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DEVELOPMENT OF NOVEL INJECTABLE FORMULATION OF CLOPIDOGREL BY QbD APPROACH

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ABSTRACT: Clopidogrel, a poorly water-soluble drug, has been the mainstay of platelet management in acute coronary syndrome (ACS) or percutaneous coronary intervention (PCI) for over a decade. Since Clopidogrel is a prodrug and requires hepatic metabolism for activation, it exhibits a slower onset of action upon oral administration. This time-lag before the onset on the action is highly undesirable for the treatment of critical conditions such as coronary intervention, which is a medical emergency during which the rapid onset of antiplatelet action is of paramount importance. To achieve rapid onset, intravenous is the preferred route of administration; however, due to poor aqueous solubility, development of the IV formulation of Clopidogrel presents a considerable challenge. In the present investigation, the liposomal formulation of Clopidogrel was developed, which enables IV administration and may potentially provide a rapid onset of action. The formulation was prepared and optimized using 3^2 full factorial designs with Design Expert 11 software and evaluated for critical quality attributes (% entrapment, particle size, PDI, zeta potential and Morphology, pH/dilution induced stability and short term stability studies. The particle size of optimized formulation was 94.5 ± 2.8 nm, with PDI of 0.126 ± 0.012 and entrapment efficiency (EE) of 89.2 ± 2.1. The developed formulation was stable over a study period of 3 months at 2°-8 °C. This formulation has great potential as rapid-acting IV formulation to fulfill the unmet need in the management of cardiovascular emergency like (ACS) and PCI.

INTRODUCTION: Clopidogrel bisulfate is a potent platelet-aggregation inhibitor and anti-thrombotic drug, currently available only as an oral dosage form. It is extensively used in the treatment of ACS to improve survival and manage thrombotic events such as myocardial infarction, stroke, and vascular death [1].

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Clopidogrel is prodrug and becomes active in the form of its thiol metabolite in the presence of cytochrome P450 enzymes in the liver while major fraction (approximately 85%) of the administered drug converts into a pharmacologically inactive metabolite, Clopidogrel carboxylic acid [2]. The thiol metabolite binds with P2Y12 receptor of platelets irreversibly and inhibits its aggregation [3].

Currently, the approved dose for Clopidogrel tablet is 300 mg loading dose followed by 75 mg as a maintenance dose once daily [4]. With oral formulation, there is a significant delay in achieving therapeutic levels of thiol metabolite in systemic circulation for an effective anti-platelet

response. This delay is attributed to various factors, including the process of disintegration, dissolution, gastric emptying, and intestinal absorption associated with the solid oral dosage form. Moreover, limited oral bioavailability (approx. 50%) also contributes to its slower onset, which is highly undesirable in acute care emergency setting [5, 6]. In an emergency situation like ACS, Clinician decision is very important and tricky while patient arrives in an emergency lab and then mobilized to the cath lab for cardiac catheterization and coronary angiography. Clinicians usually initiate Clopodogrel oral treatment early on in the emergency room to decrease the potential risk of ischemic events and cardiac death of patients prior to cardiac catheterization in cath lab [7]. However, this poses an increased risk of bleeding complication if coronary angiography indicates the requirement of coronary artery bypass graft surgery.

Early Clopidogrel oral treatment is beneficial to patients only if coronary angiography indicates no requirement of coronary artery bypass surgery [7] indicating a practical and clinical limitation of oral formulation in the acute care setting. The inability of oral formulation to achieve the desired thiol level in blood within an optimal period warrants requirement of high dose and considerable risk of bleeding. The intravenous formulation of Clopidogrel may potentially overcome these limitations as it enables Clopidogrel administration after angiography by IV route and may achieve a rapid onset of platelet aggregation inhibitory effect.

IV formulation of Clopidogrel in the parenterally acceptable vehicle could be immensely useful for treating emergency ischemic conditions or when coronary angioplasty and coronary stenting has to be undertaken. Hence, there is a clear unmet need for the development of IV formulation as there is no readily available commercial injectable formulation of Clopidogrel in the market. Development of injectable formulation of Clopidogrel, however, poses a major challenge to pharmaceutical scientists owing to its poor aqueous solubility. In the present investigation, a liposomal formulation of Clopidogrel suitable for IV administration was developed using pharmaceutically acceptable excipients with the desired quality target product profile (QTPP) by QbD. Detailed formulation optimization was carried out using the design of experiments (DoE) based on factorial design. The formulation was characterized in terms of particle size, PDI, entrapment efficiency, morphology as well as for its suitability for parenteral administration.

