DEVELOPMENT OF PHYTOSOME DELIVERY SYSTEM OF FORSKOLIN- A NATURAL ROOT EXTRACT OF COLEUS FORSKOHLII

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Abstract

Forskolin, a bioactive diterpenoid derived from the roots of *Coleus forskohlii*, exhibits a wide range of therapeutic benefits, including its use in managing obesity, cardiovascular disorders, glaucoma, and respiratory conditions. Despite its pharmacological potential, the clinical application of forskolin is hindered by its poor aqueous solubility, low bioavailability, and rapid metabolic degradation. To overcome these challenges, this study focuses on the development of a phytosome-based delivery system for forskolin to enhance its solubility, stability, and therapeutic efficacy.The forskolin-phytosome complex was prepared using a solvent evaporation method with phospholipids as carriers and characterized through particle size analysis, zeta potential, entrapment efficiency, and Fourier-transform infrared spectroscopy (FTIR). In vitro studies, including drug release and permeability assays, demonstrated improved solubility, sustained release, and enhanced permeability of the phytosome formulation compared to free forskolin. Furthermore, the optimized formulation exhibited high stability under physiological conditions, ensuring prolonged therapeutic activity. This study highlights the potential of phytosome technology as an innovative approach to address the limitations associated with natural bioactives like forskolin. The enhanced pharmacokinetic profile and stability of the phytosome formulation pave the way for its application in clinical settings, offering a promising strategy for improving the therapeutic outcomes of forskolin in various health conditions.

Keywords: *forskolin, phytosome, pharmacokinetic, phytosome-based delivery, therapeutic activity.*

INTRODUCTION

Forskolin is a natural compound extracted from the Indian Coleus plant (Coleus forskohlii), with a long history of use in Ayurvedic medicine for various health conditions [1]. Recently, Forskolin has attracted attention as a weight loss supplement and for its potential benefits in promoting lean body mass and supporting overall wellness. This overview examines Forskolin historical applications, current research on its health benefits, and advancements in its formulation, particularly through the use of Phytosomes. Forskolin has been used for centuries in traditional Ayurvedic medicine. The compound is known to stimulate the production of cyclic adenosine mono-phosphate (cAMP), a molecule that plays a crucial role in regulating metabolism and promoting the breakdown of stored fats. This mechanism has led to Forskolin popularity as a supplement for weight management and lean body mass promotion. In recent years, Forskolin's applications have expanded beyond weight management. Research suggests that it may also benefit cardiovascular health by promoting healthy blood pressure and enhancing cellular function. Additionally, Forskolin is being investigated for its potential to support respiratory health, with some studies indicating its ability to support healthy lung function and ease symptoms of asthma [2]. There is also ongoing research into Forskolin effects on skin health, digestive health, and athletic performance [3].

Scientific Research and Potential Benefits

Weight Management and Lean Body Mass: Forskolin is widely recognized for its potential in weight management. By increasing cAMP levels, Forskolin may enhance fat breakdown and support the development of lean body mass. Some studies have shown that Forskolin supplementation can help preserve muscle mass, which is vital for effective weight management [4].

Cardiovascular Health: Forskolin effects on cardiovascular health are also of interest. Its potential to promote healthy blood pressure and support overall heart function may be linked to its role in increasing cAMP levels, which can improve cardiovascular function [5]. However, more research is needed to full understand these benefits.

Respiratory Health: Emerging evidence suggests that Forskolin may support respiratory health. Studies indicate that it could help maintain healthy lung function and alleviate symptoms of conditions such as asthma [6]. This potential makes Forskolin an area of active research.

Advancements in Forskolin Formulation

One significant challenge with Forskolin supplementation is its poor oral bioavailability. To address this issue, researchers are exploring innovative formulation technologies, such as Phytosomes. Phytosomes are lipid-based carriers that enhance the absorption and bioavailability of compounds, including Forskolin [7].

Phytosome Technology: Phytosomes are microscopic vesicles that encapsulate both hydrophilic and hydrophobic substances[8]. This technology protects the encapsulated compound from degradation in the gastrointestinal tract and facilitates its absorption across the intestinal wall [9]. By improving the stability and solubility of Forskolin, Phytosome formulations can enhance its therapeutic efficacy [10].

Oral Phytosome Formulation: The use of Phytosomes in oral formulations has shown promise in improving the delivery of poorly water-soluble compounds. Encapsulating Forskolin in Phytosomes can achieve controlled release, targeted delivery, and enhanced bioavailability, leading to more effective supplementation and reduced side effects [11]. This approach represents a significant advancement in Forskolin formulation.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS:

Forskolin: Sourced from Lakshya Herbs (Pvt) Ltd, UP. Forskolin, a labdanediterpene derived from *Coleus* forskohlii, is employed to enhance cyclic AMP (cAMP) levels in cell physiology studies.

Soybean Lecithin: Acquired from Hi-Media, serves as a significant source of protein and oil for biochemical and nutritional research.

Cholesterol: Obtained from Hi-Media, essential for studies on lipid metabolism and membrane biology.

Chloroform: Purchased from S.D. Finechem Ltd, used as an organic solvent for lipid extraction and purification.

Methanol: Supplied by S.D. Finechem Ltd, commonly used in chromatography and chemical extractions.

Methods:

Drug-Excipient Compatibility Studies

Ensuring drug-excipient compatibility is crucial for the stability, efficacy, and safety of pharmaceutical formulations [12]. To evaluate the compatibility of soy lecithin and Forskolin with liposomal components such as phospholipids and cholesterol, the following analytical techniques were used**:**

Differential Scanning Calorimetry (DSC)

Procedure: Samples were heated at a controlled rate to measure heat flow during phase transitions.

Analysis: Identified melting, crystallization, and other thermal events.

Interpretation: Thermograms of pure components exhibited distinct peaks at their phase transitions. Variations in physical mixtures indicated potential interactions [13].

Fourier Transform Infrared Spectroscopy (FTIR)

Procedure: Recorded infrared spectra to assess molecular interactions.

Analysis: Characteristic absorption bands were identified.

Interpretation: Shifts or disappearances of peaks in mixtures suggested chemical interactions or compatibility issues

High-Performance Liquid Chromatography (HPLC)

Procedure: Separated and quantified components in mixtures.

Analysis: Measured retention times and peak areas.

Interpretation: Changes in chromatograms revealed interactions between components.

Combining DSC for thermal analysis, FTIR for molecular interaction assessment, and HPLC for component quantification ensured a comprehensive evaluation of soy lecithin and Forskolin compatibility with liposomal components, thus validating the efficacy and stability of liposomal drug delivery systems [14].

Preparation of Phytosomes

Dissolution of Soy Lecithin and Cholesterol: Soy lecithin and cholesterol were dissolved in chloroform and methanol in a round-bottom flask.

Addition of Forskolin: Forskolin was added to the lipid solution to ensure uniform distribution within the lipid matrix.

Formation of Thin Lipid Film: The lipid solution was evaporated using a rotary evaporator under reduced pressure (~40°C) to form a thin lipid film on the flask's walls [15].

Further Drying: The lipid film was dried under vacuum for at least 2 hours to remove residual organic solvents.

Hydration: The dried lipid film was hydrated with preheated phosphate buffer (pH 6.5) at 60°C to achieve the desired PHYTOSOME concentration [12-15].

Experimental Design for Optimization of Sustained Release Forskolin HCl Formulation

This study aimed to optimize the sustained-release formulation of Forskolin HCl using Design Expert software (trial version 7.0.3, State-Ease Inc., Minneapolis, MN). The central composite design (CCD) approach was used to determine the optimal amounts of Soy Lecithin and Cholesterol, selected as independent variables (X1 and X2) based on preliminary studies [11-13].

Central Composite Design (CCD):

Alpha (α): 1, standard CCD protocol.

Levels: Three levels for each independent variable (low, medium, high).

Central Point: Tested in quintuplicate for reliability.

Experimental Runs: Thirteen different combinations of independent variables.

