peak area observed at different drug concentrations (Kapil et al., 2009). The respective AUC values at different concentrations of the drug are presented in Table 4.2.

$$y = 10109\,x + 33403 \qquad \text{Eq… (4.1)}$$

Table 4.2. Peak areas at different ACE concentrations used for the calibration curve.

Conc. (µg/mL)	Mean peak area ± SD	RT	Calculated conc. (µg/mL)	% RSD	R^2
10	120071 ± 573	15.1 ± 0.07	9.49	0.48	0.9982
15	183245 ± 627	15.1 ± 0.05	15.71	0.34	
20	238291 ± 530	15.1 ± 0.02	21.12	0.22	
25	291314 ± 690	15.1 ± 0.03	26.34	0.24	
30	341928 ± 1223	15.1 ± 0.03	31.32	0.36	
50	560951 ± 1552	15.1 ± 0.06	52.86	0.28	
70	722183 ± 1827	15.1 ± 0.05	68.72	0.25	
90	937451 ± 1580	15.1 ± 0.09	89.90	0.17	
100	1050029 ± 1795	15.1 ± 0.08	100.97	0.17	

The linearity of ACE was observed in concentration range of 10-100 µg/mL having coefficient of correlation of R^2= 0.9982 (Figure 4.2). This high value of correlation coefficient validates the linearity of calibration graphs and adherence of this system to Beer's law. The values of relative standard deviation were ≤0.48% at the studied drug concentration levels, which were quite low and indicate high precision of measurements (Causon, 1997).

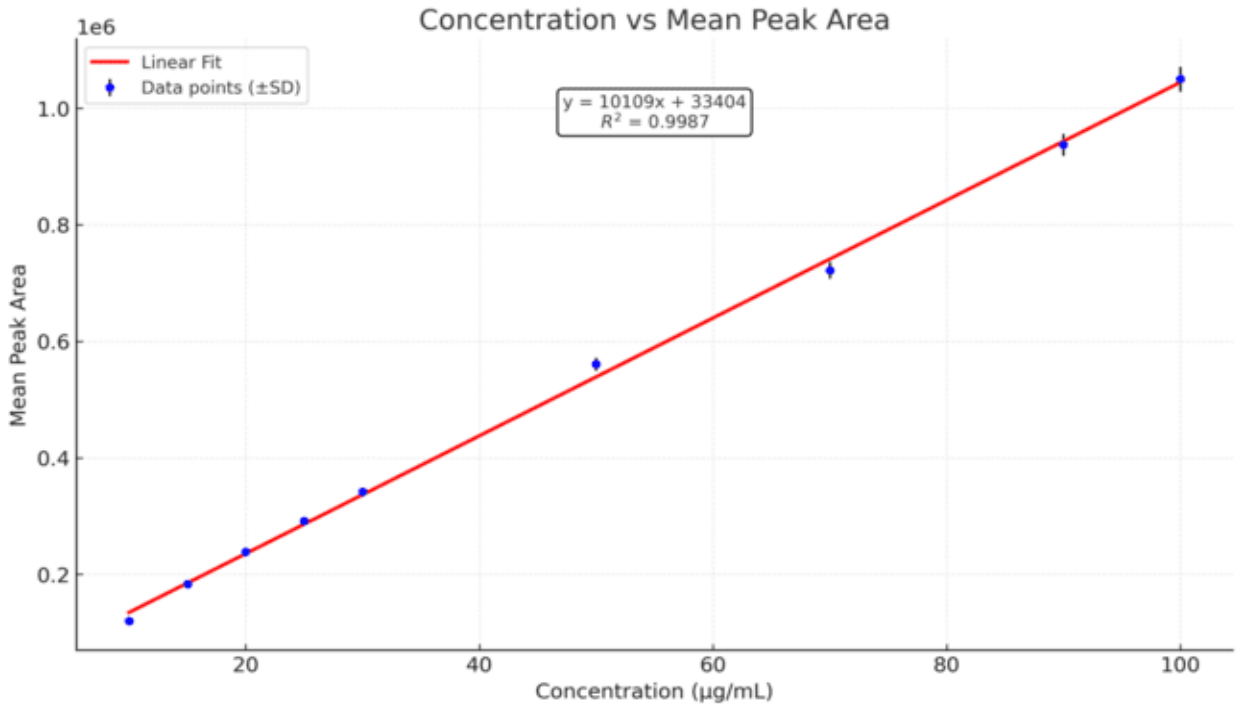

Figure 4.2: Calibration plot of ACE.

Accuracy: In the present study, calculated concentration was compared with the standard concentration to determine the accuracy. Mean accuracy for the studied drug concentrations (10, 30, 50 µg/mL) was found in the range of 98.03 % to 100.05 % and the corresponding % RSD values ranging between 0.25 and 1.41 which shows high degree of

accuracy. The values were well within the permissible limits. Table 4.3 shows the accuracy data of validation of the analytical procedure.

Table 4.3: Accuracy data for validation of ACE.

Standard Conc. (µg/mL)	Peak Area	Calculated Conc. (µg/mL)	% Accuracy	Mean Accuracy ± SD	% RSD
10	126901	9.73	97.33	98.03 ± 1.38	1.41
	127054	9.71	97.15		
	129541	9.96	99.62		
30	331256	30.04	100.15	99.84 ± 0.28	0.28
	329589	29.88	99.6		
	330112	29.93	99.77		
50	531619	49.99	99.98	100.05 ±0.24	0.25
	530898	49.92	99.84		
	533312	50.16	100.32		

Precision: The results depict the repeatability of analytical method as the corresponding % RSD values ranges between 0.08 and 0.76 for intra-day 0.08 and 1.01 for inter-day studies, respectively. The values were well within the permissible limits (Elsayed, 2006; *ICH Official Web Site : ICH*, n.d.). Table 4.4 shows intra-day and inter-day precision studies.