MATERIALS AND METHODS:

Material: Clopidogrel bisulfate (CLPD) was obtained from MSN laboratory limited, Medak, India. DiMyristoyl Phosphatidylglycerol (DMPG) from Corden pharma and DiMyristoyl Phosphatidylcholine (DMPC) and Cholesterol (Chole) was obtained from Vav life science, Mumbai, India. Citric acid anhydrous, sucrose, sodium phosphate dibasic, sodium phosphate monobasic, and sodium hydroxide were obtained from Loba Chemie, India. All chemical and solvent used were of analytical grade.

Methods:

Preparation of Clopidogrel Loaded Liposomes: Clopidogrel liposomes were prepared using high-pressure homogenization (HPH). Briefly, an aqueous phase was prepared by dissolving citric acid in water for injection (WFI) with pH adjusted to 7.4 using 0.5N sodium hydroxide solution. Lipid phase was prepared by dissolving DMPC, DMPG, and Cholesterol in ethanol at 50 °C. Clopidogrel was added and dissolved in the lipid phase. Lipid phase was then injected into the aqueous phase using syringe under stirring (800 rpm) at room temperature (RT) to form multilayered vesicles (MLVs). MLVs then subjected to particle size reduction using high-pressure homogenization (PandaPlus2000, GEA Niro Soavi, Germany) to form liposomes. The liposomal formulation was then subjected to ultrafiltration using pellicon XL mini cassette (biomax300, Millipore) to remove ethanol and unentrapped drug and final volume were made up with sucrose solution [8].

Optimization of Process Variables: Liposome containing Clopidogrel was optimized for critical process parameters (CPP) by factorial design to investigate the combined influence of two CPP in high-pressure homogenization process for the preparation of Clopidogrel liposome. CPP involved during the homogenization process were identified to be the pressure and number of cycles. These were optimized using 3^2 full factorial designs with

Design Expert 11.1.2.0 software (Stat-Ease, Inc., USA). Thirteen trials were carried out with pressure (500 to 1000 bars) and several cycles (2 to 6) as independent variables at three levels. Particle size and PDI were considered as dependent variables. Based on the factorial design, thirteen batches were prepared. The major process parameters and the formulation parameters were optimized to achieve the desired particle size and PDI. The full factorial design used for the optimization of process variables, as shown in **Table 1**. The data obtained were statistically analyzed using ANOVA to determine the significance of the effects of the variables. ANOVA for Response Surface Linear Model for particle size and Quadratic model for PDI were shown in **Table 2** and **3,** respectively.

Effects of Formulation Variables: Influence of formulation variables was studied by changing the molar ratio of DMPC: Chol: DMPG: CLPD and the EE, particle size, and zeta potential were measured for each ratio. Generally, cholesterol is added in the formulation to reduce the permeability of the bilayer membrane and better stabilization of liposome structure [9]. Incorporation of negatively charged lipid (DMPG) provides net negative electric charge/zeta potential to the particles [10, 11].

Characterization of Optimized Formulation:

Particle Size, PDI and Zeta Potential: The average particle size, PDI, and zeta potential of the liposomes were determined using Zetasizer Nano series Nano-ZS (Malvern Instruments, Malvern, UK). The measurement of particle size was based on the principle of dynamic light scattering (DLS), while zeta potential was determination was based on the principle of Laser Doppler Electrophoresis. Briefly, 1 ml formulation sample was slowly transferred into the sample port of the zeta cell in such a way that no air bubbles formed, and the cell electrode was completely covered. For particle size measurement, 2 ml samples were transferred into the measurement cells, and an air bubble formed were removed by gentle tapping of the cell. The samples were then analyzed using Zetasizer.

Transmission Electron Microscopy: Vesicle shape and surface morphology of Clopidogrel liposome (CL) were evaluated using cryo-TEM. Formulation sample, about 5 µl, was placed onthe carbon-coated side of the grid and blotted. After blotting, tweezer was plunged immediately into liquid ethane. The grid was then carefully transferred to the grid box located in the ethane container assembly using a tweezer. The grid box was maintained at liquid nitrogen temperature till the grid was transferred to cryo holder which was also maintained below -165 °C using liquid nitrogen. The sample was allowed to stabilize for about 10 min or till the drifting of sample stops. The images were taken using Cryo-TEM equipped with a camera.

