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"2^3 Full Factorialdesigns for The Formulation and Optimization of Lipid Vesicle System Containing Pipemidic Acid Using Design of Experiments"

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ABSTRACT
The present study focused on the formulation and optimization of a Pipemidic acid-loaded liposome system using a Box–Behnken design combined with Response Surface Methodology (RSM). Pre-formulation studies confirmed that Pipemidic acid is a white to off-white crystalline powder with slight alkalinity (pH 8.1), thermally stable (melting point 249 °C), and sparingly soluble in water but freely soluble in organic solvents, supporting its incorporation into lipid vesicles. The effects of soya lecithin concentration, cholesterol concentration, and sonication time on particle size and entrapment efficiency were systematically evaluated. Statistical analysis indicated that a linear model best described the responses. The optimized formulation, consisting of 500 mg soya lecithin, 125 mg cholesterol, and 60 minutes of sonication, showed a particle size of 259.2 nm, entrapment efficiency of 93.44%, and a zeta potential of –31.7 mV, confirming good colloidal stability. *In-vitro* drug release studies demonstrated sustained release over 14 hours with 92.39% cumulative release, following Zero-order kinetics (R^2 = 0.968). The study confirmed that the DoE-based optimized liposome formulation is suitable for controlled drug delivery, offering enhanced encapsulation, stability, and prolonged therapeutic action for Pipemidic acid.

Keywords: Pipemidic acid, Liposomes, Particle size, Entrapment efficiency, *In-vitro* drug release.

I. INTRODUCTION

Liposomes are colloidal particles formed as concentric bio molecular layers that are capable of encapsulating drugs. They are lipid vesicles that fully enclose an aqueous volume. These lipid molecules are usually phospholipids with or without some additives. Cholesterol may be included to improve bilayer characteristics of Liposomes, for increasing micro viscosity of the bilayer, for reducing permeability of the membrane to water soluble molecules, for stabilizing the membrane and for increasing rigidity of the vesicle **(Nsairat*et al.*, 2024).**The liposomes help the drug penetrate the cancer cells more selectively and decrease the possible side effects (nausea, hair loss and vomiting). Liposomes possess advantage over others are enhanced solubility of drug which is encapsulated, prevent the drug from biological and chemical degradation, decrease the toxic effect of drug, enhance therapeutic index and efficiency of drug entities, compatibility of drug increases with non-toxic and biodegradable materials **(Sharma *et al.*, 2021).**The separation of inner core from outer phase improves the stability of the drug enclosed inside the formulation. Encapsulation of drug leads to gain controlled or sustain release of drug. The bioavailability of poorly soluble drugs can be improved using this technique. The change in the biological characters can be achieved by modifying the ligands on the surface of liposomes **(Natarajan *et al.*, 2014).**

Pipemidic acid is a synthetic antibacterial agent belonging to the quinolone class, commonly used in the treatment of urinary tract infections. Despite its therapeutic effectiveness, its clinical utility is limited by poor aqueous solubility, rapid elimination, and variable bioavailability **(Ngoepe*et al.*, 2021).**These challenges necessitate the development of an optimized drug delivery system that can enhance its therapeutic performance. Incorporation of pipemidic acid into lipid vesicle systems may improve its pharmacokinetic profile, provide sustained drug release, and enhance its antibacterial efficacy**(Skwarczynski*et al.*, 2022).**
Incorporation of pipemidic acid into a lipid vesicle system offers a promising strategy to overcome these challenges. Encapsulation within lipid vesicles can improve the solubility of the drug, protect it from degradation, and provide sustained and controlled release. Furthermore, vesicular systems may enhance drug accumulation at the site of

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infection, thereby increasing antibacterial efficacy while minimizing systemic side effects. Such systems also have the potential to improve patient compliance by reducing dosing frequency **(Pancani*et al.,* 2018).**

The formulation and optimization of such complex delivery systems require a systematic and scientific approach. Design of Experiments (DoE) is a powerful statistical tool that enables the evaluation of multiple formulation variables simultaneously, reducing the number of experimental trials while providing reliable and reproducible results**(Politis*et al.,* 2017).**Among various experimental designs, the 2^3 full factorial design is widely used for optimization studies, as it allows the investigation of three independent variables at two levels each. This design not only identifies the main effects of each factor but also evaluates interaction effects between variables, thereby providing a comprehensive understanding of the formulation process **(Jankovic *et al.,* 2021).**In the context of lipid vesicle formulation, factors such as lipid concentration, surfactant ratio, and processing conditions significantly influence critical quality attributes like vesicle size, entrapment efficiency, and drug release profile. By applying a 2^3 full factorial design, it is possible to systematically optimize these variables to achieve an ideal formulation with desired characteristics**(Javed*et al.,* 2022).**

Therefore, the present study focuses on the formulation and optimization of a lipid vesicle system containing pipemidic acid using a 2^3 full factorial design. The objective is to develop a stable and efficient vesicular drug delivery system with enhanced entrapment efficiency, controlled release behavior, and improved therapeutic potential.

II. MATERIAL AND METHODS

2.1 Chemicals

Pipemidic acid was procured from Yarrow Chem Products, Mumbai, India, and used as the model drug for lipid vesicle formulation. Phosphatidylcholine (soy lecithin), obtained from Lipoid GmbH (India Pvt. Ltd.), Mumbai, served as the primary lipid for vesicle formation, while cholesterol from HiMedia Laboratories Pvt. Ltd., Mumbai, was used as a membrane stabilizer. Surfactants such as Tween 80 (Merck Life Science Pvt. Ltd., India) and Span 60 (HiMedia Laboratories Pvt. Ltd., Mumbai) were incorporated to enhance vesicle stability and modulate vesicle size. Chloroform and methanol, both procured from Merck Life Science Pvt. Ltd., India, were used as organic solvents for lipid dissolution and formulation processes. Phosphate buffer saline (PBS), obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, was used as the hydration medium and for release studies.