Sensitivity (LOD/LOQ): The LOD and LOQ were 0.03 µg/mL and 0.09 µg/mL respectively, determined using S/N criteria of approximately 3:1 (LOD) and 10:1 (LOQ). These low values support adequate sensitivity for trace-level detection and routine quantification within the studied range (FDA, 2014)(ICH, 1996).

Table 4.4: Validation for inter- and intra-day precision.

Conc. (µg/mL)	Mean Peak Area	Calculated conc. (µg/mL)	Mean ± SD	% RSD
Inter day				
10	127120	9.86	9.96 ± 0.10	1.01
	128202	9.96		
	129204	10.06		
30	335107	30.11	30.08 ± 0.08	0.26
	335457	30.15		
	333818	29.99		
50	539104	49.98	49.96 ± 0.04	0.08
	538450	49.91		
	539210	49.99		
Intra day				
10	126110	9.82	9.91 ± 0.07	0.76
	127342	9.94		
	127599	9.96		
30	335017	30.10	30.18 ± 0.08	0.27
	336652	30.26		
	335998	30.19		
50	539204	49.92	49.91 ± 0.04	0.08
	538650	49.86		
	539450	49.94		

4.2. Solubility in Solvents and Buffers

ACE solubility (shake-flask) was highest in methanol**,** followed by ethanol and DMSO **(**Figure 4.3**)** supporting the use of methanol as the primary organic solvent for formulation and ethanol as an auxiliary solvent for analyses. In aqueous buffers **(**Figure 4.4**),** ACE solubility increased with pH (6.0→7.4) viz consistent with ionization of the weak acid (pKa4.7).

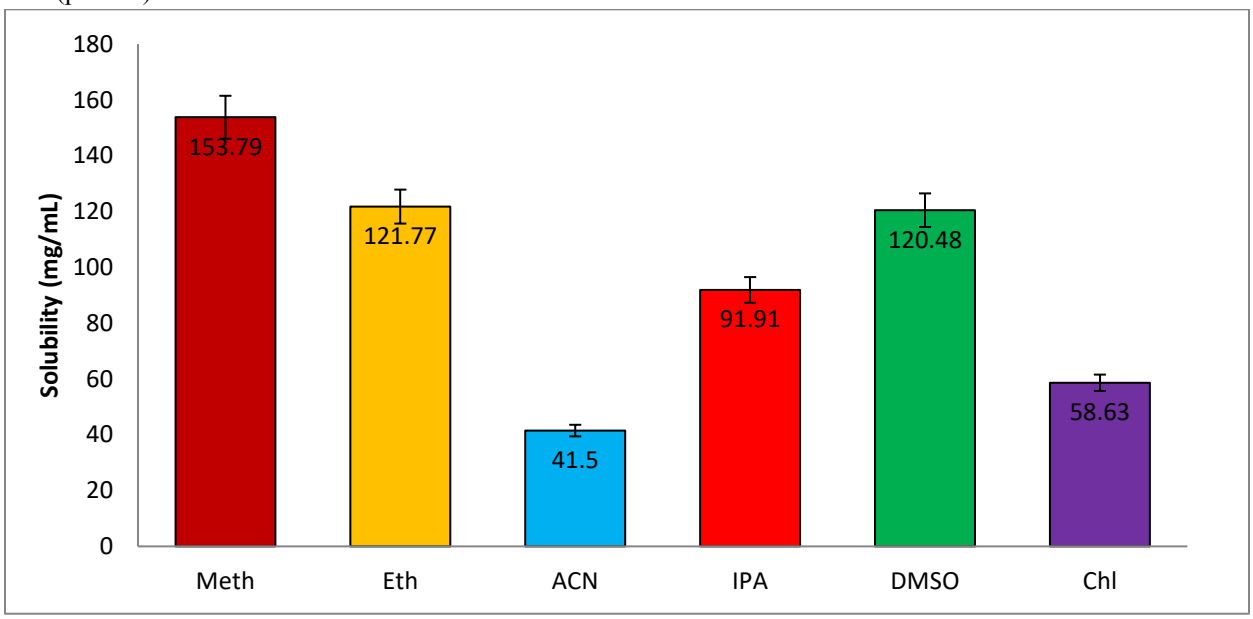

Figure 4.3: Solubility of ACE in organic solvents (mean ± SD).