Drug Entrapment: Entrapment efficiency was determined by separating free drug by centrifugation technique [12] using Amicon Ultra-15 ultrafiltration device (molecular weight cutoff was 100 K, Millipore). The Clopidogrel liposomes sample was added into Amicon Ultra-15 ultrafiltration device and centrifuged (Thermo fisher scientific) at 3500 rpm for 15 min. The filtrate was removed, and Clopidogrel content was determined by UV spectroscopy at 220 nm as free Clopidogrel (C_{free}). Total Clopidogrel (C_{total}) was determined at 220 nm by UV spectroscopy from as such sample without subjecting to free drug separation process after diluting with methanol. The EE of Clopidogrel in liposome was calculated by using the below equation.

$$EE\ (\%) = C_{total} - C_{free}) / C_{total} \times 100\% \quad \ldots. (1)$$

Where C_{Total} is the total drug concentration before filtration containing both trapped and free Clopidogrel, C_{free} is the drug concentration in the filtered solution.

pH/Dilution Induced Stability: Clopidogrel has poor solubility at physiological pH; hence, it may precipitate and cause phlebitis and embolism *in-vivo* upon administration. Potential for pH/dilution induced instability of Clopidogrel liposome was assessed at pH 7.4 (physiological pH) using phosphate buffer against Clopidogrel solution (Plain) as control.

***In-vitro* Drug Release Study (IVR):** The IVR study was performed by the dialysis bag diffusion method using a dialysis membrane (MWCO 12000 to 14000, Himedia) [13, 14]. The membrane was soaked in water at room temperature for overnight before use. Briefly, 10 ml of the formulation

containing Clopidogrel was taken in the dialysis tube, sealed and immersed in a receptor compartment having 90 mL of media (pH 7.4 phosphate buffer maintained at 37 ± 0.5 °C) and stirred continuously at 250 rpm by a magnetic stirrer. Samples (2 ml) were withdrawn at specified time intervals up to 6 h and replaced withan equal volume of fresh media to maintain the volume of the receptor compartment. Sink condition was maintained for the duration of the IVR study. The amount of drug released was estimated using UV-visible spectrophotometer at 220 nm after appropriate dilution of the samples. The % cumulative drug release (CDR) from formulation and plain drug solution were calculated.

Stability Study: Stability of optimized formulation was determined over 3 months at 2°-8°C and RT. Assay and mean particle size (z-avg) of samples were measured as an indicator of the chemical and physical stability of the formulation, respectively.

RESULTS:

Process Optimization:

Influence of CPP on CQA: Two critical process variables involved during the high-pressure homogenization process were pressure and number of cycles, which were identified to have a potential impact on CQA (particle size and PDI). Statistical tool ANOVA was applied to access the significance and the magnitude of the effects of the CPP variables and their interactions. A quadratic model was found to be significant for particle size as well as PDI. The model F-value of 135.46 implies the model is significant. There is only a 0.01% chance that F-value this large could occur due to noise indicates the model is significant.

The predicted R^2 of 0.9351 is in reasonable agreement with the adjusted R^2 of 0.9825; *i.e.*, the difference is less than 0.2. Values of "Prob > F" less than 0.05 indicated model terms are significant. Pressure (A) and Number of cycles (B) were found to be significant model terms ($p < 0.05$), while the model terms AB, $A^{2,}$ and B^2 were found to be non-significant for particle size (Table 2). Whereas Pressure (A), Number of cycles (B) and B^2 were found to be significant model terms ($p < 0.05$), while the model terms AB and A^2 were found to be non-significant for PDI as per **Table 3**.