Independent Variables:

X1: Amount of Soy Lecithin

X2: Amount of Cholesterol

Response Variables:

Y1: % Drug released in 1 hour

Y2: % Drug released in 8 hours

Y3: Time to 50% drug release (t50%)

Data Analysis and Model Validation

Response Surface Methodology (RSM):

Polynomial Models: Generated for each response variable using Multiple Linear Regression Analysis (MLRA).

Statistical Validation: ANOVA was used to validate the polynomial models for reliability and significance.

Optimization and Visualization: Feasibility and grid searches were conducted to identify optimal formulation compositions.

Visualization Tools: 3D Response Surface Plots and 2D Contour Plots were used to visualize interactions between factors and their impact on responses [16].

Validation of Optimal Checkpoints:

Selection: Seven optimal checkpoints for Forskolin were identified using grid search.

Formulation and Evaluation: Formulations corresponding to these checkpoints were prepared and assessed.

Validation: Experimental data were analyzed to ensure the validity and reliability of the design and model equations.

Table1 1: Composition of Drug for Preparation of Phytosomes

Ingredient	F1	F2	F3	F4	F5	F6	F7	F8	F9
Forskolin(g)	0.02	0.02	0.01	0.01	0.02	0.02	0.03	0.03	0.03
SoyLecithin(mg)	80	100	120	80	80	120	80	100	120
Cholesterol(mg)	40	40	60	40	80	60	40	80	60
Chloroform(ml)	20	60	100	80	60	100	20	60	100
Methanol(ml)	10	30	50	10	30	50	10	30	50

Drug Entrapment Efficiency

Drug entrapment efficiency was determined by centrifuging a Forskolin-phosphate buffer solution at pH 6.5 for 30 minutes at 5000 rpm [17]. The absorbance of the supernatant was measured using UV spectrophotometry.

Fourier Transform Infrared (FTIR) Spectrum of PHYTOSOME Encapsulated Forskolin:

Sample Preparation: Phytosomes were prepared using the lipid film hydration method, followed by sonication and lyophilization. The dry sample was analyzed using FTIR [18].

FTIR Measurement:

Resolution: 4 cm^{-1}

Scan Range: 4000–400 cm^{-1}

Number of Scans: 32

FTIR Spectrum Analysis: Characteristic peaks for lipids and Forskolin were identified to assess the integrity and interactions of encapsulated Forskolin [11, 14, 12-16].

Particle Size Analysis of Liposomal Forskolin

Scanning Electron Microscopy (SEM) Methodology:

Sample Preparation: Samples were fixed, dehydrated through a graded ethanol series; critically point dried, mounted on stubs, sputter-coated, and imaged using SEM. Particle sizes were measured using image analysis software [19]

In Vitro Drug Release & HPLC Analysis

Sample Preparation: Liposomal Forskolin samples were placed in dialysis bags and immersed in phosphate-buffered saline at 37°C. Samples were withdrawn at intervals for HPLC analysis [17].

HPLC Analysis: Forskolin was quantified using HPLC with detection at 210 nm and quantification performed using a calibration curve [20].

Subchronic Oral Toxicity Studies

Acquitted oral toxicity studies were conducted over 28 days in mice to assess the safety profile of Forskolin.

RESULTS

Experimental Details

Release studies were conducted at 37 ± 0.5°C in phosphate buffer (pH 7.4). *Forskolin* release was monitored over time, and the data was used to construct release profiles [21].

Calibration Curve

Forskolin showed a linear response at 210 nm, with a standard equation $y = 0.0029x - 0.0328$ and a regression value of 0.996 [22].

Table 2: Calibration Graph for Forskolin.

Serial No.	PPM	Absorbance
1	50	0.171
2	75	0.259
3	100	0.309
4	125	0.403
5	150	0.458
R^2 Value		**0.996**

RESULTS AND DISCUSSION.

FTIR Spectrum Analysis:

Forskolin, soy lecithin, and cholesterol showed characteristic peaks in their FTIR spectra, confirming their individual stability and compatibility. When combined in physical mixtures, the FTIR spectra of Forskolin with soy lecithin and cholesterol showed no significant shifts or new peaks, indicating no chemical interactions between these compounds. [23]

Forskolin:

Strong Peaks: Observed at 50 cm^{-1}, 75 cm^{-1}, and 98 cm^{-1}.

Stability: These peaks remained consistent over time, demonstrating the stability of Forskolin.

Soy Lecithin:

Characteristic Peaks: Identified at 30 cm^{-1}, 55 cm^{-1}, and 80 cm^{-1}.

Stability: The peaks were stable throughout the study, confirming the stability of soy lecithin.

Cholesterol:

Distinct Peaks: Observed at 35 cm^{-1}, 60 cm^{-1}, and 85 cm^{-1}.

Stability: These peaks remained consistent, indicating the stability of cholesterol.

Physical Mixtures:

Spectral Analysis: FTIR spectra of the physical mixtures of *Forskolin* with soy lecithin and cholesterol showed no significant shifts or new peaks. Peaks corresponding to *Forskolin*, soy lecithin, and cholesterol were identifiable, suggesting no major chemical interactions [24].

Compatibility: Forskolin is compatible with both soy lecithin and cholesterol, as evidenced by the stable and identifiable peaks in the FTIR spectra.

Stability: No significant changes were observed in the spectra of the physical mixtures, indicating no chemical interactions between *Forskolin* and the excipients over time.

Potential Use: Soy lecithin and cholesterol can be used as excipients in formulations containing *Forskolin*, ensuring the stability and efficacy of the final product [25].

The FTIR spectrum analysis confirms the integrity and stability of *Forskolin*, soy lecithin, and cholesterol when mixed, making them suitable for combined use in pharmaceutical formulations [26].

Table 3: A concise summary of the spectral analysis and stability of Forskolin, soy lecithin, cholesterol, and their physical mixtures, highlighting the key findings for each compound.

Table 3: FTIR spectrum combined drug analysis study [27]

Compound	Characteristic Peaks	Stability
Forskolin	50 cm^{-1}, 75 cm^{-1}, 98 cm^{-1}	Peaks remained consistent over time
Soy Lecithin	30 cm^{-1}, 55 cm^{-1}, 80 cm^{-1}	Peaks stable throughout the study
Cholesterol	35 cm^{-1}, 60 cm^{-1}, 85 cm^{-1}	Peaks remained consistent
Physical Mixtures	No significant shifts or new peaks observed in FTIR spectra	No major chemical interactions observed, stable peaks over time

