BILOSOMES AS NANOVESICULAR CARRIERS FOR PRAMIPEXOLE: DESIGN, OPTIMIZATION, AND IN-VITRO CHARACTERIZATION

1Mansi Upreti, 2Meenakshi Kandwal, 3Shivanand Patil

Research Scholar, Shree Dev Bhoomi Institute of education science and technology, Dehradun

Associate Professor, Shree Dev Bhoomi Institute of education science and technology, Dehradun

Professor, Shree Dev Bhoomi Institute of education science and technology, Dehradun

Abstract

Bilosomes represent a groundbreaking advancement in vesicular drug delivery systems, specifically engineered to overcome the limitations of conventional oral delivery for sensitive therapeutics. These bile salt-stabilized nanovesicles exhibit superior gastrointestinal stability, enhanced permeability, and the ability to encapsulate both hydrophilic and lipophilic drugs. This study aimed to develop and characterize pramipexole-loaded bilosomes for improved Parkinson's disease management. Preformulation studies, including solubility analysis, FTIR spectroscopy, and DSC, confirmed the drug's compatibility with excipients. Six bilosome formulations (F1–F6) were prepared via thin-film hydration, varying cholesterol (10–40 mg) and sodium deoxycholate (10–40 mg) concentrations. Formulation F4, optimized with 40 mg cholesterol and 10 mg bile salt, demonstrated exceptional entrapment efficiency (98.87 ± 0.12%), nanoscale particle size (146.34 ± 2.1 nm), and sustained drug release (99.15 ± 0.12% over 24 h) following zero-order kinetics (R^2 = 0.982). Accelerated stability studies (40°C ± 2°C, 75% RH ± 5%) revealed no significant degradation over six months. These findings underscore bilosomes' potential as a robust platform for oral delivery of pramipexole, addressing its bioavailability challenges while ensuring therapeutic efficacy.

Keywords: **Bilosomes, Pramipexole, Parkinson's Disease, Nanocarriers, Oral Bioavailability, Sustained Release**

1. INTRODUCTION

1.1. Neurological Disorders and Drug Delivery Challenges

Neurological disorders, including Parkinson's disease (PD), Alzheimer's disease, and epilepsy, pose significant therapeutic challenges due to the blood-brain barrier (BBB) and poor drug bioavailability. Pramipexole, a non-ergot dopamine agonist, is a first-line PD treatment but suffers from low oral bioavailability (~90%) and rapid metabolism. Conventional formulations often fail to maintain therapeutic plasma concentrations, necessitating frequent dosing and increasing side effects.

1.2. Bilosomes: A Paradigm Shift in Drug Delivery

Bilosomes, a hybrid of liposomes and bile salts, offer a transformative solution. Their unique composition—non-ionic surfactants (e.g., Span 60), cholesterol, and bile salts (e.g., sodium deoxycholate)—confers:

- **GI Stability:** Resistance to enzymatic degradation and bile salt disruption.
- **Enhanced Permeability:** Bile salts modulate tight junctions, promoting intestinal absorption.
- **Versatility:** Suitable for peptides, proteins, and small molecules like pramipexole.

This study systematically evaluates bilosomes for pramipexole delivery, addressing formulation, characterization, and stability.

2. MATERIALS AND METHOD

Bilosomes are typically composed of lipids**,** bile salts, and sometimes stabilizers or surfactants**.** These components from stable vesicular structures suitable for oral dosage form. The chemicals and solvents were purchased from authenticated suppliers and all of them were analytical and laboratory grades

2.1 Materials used for bilosome Preparation

Table No.1: List of Material used in preparation of bilosomes

Material	Function	Examples	Common Suppliers
Phospholipids	Main structural component of bilosome vesicles	- Soya lecithin - Egg	Sigma-Aldrich
Bile Salts	Provide bile resistance and mucosal penetration	taurocholate - Sodium glycocholate	- Sigma-Aldrich
Cholesterol	Enhances vesicle stability and membrane rigidity	- Cholesterol (USP grade)	Avanti Polar Lipids
Surfactants *(optional)*	Stabilize bilosome dispersion	- Tween 80	- SD Fine-Chem
Buffer Components	Maintain pH during formulation	- Phosphate-buffered saline (PBS) - Tris buffer	- SRL
Solvents	Used in film hydration or solvent evaporation techniques	- Chloroform - Methanol - Ethanol	- SD Fine-Chem
Active Pharmaceutical Ingredient (API)	Drug or antigen to be encapsulated	Varies depending on the application	Custom or pharma-grade suppliers

2.2.Preformulation Studies for Pramipexole-Loaded Bilosomes”

2.2.1.Organoleptic Properties

The **organoleptic features** of pramipexole were examined through **visual inspection**. Observations of **stigmasterol's sensory characteristics**, such as **color**, **smell**, **physical form**, and other general **appearance attributes**, were also recorded.

2.2.2 Solubility Assessment

The **qualitative solubility** of pramipexole in various **solvents** was determined in accordance with **USP NF, 2007 guidelines**. Approximately **1 mg** of the drug was placed in a **10 mL test tube** and dissolved in **1 mL each** of different solvents, including **water**, **methanol**, **ethanol**, **chloroform**, and **phosphate buffer** (pH **6.8–7.4**).

2.2.3 Melting Point Determination

The **melting point** of pramipexole was analyzed using the **open capillary method** with a **Thiele's tube** setup. A small quantity of the sample was packed into a **thin-walled capillary tube** (10–15 mm in length, ~1 mm internal diameter, sealed at one end). **Liquid paraffin oil** was added to the **Thiele's tube**, which was then exposed to a **heat source**. The capillary tube was suspended in the oil, and the sample was gradually heated. A **thermometer** was used to monitor the temperature, and the point at which **melting began** was recorded as the **melting point**.

2.2.4 Drug Characterization

2.2.4.1 Determination of λmax and Calibration Curve

About **5 mg** of pramipexole was accurately weighed and transferred into a **5 mL volumetric flask**. Methanol was added to reach the **5 mL mark**, forming a solution with a **concentration of 1000 µg/mL**. From this, **1 mL** was pipetted and diluted to **10 mL** with methanol to obtain a **100 µg/mL standard stock solution**.

2.2.4.2 λmax Measurement

From the standard stock, **1 mL** was taken and diluted to **10 mL** using methanol to produce a **10 µg/mL solution**. The sample was scanned using a **double-beam UV-VIS spectrophotometer (Shimadzu-1700)** across the **200–800 nm** range, with methanol as the **blank**. The **maximum absorbance wavelength (λmax)** was recorded.

2.2.4.3 Linearity (Calibration Curve)

Aliquots of **10, 20, 30, 40, 50, and 60 µg/mL** were prepared from the **100 µg/mL working standard**. Each was transferred into a **5 mL volumetric flask**, and the volume was adjusted with methanol. The **absorbance** of these solutions was measured at **250.0 nm** against methanol as the **reference**. A **calibration curve** was plotted using **absorbance versus concentration**, and a **six-point curve** was established across the **10–60 µg/mL** range.

2.2.4.4 FTIR of Drug (Fourier Transform Infrared Spectroscopy)

FTIR measures the absorption of infrared radiation by the molecule at various wavelengths, which corresponds to the vibrational frequencies of the chemical bonds in the compound. Each functional group in a molecule absorbs infrared radiation at a characteristic frequency, generating a unique absorption spectrum.

2.2.4.5 Drug-Excipient Compatibility Studies by DSC

Differential Scanning Calorimetry (DSC) thermograms of pure pramipexole and its physical blend with excipients were analyzed to examine any potential interactions between the active pharmaceutical ingredient and the formulation components. The analysis was conducted over a temperature range of 50°C to 300°C, with a heating rate of 20°C per minute, using a DSC instrument from Shimadzu Corporation, Japan.**Partition Coefficient**

Quantification: Calculate the concentration of each phase using following

Equation Eq.1

$$\textbf{Partition coffiecient} = \frac{\textbf{Concentartion of drug in organic phase}}{\textbf{Concentration of drug in aqueous phase}} \quad \text{....Eq.1}$$

3. Formulation development of bilosomes

The details of Formulation of six bilosome formulations using pramipexole as the model drug are shown in **Table No.2** The formulations contain the different types of excipeints which are essentially used for the development of bilosomes .