TABLE 1: FULL FACTORIAL DESIGN WITH CODED AND ACTUAL VALUES USED FOR OPTIMIZATION OF PROCESS VARIABLES. (INDEPENDENT VARIABLE: PRESSURE AND NUMBER OF CYCLES; DEPENDENT VARIABLE: MEAN PARTICLE SIZE AND POLYDISPERSITY INDEX-PDI)

S. no.	Batch no.	Coded Values		Actual Values		Responses	
		A: Pressure	B: No. of cycles	A: Pressure	B: No. of cycles	Mean particle size	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 2: 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 3: 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	-	-	-
Cor Total	0.0685	12	-	-	-	-

Full model equations for particle size and PDI in terms of coded factors were obtained as

Mean particle size = +221.87-112.37A-42.70B+12.70AB-9.34A^2+2.26B^2 …..(2)

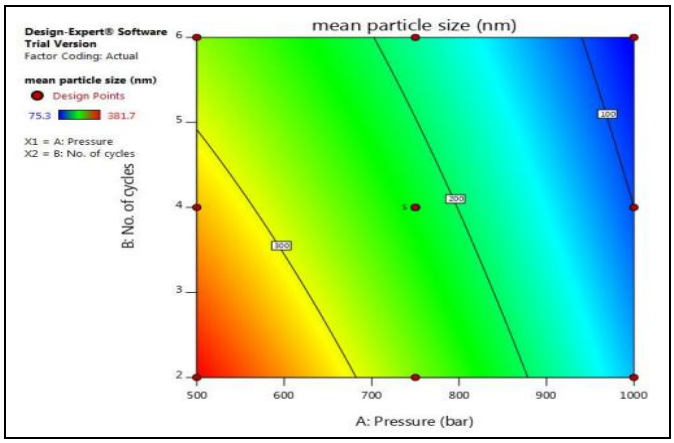
PDI = +0.2353-0.0620A-0.0673B-0.0127AB-0.0160A^2+ 0.0570B^2 …..(3)

Final Reduced polynomial model equations for particle size and PDI in terms of coded factors were obtained as

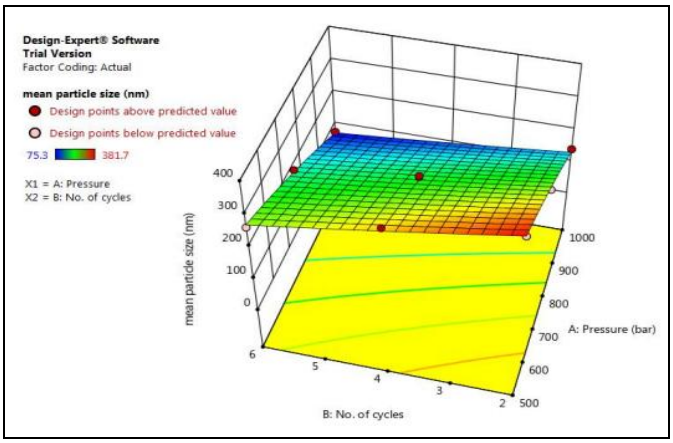
Mean particle size = +218.60-112.37A-42.70B …..(4)

PDI = +0.2307-0.0620A-0.0673B+0.0510B^2 …..(5)

The regression model was also used to generate the contour plots, 3D surface plots, and the overlay plot for particle size for analyzing interactions of the independent factors **Fig. 1**. As evident from the 3D surface plots, with increasing the pressure and a number of cycles, the particle size and PDI were reduced as indicated in **Fig. 1B,** and **Fig. 1D**.



A



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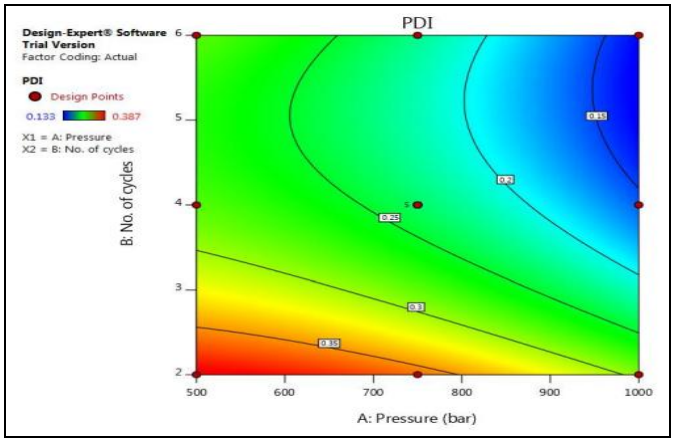