2.2 Pre-formulation studies

Pre-formulation studies of drug were conducted to evaluate its basic physical and chemical characteristics prior to formulation development. The studies included assessment of solubility, melting point, pH, and stability of drug. Drug–excipient compatibility studies were performed to ensure the absence of any undesirable interactions. UV spectrophotometry was used to assess drug purity and for analytical method development. FTIR spectroscopy was used to identify functional groups and to study possible interactions between drug and excipients. The data obtained from these studies aided in understanding the drug's behavior and stability. The results guided the selection of suitable excipients and formulation components for developing stable and effective lipid vesicle system**(Vilegave*et al.,* 2013).**

2.2.1 Organoleptic Properties

A small amount of Pipemidic Acid was examined on a clean watch glass for its color, odor, and physical appearance. The sample was visually inspected under daylight, its odor was assessed by gentle wafting, and its texture (crystalline or powder form) was noted. All observations were recorded systematically**(Fantini*et al.,* 2020).**

2.2.2 Solubility study

Pipemidic acid (10 mg) was added to test tubes containing 1 mL of different solvents (methanol, ethanol, chloroform, distilled water, and DMSO), mixed thoroughly, and allowed to stand for 24 hours with intermittent shaking. The solutions were then visually examined for solubility, and the results were recorded as soluble, sparingly soluble, slightly soluble, or insoluble**(Ansari *et al.,* 2023).**

2.2.3 Melting Point Analysis

A small amount of pipemidic acid (2–3 mg) was powdered and filled into a capillary tube, which was then placed in a melting point apparatus. The sample was heated gradually, and the temperatures at the onset and complete melting were recorded. The procedure was repeated three times, and the average melting point was calculated and compared with literature values to assess purity**(McCullough *et al.,* 1957).**

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2.2.4 pH determination
A solution of pipemidic acid (1 mg/mL) was prepared in distilled water, and the pH was measured using a calibrated digital pH meter (buffers pH 4.0, 7.0, and 9.0). The electrode was immersed in the solution, readings were recorded after stabilization, and the measurement was repeated three times to obtain the average pH, which was compared with the expected range for formulation suitability**(Avnir*et al.*, 2005).**

2.2.5 Determination of Maximum Wavelength (λmax)

2.2.5.1 Preparation of Pipemidic Acid Standard Stock Solution in Methanol
Pipemidic acid (10 mg) was dissolved in methanol and diluted to 10 mL to prepare a 1000 µg/mL stock solution. From this, 1 mL was further diluted to 10 mL with methanol to obtain a 100 µg/mL working solution. The prepared solutions were stored in amber-colored vials to protect from light and ensure stability**(Mestre *et al.*, 2001).**

2.2.5.2 Determination of λmax of Pipemidic Acid
A standard solution of pipemidic acid (10 µg/mL) was prepared in methanol, and methanol was used as the blank. The UV–Visible spectrophotometer was set to scan between 200–400 nm after proper calibration. The sample was scanned in a quartz cuvette, and the wavelength of maximum absorbance (λmax) was recorded. The measurement was repeated three times for accuracy and used for further analysis**(Iacovino*et al.*, 2013).**

2.2.5.3 Linearity and Calibration Curve
A 100 µg/mL stock solution of pipemidic acid was prepared in methanol and further diluted to obtain working standards (2–12 µg/mL). The absorbance of each solution was measured at the predetermined λmax using methanol as blank. A calibration curve of absorbance versus concentration was plotted, and linear regression analysis was performed to determine the equation and correlation coefficient (R^2), ensuring linearity for quantitative analysis**(Moosavi*et al.*, 2018).**

2.2.6 Functional group identified by FTIR
A small amount of pipemidic acid (2–3 mg) was mixed with potassium bromide and compressed into a thin pellet for FTIR analysis. The sample was scanned in the range of 4000–400 cm^{-1}, and the spectrum was recorded to identify characteristic functional group peaks. The observed peaks were compared with literature values to confirm the identity and purity of the drug**(Enders *et al.*, 2021).**

2.3 Preparation of Liposomes formulation by DoE
Pipemidic acid-loaded liposomes were prepared by the thin film hydration method using a rotary evaporator. The drug, soya lecithin, and cholesterol were dissolved in a chloroform–ethanol mixture and evaporated at 40°C to form a thin lipid film. This film was hydrated with phosphate buffer (pH 6.8) to obtain a milky liposomal suspension, which was then sonicated to reduce particle size. The formulation was centrifuged to separate unentrapped drug, and the collected liposomes were evaluated for particle size and entrapment efficiency, with the optimized formulation stored for further studies**(Lonkar, 2016).**

Table 1: Composition of Liposome formulation by DoE

Formulation Code	Pipemidic Acid (mg)	Factor 1 Soya Lecithin (mg)	Factor 2 Cholesterol (mg)	Chloroform (ml)	Ethanol (ml)	Buffer pH 6.8 (ml)	Factor 3 Sonication Time (min)
LF 1	200	350	50	8.0	2.0	10.0	60
LF 2	200	500	50	8.0	2.0	10.0	40
LF 3	200	500	125	8.0	2.0	10.0	60
LF 4	200	500	125	8.0	2.0	10.0	20
LF 5	200	350	200	8.0	2.0	10.0	20
L F 6	200	500	200	8.0	2.0	10.0	40
LF 7	200	200	50	8.0	2.0	10.0	40
LF 8	200	200	200	8.0	2.0	10.0	40
LF 9	200	200	125	8.0	2.0	10.0	60
LF 10	200	200	125	8.0	2.0	10.0	20
LF 11	200	350	200	8.0	2.0	10.0	60
LF 12	200	350	50	8.0	2.0	10.0	20