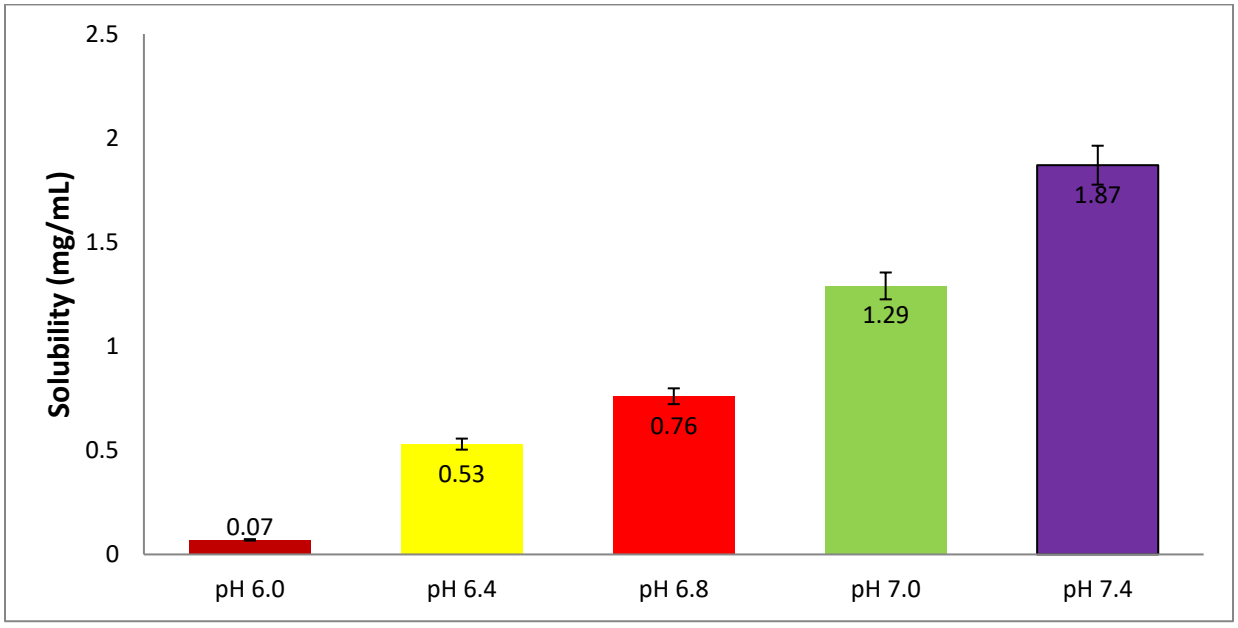

Figure 4.4: Solubility of ACE in phosphate buffers (pH 6.0–7.4; mean ± SD).

4.3. Selection of *in vitro* release medium

To ensure sink conditions for in-vitro release, PBS pH 7.4 and ethanol mixtures were evaluated. Solubility (g/100 mL) increased with ethanol content (Table 4.5) although 60:40 provided higher solubility, 70:30 (PBS:ethanol, pH 7.4) was selected as the release medium to maintain physiological pH control while still achieving sink conditions.

Table 4.5: Solubility study for selection of media for In vitro release studies.

pH	PBS (pH 7.4): Ethanol	Solubility (g/100mL)
7.4	60:40	1.86 ± 0.05
	70:30	1.43 ± 0.07
	80:20	0.89 ± 0.03
	90.10	0.65 ± 0.05

4.4. Formulation Optimization

Different liposomal formulations (F1–F9) were prepared by varying phospholipid, cholesterol and solvent volume in accordance with the three-factor factorial design. The composition of each batch and the measured vesicle size, polydispersity index (PDI) and entrapment efficiency are summarized in **Table 4.6.** Among all batches, formulation F5 demonstrated the most favourable balance of small vesicle size, narrow distribution and high drug entrapment efficiency, thereby serving as the optimized formulation for further characterization.

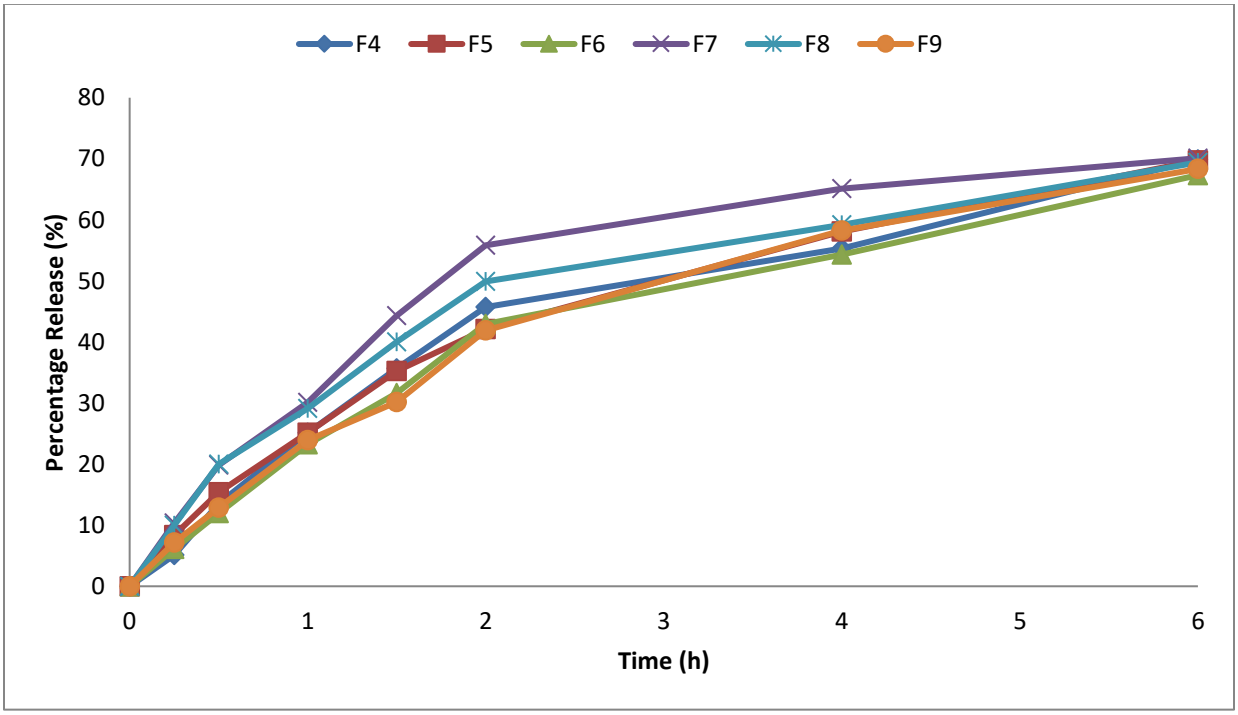

Figure 4.6: *In vitro* release profile of different liposomal formulations.