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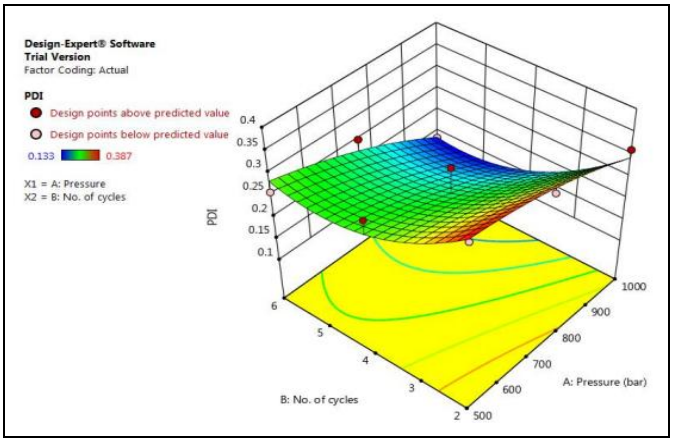

D

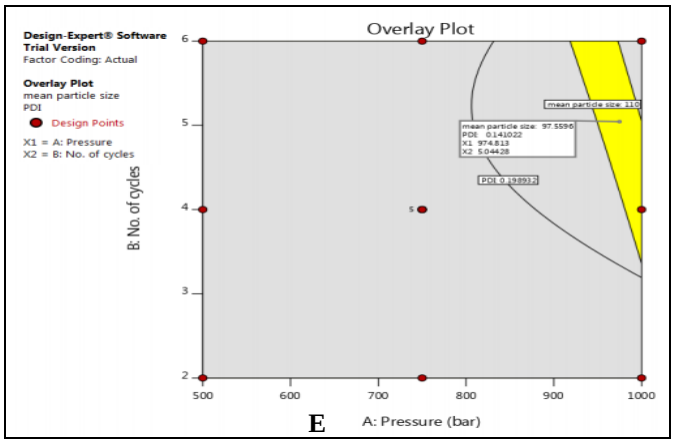

FIG. 1: CONTOUR PLOTS, 3D SURFACE PLOTS AND OVERLAY PLOT FOR PROCESS OPTIMIZATION: (A) CONTOUR PLOTS FOR PARTICLE SIZE, (B) 3D SURFACE PLOT FOR PARTICLE SIZE, (C) CONTOUR PLOT FOR PDI, (D) 3D SURFACE PLOT FOR PDI, (E) OVERLAY PLOT FOR OPTIMIZATION

Experimental validation of DoE trials was undertaken by the preparation and characterization of liposome at the checkpoint batch suggested by the software. The observed values (Particle size 92.8 nm and PDI 0.126) were in close agreement with the predicted values (Particle size 97.6 nm and PDI 0.141) establishing the reliability of the optimization procedure.

Influence of Formulation Variables: Influence of level of Cholesterol and DMPG on EE, Particle size, and Zeta potential were determined by changing their concentration in the formulation as mentioned **Table 4**.

Increasing cholesterol concentration (0.5 mole %) resulted in a reduction of EE from 89.2 ± 2.1% to 76.1 ± 1.7% while the presence of DMPG imparted negative charge (up to -24.4 ± 1.1 mV) of the particles.

TABLE 4: INFLUENCE OF FORMULATION VARIABLES ON CQA

S. 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	92.1±3.3
2	2.7:1:0.2:0.3	89.2±2.1	-24.4±1.0	94.5±2.8
3	2.3:1.5:0.2:0.3	76.1±1.7	-21.4±1.1	98.7±4.1

Appearance and pH: The formulation was white translucent and homogeneous with pH 6.6.

Particle Size, Polydispersity Index and Zeta Potential: The mean particle size, PDI and Zeta potential of optimized formulation were measured and found to be 94.5 ± 2.8, 0.126 ± 0.012 and -24.4 ± 1.0 mV respectively, as shown in **Fig. 2** and **3**.