Table No.2: Formulation development of bilosomes

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Drug	5	5	5	5	5	5
Soya lecithin	100	100	100	100	100	100
Cholesterol (mg)	30	10	20	40	35	15
Sodium deoxycholate (bile salt)	20	40	30	10	15	35
Tween 80	10	10	10	10	10	10

3.1 Method of Preparation: Thin Film Hydration Technique

The thin film hydration technique was used for development of bilosomes. First of all, dissolved the required quantity of Soya lecithin, cholesterol, and drug in a mixture of solvents chloroform and methanol in a ratio of 2:1 in a round-bottom flask. Further, the solvent was evaporated using a rotary evaporator under reduced pressure at 40°C to form a thin lipid film. Hydrated the dry film with phosphate buffer (pH 7.4) containing the appropriate Sodium deoxycholate which is already pre-warmed to 40–45°C.Now added the Tween 80, in the hydration medium as a surfactant. In the next step sonicated the dispersion using probe sonicator to reduce vesicle size. Finally, Stored bilosome suspension at 4°C in amber-colored vials to prevent degradation.

3.1.1 Evaluation Parameters

Evaluation parameters are most important aspect of any dosages form in order to check whether the dosage form is having all the standards as per official compendia or not. Rather, it indicates the efficacy, efficiency and therapeutic value as per standards.

The following evaluation parameters were evaluated of bilosomes:-

- **Vesicle size and PDI** (Dynamic Light Scattering)
- **Zeta potential**
- **Entrapment efficiency (%EE)**
- **Drug loading**
- **In-vitro drug release**
- **Stability studies**

3.1.2 Vesicle size and PDI (Dynamic Light Scattering)

DLS measures the hydrodynamic size of bilosomes in solution. It provides information about the average vesicle size and size distribution. First of all, prepared a colloidal suspension of bilosomes in chloroform which is free of aggregates, and diluted appropriately for achieving a scattering intensity within the instrument's range. Turned on the DLS instrument and allowed it to warm up and stabilize. Selected the measurement parameters, including temperature, scattering angle (typically 90 degrees), and laser wavelength in the visible range. Calibrated the instrument using a standard reference material with known particle size and zeta potential. Placed the sample in a suitable cuvette and placed into the DLS instrument and start the measurement. The instrument emitted a laser beam into the sample and they scattered light due to Brownian movement of the bilosomes in the suspension .The scattered light was collected at the specified scattering angle, and autocorrelation function of intensity fluctuations was calculated.

3.1.3 Zeta potential

Zeta potential measures the surface charge of bilosomes. It is important to determine the stability of bilosomes. High zeta potential indicates the non intended to aggregates the particles. Prepared uniform colloidal suspension of bilosomes in chloroform and it is further diluted to an appropriate concentration to ensure that the zeta potential measurement falls within the instrument detection range from 10 to 100. Turned on the instrument and allow it to warm up, stabilize and calibrate. Placed the zeta potential cell into the instrument and omes in the suspension, causing them to move. The velocity of particle movement is directly started the measurement. The zeta potential analyser applied an electric field to the bilos related to their surface charge. The instrument records the electrophoretic mobility of the bilosomes and calculates the zeta potential using the Henry equation.

3.1.4 Entrapment efficiency (%EE)

The amount of bilosomes (10 mg) and 5 ml of methanol in a volumetric flask were precisely weighed and agitated for one minute using a vortex mixer in order to determine the entrapment efficiency. Ten milliliters was the final volume. After then, the mixture was diluted and filtered. A twin beam spectrophotometer was used to measure the drug concentration at 262 nm.

$$\textbf{Loading Efficiency} = \textbf{Actual drug content present in bilosomes/ Theoretical drug content } X100$$

3.1.5 Drug loading

In order to determine the drug loading in the bilosomes, taken 10 mL of bilosome dispersion and centrifuged at 15,000–20,000 rpm for 1 hour at 4°C. After that collected the supernatant**,** this contains the free drug which was measured the absorbance by UV at ~262 nm to determine the concentration of drug in supernatant**.** The following formula was used to calculate the amount of free drug present in the dispersion.

Drug Loading Capacity (%DL)

$$\%DL = \frac{(Dt - Df)}{Wb} x100 \ldots\ldots Eq.3$$

- DT: Total amount of drug used (mg)

- DF: Free (unentrapped) drug (mg)
- DE: Entrapped drug (DT−DF)
- WB: Weight of total bilosome formulation (mg)

3.1.6 In-vitro drug release

The drug-loaded bilosomes were placed inside a dialysis bag, which is immersed in a release medium. The drug diffuses out through the membrane into the medium and was sampled at regular time intervals. The amount of drug released was analyzed using UV- Visible spectroscopy at 262 nm. The following **Table No. 3** shows the requirement to accomplish the method of *in-vitro* drug release studies:

Table No.3: Requirement to perform the in-vitro release of drug

Item	**Details**
Pramipexole-loaded bilosomes	Test formulation
Dialysis membrane	MW cut-off 12,000–14,000 Da (pre-treated)
Release medium	Phosphate buffer pH 7.4
USP-I dissolution apparatus	Temperature-controlled at 37.4±2
Beakers, stirrers, sampling pipettes, clamps	
UV-Vis Spectrophotometer	

This is very important aspect of any release study of drug, keeping in view, first of all, soaked the dialysis membrane in distilled water for 12–24 hours than rinsed thoroughly it to remove preservatives and other debris. Further, tied one end of the membrane and filled it with a 10 mL volume of the pramipexole-loaded bilosome suspension than tied the other end securely to make a sealed pouch. After that, filled the USP dissolution vessel with 100–200 mL of phosphate buffer (pH 7.4) maintained at 37 ± 0.5°C. Thereafter Suspended the dialysis bag in the medium, stirred the medium continuously with a magnetic stirrer at 50–100 rpm. The samples were withdrawal at definite time intervals i.e. 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours**),** withdraw 2 mL of the release medium. Simultaneously, the fresh solution was added into medium for marinating sink conditions. The samples were Measured at λmax ~262 nm using a UV-Vis spectrophotometer to determine the concentration using the standard calibration curve of pramipexole. Calculated **%** drug released at each time point using following formula:

$$\%\textbf{Cumulative Drug Release} = \frac{(\textbf{Amount of drug released at time t})}{\textit{Total Drug Loaded}} \textbf{x100}\ldots\textbf{..Eq.4}$$

A % cumulative drug release vs. time graph was plotted to generate the release profile. The obtained data were used to analyze the release kinetics models of drug like, Zero-order, First-order, Higuchi, Korsmeyer-Peppas to interpret the mechanism.

Zero-order release: $C(t) = k0t$

First-order release: $\ln(C(t)/C0) = -kt$

Higuchi release: $C(t) = kH\sqrt{t}$

Korsmeyer-Peppas release (for non-Fickian diffusion): $M(t)/M\infty = kKPtn$

.

3.1.7 Stability studies

Stability studies were conducted by storing the bilosomal formulations under stress conditions (high temperature and humidity), and analyzing them at specified intervals to assess:

- Drug content
- Particle size
- Zeta potential
- Entrapment efficiency
- Physical appearance

The International Conference on Harmonisation (ICH) guidelines are followed, especially ICH Q1A (R2).

Table No.4: Study Design for stability study

Condition	Accelerated Storage (ICH)
Temperature	40 ± 2°C
Relative Humidity (RH)	75 ± 5%
Duration	Up to 6 months
Sampling Intervals	0, 1, 2, 3, and 6 months
Packaging	Glass vials / sealed containers

Procedure

First of all, fill the bilosome formulation in airtight glass vials. Labelled and stored them in a stability chamber at 40°C ± 2°C and 75% RH ± 5%**.** At time points 0, 1, 2, 3, and 6 months, withdraw samples. Evaluated all listed parameters and noted any changes in color, odor, sedimentation, or turbidity in the samples. Measured and compared all values against the initial baseline (0 month)**.**

4.RESULT AND DISCUSSION

4.1 Organoleptic properties

Result are shown of organoleptic properties of drug pramipexole are in **Table No.5.**

Organoleptic properties of drug pramipexole

Organoleptic properties	Observation
Color	White
Odor	Odorless
Taste	Bitter
Appearance	White to almost white crystalline powder
Texture	Fine and smooth powder

Organoleptic properties of the pramipexole including taste, color, odor and appearance were carried out. The drug was observed to be having a white color, odorless, bitter in taste and has a solid white crystalline powder. All characteristics were found as per official compendia so comes a across the crystalline powder is having same characteristic as drug pramipexole has.