2.4 Evaluation parameter of drug loaded liposome formulation

2.4.1 Physical Appearance

The drug-loaded liposomal formulation was visually evaluated for color, odor, and overall appearance, including clarity, uniformity, and absence of aggregation or phase separation, to ensure its quality and acceptability**(Maritim*et al.*, 2021).**

2.4.2 Size Distribution

The liposomal formulation was diluted with distilled water or buffer and analyzed using a DLS/Zeta sizer to determine particle size, polydispersity index (PDI), and size distribution. Multiple readings were taken, and results were recorded, with vesicle size below 200 nm and PDI < 0.3 indicating a uniform and optimized formulation**(Ramachandran *et al.*, 2011).**

2.4.3 Zeta potential

The liposomal formulation was diluted with distilled water or buffer and analyzed using a zeta sizer to determine zeta potential. The sample was introduced into the electrokinetic cell, and multiple readings were taken to record the mean zeta potential and distribution. Values greater than +30 mV or less than –30 mV indicated good physical stability of the formulation**(Lunardi *et al.*, 2021).**

2.4.4 Scanning Electron Microscopic (SEM)

A small amount of the liposomal formulation was placed on a metal stub, dried under vacuum, and coated with gold or platinum to ensure conductivity. The sample was then analyzed using a scanning electron microscope to observe vesicle shape, size, and surface characteristics. Micrographs were captured at different magnifications to assess morphology and detect any aggregation or deformation**(Datye, 2023).**

2.4.5 Entrapment efficiency

Take a known volume of the drug loaded liposome formulation. Separate the unencapsulated (free) drug by centrifugation at 10,000–15,000 rpm for 30–60 minutes. Collect the supernatant containing the free drug. Analyze the amount of free drug in the supernatant
using a UV–Visible spectrophotometer at the λmax of Pipemidic Acid. Determine the Entrapment Efficiency using the formula:

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

Perform the measurement in triplicate for accuracy and reproducibility. Record the EE% along with mean ± standard deviation. Compare EE% values for different formulations to optimize lipid concentration, surfactant ratio, and preparation method**(Lv*et al.*, 2018).**

2.4.6 *In-vitro* drug release

The in-vitro drug release of Pipemidic acid-loaded liposomes was evaluated using the dialysis bag diffusion method in phosphate buffer (pH 7.4) at 37 ± 2 °C with constant stirring. Samples were withdrawn at specific intervals, replaced with fresh buffer, and analyzedspectrophotometrically at 250 nm. The release data were fitted to kinetic models such as zero-order, first-order, and Higuchi to understand the drug release behavior and mechanism**(Abbasnezhad*et al.*, 2022).**

Drug release from the liposomal formulation was analyzed using various kinetic models.

In zero-order kinetics, cumulative drug release is directly proportional to time, indicating a constant release rate.

$$A_t = A_0 - K_0 t$$

First-order kinetics describes a concentration-dependent release, where a plot of log cumulative drug remaining versus time gives a straight line.

$$\log C = \log C_0 - K_t / 2.303$$

The Higuchi model explains drug release as a diffusion-controlled process, with release proportional to the square root of time.

$$Q = [D\text{€} / \tau \, (2A - \text{€}Cs) \, Cst\,]^{1/2} \text{.......................... eq (4)}$$

The Korsmeyer–Peppas model

Korsmeyer et al. (1983) derived a simple relationship which described drug release from a polymeric system equation (5).

$$(Mt/M\infty = Kt^n)$$

Where Mt / M∞ are a fraction of drug released at time t, k is the release rate constant and n is the release exponent.

III. RESULTS AND DISCUSSION

3.1 Pre-formulation study of Pipemidic Acid

3.1.1 Organoleptic evaluation

Table 2: Organoleptic evaluation of Pipemidic Acid

Physical parameter	Observation
Color	White to off-white or pale yellow

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Odor	Odorless to slightly characteristic
State	Crystalline powder
Appearance	Fine, free-flowing powder

3.1.2 Determination of pH and Melting Point

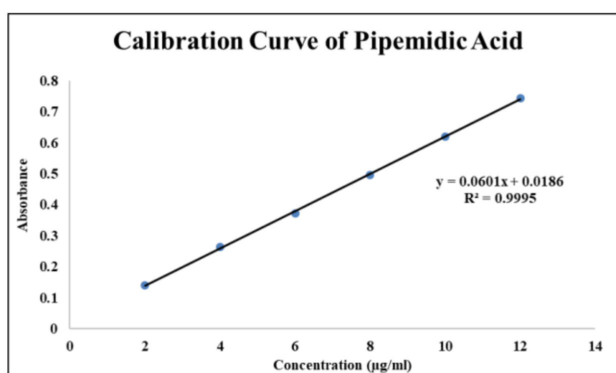
Table 3: pH and Melting Point determination

Drugs	Observed (Melting point)	Reference (Melting point)	Observed (pH)
Pipemidic Acid	249 ^{0}C	251-255°C	8.1

3.1.3 Standard calibration curve

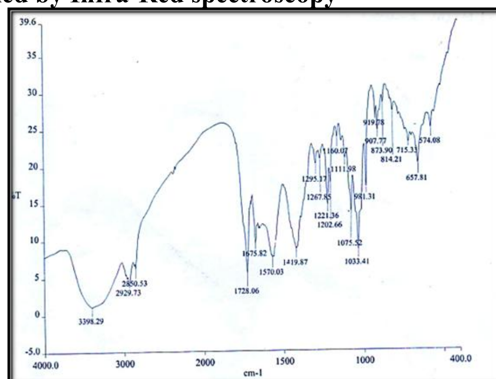
Table 4: Calibration Curve of Pipemidic Acid in Methanol