Table 4.6: Selection criteria for liposomes with varying concentrations of formulation components.

Formulation Code	Phospholipid (w/w)	Cholesterol (w/w)	Organic Solvent (mL) (Chloroform: Methanol, 2:1)	Hydration media (%)	Physical Stability		Vesicle Size (nm)	PDI	No. of Vesicles (x10^4/mm3)	*In vitro* drug Release (%)	Entrapment Efficiency (%)
					Aggregation	Sedimentation					
F1	70	30	10	q.s.	YES	YES	-	-	-	-	-
F2	80	20			YES	NO	-	-	-	-	-
F3	90	10			YES	NO	-	-	-	-	-
F4	70	30	15		NO	NO	240.3	0.367	3.2	69.8	77.2

F5	80	20			NO	NO	243.8	0.311	3.9	69.7	79.3
F6	90	10			NO	NO	250.1	0.391	3.5	67.3	79.5
F7	70	30	20		NO	NO	460.7	0.315	2.3	70.1	65.9
F8	80	20			NO	NO	562.3	0.394	2.7	69.4	68.2
F9	90	10			NO	NO	578.1	0.413	2.4	68.3	71.1

Hydration media was selected as PBS (pH 7.4)
q.s.: Quantity Sufficient

4.5. Physicochemical and structural characterisation.

4.5.1 Physical stability: Liposomes prepared with low organic solvent volumes showed visible aggregation across all phospholipid and cholesterol levels whereas intermediate and higher solvent volumes yielded physically stable and uniformly dispersed suspensions.

4.5.2 Drug release behaviour: Cumulative release decreased with increasing phospholipid content which shows that greater bilayer loading lowers the freely diffusible fraction. Drug release also increased with higher organic solvent volume viz. consistent with a higher proportion of surface associated or free drug. Profiles were biphasic with an initial burst followed by sustained release.

4.5.3 Entrapment efficiency (EE%): EE rose with phospholipid concentration which explains the expanded hydrophobic domain for ACE partitioning and declined at higher solvent volumes which is due to thinner films and larger vesicles increasing the unencapsulated fraction.

4.5.4 Vesicle count: Counts peaked at intermediate phospholipid levels. Low phospholipid limited vesicle formation, while high phospholipid shifted the population toward larger vesicles with reduced number density.

Based on these above discussed factors (Table 4.6), formulation F5 was found suitable and carried forward for further characterization

4.5.5 Hydrodynamic Size and Surface Charge Characteristics (DLS and Zeta Potential)

DLS showed a Z-average of 243.8 nm (PDI 0.311) average diameter of 243.8 nm (PDI 0.311), with approx. 260 nm vesicles accounting for 91.9% of the intensity. Zeta potential was near-neutral (−3.59 ± 7.55 mV, unimodal), indicating steric rather than electrostatic stabilization. Collectively, these metrics evidence a homogeneous, colloidal and stable liposomal dispersion suitable for controlled ACE release.

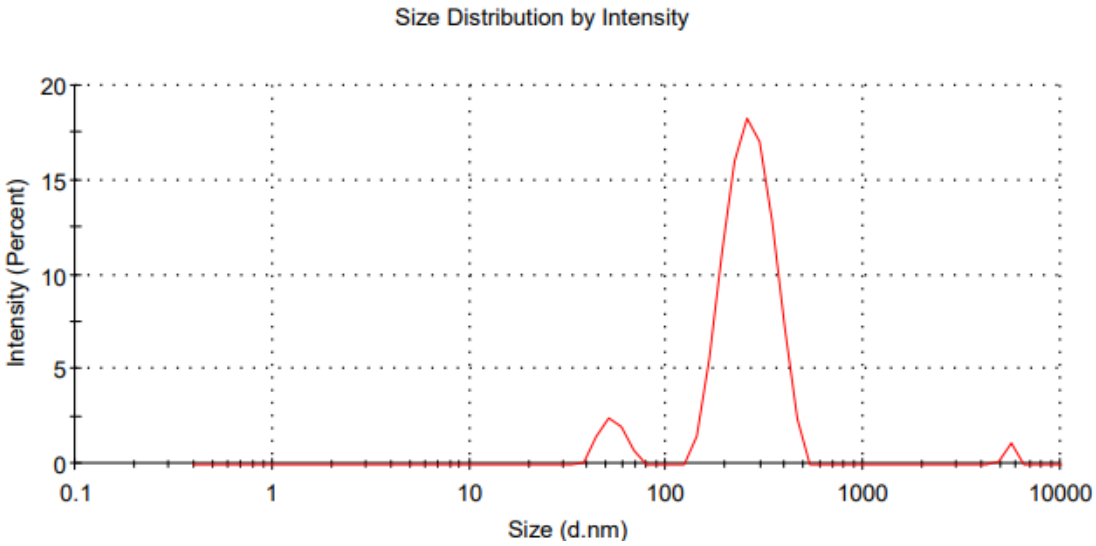

Figure 4.7: Particle size distribution and PDI of ACE loaded Liposomes.

