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Formulation and Optimization of Clopidogrel Liposomes For Acute Coronary Syndrome

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ABSTRACT

Clopidogrel, a widely used antiplatelet drug, is limited by its poor solubility and delayed onset of action when administered orally, particularly in acute coronary syndrome (ACS) settings. This study aimed to develop an intravenous (IV) liposomal formulation of Clopidogrel to achieve rapid drug concentration in the blood and immediate platelet aggregation inhibition. A liposomal formulation was prepared using a high-pressure homogenization technique, optimized via a 3^2 factorial design, and characterized for particle size, polydispersity index (PDI), zeta potential, entrapment efficiency (EE), and in vitro release. The optimized formulation exhibited a mean particle size of 91.71 ± 2.8 nm, PDI of 0.115 ± 0.009, zeta potential of -24.4 ± 1.1 mV, and EE of 89.2 ± 2.1%. The formulation demonstrated stability upon dilution with common IV fluids and showed a significant improvement in drug release compared to plain Clopidogrel solution. Stability studies indicated a shelf life of 5.1 months at 2-8°C. This study presents a promising IV liposomal formulation of Clopidogrel for rapid onset of action in ACS management.

INTRODUCTION

Clopidogrel is a potent antiplatelet agent used for the prevention of thrombotic events in patients with atherosclerotic vascular diseases. However, its poor aqueous solubility and delayed onset of action when administered orally limit its use in acute emergency settings, such as ACS. Intravenous formulations of Clopidogrel are highly desirable for rapid therapeutic effects, but the drug's poor solubility poses significant challenges. Various approaches, including nanoemulsions, cyclodextrin complexes, and liposomes, have been explored to overcome these limitations. This study focuses on developing a liposomal formulation of Clopidogrel to enhance its solubility, stability, and rapid onset of action.

Materials:
Clopidogrel bisulfate (MSN Laboratories Pvt. Ltd., India),DMPC, DMPG, Cholesterol (Vav Life Science Pvt. Ltd., India),Polysorbate 80, Poloxamer 188 (BASF, Germany),Sucrose, Citric acid, Sodium citrate (Loba Chemie Pvt. Ltd., India), Methanol, Acetonitrile (Fisher Scientific, India)

Preparation of Clopidogrel Liposomes (CL):
Liposomes were prepared using the ethanol injection method followed by high-pressure homogenization (HPH).

Lipid phase (DMPC, DMPG, Cholesterol, and Clopidogrel) was dissolved in ethanol and injected into an aqueous phase (citric acid buffer, pH 7.4) under stirring. The mixture was subjected to HPH for particle size reduction.

Optimization of Process Variables:
A 3^2 factorial design was employed to optimize homogenization pressure (500-1000 bars) and number of cycles (2-6).Particle size and PDI were the dependent variables.

Characterization of Liposomes:
Particle Size and Zeta Potential: Measured using Zetasizer Nano-ZS (Malvern Instruments, UK).
Entrapment Efficiency (EE): Determined by ultrafiltration and UV spectrophotometry.
Transmission Electron Microscopy (TEM): Used to assess vesicle morphology.
In Vitro Release (IVR): Conducted using a dialysis bag method in phosphate buffer (pH 7.4).
Stability Studies: Evaluated at 2-8°C and room temperature (RT) for 3 months.

RESULTS AND DISCUSSION

Identification of Drug

Table 1: Identification tests for CLPD with the inferences

Parameters	Observation	Reported	Inferences
Appearance	White powder	White to off-white powder	Complies
pH of 1% w/v aqueous solution	1.7	Acidic	Complies
Major IR peak	1751	1751	C=O stretching vibration
(cm^{-1})	1191	1190	C-O stretching
	839, 2954	839,2954	C-H bond