S. No	Concentration (µg/ml)	Mean Absorbance
1	2	0.140
2	4	0.265
3	6	0.372
4	8	0.496
5	10	0.620
6	12	0.744
Mean		**0.4395**
SD		**0.225033**
%RSD		**51.252**



Graph 1: Calibration curve of Pipemidic Acid

3.1.4 Functional group identified by Infra-Red spectroscopy



Graph 2: FTIR study of Pipemidic Acid

Table 5: Interpretation of IR spectrum of Pipemidic Acid

Peak obtained	Reference peak	Functional group	Name of functional group
3398.29	3400-3300	N-H Stretching	Aliphatic primary amine
2929.73	3000-2840	C-H Stretching	Alkane
1728.06	1740-1720	C=O Stretching	Aldehyde
1675.82	1690-1640	C=N Stretching	Imine / oxime

1570.03	1650-1566	C=C Stretch	Cyclic alkene

3.2 Build Information

Table 6: Build information of DOE software

File Version	12.0.1.0		
Study Type	Response Surface	**Subtype**	Randomized
Design Type	Box-Behnken	**Runs**	12
Design Model	Quadratic	**Blocks**	No Blocks

3.2.1 Formulation trials as per Box–Behnken design

Table 7: Formulation trials as per Box–Behnken design

Formulation code	Soya lecithin	Cholesterol	Sonication time	PS	EE
LF 1	350	50	60	238.23	89.56
LF 2	500	50	40	453.31	82.22
LF 3	500	125	60	255.8	92.39
LF 4	500	125	20	608.45	71.52
LF 5	350	200	20	588.63	66.51
L F 6	500	200	40	404.56	79.33
LF 7	200	50	40	417.27	80.02
LF 8	200	200	40	376.32	77.53
LF 9	200	125	60	220.41	89.4
LF 10	200	125	20	562.74	68.36
LF 11	350	200	60	198.11	90.62
LF 12	350	50	20	596.29	68.77

3.2.2 Limits of Variables (Constraints)

Table 8: Variables operating range for Liposome formulation

Name	Goal	Lower Limit	Upper Limit	Importance
A: Soya lecithin	is in range	200	500	3
B: Cholesterol	is in range	50	200	3
C: Sonication time	is in range	20	60	3
B1 (Particle size)	none	198.11	608.45	3
B2 (Entrapment efficiency)	none	66.51	92.39	3

3.2.3 Fit Summary

Table 9: Response 1: Particle size

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**	**0.9946**	**0.9911**	**Suggested**
2FI	0.6012	0.9938	0.9838	
Quadratic	0.7433	0.9916	0.9631	Aliased

3.3 Effect of formulation variables on particle size (ANOVA for Linear model) and Entrapment efficiency

3.3.1 Response 1: particle size

Table 10: Response 1: particle size (ANOVA for linear model)

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	2.655E+05	88495.90	671.88	< 0.0001	significant
A-Soya lecithin	2641.92	2641.92	20.06	0.0021	
B-Cholesterol	2362.59	2362.59	17.94	0.0029	
C-Sonication time	2.605E+05	2.605E+05	1977.64	< 0.0001	
Residual	1053.71	131.71			
Cor Total	2.665E+05				

Factor coding is Coded. Sum of squares is Type III – Partial.

The Model F-value of 671.88 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

Table 11: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	Standard Error	95% CI Low	95% CI High	VIF
Intercept	410.01	3.31	402.37	417.65	
A-Soya lecithin	18.17	4.06	8.82	27.53	1.0000
B-Cholesterol	-17.19	4.06	-26.54	-7.83	1.0000
C-Sonication time	-180.45	4.06	-189.80	-171.09	1.0000

3.3.2Final Equation in Terms of Coded Factors

Particle size (B1) = +410.01 Intercept +18.17A -17.19B -180.45 C. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

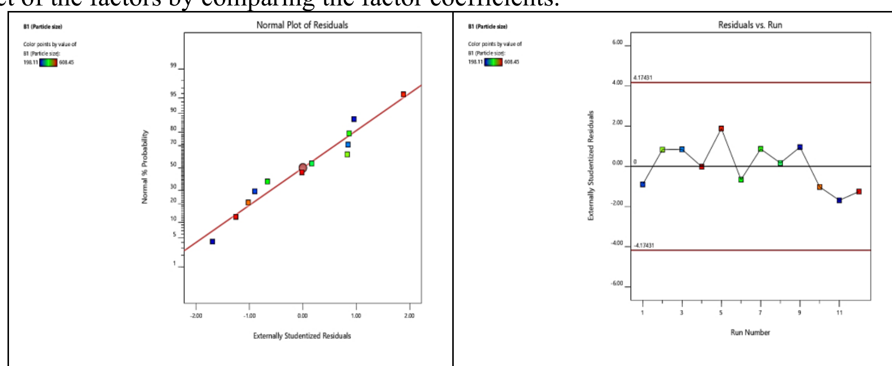
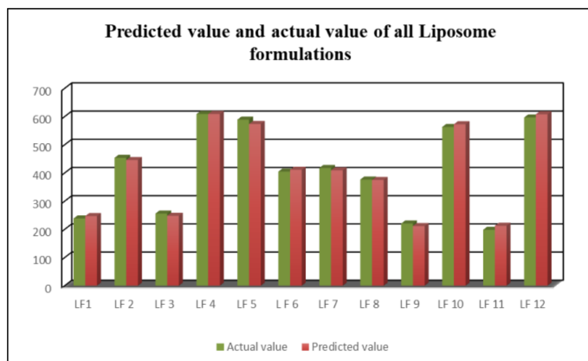


Figure1: Graphical representation of Normal plot of Residuals, Residuals vs run of Liposome formulation on Particle size