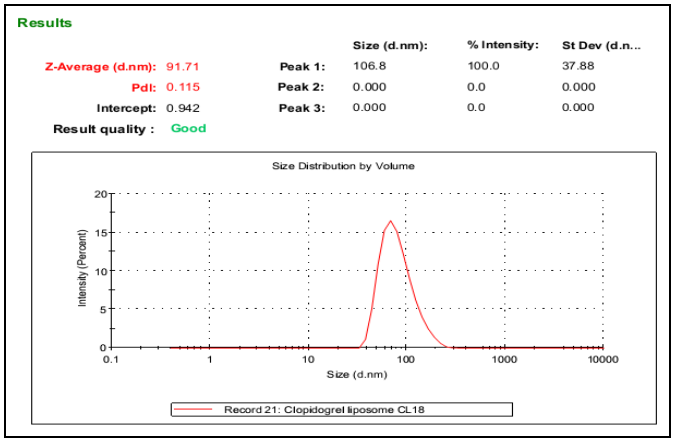

FIG. 2: PARTICLE SIZE DISTRIBUTION FOR OPTIMIZED CLOPIDOGREL LIPOSOME

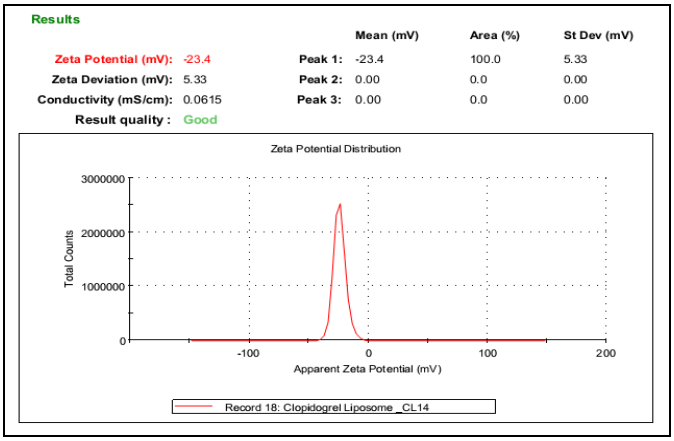

FIG. 3: ZETA POTENTIAL FOR OPTIMIZED CLOPIDOGREL LIPOSOME

The mean particle size of less than 150 nm indicates suitability for sterile filtration and PDI less than 0.2 indicates monodispersity of the formulation. If the zeta potential falls below a certain level, colloidal particles tend to aggregate due to the attractive forces. High zeta potential (either positive or negative), maintains good physical stability of system [15, 16].

Morphological Characterization: Liposome morphology was studied using Transmission electron microscopy (TEM) **Fig. 4**.

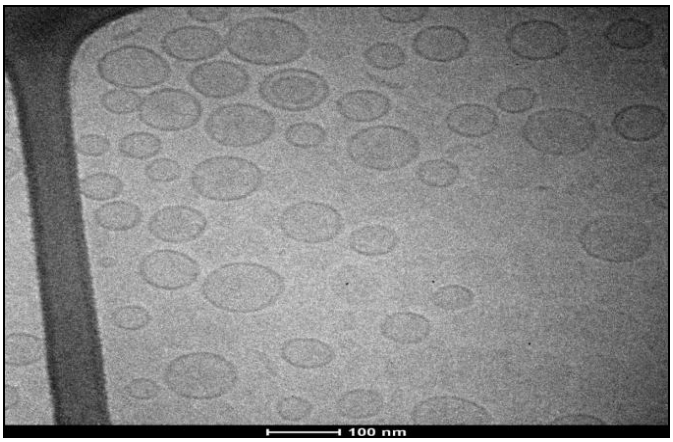

FIG. 4: TRANSMISSION ELECTRON MICROSCOPIC IMAGE OF CLOPIDOGREL LIPOSOME

TEM image showed that the prepared liposomes were unilamellar and spherical in shape, and particle size ranging between 80 to 120 nm.

Entrapment Efficiency: Entrapment efficiency was calculated for optimized formulation, and it found to be 89.2 ± 2.1% as mentioned in **Table 4**.

pH/Dilution Induced Stability: Clopidogrel liposome evaluated for pH/dilution induced stability in phosphate buffer pH 7.4 and found to be stable upon dilution, and no precipitation observed for entrapped Clopidogrel while plain drug formed cloudy precipitates.

IVR Study: The IVR profile from the liposome and plain solution are given in **Fig. 5**.