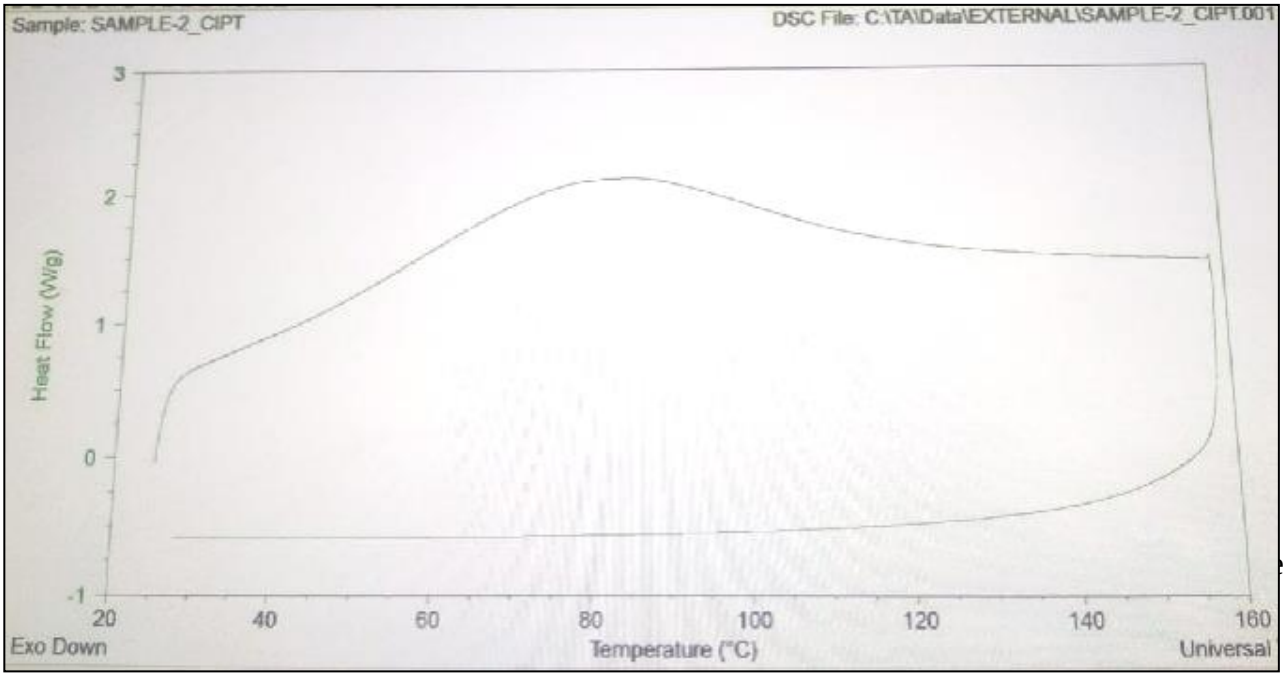

Fig 1: DSC Thermogram of Forskolin Phytosome

DSC of Soy lecithin and Cholesterol were compatible within the liposomal formulation.

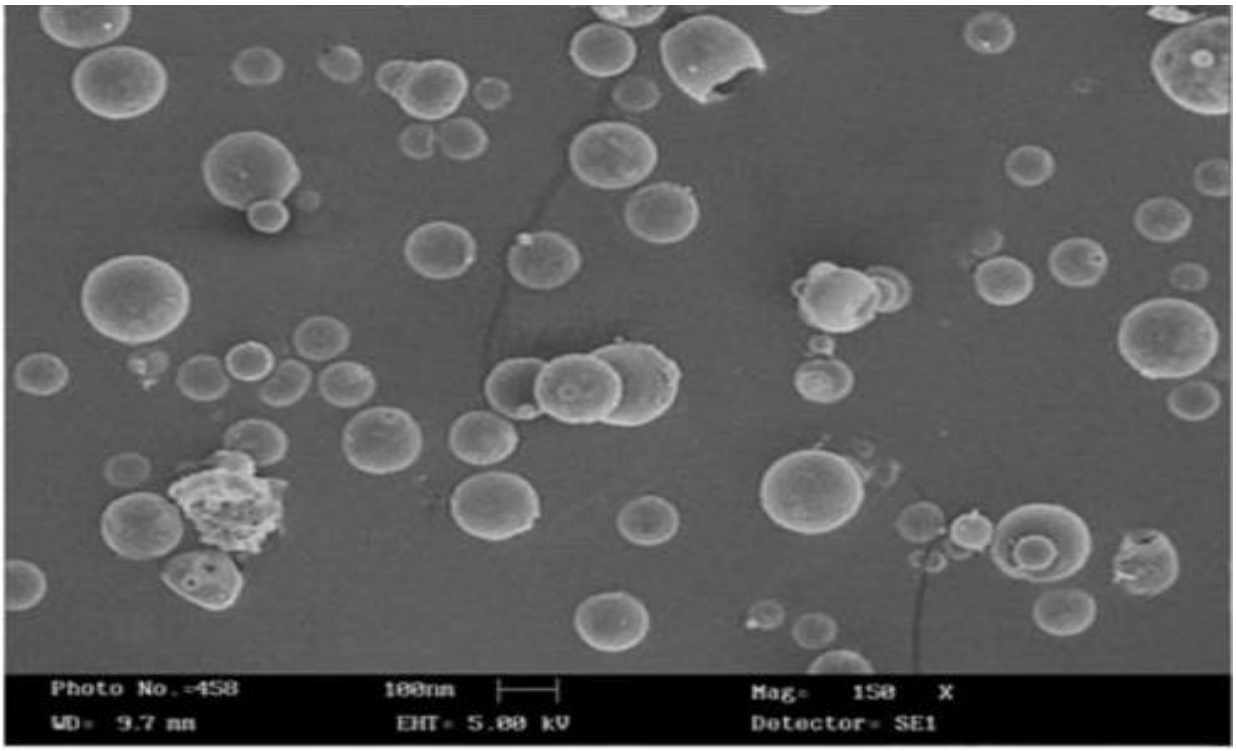

Fig 2: SEM Practical size of forskolin .

Optimization of Sustained Release Formulation: Central composite design and response surface methodology were used to identify optimal formulations.

The quadratic model was selected based on **ANOVA** results and fit statistics [28].

Table 4: Release Data for Different Formulations.

Run	(R1) (Release at 1h %)	(R2)(% Drug released in 8 hours)	R3 (t50%)
1	10	4	81
2	9	4.5	79
3	7	6	74
4	8	5	74
5	12	3.5	85
6	6	6.5	65
7	14	3	71
8	9	5.2	65
9	9	5.3	66
10	8	5.3	67
11	8.5	5.3	62
12	8.5	5.4	64
13	6	6.3	65

Statistical Analysis (ANOVA for Quadratic Model)

The model showed an F-value of 2.31 ($p = 0.14$), indicating model significance and adequacy.

Dissolution studies:

The table provided showed the percentage of dissolution at different time intervals (10, 15, 20, 30, 45, and 60 minutes) for 12 units of the raw drug. The average dissolution rates at each time interval were also provided [29]

Table 5: Dissolution studies of raw drug.

For Raw drug						
	10 MIN	**15 MIN**	**20 MIN**	**30 MIN**	**45 MIN**	**60 MIN**
UNIT 01	50	70	84	89	93	92
UNIT 02	50	73	86	91	89	97
UNIT 03	46	72	84	89	90	95
UNIT 04	56	71	83	88	89	94
UNIT 05	40	75	87	92	94	98
UNIT 06	45	66	82	87	86	93
UNIT 07	37	69	85	90	91	96
UNIT 08	55	89	81	86	94	92
UNIT 09	71	86	82	87	92	93
UNIT 10	65	83	81	86	91	92
UNIT 11	55	77	86	91	95	97
UNIT 12	49	75	78	83	90	89
mean	51.58333	75.5	83.25	88.25	91.16667	94

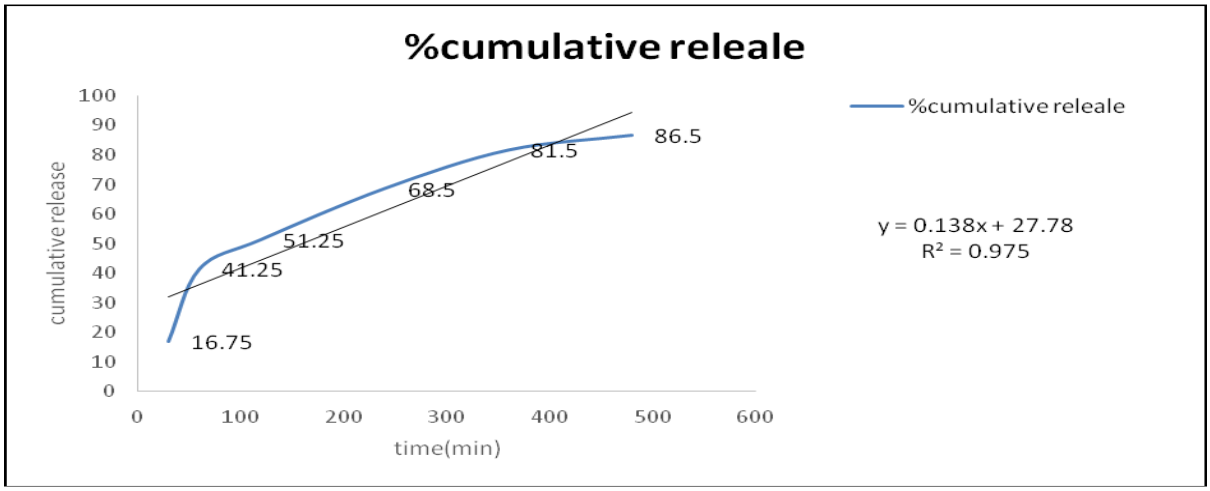

Fig 3: Cumulative drug release for raw drug

Table 6: Dissolution Studies for Phytosome Formulation

Time(min)	F1	F2	F3	F4	F5	F6	F7	F8	F9
30	25.125	9.5475	16.75	13.4	28.475	31.825	10.05	9.246	11.0952
60	61.875	23.5125	41.25	33	70.125	78.375	24.75	22.77	27.324
120	76.875	29.2125	51.25	41	87.125	97.375	30.75	28.29	33.948
240	102.75	39.045	68.5	54.8	99.12		41.1	37.812	45.3744
360		46.455	81.5	65.2			48.9	44.988	53.9856
480		49.305	86.5	69.2			51.9	47.748	57.2976