4.2 Solubility study

Solubility studies were carried out in different medium as shown in **Table No.5**

Table No.5: Solubility of drug in different solvent.

Medium	Observation
Water	Highly soluble
Ethanol	Partially soluble

Chloroform/Methanol	Soluble (for lipid film method)
Phosphate buffer (pH 6.8–7.4)	Soluble

Table No.5 depicts drug is highly soluble in water, soluble in phosphate buffer (pH 6.8- 7.4) and partially soluble in ethanol. The results indicate the good release of drug in dissolution studies. Results were compared to their official standard parameters and that was found to be satisfactory and as per standards.

4.3 Melting Point

The melting of drug was observed as given in following **Table No.6**

Table No.6: Melting point of drug Pramipexole

Drug	**Observed**	**Reference**
Pramipexole	298±0.5°C	296-301°C

Table No.6 indicates the observed value of melting point of the drug fallen within the range of reference value of drug indicates crystalline powder was pure pramipexole drug.

4.4 Characterization of drug

4.4.1 λ_{max} of drug

Following spectrum shown the λ_{max} of drug as shown in **Figure No. 1 and 2** respectively.

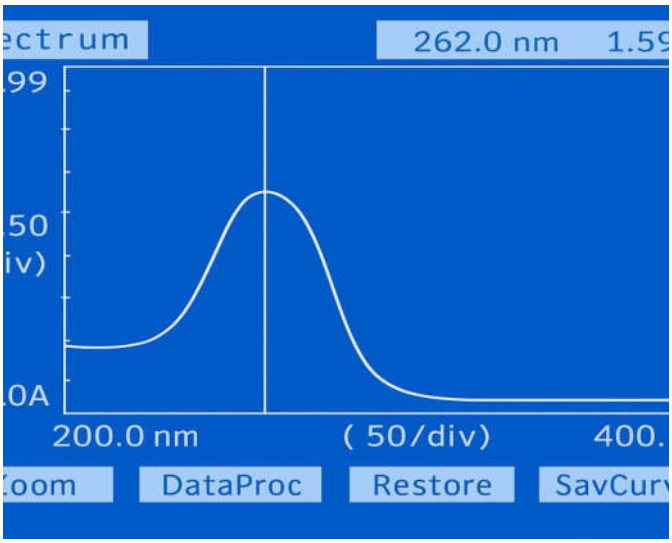


Figure No.1: Standard spectrum of ƛ max of drug pramipexole

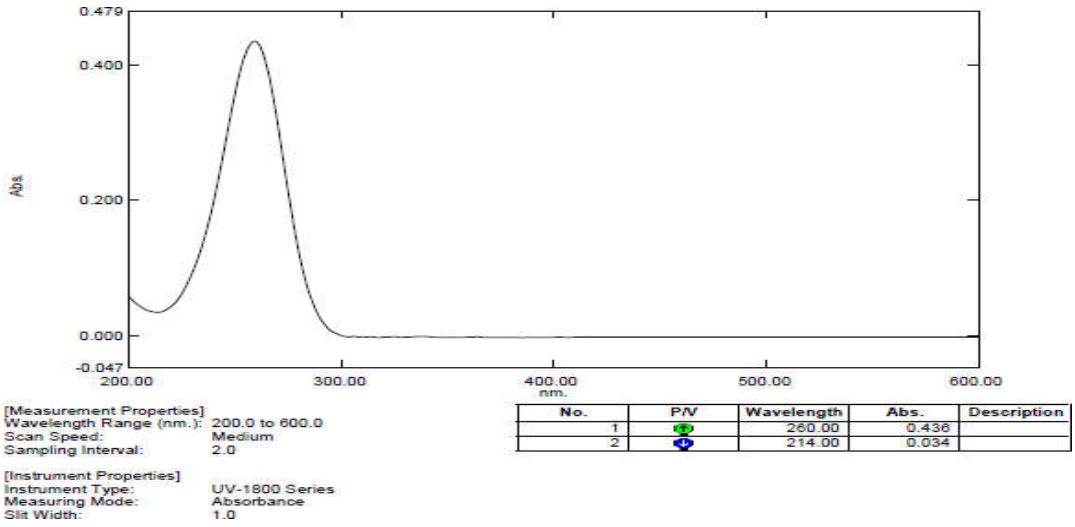


Figure No.2 : ƛ max of drug pramipexole

Discussion: Spectrum shown the ƛ max of drug is 260 nm at 0.430 which is almost equal to the official standards of drug indicates the white crystalline powder is pure drug pramipexole.

4.4.2. Calibration curve: Calibration curve was plotted between concentrations versus absorbance. The values are given in **Table No.7 and the graph is shown as Figure No.3.**

Table No.7. Calibration curve of drug

S.No	Concentration (µg/ml)	Absorbance
1	0	0
2	10	0.425
3	20	0.716
4	30	0.979
5	40	1.257
6	50	1.645
7	60	1.874

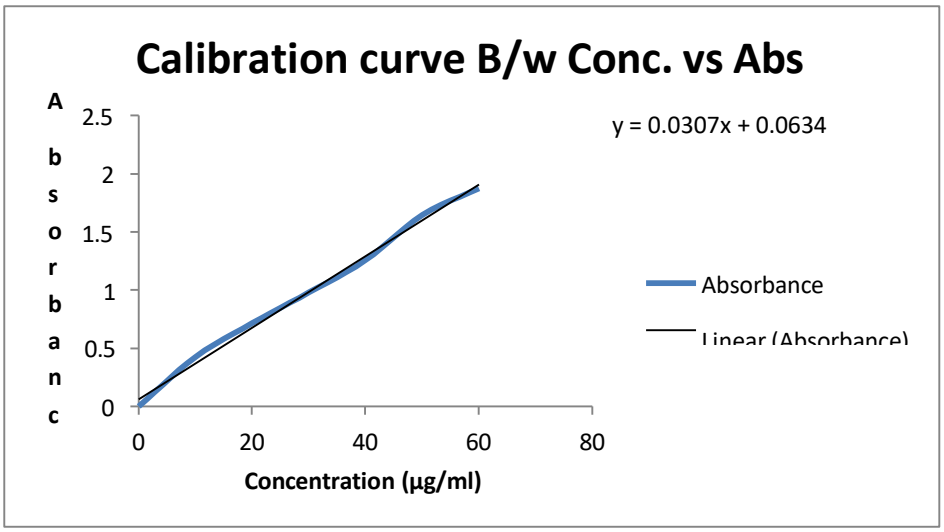
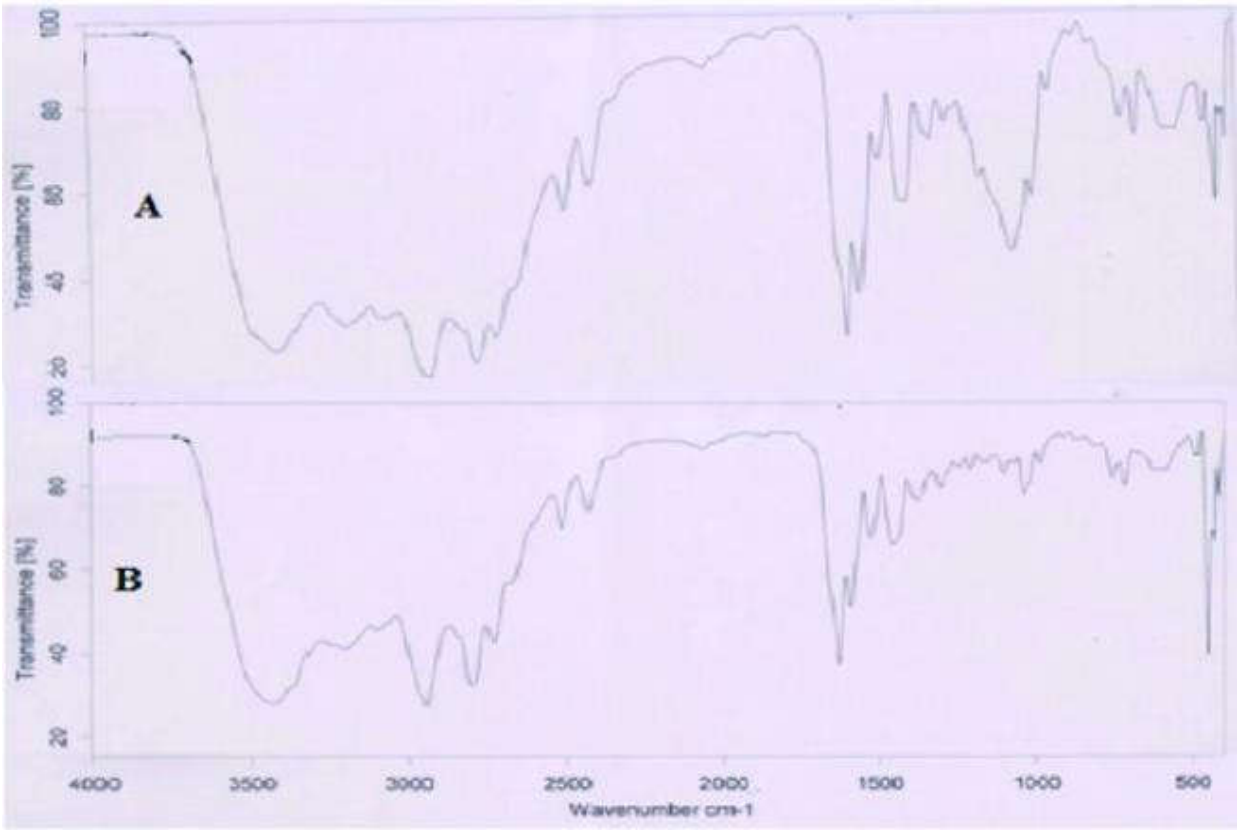