TABLE 5: INFLUENCE OF pH/DILUTION ON STABILITY

S. no.	Sample name	Dilution media	Observation
1	Clopidogrel Solution-pH 1.8	Phosphate buffer pH 7.4 (1:100)	Cloudy, Milky, Drug precipitated that may cause *in-vivo* phlebitis and embolism
2	Clopidogrel liposome -pH 7.4	Phosphate buffer pH 7.4 (1:100)	Clear solution

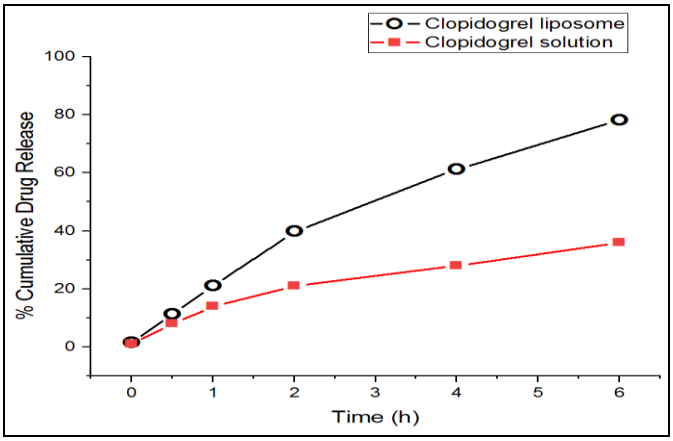

FIG. 5: *IN-VITRO* RELEASE STUDY

The CDR was 60% from Clopidogrel liposome whereas only 28% from plain solution at 4 h in phosphate buffer pH 7.4 at 37 °C.

Stability Study: Optimized formulation was assessed for its physical and chemical stability at 2°-8°C and RT. Liposome was found to be unstable at RT as indicated by a significant increase in particle size and decrease drug assay over some time.

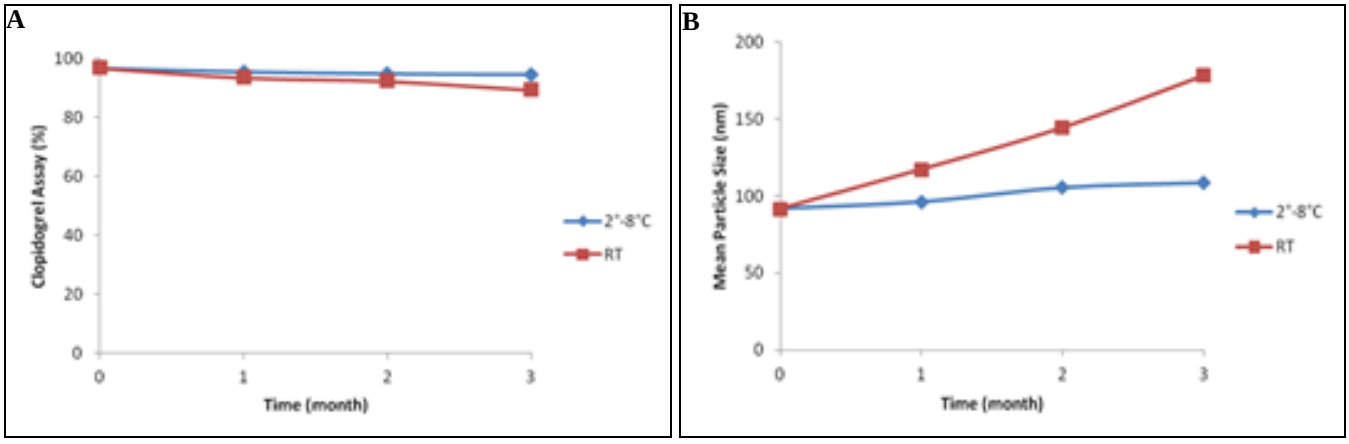

FIG. 6: STABILITY OF FORMULATION, ASSAY (A) AND PARTICLE SIZE (B)

The formulation was found to be physically and chemically stable for 3 months at 2-8°C, as indicated by particle size and assay data, as shown in **Fig. 6**.