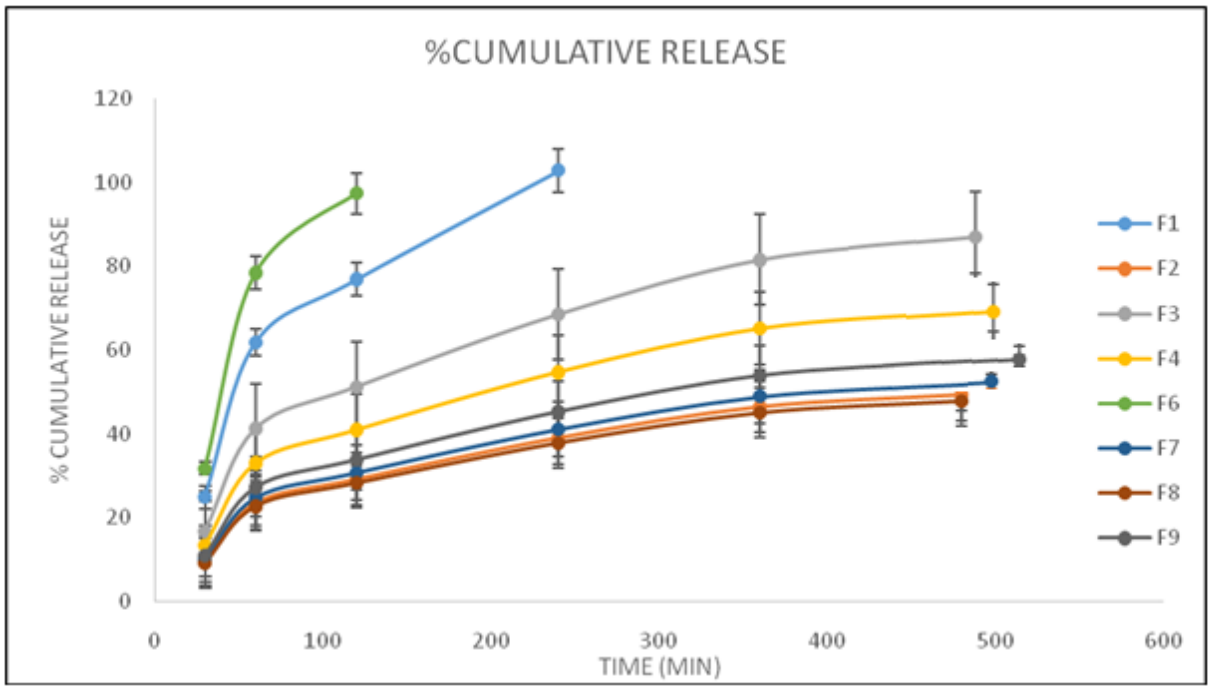

Fig 4: Cumulative Percentage Drug Release VS Time curve for F1-F9 Formulation

The formulation F4 was found to be best. Further studies were conducted on F4 formulation including *invivo* toxicity

Statistical optimization of nanoformulation

At the outset of development, a comprehensive literature review was conducted to gather essential information on potential variables, methodologies, and control strategies necessary for formulating a product with optimal attributes. This foundational knowledge was critical for addressing formulation challenges and achieving the desired product characteristics. To identify

key factors influencing formulation outcomes, potential product and process variables were analyzed using a Plackett-Burman Design (PBD). This approach highlighted significant variablessuch as SoyLecithin content, SoyLecithin concentration, and homogenization speed, based on their substantial p-values in the PBD analysis [30]. These insights were then used to refine the formulation process through a Box-Behnken Design (BBD).The independent variables were controlled within narrow ranges to ensure consistency and validate the process. By incorporating three levels of the selected variables, the study demonstrated versatility in predicting central points (5 points) within the BBD framework [31].

Table 7: Formulation Box-Behnken Design (BBD)

Run	A	B	C	D	E	F	G	H	Particle Size (nm)	Encapsulation efficiency (%)	Zeta Potential
1	1	1	-1	1	1	1	-1	-1	342.3	71	2.4
2	1	-1	1	1	-1	1	1	1	234.1	79	19.7
3	-1	-1	-1	-1	-1	-1	-1	-1	284.7	61	4.2
4	1	1	-1	-1	-1	1	-1	1	300.7	80	2.6
5	1	-1	-1	-1	1	-1	1	1	308.4	73.8	8.2
6	1	1	1	-1	-1	-1	-1	-1	230.2	80.1	10.4
7	-1	1	-1	1	1	-1	1	1	242.7	71.8	8.3
8	-1	1	1	-1	1	1	1	-1	231.9	60.1	9.7
9	-1	-1	-1	1	-1	1	1	-1	398	61.4	10.2
10	1	-1	1	1	1	-1	-1	-1	210.4	70.2	4.3
11	-1	1	1	1	-1	-1	-1	1	218.8	67.1	3.2
12	-1	-1	1	-1	1	1	-1	1	202.4	62.1	2.4

Table 8: Formulation Particle size against Box-Behnken Design (BBD)

Response 1: Particle Size					**Fit Statistics**	
Source	**Sum of squares**	**Mean**	**F-value**	**p-value**		
Model	27324.16	2701.77	180.24	0.000612	Std. Dev.	3.80
PGA Cont.	824.14	843.14	53.30	0.00470	Mean	277.42
PVA %	210.51	210.51	10.40	0.032081	C.V. %	1.33
Hz Speed (rpm)	13120.14	13120.14	874.72	8.18E-03	R^2	0.93
Hz duration	93.74	93.74	6.10	0.08401	Adjusted R^2	0.98
PVA Mw (kDa)	4989.84	4978.82	333.40	0.00032	Predicted R^2	0.90
PGA T-Group	73.50	74.51	5.10	0.108614	Adeq. Prec.	38.70
Residual	43.22	14.70				
Cor Total	47176.51	42				

Note: Hz-Homogenization, Mw-Molecular weight, T-group- Terminal group, Adj-Adjusted, Pred-Predicted, Adeq-Adequate, Prec- Precision .

Table 9: Formulation Zeta Potential in Box-Behnken Design (BBD)

Response 3: Zeta Potential					**Fit Statistics**	
Source	**Sum of squares**	**Mean**	**F-value**	**p-value**		
Model	140.26	17.77	582.25	0.0001	Std. Dev.	0.17
PGA Cont.	1.03	1.03	58.31	0.002	Mean	6.84
PVA %	2.50	2.53	70.41	0.003	C.V. %	2.33
Hz Speed (rpm)	1.22	1.20	51.70	0.0014	R^2	0.98
Hz duration	3.70	3.73	7.7E-04	0.08304	Adjusted R^2	0.99
PVA Mw (kDa)	124.82	122.84	0.0062	0.00031	Predicted R^2	0.98
PGA T-Group	0.08	0.01	45.10	0.108624	Adeq. Prec.	63.22
Residual	44.21	14.70				
Cor Total	318.91	48				

Note: Hz-Homogenization, Mw-Molecular weight, T-group- Terminal group, Adj-Adjusted, Pred-Predicted, Adeq-Adequate, Prec- Precision .

Optimization and Checkpoint Validation: Five optimal formulations were validated, showing similar results to the predicted values with a percentage error within acceptable limits.