Figure No.3: Calibration curve of drug pramipexole discussion

The curve was plotted between concentrations of drug in micro gram per ml versus concentration. It has found the linear regression equation was y = 0.030x + 0.06 with correlation coefficient R^2 = 0.995. The value of R^2 = 0.995 is very close to one indicates the linearity of the equation which signifies that the figures of equation support the result and authenticity of the dilution procedure and aliquots concentrations.

4.3 FTIR of Drug (Fourier Transform Infrared Spectroscopy)

The following table is indicating the important peaks of drugs which help which are the important functional groups and play a vital role imparting in the therapeutic action. **Figure No.4** shows the standard spectra of drug pramipexole whereas Figure **No.5** shows the FTIR spectra of drug pramipexole.



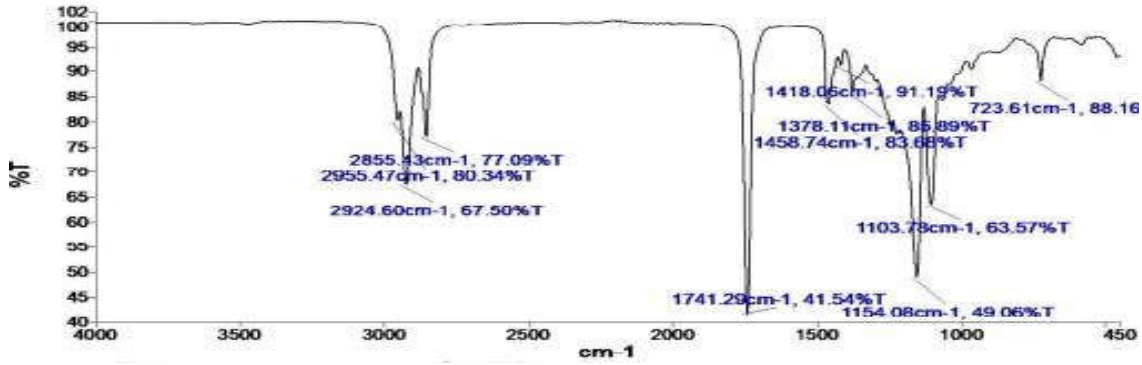


Figure No.4 : Standard FTIR of drug pramipexole

Figure No.5: FTIR graph of drug pramipexole

Table No. 8: FTIR Peaks for Pramipexole

Wavenumber (cm^{-1})	Type of Vibration	Functional Group / Assignment
~3400 – 3300	N–H stretching (broad)	**Secondary amine** (–NH)
~3050 – 3000	Aromatic C–H stretching	**Aromatic rings**
~2950 – 2850	Aliphatic C–H stretching	**Methyl ($–CH_3$)** and **methylene ($–CH_2$)** groups
~1600 – 1580	C=C stretching	**Aromatic ring** vibration
~1450 – 1350	C–H bending	Aliphatic **CH_2/CH_3 groups**
~1250 – 1050	C–N stretching	**Amines**, including part of the amine salt
~700 – 600	C–H bending (out-of-plane)	**Mono-substituted aromatic ring**

Table No.8 depicts the main functional stretching and bonding peaks were presented in the spectra clearly indicating the identification, and purity of drug. The figure no. also indicating regarding the no any interaction was observed among drug and other expedients as there is no significant shifting of peaks in the spectra.

4.4 DSC of pure drug and drug excipient mixture

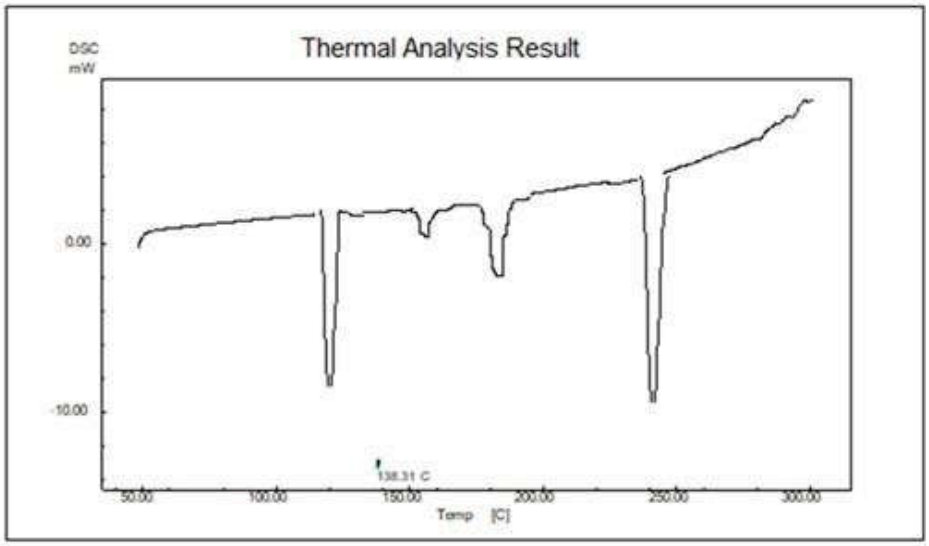


Figure No.6 (a): DSC of pure drug pramipexole

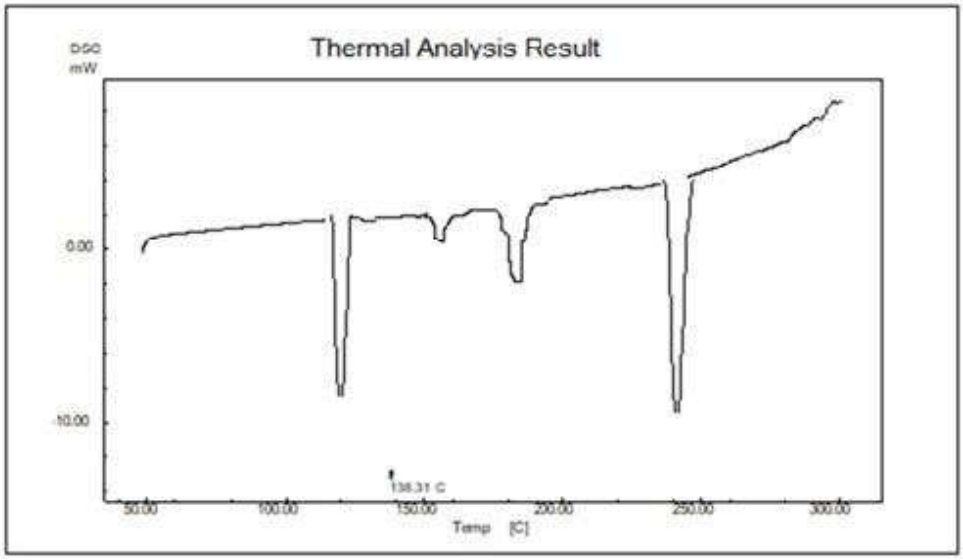


Figure No.6 (b): DSC of pure drug pramipexole and excipient Discussion

Figure No.6 (a) and (b) demonstrate that there are no significant change in the thermogram indicates that there is no interaction between drug and excipients.