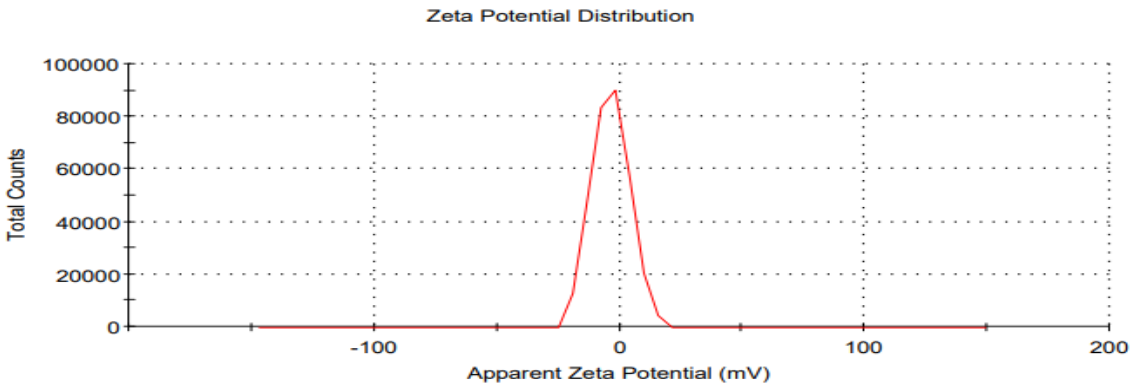

Figure 4.8: Zeta potential of ACE loaded Liposomes.

4.5.6 Morphological Validation of Liposomal Structure (FESEM, HR-TEM)

Electron microscopy established nanoscale vesicular architecture and complete drug incorporation. FESEM distinguished irregular 1–5 µm crystalline ACE from the smooth, spherical 250–300 nm liposomes without adherent drug crystals, indicating encapsulation within bilayers. HR-TEM revealed uniform, well-defined vesicles (250–300 nm) with continuous membranes and clear core–shell contrast, with no electron-dense ACE crystallites inside or outside vesicles—confirming intact bilayers and molecular-level drug association with the lipid phase.

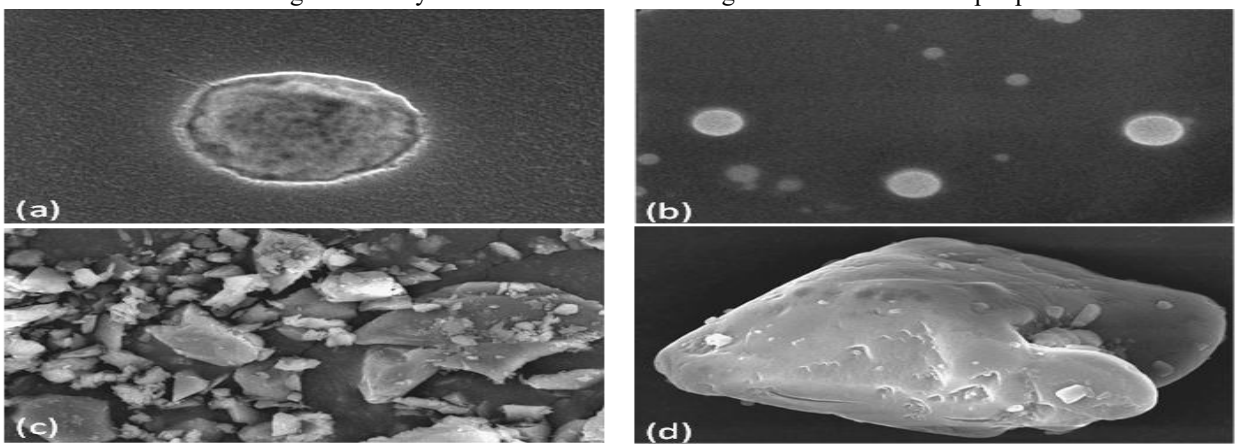

Figure 4.9: FESEM photomicrographs of (a) ACE-loaded spherical liposome at 60,000X, (b) distribution of liposomes at 18000X, (c) Pure Aceclofenac drug at 4000X and (d) irregular shaped drug particle at 12000X.

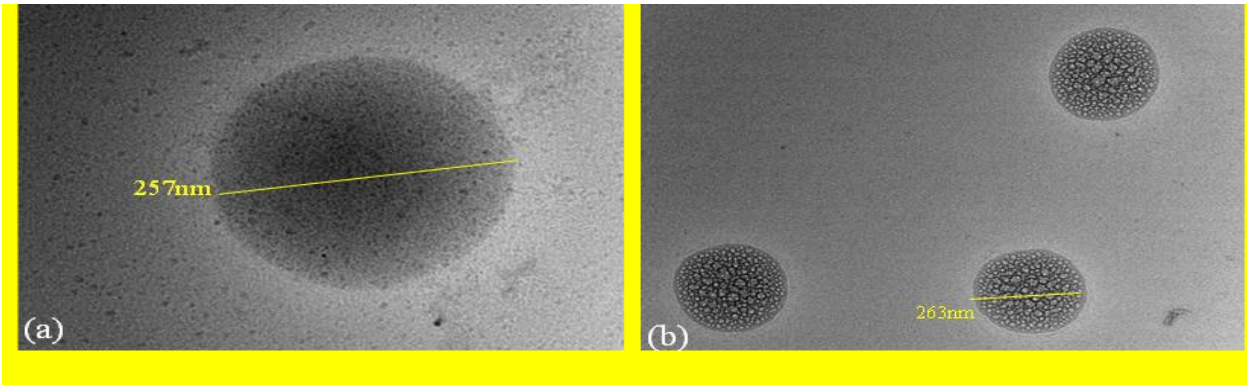

Figure 4.10: HR-TEM images of ACE-loaded liposomes of (a) well-defined spherical vesicle with smooth bilayer morphology and (b) uniform size distribution and structural integrity of the liposomal formulation