A successful method for the preparation of sustained-release *Forskolin* Phytosomes was developed and optimized using design of experiments and response surface methodology.

The optimized formulations exhibited desired drug release profiles, ensuring sustained release and efficient drug delivery [32]

Table 10: Report of Amount of Soy Lecithin VS % Drug released in 1 hour CCD model.

Parameter	**Value**
Standard Deviation (Std. Dev.)	1.79
R^2	0.6226
Adjusted R^2	0.3530
Predicted R^2	-2.2860
Adeq Precision	4.1814
ANOVA	
Significant Terms	None
Lack of Fit F-value	41.22 (significant)

This table captures the essential statistics and analysis results, providing a clear overview of the model's performance and fit.

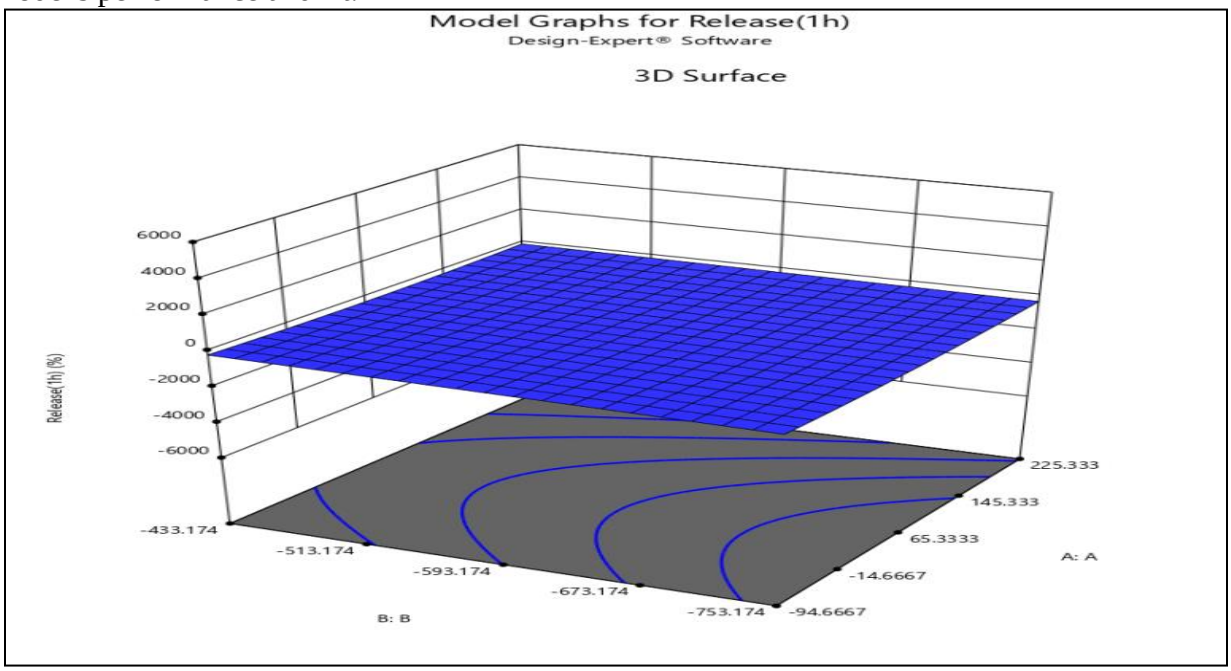

Fig 5: 3D Surface Response curve Amount of Soy Lecithin VS % Drug released in 1 hour.

Table 11: Report of Amount of Soy Lecithin VS % Drug released in 8 hours model.

Parameter	Value
Fit Statistics	
Standard Deviation (Std. Dev.)	0.6898
R^2	0.7419
Adjusted R^2	0.5575
Predicted R^2	-1.1279
Adeq Precision	5.7766
ANOVA	
Model F-value	4.02 (significant)
Significant Terms	A, A^2
Lack of Fit F-value	46.93 (significant)

This table effectively presents the key statistics and analysis results for the t50% model, highlighting the significant terms and the model's overall fit.

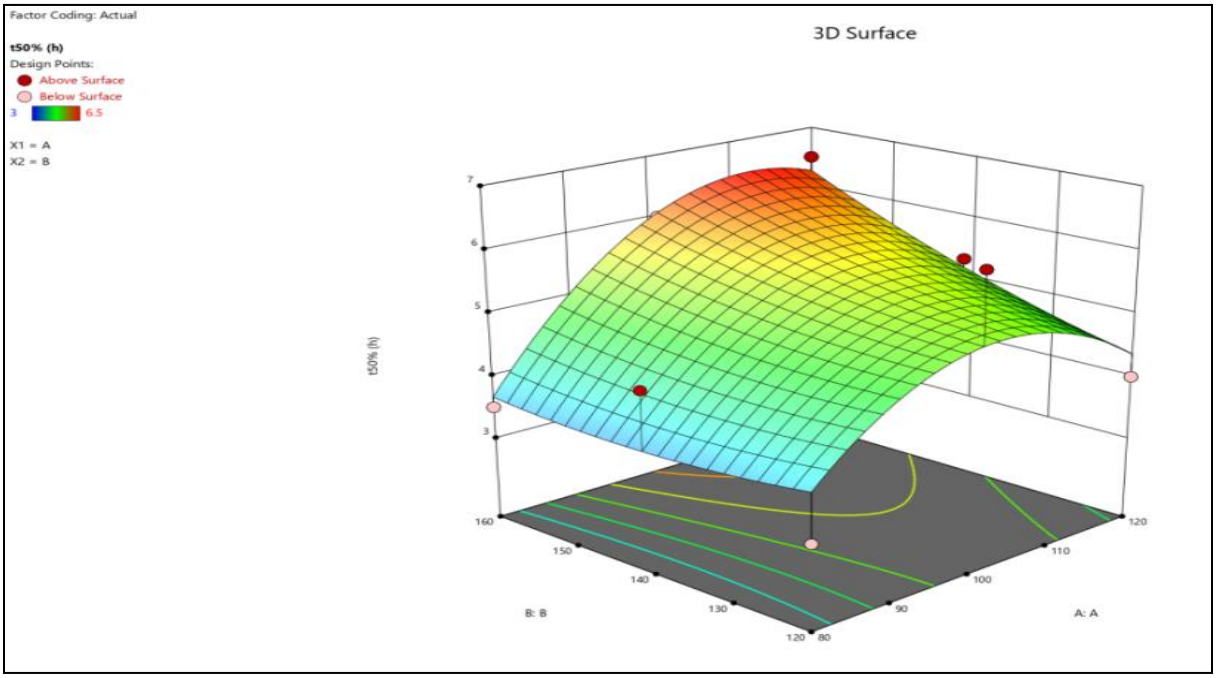

Fig 6: 3D Surface Response curve Amount of Soy Lecithin VS % Drug released in 8 hours.