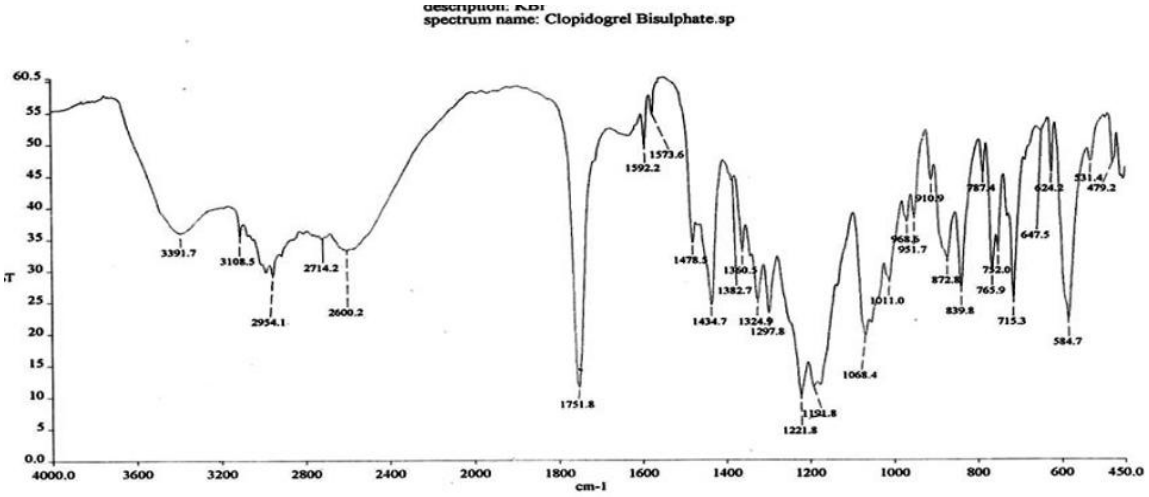


Figure 1 : IR spectrogram of Clopidogrel bisulfate.

ANALYTICAL METHODOLOGY

UV Spectrophotometric estimation of Clopidogrel

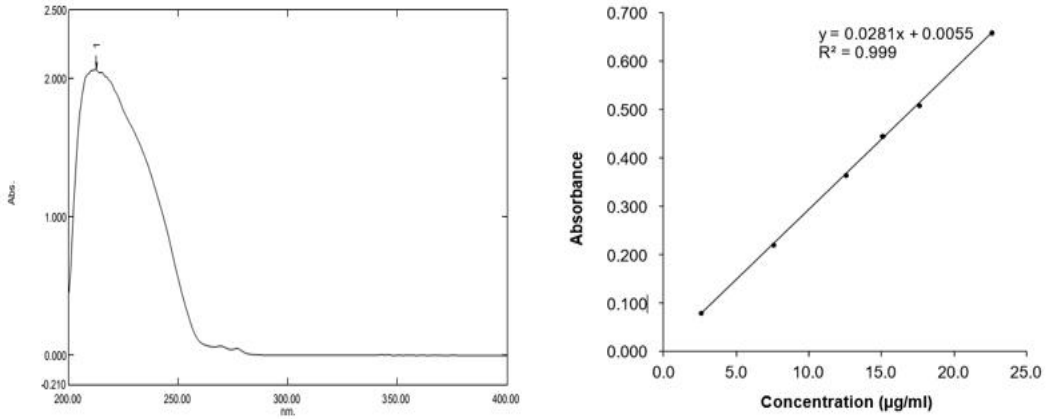


Figure 2 : UV Absorbance spectra and calibration curve of Clopidogrel in methanol

Accuracy

Table 2: Accuracy of Clopidogrel bisulfate estimation in UV-Visible spectroscopy

Sr. No.	Level	Concentration (µg/ml)	Amount recovered (%)	% Accuracy	% Accuracy Mean ±SD	RSD (%)
1	LQC	7.5	49.25	98.49		
2	MQC	14.9	98.95	98.95	99.27±1.16	1.17
3	HQC	22.4	150.53	100.35		