4.5.7 Thermal, Structural and Spectroscopic Confirmation of Encapsulation(DSC, XRD, FT-IR, ^{1}H NMR)

Thermal and diffraction signatures demonstrated loss of drug crystallinity upon encapsulation. DSC endotherms of the components (ACE 153.5 °C, cholesterol 148.3 °C, PL 168.6 °C) contrasted with the formulation's broader events (149.5 °C, 161 °C) and absence of ACE's sharp melting peak, consistent with molecular dispersion in the bilayer. XRD likewise showed disappearance of ACE's crystalline peaks (2θ at 14.4°, 18.44°, 19.38°, 22.22°, 25.91°) and emergence of a broad 16–22° halo, characteristic of an amorphous lipid matrix. FT-IR retained lipid-specific bands (ester C=O, phosphate) with minor shifts and partial masking of ACE peaks which indicated physical entrapment without new covalent interactions. ^{1}H-NMR preserved lipid resonances (e.g., choline headgroup shift 3.362→3.133 ppm) while ACE aromatic multiplets shifted (7.58–6.74→7.40–6.91 ppm) and the -COOH proton (13.54–12.46 ppm) was not observed that is consistent with proton exchange at the lipid-water interface and restricted drug mobility within the bilayer microenvironment.

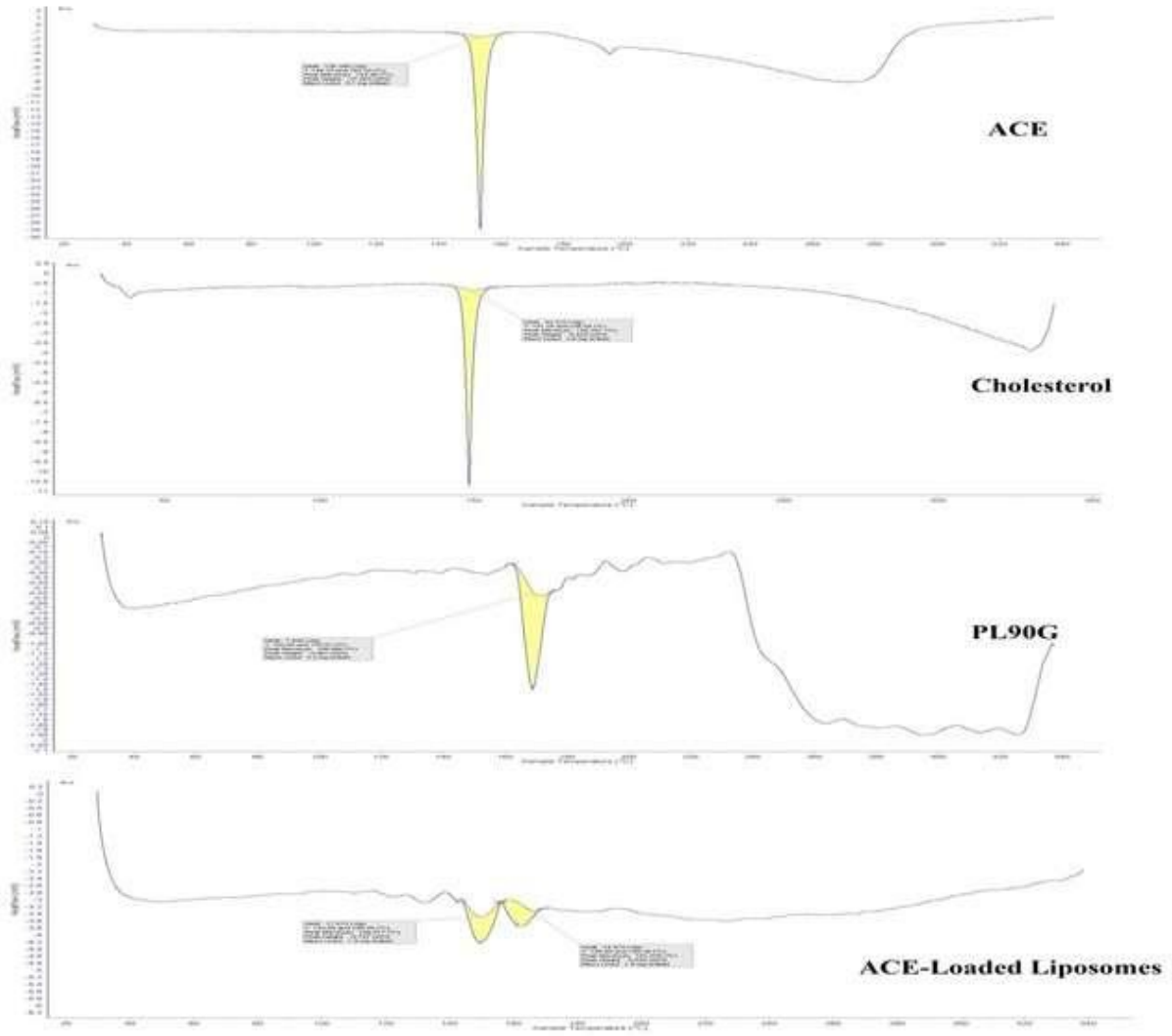

Figure 4.11: DSC thermogram of ACE, Cholesterol, phospholipid (PL90G) and liposomes.