4.5 Characterization of Bilosomes

4.5.1 Physical appearance

Bilosomes were visually checked upon the following parameters as shown in **Table No.9**

Table No.9:Physical appearance of drug

Formulations	Form	Colour	Consistency	Clarity
F1	Suspension	Pale yellow	Uniform	Opalescent
F2	Phase separation	Pale yellow	Irregular	Opalescent
F3	Phase separation	Pale yellow	irregular	Opalescent
F4	Suspension	Pale yellow	Uniform	Opalescent
F5	Suspension	Pale yellow	Uniform	Opalescent
F6	Phase separation	Pale yellow	irregular	Opalescent

Table No.9 depicts about the physical appearance of formulation F1 to F6. F2, F3 and F6 have shown the phase separation in the dispersion media. It may be due to lack amount of cholesterol added in the formulation, 10, 20, 15 mg respectively. Therefore all three formulations will not be carrying forward for further study.

4.5.2 Particle size of Bilosomes

		Size (d.n...	% Number:	St Dev (d.n...
Z-Average (d.nm): 317.22	Peak 1:	387.6	84.4	53.4
PdI: 0.387	Peak 2:	3252	13.8	1227
Intercept: 0.808	Peak 3:	52.42	1.8	9.351
Result quality Good				

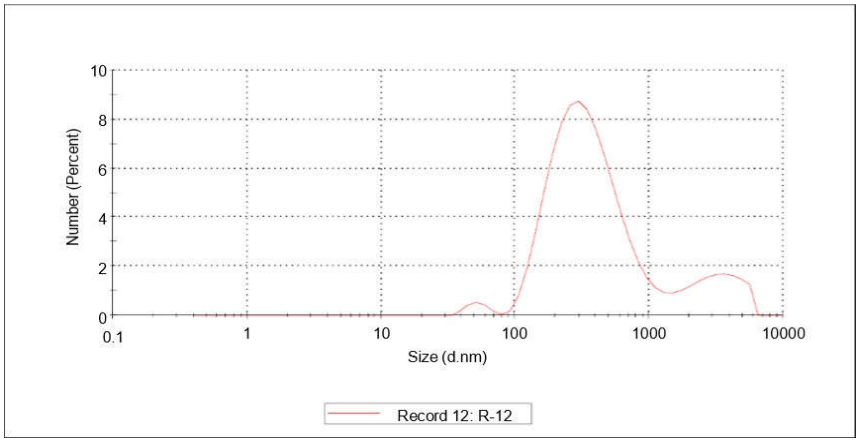


Figure No.7: Particle size of formulation (F1)

		Size (d.n...	% Number:	St Dev (d.n...
Z-Average (d.nm): 146.34	Peak 1:	133.6	100.0	63.12
PdI: 0.223	Peak 2:	0.000	0.0	0.000
Intercept: 0.892	Peak 3:	0.000	0.0	0.000
Result quality Good				

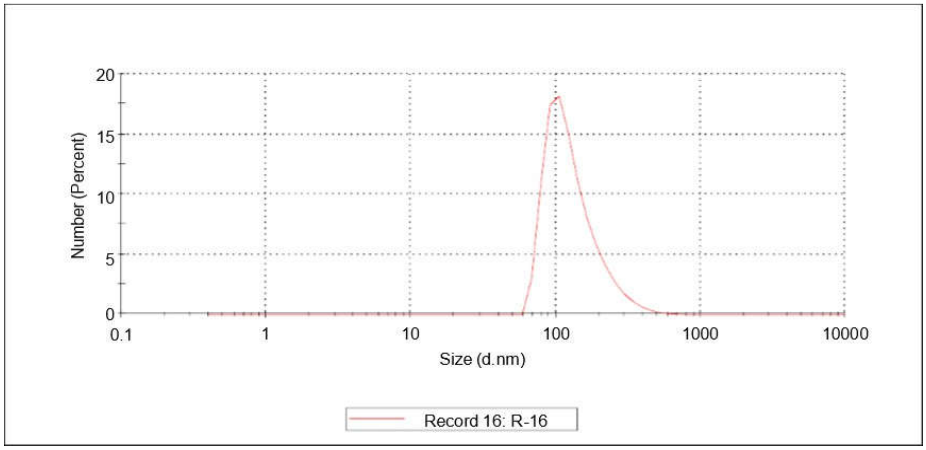


Figure No.8: Particle size of formulation (F4)

		Size (d.n...	% Number:	St Dev (d.n...
Z-Average (d.nm): 224.01	Peak 1:	235.0	100.0	36.0
PdI: 0.367	Peak 2:	0.000	0.0	0.000
Intercept: 0.834	Peak 3:	0.000	0.0	0.000
Result quality Good				

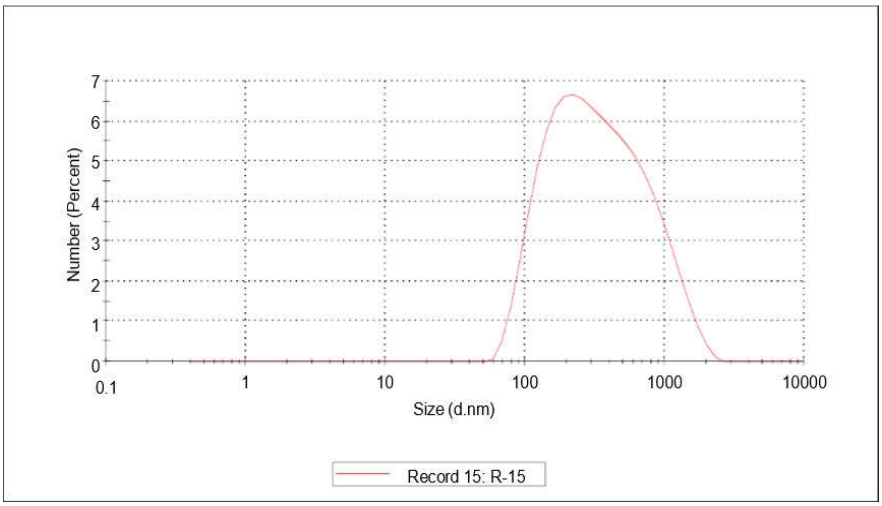


Figure No.9: Particle size of formulation (F5)

Table No. 10: Particle size of Pramipexole loaded Bilosomes

S. No	Formulation code	Particle size (nm)	PDI Value
1.	F1	317.22 nm	0.387
2.	F4	146.34 nm	0.223
3.	F5	224.01 nm	0.367

Discussion

Table No.10 **Particle size** is considered a **crucial parameter** in the **characterization** of **bilosomal formulations**. The **mean particle size** of the developed **pramipexole-loaded bilosomes** was determined using a **Malvern Zetasizer**. The analysis revealed that the **average particle size** of all formulations ranged from **146.34 nm to 317.51 nm**, and all values were **within the acceptable range for nanocarrier systems**.

4.5.3 Zeta potential

Zeta potential indicates the stability of the formulation. Formulations F1, F4 and F5 have gone through for determination of stability parameters and their results in terms of zeta potential were obtained in the manner of different graphs and values which are shown in following figures.