DEVELOPMENT OF CLOPIDOGREL LIPOSOME

Drug-Excipients compatibility

a) FTR spectra of Clopidogrel bisulfate b)Clopidogrel –Excipients mixture

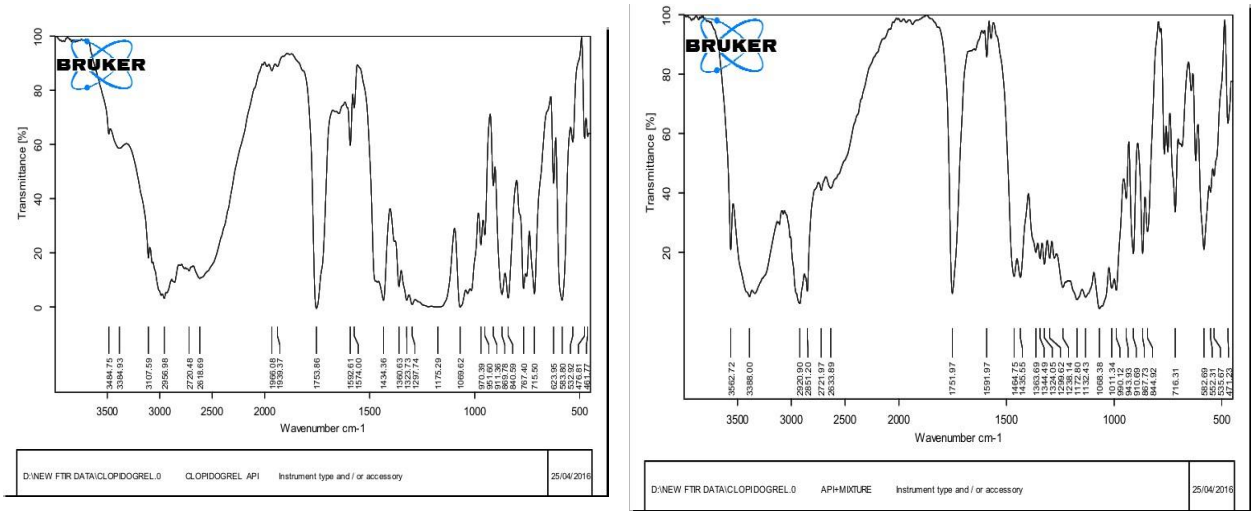


Figure 3: FTR spectra: Clopidogrel (A); Clopidogrel –Excipients mixture (B)

PREPARATION OF CLOPIDOGREL LIPOSOME (CL)

Optimization of liposome process variables (CPP)

Table 3: Full factorial design with coded and actual values used for optimization of formulation variables

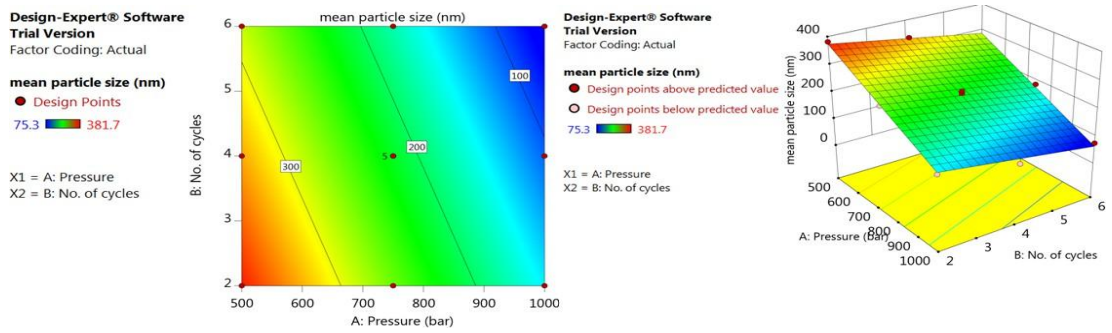
Sr. no	Batch no.	A:Pressure	B:No. of cycles	A:Pressure	B:No. of cycles	Mean particle	PDI
1	CL1	-1	-1	500	2	381.7	0.387
2	CL2	0	-1	750	2	257.8	0.348
3	CL3	+1	-1	1000	2	142.3	0.312
4	CL4	-1	0	500	4	333.6	0.312
5	CL5	0	0	750	4	219.7	0.292
6	CL6	+1	0	1000	4	87.4	0.141
7	CL7	-1	+1	500	6	263.9	0.259
8	CL8	0	+1	750	6	186.4	0.251
9	CL9	+1	+1	1000	6	75.3	0.133
10	CL10	0	0	750	4	208.7	0.193
11	CL11	0	0	750	4	229.7	0.215
12	CL12	0	0	750	4	221.1	0.235
13	CL13	0	0	750	4	234.2	0.227

Table 4: Response Surface Linear Model for particle size (p < 0.0001).