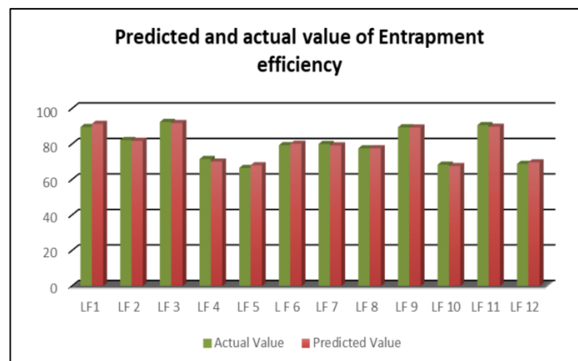
3.3.3 Predicted value and actual value of all formulations and Entrapment efficiency

Table 12: Predicted value and actual value of all Liposome formulations and Entrapment efficiency

Formulations	Actual Value (Particle size)	Predicted Value (Particle size)	Actual Value (Entrapment efficiency)	Predicted Value (Entrapment efficiency)
LF1	238.23	246.75	89.56	91.36
LF 2	453.31	445.37	82.22	81.78
LF 3	255.80	247.74	92.39	91.81
LF 4	608.45	608.63	71.52	70.10
LF 5	588.63	573.27	66.51	68.01
L F 6	404.56	411.00	79.33	80.13
LF 7	417.27	409.02	80.02	79.24
LF 8	376.32	374.65	77.53	77.59
LF 9	220.41	211.39	89.40	89.27
LF 10	562.74	572.28	68.36	67.57
LF 11	198.11	212.38	90.62	89.71
LF 12	596.29	607.64	68.77	69.66



Graph 3: Graphical representations of Predicted value and actual value of all Liposome formulations



Graph 4: Graphical representations of Predicted and actual value of Entrapment efficiency

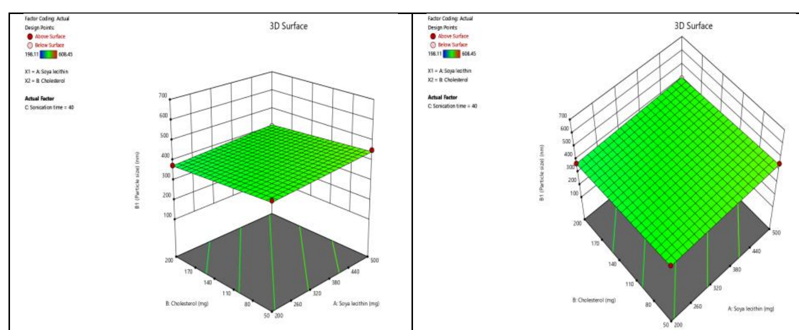


Figure 2: Response surface plot showing combined effect of soya lecithin and Cholesterol on particle size of liposomes

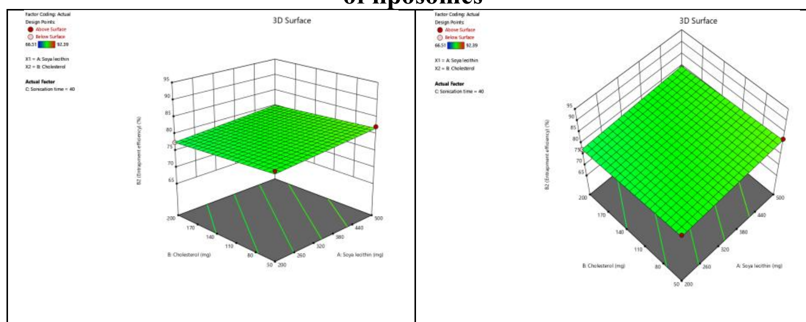


Figure 3: Response surface plot showing combined effect of soya lecithin and Cholesterol on entrapment efficiency Liposome formulation (Three dimensional)

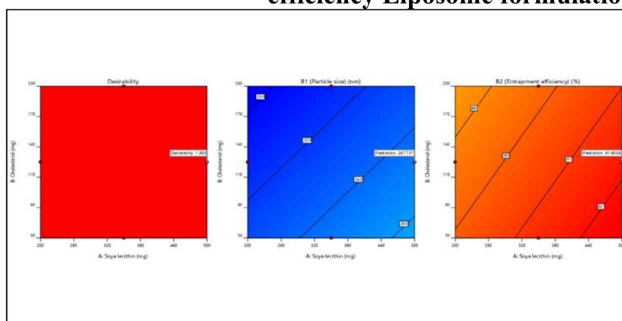


Figure 4: Response surface plot showing prediction data for optimization

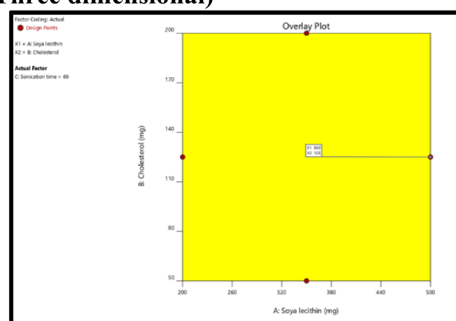


Figure 5: Overlay plot for optimization formulation

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3.3.4 Effect of formulation variables on Entrapment efficiency

Table 13: Response 2: Entrapment efficiency (Fit Summary)

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**	**0.9837**	**0.9733**	**Suggested**
2FI	0.6788	0.9802	0.9482	
Quadratic	0.2953	0.9854	0.9361	Aliased

3.4 ANOVA for linear model

3.4.1 Response 2: EE (ANOVA Linear)

Table 14: Response 2: Entrapment efficiency

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	960.29	320.10	221.70	< 0.0001	significant
A-Soya lecithin	12.88	12.88	8.92	0.0174	
B-Cholesterol	5.41	5.41	3.75	0.0889	
C-Sonication time	942.00	942.00	652.44	< 0.0001	
Residual	11.55	1.44			
Cor Total	971.84				