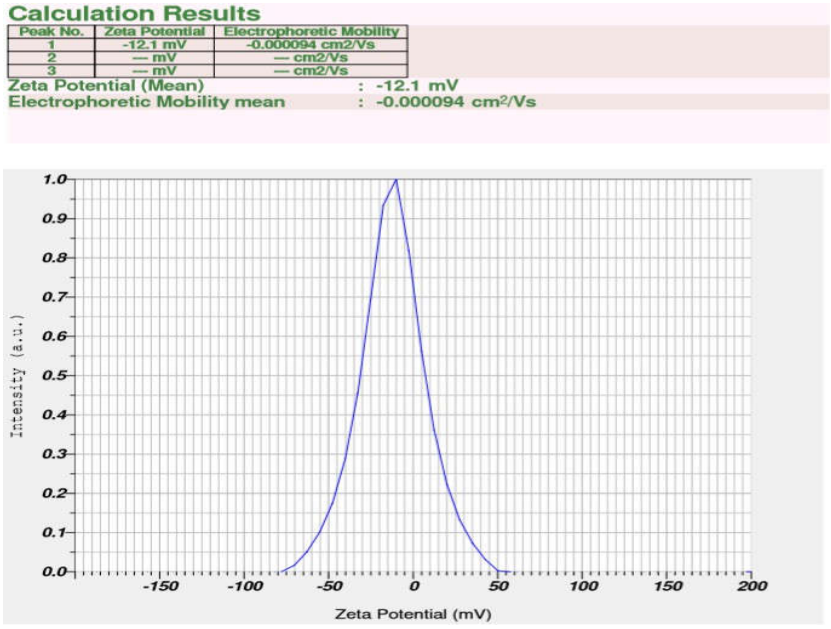


Figure No.10: Particle size of formulation (F1)

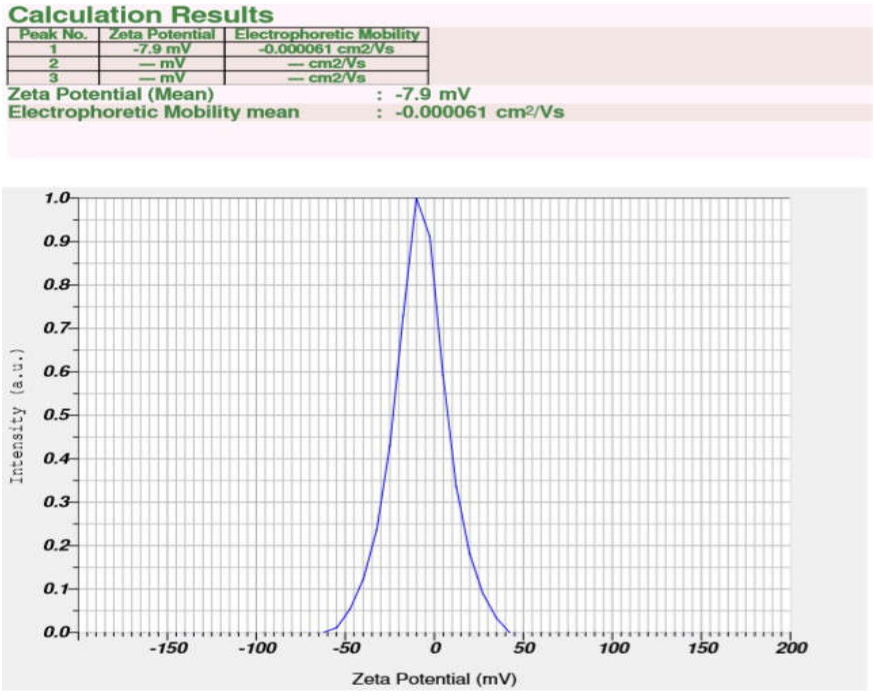


Figure No.11: Particle size of formulation (F4)

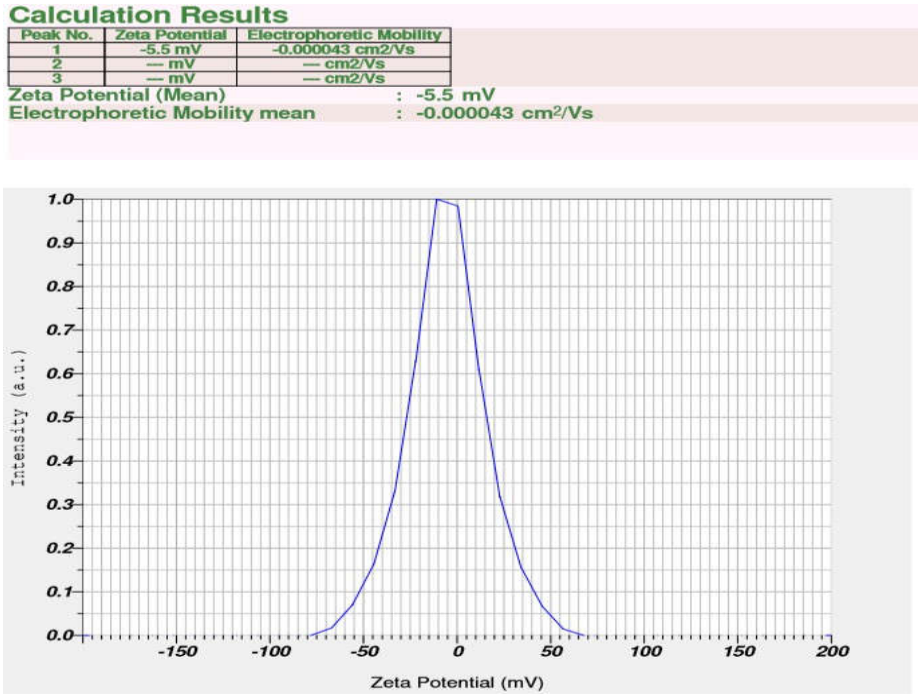


Figure No.12: Particle size of formulation (F5)

The values of zeta potential are given in **Table No.11**
Table No 11: Zeta potential of different formulations

S. No	Formulation	Zeta potential (mV)
1.	F1	-12.1
4.	F4	-7.9
5.	F5	-5.4

4.5.4 Entrapment efficacy

Entrapment efficiency of drug indicates the amount of drug within the vesicles. Entrapment efficiency also affects the therapeutic action of drugs. The entrapment efficiency of all the formulation is given in **Table No.12**

Table No.12: Entrapment efficiency of bilosomes formulation

S. No	Formulation (F1 to F2)	Entrapment efficacy (%)
1.	F1	97.45

4.	F4	98.87
5.	F5	98.44

Discussion

4.5.5 Scanning electron microscope (SEM) of bilosomes

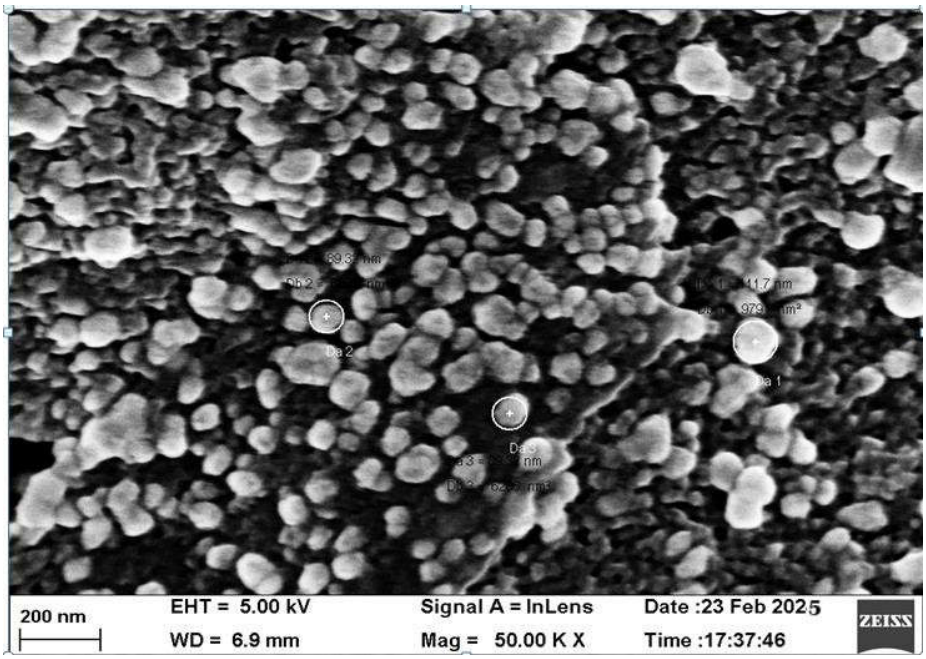


Figure No. 13: Scanning electron microscope of bilosomes Discussion

4.5.6 In-vitro Drug release study

The in-vitro drug release of Formulations F1, F4 and F5 were evaluated and their results are giben in **Table No.13. The Figure No.14** shown the cumulative release of drug

Table No.13: In-vitro drug release study of bilosome

Time (hrs)	**F1**	**F4**	**F5**
0.25	2.118±0.073	3.127±0.024	3.564±0.041
0.5	5.357±0.073	7.234±0.027	8.147±0.054
1	8.487±0.036	10.624±0.087	11.413±0.037
2	12.761±0.036	16.745±0.150	22.374±0.114
3	17.410±0.055	24.845±0.212	29.504±0.317
4	24.543±0.055	28.214±0.315	34.463±0.241