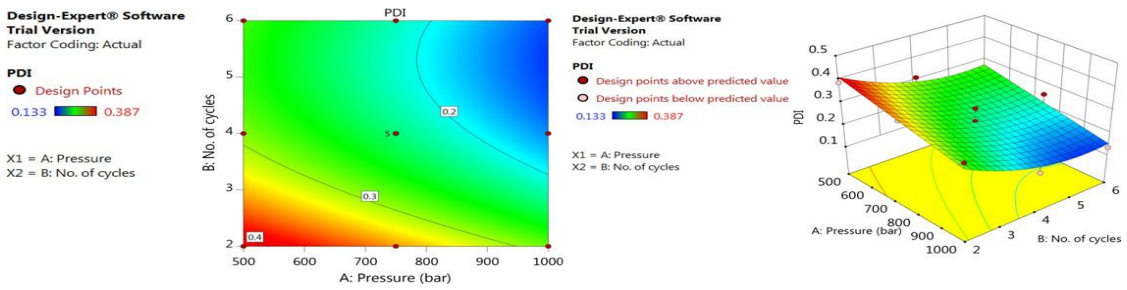
ANOVA for Response Surface Linear Model (Partial sum of squares—Type III)					
Source	Sum of Square	df	Mean sum of square	F value	P value
Model	87588.97	5	17517.79	135.46	< 0.0001 Significant
A-Pressure	75757.61	1	75757.61	585.80	< 0.0001 Significant
B-No. of cycles	10939.74	1	10939.74	84.59	< 0.0001 Significant
AB	645.16	1	645.16	4.99	0.0607 not Significant
A^2	241.01	1	241.01	1.86	0.2145 not Significant
B^2	14.09	1	14.09	0.1089	0.7510 not Significant
Residual	905.27	7	129.32	-	- -
Lack of Fit	516.46	3	172.15	1.77	0.2915 not Significant
Pure Error	388.81	4	97.20	-	- -
Core Total	88494.24	12	-	-	- -

Table 5: Response Surface Quadratic Model for PDI (p= 0.0044)

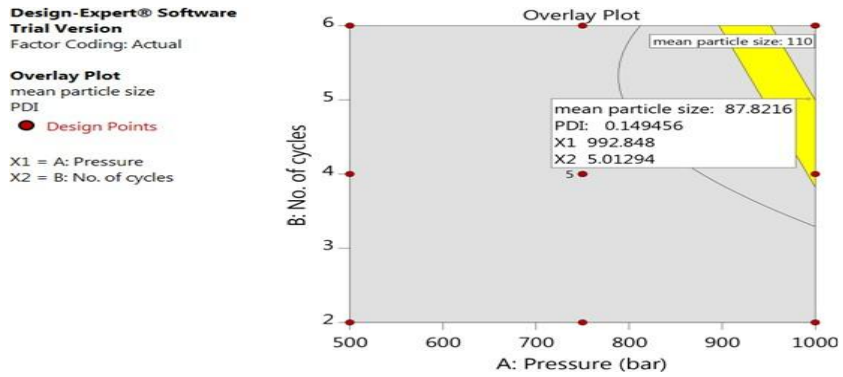
ANOVA for Response Surface Quadratic Model (Partial sum of squares—Type III)					
Source	Sum of Square	df	Mean sum of square	F value	P value
Model	0.0600	5	0.0120	9.93	0.0044 Significant
A-Pressure	0.0231	1	0.0231	19.08	0.0033 Significant
B-No. of cycles	0.0272	1	0.0272	22.50	0.0021 Significant
AB	0.0007	1	0.0007	0.5378	0.4872 not Significant
A^2	0.0007	1	0.0007	0.5822	0.4704
B^2	0.0090	1	0.0090	7.43	0.0295 Significant
Residual	0.0085	7	0.0012	-	- -
Lack of Fit	0.0030	3	0.0010	0.7399	0.5811 not Significant
Pure Error	0.0054	4	0.0014	-	- -
Core Total	0.0685	12	-	-	- -



(A) **(B)**



(C) **(D)**



(E)

Figure 4 : Process optimization of CL: Contour plots (A) & 3D surface plot (B) for particle size, Contour plot (C) & 3D surface plot (D) for PDI, Overlay plot (E).