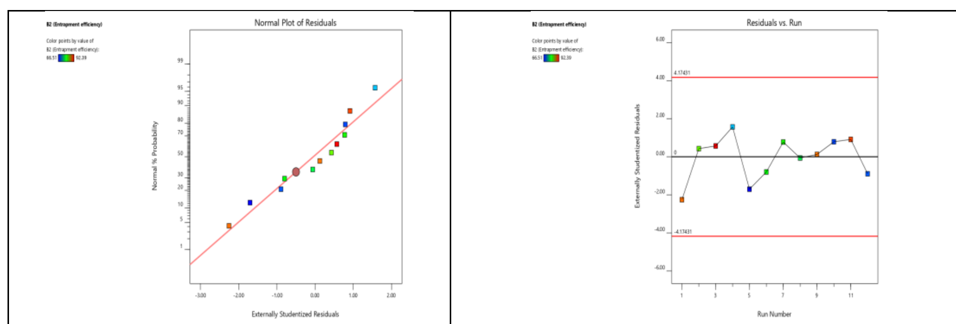
Table 15: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	Standard Error	95% CI Low	95% CI High	VIF
Intercept	79.69	0.3469	78.89	80.49	
A-Soya lecithin	1.27	0.4248	0.2891	2.25	1.0000
B-Cholesterol	-0.8225	0.4248	-1.80	0.1571	1.0000
C-Sonication time	10.85	0.4248	9.87	11.83	1.0000

3.4.2Final Equation in Terms of Coded Factors

Entrapment efficiency (B2) = +79.69 + 1.27A − 0.8225B + 10.85C. This coded equation can be used to predict the response at different levels of each factor. In this system, the high level of each variable is represented as +1, while the low level is represented as −1. Using coded values simplifies interpretation and comparison, as it allows the direct assessment of the relative influence of each factor.

From the coefficients, it is evident that C (sonication time) has the greatest positive effect on entrapment



efficiency, followed by A (soya lecithin), whereas B (cholesterol) shows a slight negative influence.

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Figure 6: Graphical representation of Residuals vs run, Normal plot of Liposome formulation on Entrapment efficiency

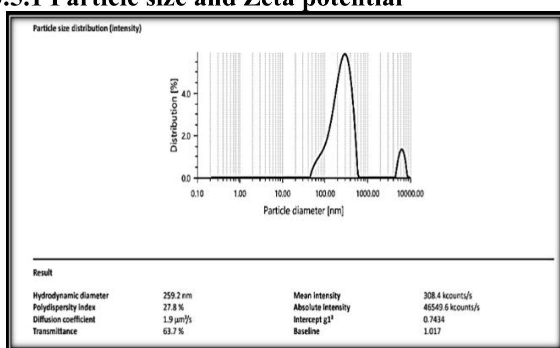
3.4.3 Optimized formula of Liposome formulation

Table 16: Optimized formula of Liposome formulation

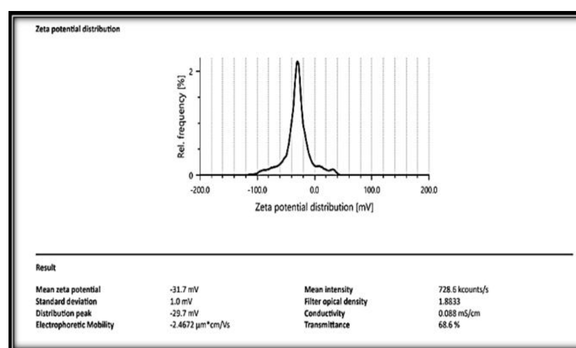
Polymer Eudragit RS100 (mg)	Surfactant Tween 80 (%)	Stirring time (hrs.)	Particle size (nm)	Entrapment efficiency (%)	Desirability	
350.000	50.000	20.000	607.640	69.657	1.000	
200.000	50.000	40.000	409.022	79.240	1.000	
500.000	**125.000**	**60.000**	**247.738**	**91.806**	**1.000**	**Selected**

3.5 Characterization of optimized Liposome formulation

3.5.1 Particle size and Zeta potential



Graph 5: Particle size of optimized formulation



Graph 6: Zeta potential of optimized formulation

Table 17: Particle size of Liposome formulation

Formulation	Particle size (Predicted value)	Particle size (Actual value)	Entrapment efficacy (Predicted value)	Entrapment efficacy (Actual value)
Liposome	247.738nm	259.2 nm	91.806%	93.438 %

3.5.2 SEM analysis of Liposome formulation

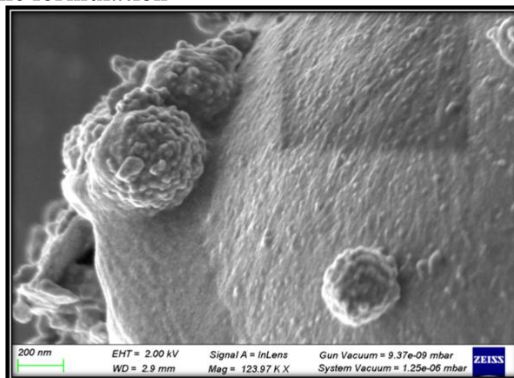


Figure 7: SEM analysis of Liposome formulation

3.6 *In-vitro* drug release studies

Table 18: *In-vitro* drug release studies

Time (Hr)	cumulative % drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time	log Cumu % drug released
0	0	100	0.000	2.000	0.000	0.000
1	21.28	78.72	1.000	1.896	0.000	1.328
3	33.24	66.76	1.732	1.825	0.477	1.522
4	41.29	58.71	2.000	1.769	0.602	1.616
6	52.2	47.8	2.449	1.679	0.778	1.718
8	60.23	39.77	2.828	1.600	0.903	1.780
10	71.31	28.69	3.162	1.458	1.000	1.853