5	29.340±0.554	32.312±0.247	42.525±0.178
6	35.634±0.554	35.214±0.302	48.347±0.214
8	41.294±0.554	40.547±0.119	55.247±0.047
10	47.374±0.363	48.841±0.289	62.547±0.074
12	54.257±0.363	56.115±0.245	69.245±0.342
24	62.435±0.363	99.148±0.120	79.504±0.741

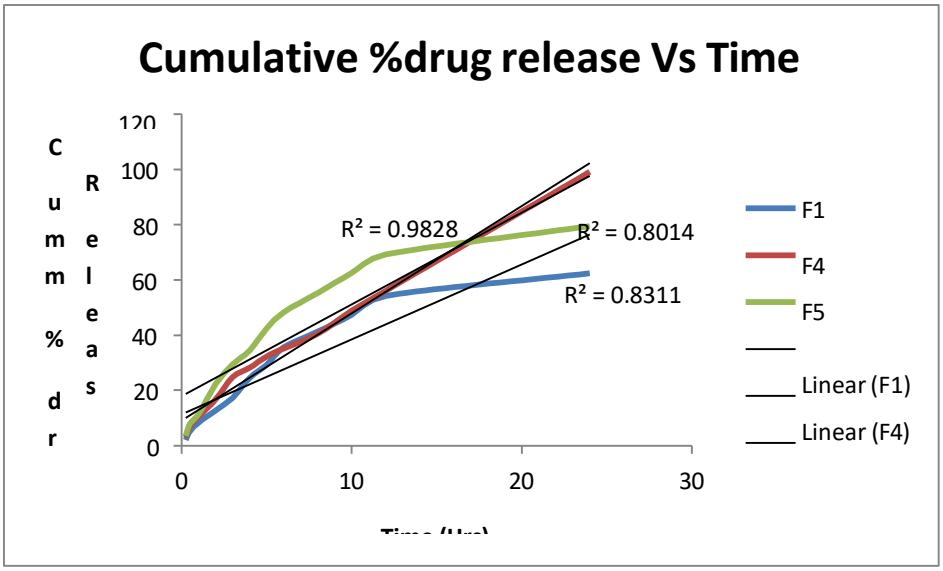


Figure No. 14: Cumulative release of drug versus time

The cumulative percentage of drug release over time is displayed in Figure No. The graph shows that the medicine is released at varying times by all three formulations (F1, F4, and F5). The formulation F4 showed the best drug release pattern out of the three graphs and sustained the drug release activity for a considerable amount of time. The drug's linearity and persistent effect are demonstrated by the R2 value of the drug's release from formulation F4, which is 0.982 and almost 1. Zero-order release of a drug is another name for this type of release pattern.

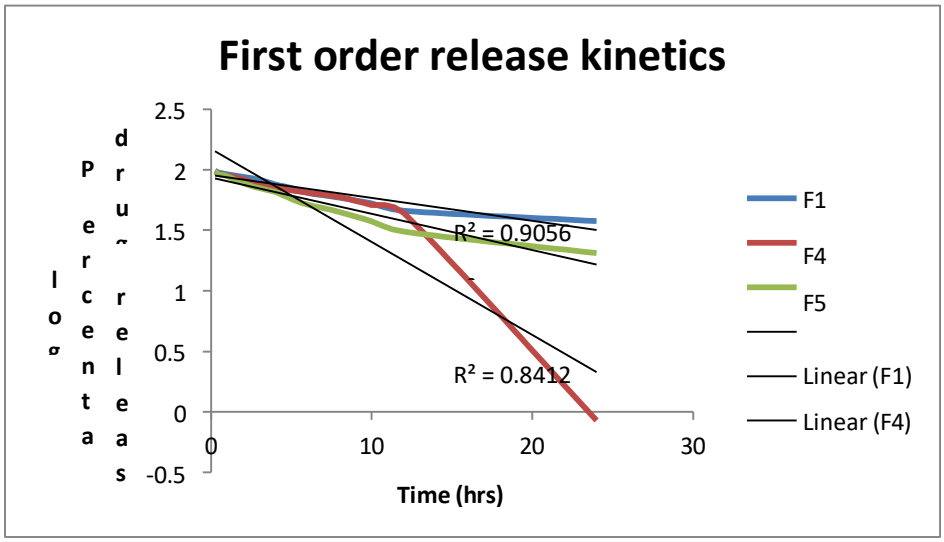
4.5.6.1 First Order Release Kinetics

The values are given in **Table No.14** and a graph was plotted between percentage log drugs remaining v/s time

Table No.14: First order release kinetics of drug

Time (hrs)	F1	F4	F5
0.25	1.990702835	1.986203	1.984239
0.5	1.976088498	1.967389	1.963093
1	1.961482793	1.951221	1.94737
2	1.940710679	1.92041	1.890007
3	1.916927466	1.875958	1.848164
4	1.877699535	1.85604	1.816487
5	1.849173633	1.830512	1.759479
6	1.808656521	1.811481	1.713096
8	1.76868249	1.774174	1.650822
10	1.721200361	1.708922	1.573487
12	1.660324644	1.642316	1.487916
24	1.574783393	-0.06956	1.311669

A graph was plotted between Log % drug release vs time which is shown in **Figure No. 15**



4.5.6.2 Higuchi Order: The following Table No. 15 given the values of Higuchi model.

Time (SQRT)	F1	F4	F5
0.5	2.118	3.127	3.564
0.707106781	5.357	7.234	8.147
1	8.487	10.624	11.413
1.414213562	12.761	16.745	22.374
1.732050808	17.41	24.845	29.504
2	24.543	28.214	34.463
2.236067977	29.34	32.312	42.525
2.449489743	35.634	35.214	48.347
2.828427125	41.294	40.547	55.247
3.16227766	47.374	48.841	62.547
3.464101615	54.257	56.115	69.245
4.898979486	62.435	99.148	79.504
4.898979486	62.435	99.148	79.504

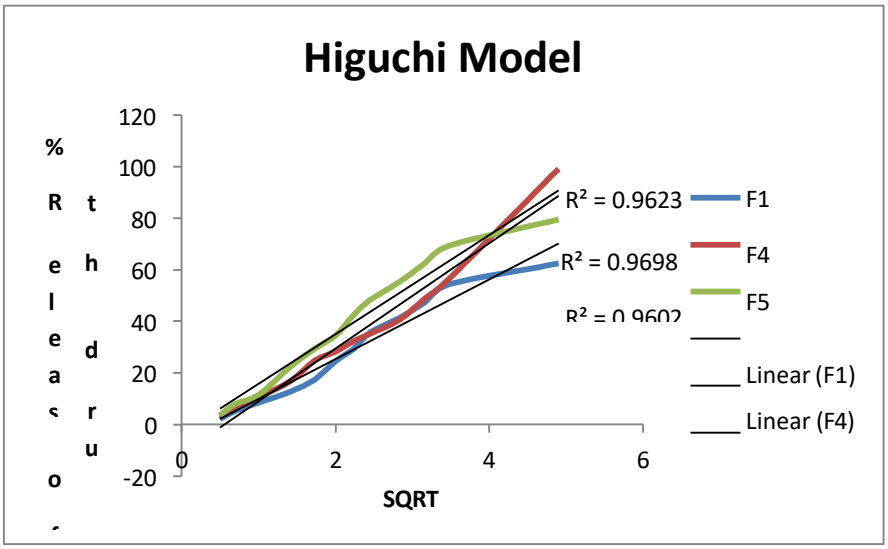


Figure No.16: Higuchi order graph

4.5.6.3.Korsemeyer peppas Model

Table No. 16: Korsemayer pappas model of drug

log time (hrs)	F1	F4	F5
-0.60206	0.325926	0.495128	0.551938
-0.30103	0.728922	0.859379	0.910998
0	0.928754	1.026288	1.0574
0.30103	1.105885	1.223885	1.349744
0.477121	1.240799	1.395239	1.469881
0.60206	1.389928	1.450465	1.537353
0.69897	1.46746	1.509364	1.628644
0.778151	1.551865	1.546715	1.68437
0.90309	1.615887	1.607959	1.742309
1	1.67554	1.688785	1.796206
1.079181	1.734456	1.749079	1.840388
1.380211	1.795428	1.996284	1.900389