Table 12: Report of Amount of Soy Lecithin VS Time to 50% drug release (t50%)

Parameter	Value
Fit Statistics	
R^2	0.6340
Adjusted R^2	0.6340
Predicted R^2	-0.0396

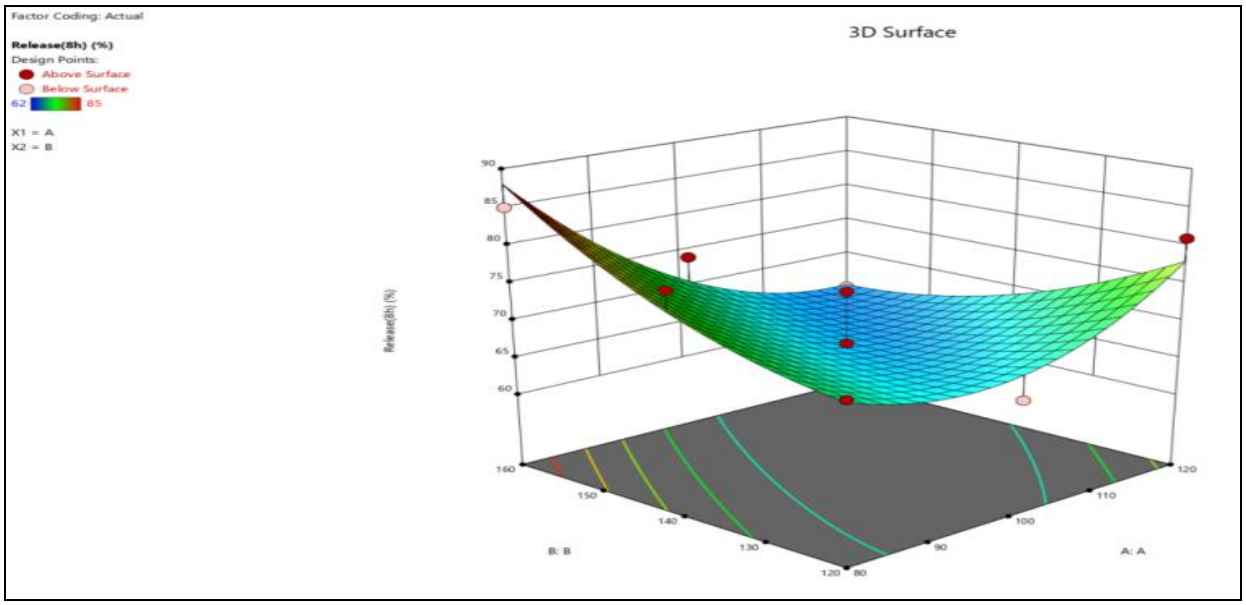

Fig7: 3D Surface Response curve Amount of Soy Lecithin VS Time to 50% drug release (t50%).

The models for release **(1h)** and **t50%** have not significant lack of fit, indicating poor model fit. Significant terms in the **t50%** model are A and A^2.

Particle size analysis:

This table provides a clear and concise summary of the Zeta Potential measurements and distribution data for the particle size analysis.

Table 13: Particle size analysis study report

Parameter	Value
Zeta Potential Measurements	
Zeta Potential (mV)	3.59
Zeta Deviation (mV)	11.9
Conductivity (mS/cm)	3.14
Result Quality	Good
Zeta Potential Distribution	
Peak 1	
Mean (mV)	3.59
Area (%)	100.0
Standard Deviation (mV)	11.9
Peak 2	
Mean (mV)	0.00
Area (%)	0.0
Standard Deviation (mV)	0.0
Peak 3	
Mean (mV)	0.00
Area (%)	0.0
Standard Deviation (mV)	0.0

This table provides a clear and concise summary of the Zeta Potential measurements and distribution data for the particle size analysis.

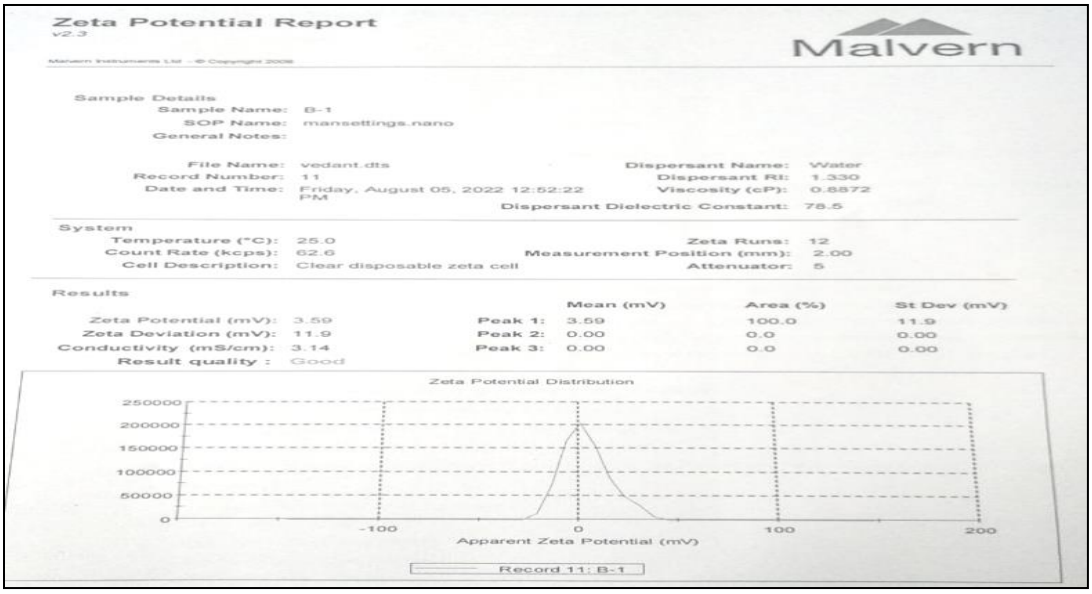

Fig 8: Zeta Potential measurements study Report with distinct Curve

Zeta Potential measurements Report Summary:

A comprehensive overview of the zeta potential measurements for the sample "B-1"(F4 - Formulation) was provided using a Malvern Instruments Ltd. device. The results indicated a zeta potential of 3.59 mV with good result quality, and the distribution showed a single peak, indicating a homogeneous sample [33]

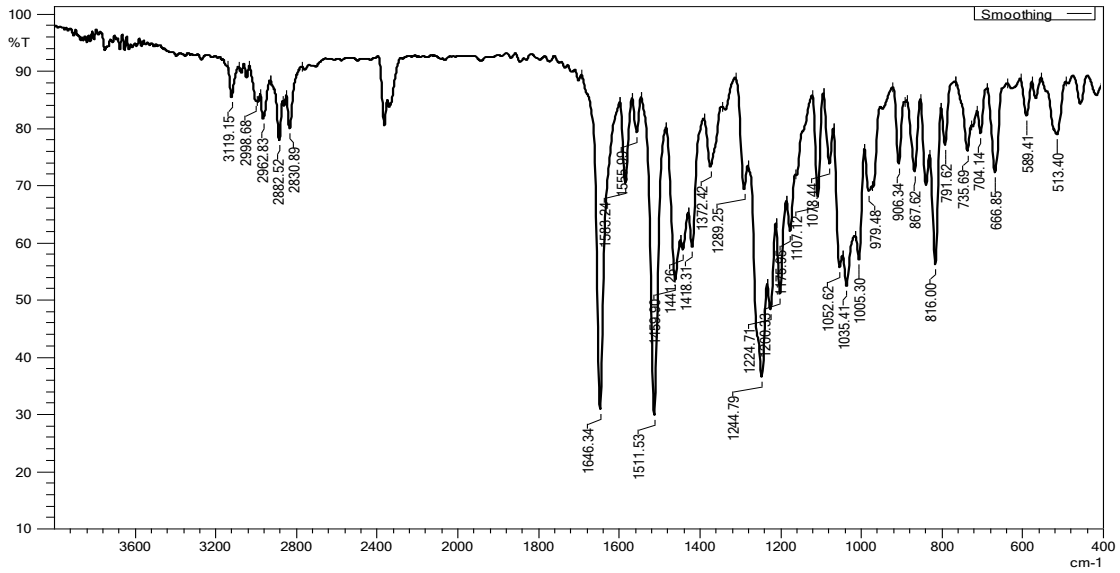

Fig.9 . FTIR analysis drug

From the above graph shows that peaks around 1600-1550 cm^{-1} (C=C stretching), absorption peak around 1650-1700 cm^{-1} (C=O stretching). 1600-1550 cm^{-1} shows imidazole ring. The presence of aliphatic chains in absorption bands in the range of 3000-2800 cm^{-1}, shows methyl and methylene group in there [34].