12	84.42	15.58	3.464	1.193	1.079	1.926
14	92.39	7.61	3.742	0.881	1.146	1.966

Table 19: Regression coefficients (R^2 value)

Formulation	Model	Kinetic parameter values
Liposome Formulation	Zero Order	$R^2 = 0.968$
	First Order	$R^2 = 0.903$
	Higuchi	$R^2 = 0.802$
	Korsmeyerpeppas	$R^2 = 0.660$

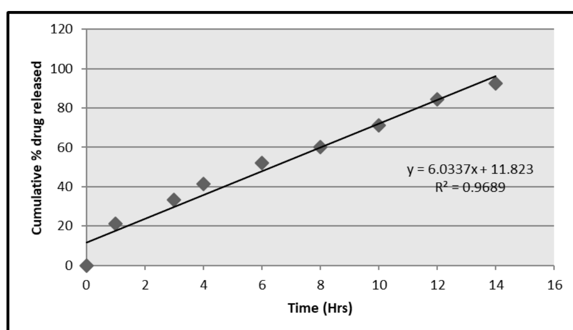


Figure 28: Zero order

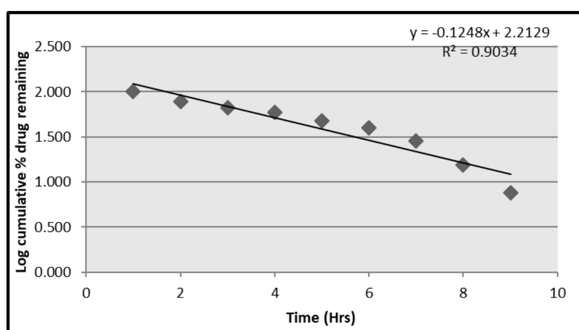


Figure 29: First order

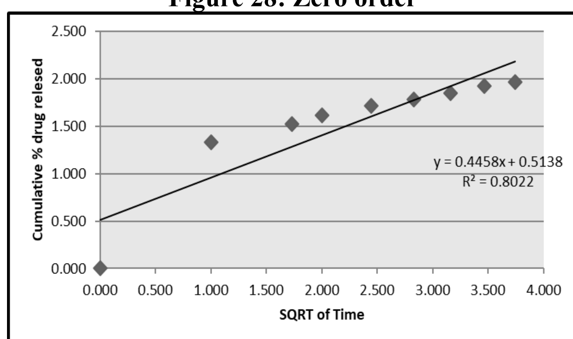


Figure 30: Higuchi

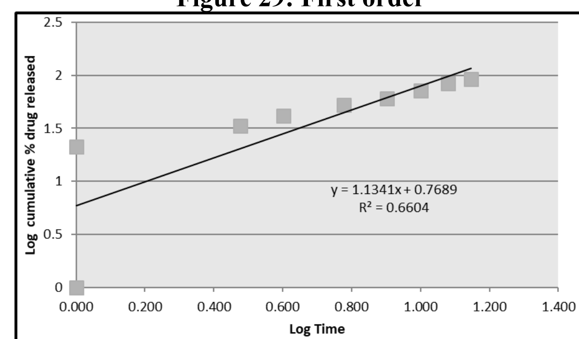


Figure 31: Korsmeyerpeppas

Discussion

The present study successfully demonstrated the formulation and optimization of Pipemidic acid-loaded liposomes with desirable physicochemical and drug delivery characteristics. Preformulation studies confirmed the purity, stability, and suitability of the drug for formulation, with acceptable organoleptic properties, melting point, pH, and solubility profile favoring lipid-based systems. The calibration curve showed excellent linearity ($R^2 = 0.9995$), validating the analytical method, while FTIR analysis confirmed the structural integrity of the drug. Optimization using a Box–Behnken design effectively identified the influence of formulation variables on particle size and entrapment efficiency. Sonication time was found to significantly reduce particle size and enhance entrapment efficiency, while lipid composition also played a key role. The optimized formulation exhibited nanosized vesicles (~259 nm), high entrapment efficiency (~93%), and good stability with a zeta potential of –31.7 mV. SEM analysis confirmed spherical, well-defined vesicles with minimal aggregation.

The in-vitro drug release study showed a sustained release profile with an initial burst followed by controlled release up to 14 hours. Kinetic modeling revealed that the release followed zero-order kinetics, indicating a constant and controlled drug release pattern. Overall, the optimized liposomal formulation demonstrated improved stability, high drug loading, and sustained release behavior, highlighting its potential as an effective drug delivery system for enhanced therapeutic performance.

IV. CONCLUSION

Based on the selected DoE-optimized formulation, the lipid vesicle system containing Pipemidic acid was successfully developed with

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desirable physicochemical and release characteristics. The statistical optimization approach effectively identified the ideal combination of formulation variables required to achieve minimum particle size and maximum entrapment efficiency. The close agreement between predicted and actual values confirmed the robustness and reliability of the DoE model.

The optimized liposomal formulation exhibited nanosized vesicles, high drug encapsulation, good stability, and sustained Zero-order drug release, indicating its potential as an efficient controlled drug delivery system. Therefore, the DoE-based selected formulation can be considered a promising strategy for enhancing the therapeutic performance and bioavailability of Pipemidic acid.