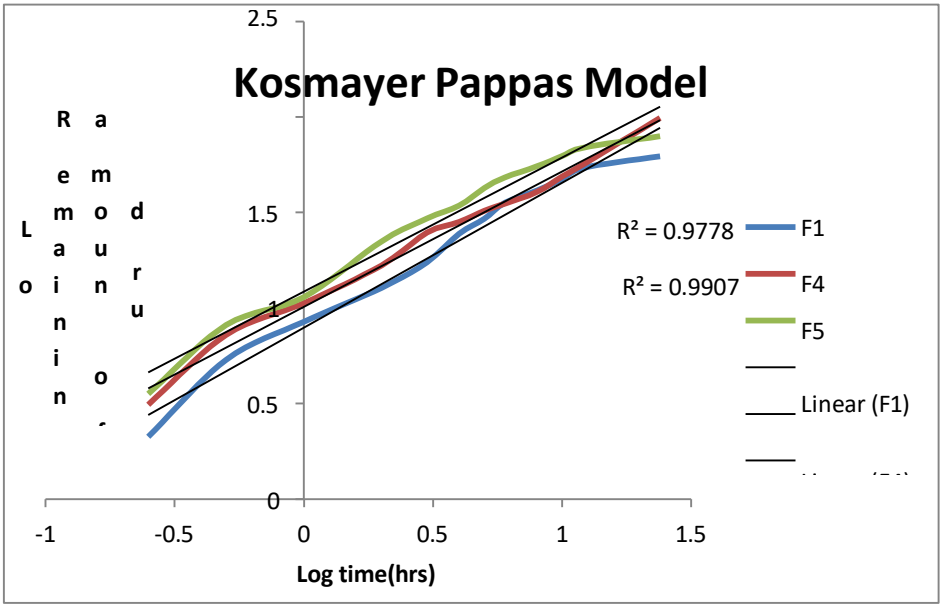


Figure No. 17 : Korsemeyer peppas graph of formulation

The values of R^2 of all formulation are given in **Table No. 17** R^2 value of F4 in zero order and Kosmayer papas is very close to one, which indicates that the drug release is following the same models**.**

Table No.17: R^2 value of all formulations

Formulation	**Zero order (R^2)**	**First order (R^2)**	**Higuchi (R^2)**	**Korsmeyer-Peppas (R^2)**
F1	0.831	0.905	0.962	0.977
F4	0.982	0.841	0.969	0.990
F5	0.801	0.932	0.960	0.973

Therefore, F4 formulation is suited for best formulation among all three so that it has considered for stability studies.

4.7 Stability Studies

Formulation F4 was considered for stability studies as it was found as best formulation among all. An accelerated study was performed at 40°C ± 2°C and 75% RH ± 5% as per guidelines. Results are given in the following **Table No.18.**

Table No.18: Stability studies of Formulation F4.

Parameters	**Time (Months)**				
	0	1	2	3	6
Colour	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Particles size	146.34 (nm)	146.34 (nm)	146.34 (nm)	146.34 (nm)	146.34 (nm)
Entrapment Efficiency	98.87	98.87	98.87	98.86	98.86
Drug release	99.148±0.120	99.146±0.120	99.145±0.112	99.145±0.112	99.144±0.110

Table No.26 depicts that during the six-month extensive stability studies of formulation F4, it was observed that there were no significant changes in the formulation. Therefore, it has been determined that the formulation F4 is the best formulation for this research study, which can be used for future prospects and considered as marketed preparations after getting proper in vivo studies and necessary approval from regulatory agencies

Conclusion

Bilosomes are advanced drug delivery vesicles containing bile salts, enhancing stability and permeability, making them ideal for oral delivery of sensitive drugs like peptides and vaccines. The study involved extensive research from peer-reviewed journals over ten years, using analytical-grade chemicals. Six bilosome formulations were developed via the thin-film hydration technique and evaluated for parameters like vesicle size, entrapment efficiency (highest in **F4: 98.87%**), drug release (**99.148% in 24 hours**), and stability. **F4** showed optimal performance, following zero-order and Korsmeyer-Peppas release kinetics, indicating sustained diffusion-based release. Accelerated stability tests confirmed **F4** remained stable under regulatory conditions (40°C, 75% RH).

REFERENCES

1. Kawabata Y, Wada K, Nakatani M, et al. (2011). Formulation dZafar A., Alsaidan O.A., Imam S.S., Yasir M., Alharbi K.S., Khalid M. Formulation and Evaluation of Moxifloxacin Loaded Bilosomes In-Situ Gel: Optimization to Antibacterial Evaluation. Gels. 2022;8:418. doi: 10.3390/gels8070418.
2. Shukla A., Mishra V., Kesharwani P. Bilosomes in the context of oral immunization: Development, challenges and opportunities. Drug Discov. Today. 2016;21:888–899. doi: 10.1016/j.drudis.2016.03.013.
3. Shukla A., Katare O.P., Singh B., Vyas S.P. M-cell targeted delivery of recombinant hepatitis B surface antigen using cholera toxin B subunit conjugated bilosomes. Int. J. Pharm. 2010;385:47–52. doi: 10.1016/j.ijpharm.2009.10.027.
4. Jain S., Indulkar A., Harde H., Agrawal A.K. Oral mucosal immunization using glucomannosylated bilosomes. J. Biomed. Nanotechnol. 2014;10:932–947. doi: 10.1166/jbn.2014.1800.
5. Ahmad J., Singhal M., Amin S., Rizwanullah M., Akhter S., Kamal M.A., Haider N., Midoux P., Pichon C. Bile Salt Stabilized Vesicles (Bilosomes): A Novel Nano-Pharmaceutical Design for Oral Delivery of Proteins and Peptides. Curr. Pharm. Des. 2017;23:1575–1588. doi: 10.2174/1381612823666170124111142.
6. Hu S., Niu M., Hu F., Lu Y., Qi J., Yin Z., Wu W. Integrity and stability of oral liposomes containing bile salts studied in simulated and ex vivo gastrointestinal media. Int. J. Pharm. 2013;441:693–700. doi: 10.1016/j.ijpharm.2012.10.025.
7. Panda DS, Eid HM, Elkomy MH, et al. (2021). Berberine encapsulated lecithin-chitosan nanoparticles as innovative wound healing agent in type II diabetes. Pharmaceutics 13:1197.
8. Qumbar M, Imam SS, Ali J, et al. (2017). Formulation and optimization of lacidipine loaded niosomal gel for transdermal delivery: in-vitro characterization and in-vivo activity. Biomed Pharmacother 93:255–66.
9. Roger E, Lagarce F, Garcion E, et al. (2010). Biopharmaceutical parameters to consider in order to alter the fate of nanocarriers after oral delivery. Nanomedicine (Lond) 5:287–306.
10. Shaker S, Gardouh AR, Ghorab MM. (2017). Factors affecting liposomes particle size prepared by ethanol injection method. Res Pharm Sci 12:346–52. [
11. Soetaert K. plot3D: Plotting Multi-Dimensional Data, R package version 1.1. 1. 2017.
12. Souza MP, Vaz AFM, Correia MTS, et al. (2014). Quercetin-loaded lecithin/chitosan nanoparticles for functional food applications. Food Bioprocess Technol 7:1149–59.
13. Tian CM, Jiang X, Ouyang XX, et al. (2016). Berberine enhances antidiabetic effects and attenuates untoward effects of canagliflozin in streptozotocin-induced diabetic mice. Chin J Nat Med 14:518–26.

14. Waglewska E, Pucek-Kaczmarek A, Bazylińska U. (2020). Novel surface-modified bilosomes as functional and biocompatible nanocarriers of hybrid compounds. Nanomaterials (Basel) 10:2472.

15. Wang L, Li H, Wang S, et al. (2014). Enhancing the antitumor activity of berberine hydrochloride by solid lipid nanoparticle encapsulation. AAPS PharmSciTech 15:834–44.

16. Jhong C-H, Riyaphan J, Lin S-H, et al. (2015). Screening alpha-glucosidase and alpha-amylase inhibitors from natural compounds by molecular docking in silico. Biofactors 41:242–51.

17. Karthikeyan R, Marimuthu G, Spence DW, et al. (2014). Should we listen to our clocesign for poorly water-soluble drugs based on biopharmaceutics classification system: basic approaches and practical applications. Int J Pharm 420:1–10.

18. Lee YS, Kim WS, Kim KH, et al. (2006). Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states. Diabetes 55:2256–64