Effects of formulation variables

Table 6: Influence of formulation variables on CQA (n=3)

Sr. No.	Molar ratio DMPC:Chol:DMPG:CLPD	EE (%)	Zeta potential (mV)	Mean Particle Size (nm)
1	2.8:1.1:0:0.3	87.3±0.9	5.7±1.6	92.12±3.3
2	2.7:1:0.2:0.3	89.2±2.1	-24.4±1.0	94.53±2.8
3	2.3:1.5:0.2:0.3	76.1±1.7	-21.4±1.1	98.71±4.1

Characterization of Clopidogrel Liposome (CL)

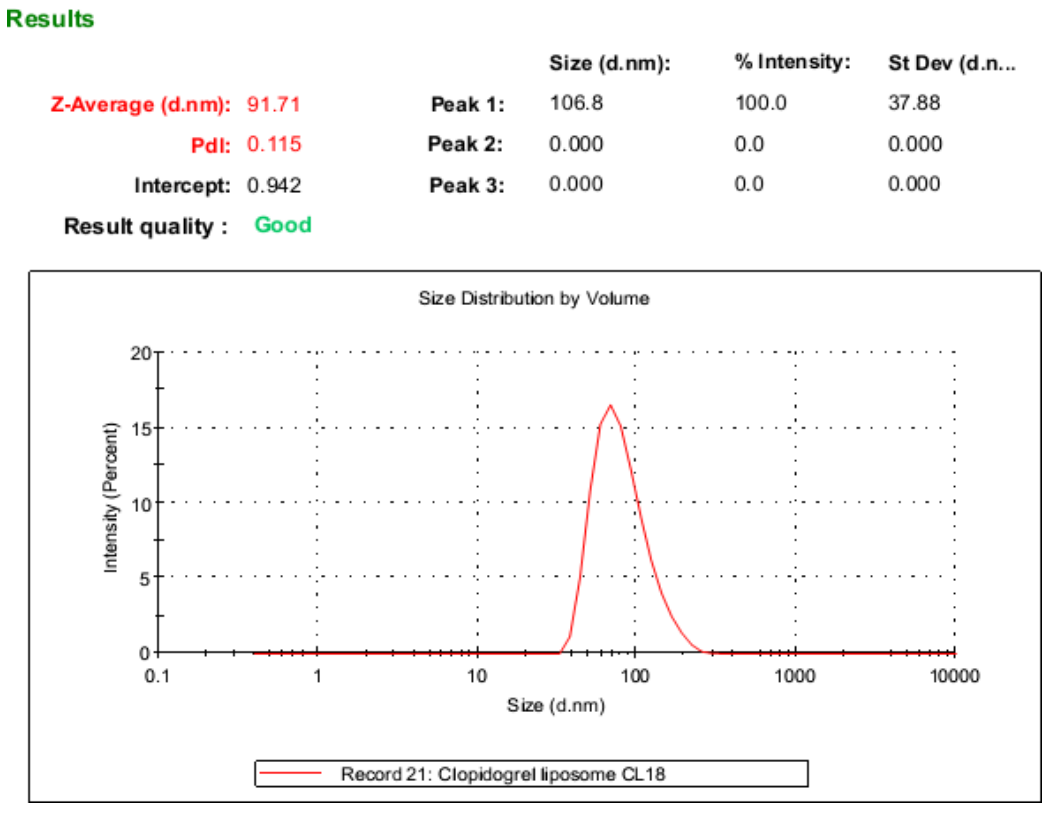


Figure 5: Particle size distribution for optimized Clopidogrel liposome