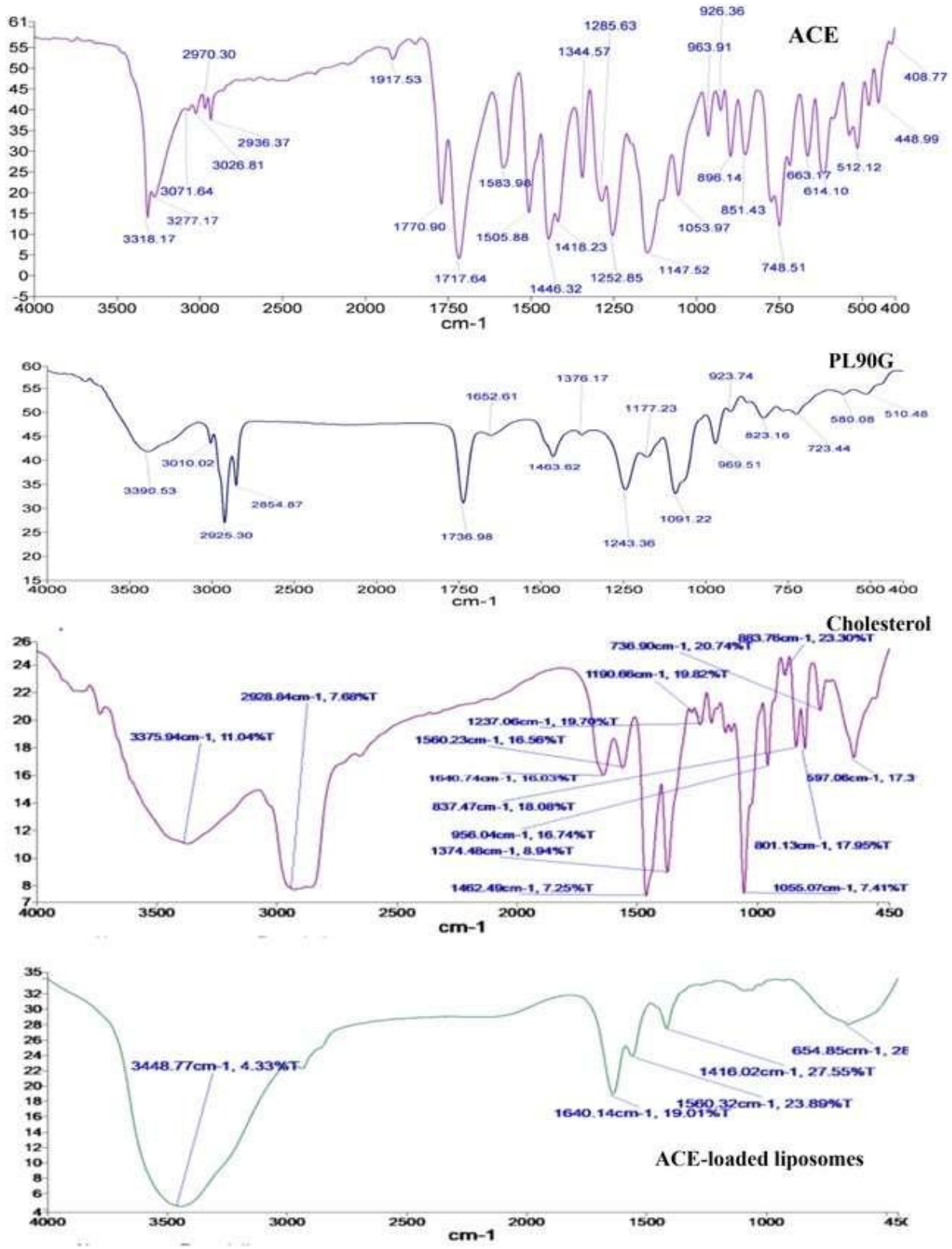

Figure 4.12: FTIR spectra of ACE, phospholipid, Cholesterol and ACE-loaded liposomes.