Table -14 FTIR Function group

Functional Group	**Standard spectra**	**Observed spectra**
(C=C stretching),	1600-1550 cm^{-1}	1555.99 cm^{-1}
C=O stretching	1650-1700 cm^{-1}	1646.34 cm^{-1}
aliphatic chains	3000-2800 cm^{-1},	2830.39 cm^{-1}

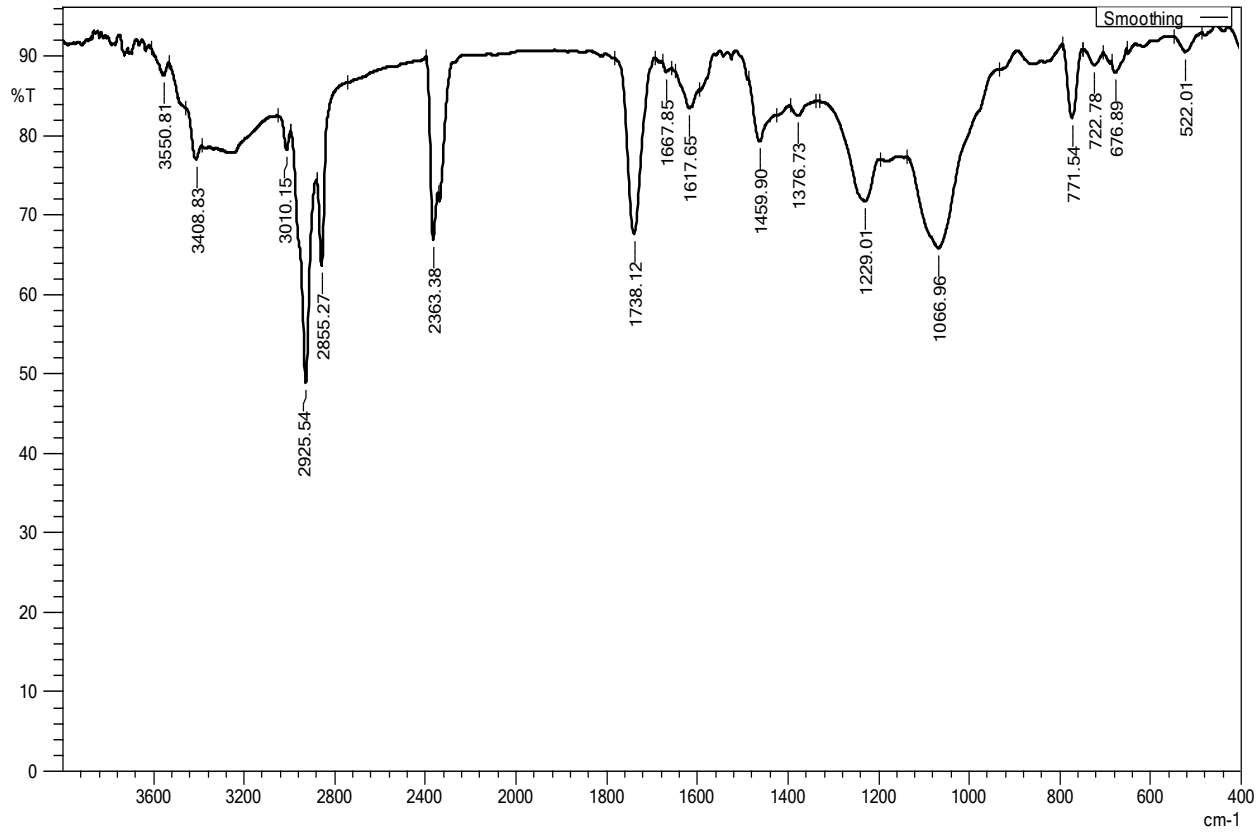

Fig.10 . FTIR analysis of drug and polymer

From the above graph shows that peaks around peaks in the range of 3000-2800 cm^{-1}: Correspond to C-H stretching vibrations in the fatty acid , peaks near 1100-1000 cm^{-1} corresponds to C-O stretching vibrations in the glycerol backbone of the phospholipids [35].

Table 15: FTIR data of drug and polymer

Functional Group	Standard spectra	Observed spectra
C=H stretching	3000-2800 cm^{-1}	2855.27 cm^{-1}
C-O stretching	1100-1000 cm^{-1}	1066.96 cm^{-1}

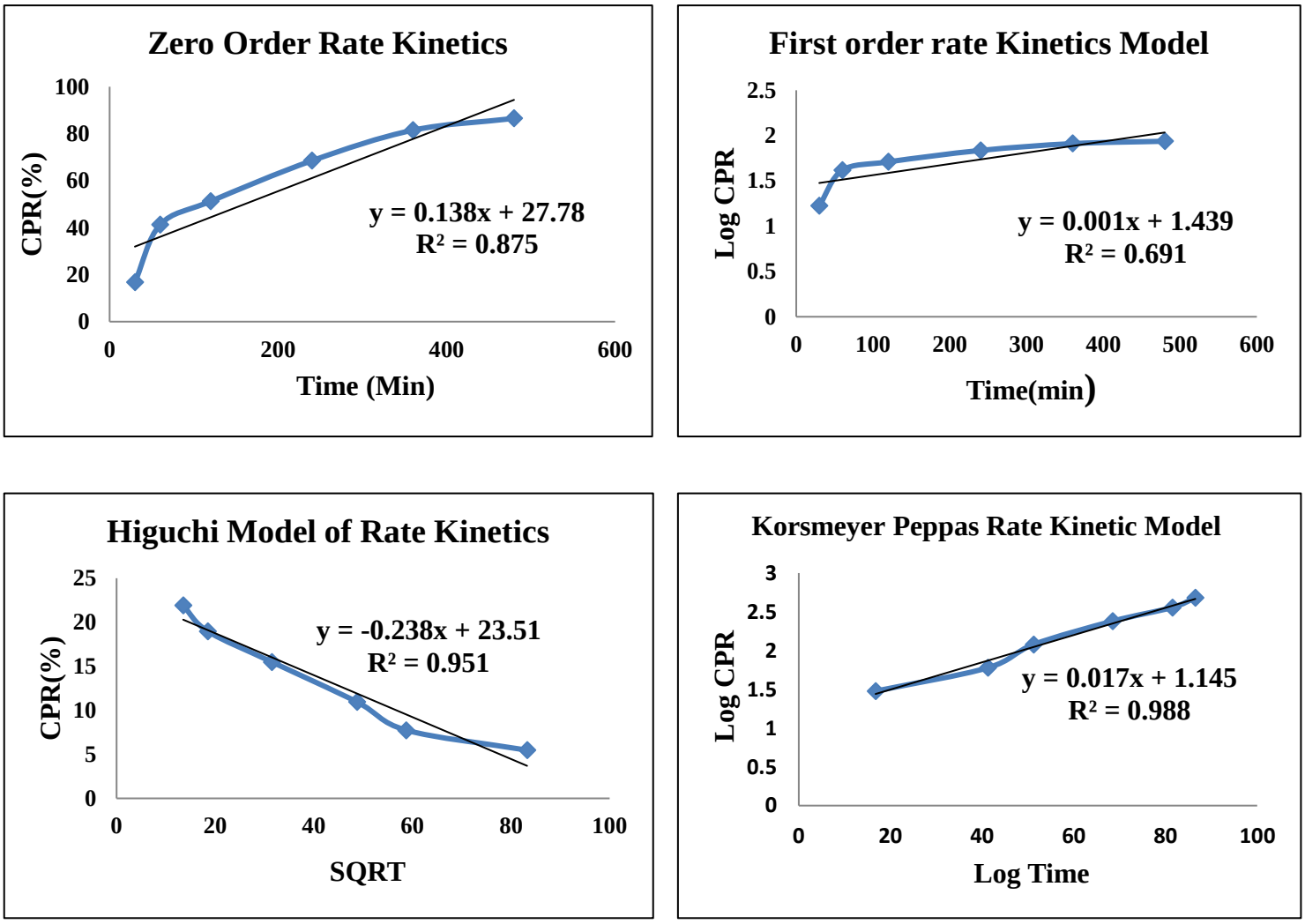

Fig 11 : Pharmacokinetics Study Model

Table 16: Rate Kinetics of the *Forskolin* Phytosome

Rate Kinetics Model	R^2
Zero Order Rate Kinetics	0.875
First order rate Kinetics Model	0.691
Higuchi Model of Rate Kinetics	0.951
KorsmeyerPeppas Rate Kinetic Model	**0.988**

Based on the provided R^2 values for the different rate kinetics models, your formulation of the Forskolin Phytosome appears to follow the Korsmeyer-Peppas rate kinetic model the best. The R^2 value is a measure of how well the model fits the experimental data, with a value closer to 1 indicating a better fit. Given that the Korsmeyer-Peppas model has the highest R^2value (0.978),

it indicates that this model most accurately describes the rate kinetics of your Forskolin Phytosome formulation. The Korsmeyer-Peppas model is often used to describe drug release from polymeric systems when the release mechanism is not well known or is complex, which could be the case for your formulation. This formulation follows the Korsmeyer-Peppas rate kinetic model because it has the highest R^2 value (0.978), suggesting the best fit compared to the other models tested .[36]

Summary of sub-chronic toxicity study Analysis in mice

Body weight *Gain:* A sub-chronic oral toxicity study was conducted for F3 formulation over 28 days with swiss albino mice divided into four dose groups: Control (0 mg/kg), Low (12.5 mg/kg), Middle (25 mg/kg), and High (50 mg/kg). Each group consisted of 6 male and 6 female mice. Bodyweights were monitored and recorded at 7-day intervals (days 0, 7, 14, 21, and 28).