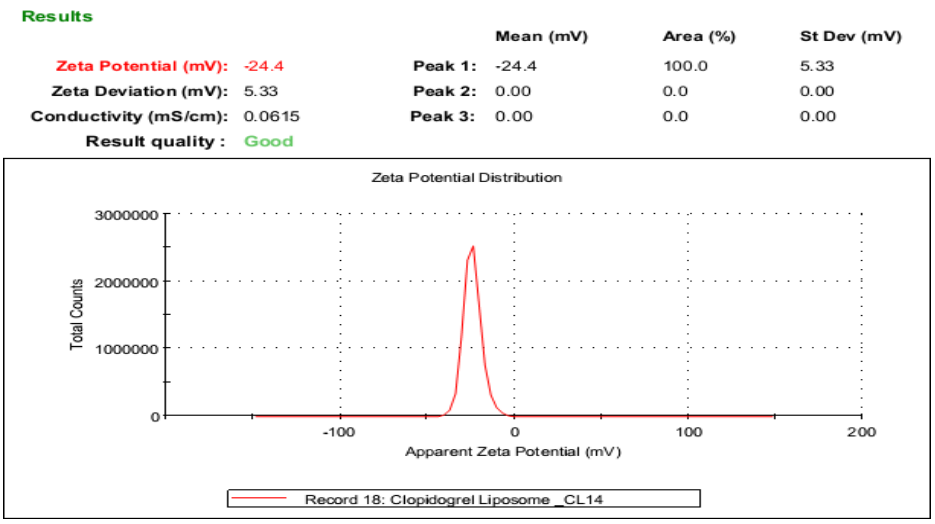


Figure 6: Zeta potential for optimized Clopidogrel liposome

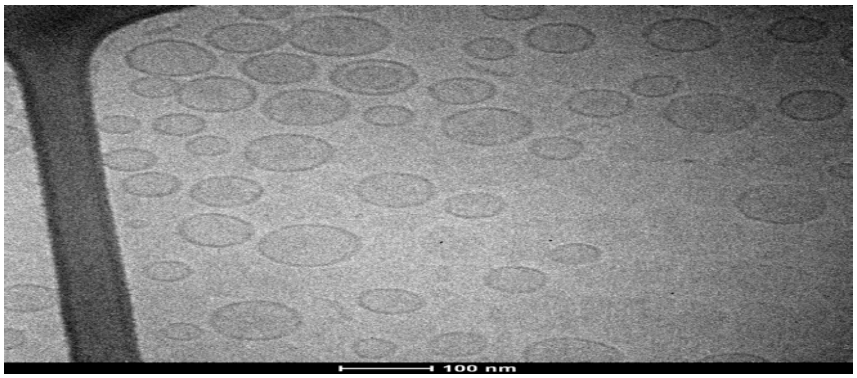


Figure 7: Transmission Electron Microscopic image of CL using TEM with CCD camera (TEM Philips Tecnai 20, Holland).

Admixture stability study

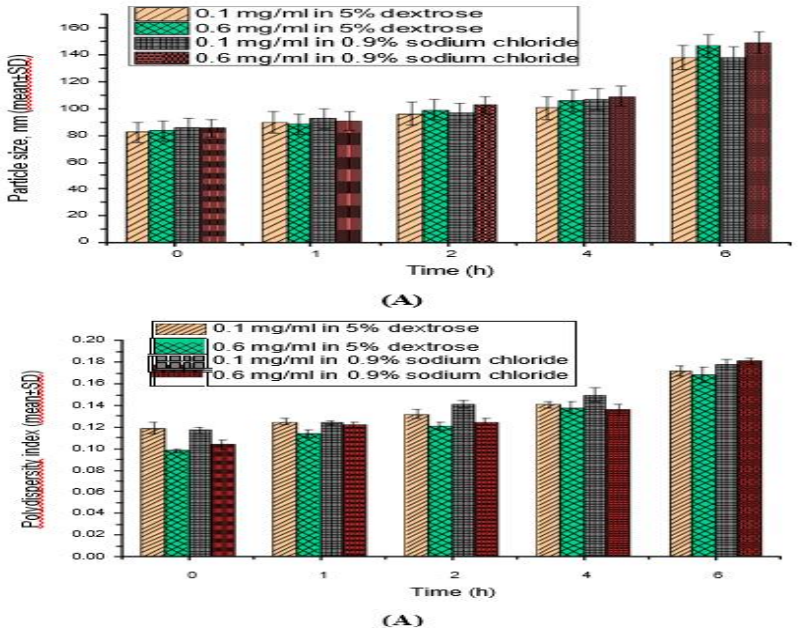


Figure 8 : Admixture stability study with 5% dextrose and 0.9% sodium chloride injection at RT (n=3); Particle size (A) and PDI (B).