REFERENCES

[1]. Nsairat H, Ibrahim AA, Jaber AM, Abdelghany S, Atwan R, Shalan N, Abdelnabi H, Odeh F, El-Tanani M, Alshaer W. Liposome bilayer stability: emphasis on cholesterol and its alternatives. Journal of liposome research. 2024 Jan 2;34(1):178-202.
[2]. Sharma S, Sudhakara P, Singh J, Ilyas RA, Asyraf MR, Razman MR. Critical review of biodegradable and bioactive polymer composites for bone tissue engineering and drug delivery applications. Polymers. 2021 Aug 6;13(16):2623.
[3]. Natarajan JV, Nugraha C, Ng XW, Venkatraman S. Sustained-release from nanocarriers: a review. Journal of Controlled Release. 2014 Nov 10;193:122-38.
[4]. Ngoepe MP, Moeno S. Evaluation of Pipemidic Acid Derivatives for Potential Antimicrobial Activity Application: In silico Studies on Bioactivity. Letters in Drug Design & Discovery. 2021 Feb 1;18(2):172-82.
[5]. Skwarczynski M, Bashiri S, Yuan Y, Ziora ZM, Nabil O, Masuda K, Khongkow M, Rimsueb N, Cabral H, Ruktanonchai U, Blaskovich MA. Antimicrobial activity enhancers: Towards smart delivery of antimicrobial agents. Antibiotics. 2022 Mar 18;11(3):412.
[6]. Pancani E, Menendez-Miranda M, Pastor A, Brisset F, Bernet-Camard MF, Desmaële D, Gref R. Nanoparticles with high payloads of pipemidic acid, a poorly soluble crystalline drug: Drug-initiated polymerization and self-assembly approach. Actapharmaceuticasinica B. 2018 May 1;8(3):420-31.
[7]. N. Politis S, Colombo P, Colombo G, M. Rekkas D. Design of experiments (DoE) in pharmaceutical development. Drug development and industrial pharmacy. 2017 Jun 3;43(6):889-901.
[8]. Jankovic A, Chaudhary G, Goia F. Designing the design of experiments (DOE)–An investigation on the influence of different factorial designs on the characterization of complex systems. Energy and Buildings. 2021 Nov 1;250:111298.
[9]. Javed S, Mangla B, Almoshari Y, Sultan MH, Ahsan W. Nanostructured lipid carrier system: A compendium of their formulation development approaches, optimization strategies by quality by design, and recent applications in drug delivery. Nanotechnology reviews. 2022 Apr 22;11(1):1744-77.
[10]. Vilegave K, Vidyasagar G, Chandankar P. Preformulation studies of pharmaceutical new drug molecule and products: An Overview. The American Journal of Pharmacy. 2013;1(3):1-20.
[11]. Fantini P, Pinzone M, Taisch M. Placing the operator at the centre of Industry 4.0 design: Modelling and assessing human activities within cyber-physical systems. Computers & Industrial Engineering. 2020 Jan 1;139:105058.
[12]. Ansari E, Honarvar B, Sajadian SA, Aboosadi ZA, Azizi M. Experimental solubility of aripiprazole in supercritical carbon dioxide and modeling. Scientific Reports. 2023 Aug 17;13(1):13402.
[13]. McCullough JP, Waddington G. Melting-point purity determinations: Limitations as evidenced by calorimetric studies in the melting region. AnalyticaChimicaActa. 1957 Jan 1;17:80-96.
[14]. Avnir Y, Barenholz Y. pH determination by pyranine: medium-related artifacts and their correction. Analytical biochemistry. 2005 Dec 1;347(1):34-41.
[15]. Mestre YF, Zamora LL, Calatayud JM. Spectrophotometric determination of nalidixic and pipemidic acids in a flow injection assembly with a solid-phase reactor as a highly stable reagent source. Analyticachimicaacta. 2001 Jul 3;438(1-2):93-102.
[16]. Iacovino R, Rapuano F, Caso JV, Russo A, Lavorgna M, Russo C, Isidori M, Russo L, Malgieri G, Isernia C. β-Cyclodextrin

inclusion complex to improve physicochemical properties of pipemidic acid: Characterization and bioactivity evaluation. International journal of molecular sciences. 2013 Jun 25;14(7):13022-41.
[17]. Moosavi SM, Ghassabian S. Linearity of calibration curves for analytical methods: a review of criteria for assessment of method. Calibration and validation of analytical methods: a sampling of current approaches. 2018 Apr 25;109.
[18]. Enders AA, North NM, Fensore CM, Velez-Alvarez J, Allen HC. Functional group identification for FTIR spectra using image-based machine learning models. Analytical Chemistry. 2021 Jun 30;93(28):9711-8.
[19]. Lonkar vg, shinde ad. Formulation and evaluation of liposomal drug delivery system of metformin hydrochloride. Pharmacophore. 2016;7(4-2016):269-79.
[20]. Maritim S, Boulas P, Lin Y. Comprehensive analysis of liposome formulation parameters and their influence on encapsulation, stability and drug release in glibenclamide liposomes. International journal of pharmaceutics. 2021 Jan 5;592:120051.
[21]. Ramachandran G, Cooper DW. Size distribution data analysis and presentation. Aerosol measurement. 2011 Jul 7:479-506.
[22]. Lunardi CN, Gomes AJ, Rocha FS, De Tommaso J, Patience GS. Experimental methods in chemical engineering: Zeta potential. The Canadian Journal of Chemical Engineering. 2021 Mar;99(3):627-39.
[23]. Datye A, DeLaRiva A. Scanning electron microscopy (SEM). InSpringer handbook of advanced catalyst characterization 2023 May 18 (pp. 359-380). Cham: Springer International Publishing.
[24]. Lv Y, He H, Qi J, Lu Y, Zhao W, Dong X, Wu W. Visual validation of the measurement of entrapment efficiency of drug nanocarriers. International journal of pharmaceutics. 2018 Aug 25;547(1-2):395-403.
[25]. Abbasnezhad N, Zirak N, Champmartin S, Shirinbayan M, Bakir F. An overview of *in vitro* drug release methods for drug-eluting stents. Polymers. 2022 Jul 5;14(13):2751.