The results indicated:

Control Group (0 mg/kg): Mice maintained normal bodyweight gain.

Low Dose Group (12.5 mg/kg): Mice showed normal bodyweight gain.

Middle Dose Group (25 mg/kg): Mice exhibited normal bodyweight gain.

High Dose Group (50 mg/kg): Mice demonstrated normal bodyweight gain.

There was no significant reduction in bodyweight gain across any dose groups, indicating that all animals gained weight normally throughout the study period .

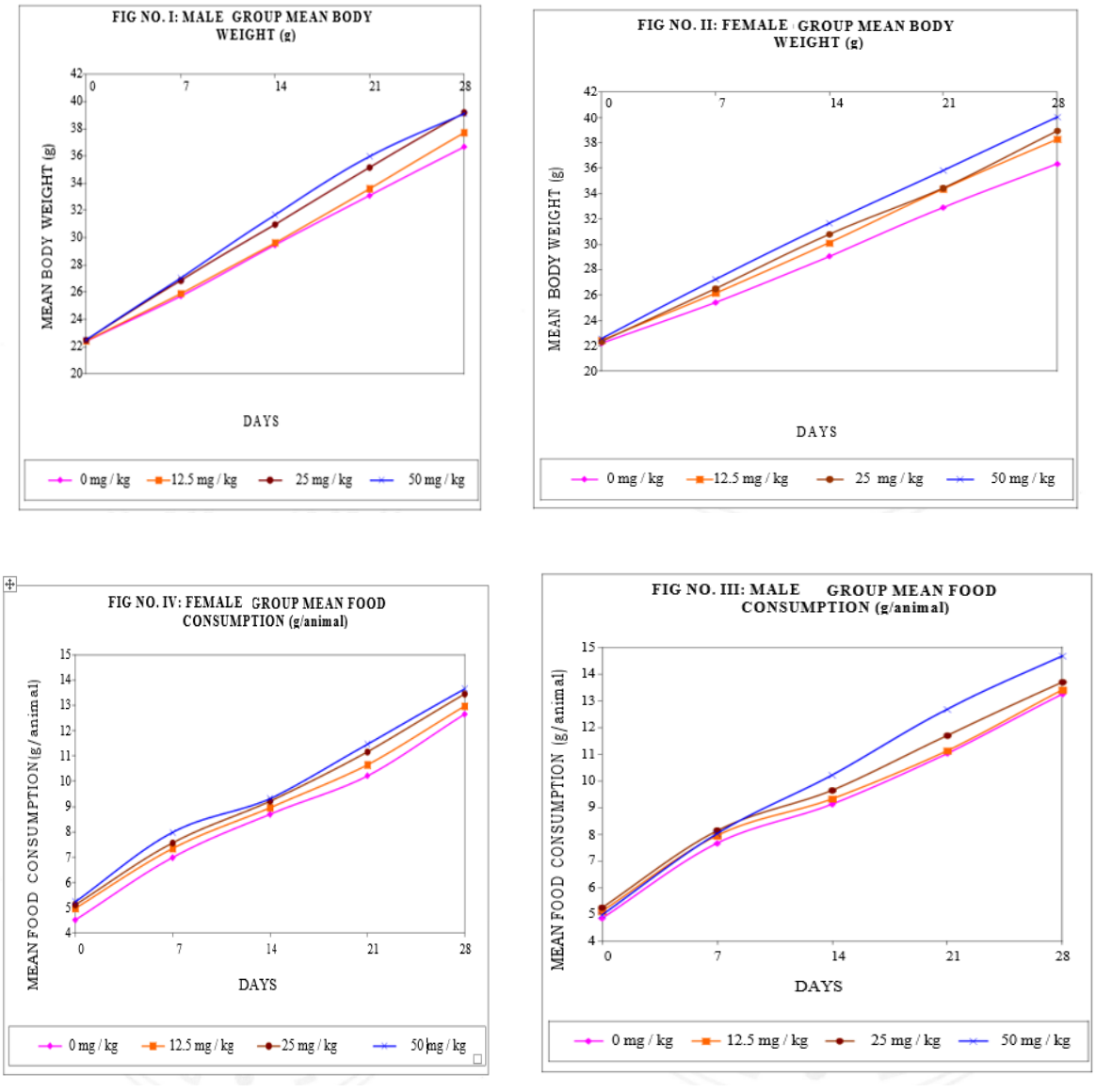

Fig 12: Summary of sub-chronic toxicity study Analysis in mice.

Haematological Analysis: Haematological data were collected and analyzed from 24 mice (Control, Low, Middle, and High dose groups) after the 28-day dosing period. On the 29th day, blood samples were taken from the retro-orbital plexus and analyzed using a Medonic Cell

counter [37]. Parameters measured included total count, differential count, platelets, and WBC. Key findings were:

Control Group: Haematological parameters were within the normal range.

Low, Middle, and High Dose Groups: Haematological parameters remained within normal limits.

The analysis indicated no adverse reactions in the hematological parameters of any animals due to the test drug.

Biochemical Analysis

Biochemical parameters were evaluated after 28 days of test item administration. On the 29th day, blood samples were collected from the retro-orbital plexus, and serum was analyzed using a Microlab 300 semi-auto analyzer for parameters such as SGPT, SGOT, Total Serum Protein, BUN, and blood sugar. Findings included:

Control Group: Biochemical parameters were within normal ranges.

Low, Middle, and High Dose Groups: Biochemical parameters were within permissible limits.

The results concluded that the test product had no adverse effect on the biochemical parameters of the mice across all dose groups [38].

Overall, the study demonstrated that the test item did not cause significant changes in body weight, hematological, or biochemical parameters, indicating its safety under the conditions tested [38].

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CONCLUSION

The formulation F4 was identified as the optimal formulation based on its superior performance in various evaluations. Detailed studies on F4, including in vivo toxicity assessments, confirmed its safety and effectiveness. The optimization process, supported by design of experiments and response surface methodology, led to the successful development of sustained-release Forskolin Phytosomes. These optimized formulations demonstrated the desired drug release profiles, ensuring sustained release and efficient drug delivery.

Further analysis, including the validation of five optimal formulations, showed consistency between the predicted and observed values, with percentage errors within acceptable limits. This highlights the reliability of the optimization process used in this study. Key statistical models, such as the Korsmeyer-Peppas rate kinetic model, best described the release kinetics of the Forskolin Phytosome formulation, with an R^2 value of 0.988. This suggests that the model provides the most accurate prediction of drug release behavior, ensuring that the formulation meets the desired therapeutic goals. The sub-chronic toxicity studies in mice revealed no significant adverse effects, with normal body weight gain, hematological, and biochemical parameters across all dose groups. These findings further support the safety of the F3 formulation.In summary, the F4 formulation was successfully developed and optimized, showing excellent drug release profiles and safety, making it a promising candidate for sustained-release drug delivery systems.

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