DISCUSSION: Clopidogrel is hydrophobic drug and difficult to formulate it by conventional injectable dosage form. Here we developed a liposomal injectable formulation of such lipophilic drug by passive loading method in which clopidogrel entrapped in liposome bilayer as similar to other hydrophobic drugs entrapped in phospholipid bilayer in line with the literature. Liposome was prepared using HPH process, optimized for CPP and characterized for CQA using DoE approach. The aim of the process optimization is specifically to identify the levels of the process variable that affect the chosen responses and determine the levels of the variable to obtain a robust process with desired quality characteristics. Here homogenization pressure and no. of homogenizing cycles was optimized as a CPP for high-pressure homogenization process as per DoE approach. The level of both CPP and their effect on CQA (Particle size and PDI) was optimized using a statically significant model, and their relationship was established using 3D response surface plots. As evident from the 3D surface plots, with increasing the pressure and several cycles, the particle size and PDI were reduced. Furthermore reliability of optimization model was also checked by preparation and characterization of liposome at the checkpoint batch (Homogenization pressure: 1000 bar & No. of cycles: 5) in overlay plot and found satisfactory with close agreement of observed value (Particle size 92.8 nm and PDI 0.126) to model predicted values (Particle size 97.6 nm and PDI 0.141).

The liposome composition was also optimized to get high EE% and surface charge, which determine the physical stability of particles. It was observed that increasing cholesterol level, EE% of clopidogrel was reduced from 87.3 ± 0.9% to 76.1 ± 1.7% because of displacement of the drug from bilayer by cholesterol as the similar negative influence of cholesterol on entrapment of hydrophobic drug was observed by Zaru and Antimisiaris [17], Deniz and Banerji [18]. Liposome bilayer composed of phospholipid and cholesterol, as cholesterol is an integral part of liposomal bilayer structure it competes with clopidogrel for lipophilic space in the phospholipid bilayer and hence reduced the entrapment of drug in bilayer which is in good agreement with the characteristic of drug to be encapsulated as described by Talsma and crommelin [19]. TEM image supports unilamellar structure and incorporation of clopidogrel in the phospholipid bilayer.

Primarily prepared liposome consists of DMPC and cholesterol, which showed +5.7 mV surface charge, which is not sufficient to prevent aggregation and may lead to physical instability upon storage. For physical stability, Zeta potential should be approximately ±30 mV at least [16, 20]. Negatively charged phospholipid (DMPG) has been used widely to impart negative charge in a liposome as pure DMPG liposome has -38.4 mV zeta potential value [21]. Eventually, incorporation of negatively charged DMPG in clopidogrel liposome composition resulted in high negative zeta potential value -24.4 mV that is sufficient to prevent particle aggregation and impart good physical stability of formulation during storage. Result of EE% and TEM image indicates incorporation/association of Clopidogrel molecules with phospholipid bilayer and resembles spherical, unilamellar liposome structure with uniform particle shape and mean particle size was around 100 nm, in good agreement with particle size distribution and narrow PDI results. Optimum mean particle size (91.7) and PDI value (0.115) indicates the monodisperse and homogeneous distribution of liposome size less than 100 nm which further provides suitability for sterile filtration of parenteral formulation through 0.22-micron filter.

Clopidogrel plain solution found very acidic (pH 1.8 < pKa 4.6) which cause pain, irritation, and embolism at physiological pH 7.4, which is above its pKa during IV administration. pH/dilution induced stability study showed Clopidogrel plain solution forms cloudy precipitates while liposomal entrapment of Clopidogrel prevents its precipitation /aggregation and provides clear solution upon dilution with phosphate buffer pH 7.4 that characteristic of novel aqueous based developed formulation may potentially help to avoid injection site pain, irritation, phlebitis and embolism during IV administration. IVR study carried out to predict the release profile of entrapped drug against unentrapped drug (plain solution) at physiological pH showed that entrapped Clopidogrel released faster than plain solution (unentrapped) which support molecular solubilization upon slow diffusion of the drug while plain drug aggregates

and diffuse slowly through dialysis membrane due to poor solubility upon sudden exposure at physiological pH. Short term storage stability of optimized formulation showed a significant change in particle size and assay at RT condition and found physically and chemically unstable at a higher temperature. At 2°-8°C condition, there is no significant change in assay and particle size of formulation, it found chemically and physically stable for 3 month storage period.

CONCLUSION: A novel injectable drug delivery system was successfully developed for Clopidogrel using safe excipients. Developed Clopidogrel liposome as a parenteral drug delivery could be an alternative to the oral dosage form owing to its ability to provide better drug release without causing any precipitation upon exposure to blood pH enabling IV administration and may potentially overcome the limitation of oral formulation providing rapid onset of action in acute emergency settings like PCI/ACS**.**

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