In vitro release study

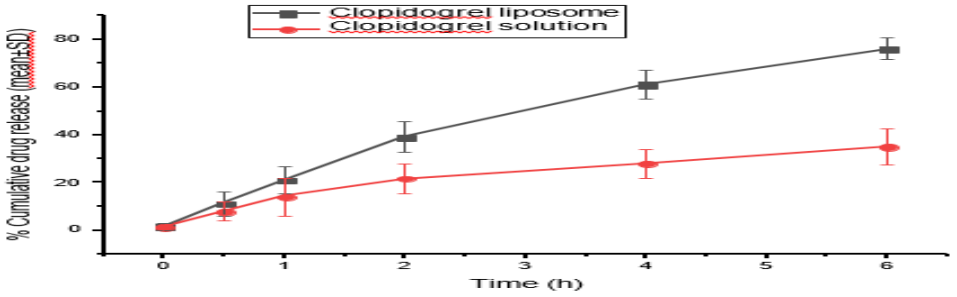


Figure 9: *In vitro* release of CL formulation at pH 7.4 (n=3)

Stability Study

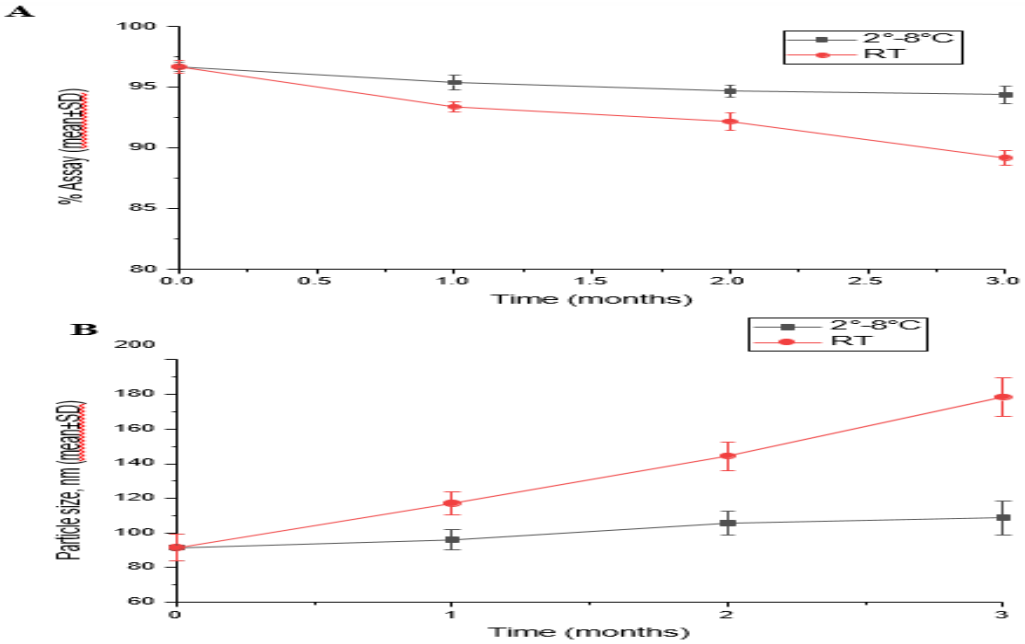


Figure 10: Stability of formulation (n=3); Assay (A) and particle size (B)

Shelf Life Estimation

Table 7: Regression model for shelf life estimation of CL formulation

Source	DF	Seq SS	Seq MS	F-Value	P-Value
Month	1	2.8880	2.8880	22.92	0.041
Error	2	0.2520	0.1260		
Total	3	3.1400			

Model Summary

Table 8: Co-efficient data for shelf life estimation of CL formulation

Term	Coef	SE Coef	T-value	P-Value	VIF
Constant	96.440	0.297	324.73	0.000	
Month	-0.760	0.159	-4.79	0.041	1