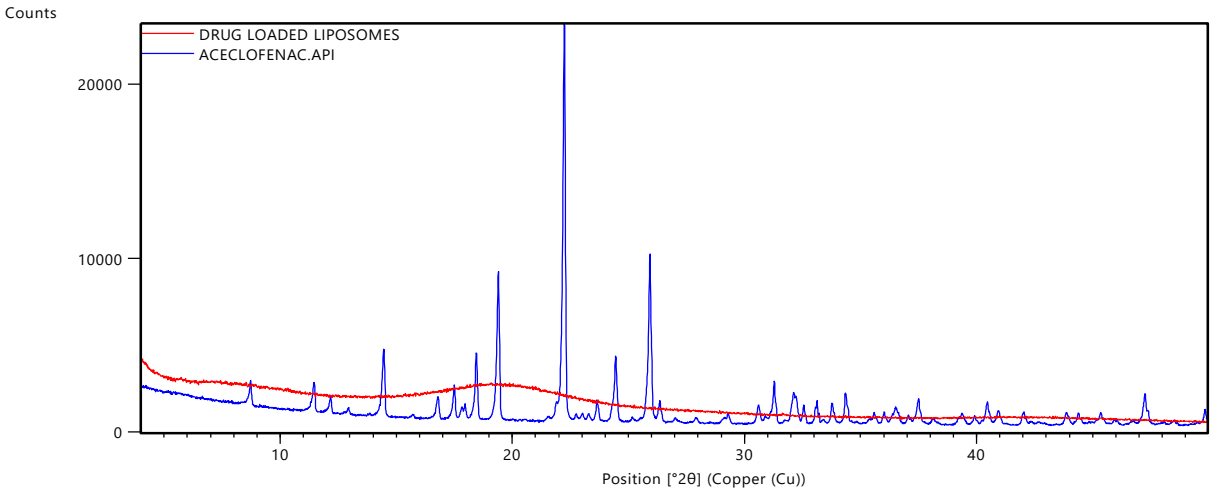

Figure 4.13: Overlay of X-ray diffraction patterns for pure aceclofenac and aceclofenac-loaded liposomes.

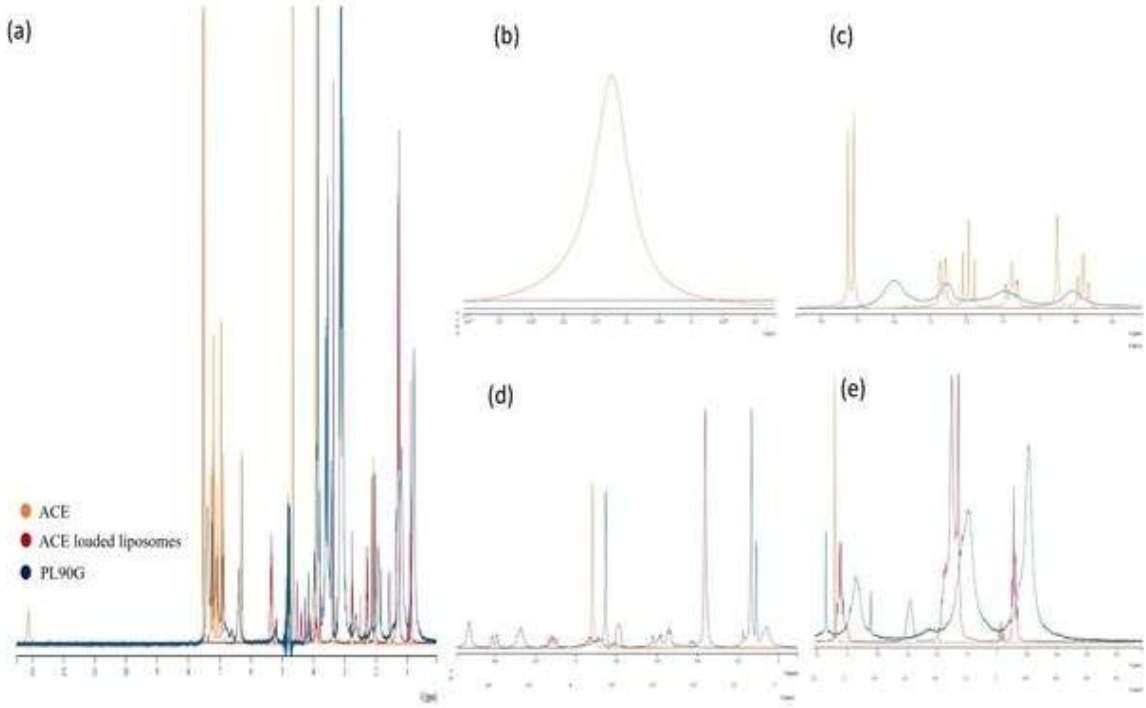

Figure 4.14: Overlayed ^{1}H NMR spectra of pure Aceclofenac (ACE), PL90G and ACE-loaded liposomes (a) full spectra, (b) carboxylic proton region, (c) aromatic region, (d) choline and glycerol resonances and (e) methoxy and aliphatic chain region.

5. CONCLUSION

The results of this study collectively demonstrate the successful development, optimization and characterization of ACE-loaded liposomes with potential application as a controlled drug delivery system. Analytical method validation established a robust, accurate and precise HPLC procedure for quantitative estimation of ACE that ensures reliable assessment of formulation parameters. Solubility profiling confirmed the suitability of methanol and ethanol as effective solvents. It highlighted the influence of pH on drug solubility, providing a rational basis for excipient selection and release medium

optimization. Moreover, optimization studies revealed that a Phospholipid: cholesterol ratio of 80:20 with 15 mL of organic solvent produced liposomes of optimal size, stability, entrapment efficiency and release kinetics.

Comprehensive physicochemical characterization such as DLS, zeta potential, microscopy (optical, FESEM, HR-TEM), DSC, FTIR, XRD and NMR confirmed the formation of nanosized, spherical, multilamellar vesicles with high encapsulation efficiency, structural integrity and absence of crystalline drug deposits. The biphasic release pattern observed *in vitro,* characterized by an initial burst followed by sustained release that underscored the suitability of the system for prolonged therapeutic action. Thermal and spectroscopic analyses further verified the molecular dispersion of ACE within the lipid bilayer without evidence of deleterious drug-excipient interactions.

In conclusion, the study emphasizes that instrumental approaches are an integral part of pharmaceutical research, as each method, whether chromatographic, spectroscopic, thermal, or microscopic, delivers distinct and complimentary insights into the formulation. The combined use of these analytical tools not only confirmed successful drug encapsulation and stability but also demonstrated how instrumental characterization is indispensable across all branches of science for detecting, validating and understanding material behavior at both molecular and structural levels.

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