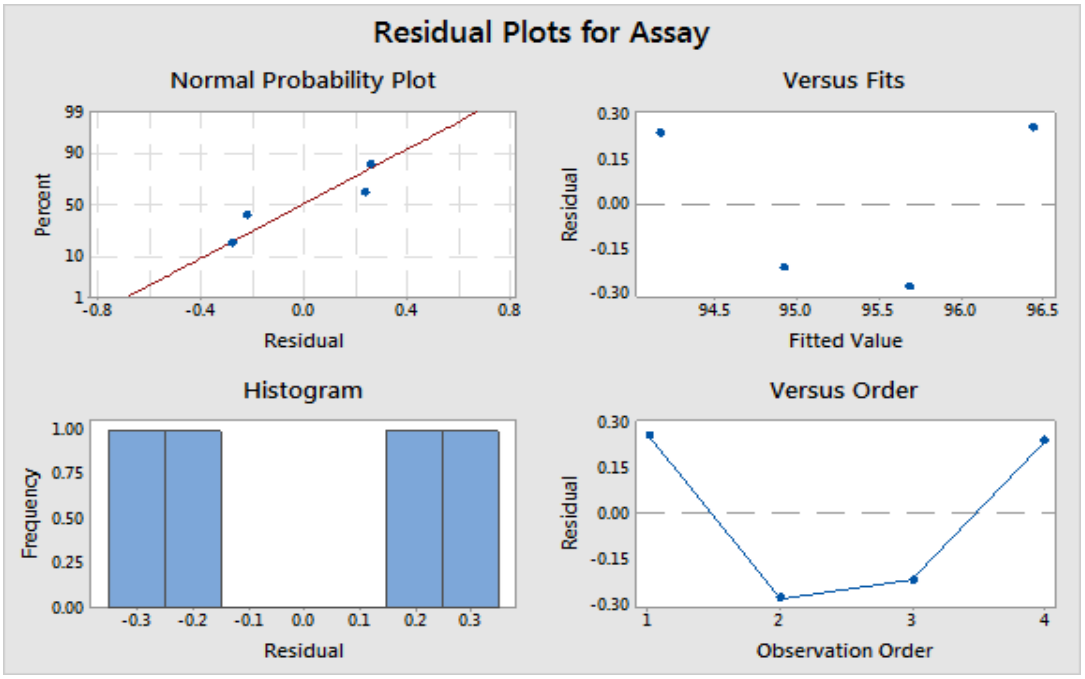


Figure 11: Residual plots for Assay shelf life estimation

Shelf Life

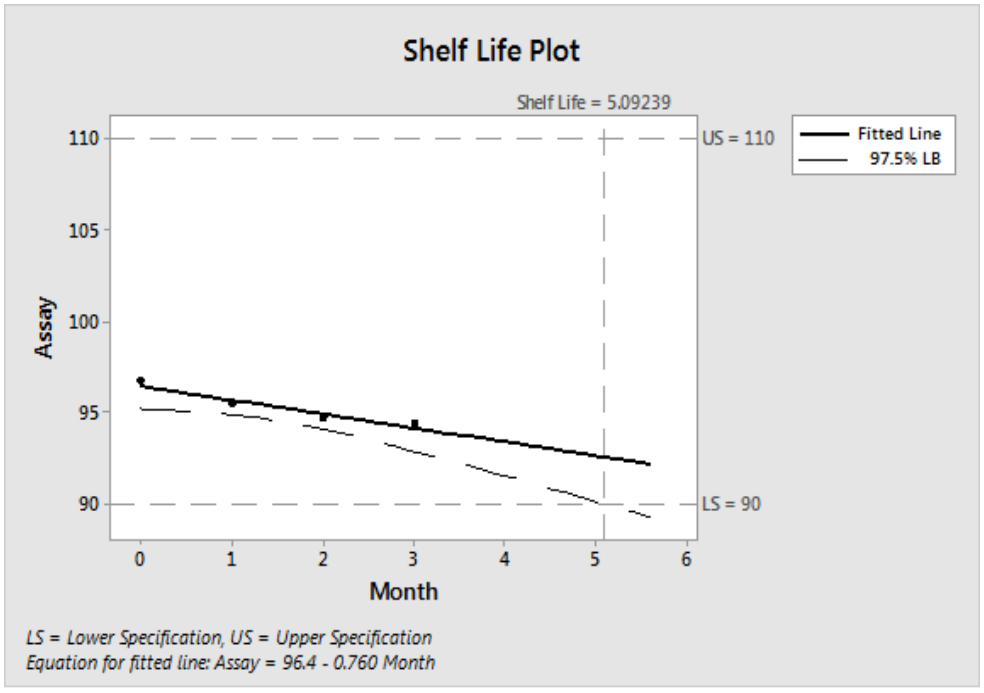


Figure 12 : Shelf life estimation plots

CONCLUSION

This study successfully developed an intravenous liposomal formulation of Clopidogrel with rapid onset of action, suitable for use in acute coronary syndrome. The optimized formulation exhibited excellent physicochemical properties, stability, and in vitro release characteristics.

The liposomal approach effectively addressed the challenges associated with Clopidogrel's poor solubility and delayed onset of action, offering a promising alternative for emergency care settings. Further in vivo studies are warranted to confirm the clinical efficacy and safety of this formulation.

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