FORMULATION, OPTIMIZATION AND CHARACTERIZATION OF TICAGRELOR-LOADED SOLID LIPID NANOPARTICLES USING CENTRAL COMPOSITE DESIGN

Shailaja Pashikanti[1], Aswini Lavanya Pudi[2*], Dinesh Kumar Chandanapalli[3]

Abstract:

Background: Ticagrelor (TGL) is an antiplatelet agent that belongs to BCS class IV and has an oral bioavailability of 36%.

Objectives: The intention of my present study is to develop an optimized formulation of Solid lipid nanoparticles that increase the solubility and permeability of ticagrelor by using a central composite design.

Materials and methods: The PSLNs are prepared using the hot homogenization method, GMS, Poloxamer407 and Tween 80. The lipid to drug ratio (X_1), the concentration of surfactant (X_2) and the amount of PEG 2000 (X_3) are considered as independent variables and particle size, Zeta potential, % Encapsulation efficiency and PDI are considered as the dependent variables. The optimized PSLNs are further characterized for an *in-vitro, ex vivo,* DSC, SEM and TEM analysis.

Results: Initial trail runs are done for selecting the ranges for optimization. The design provided 16 runs, the particle size, zeta potential, %EE and PDI ranges from 323nm to 648nm; -21.45mV to -37.63mV; 83.28% to 92.00% and 0.42 to 0.52 respectively. The optimized formulation developed from the design with 0.548 probability showed 468.9nm particle size, -29.37mV zeta-potential, 88.87% EE and 0.36 PDI. This showed *in-vitro* release up to 24h with 93.42% drug release and it showed non-fickian diffusion as the n value is 0.52. The *ex-vivo* has a steady-state flux of 0.0096mg/cm^2 min The SEM and TEM studies showed that the formulation was pegylated and the drug was encapsulated.

Keywords: Ticagrelor, PEGylated SLNs, Optimization, Central, Composite design

[1,2*,3]Department of Pharmaceutical Technology, A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India, Pincode 530003

***Corresponding Author:** Aswini Lavanya Pudi
Department of Pharmaceutical Technology, A.U College of Pharmaceutical Technology, Andhra University, Visakhapatnam, Andhra Pradesh, India – 530003, E-mail: aswini.pudi0039@gmail.com

DOI: 10.48047/ecb/2023.12.7.305

INTRODUCTION:

Hemostasis is a highly complex and tightly regulated process involving the blood cells soluble plasma proteins and vessel walls for preserving the integrity of the circulatory system. Damage of endothelium and exposure of the sub-endothelium matrix activates hemostasis. Primary hemostasis and secondary hemostasis are the two stages of blood clotting. Primary hemostasis involves vasoconstriction, platelet adhesion, platelet activation and platelet aggregation. The platelet activation is mediated by involving the release of serotonin, platelet-activating factor and adenosine phosphate from the platelet granules. ADP is stored specifically in the dense granules of platelets. The released ADP binds two purinergic receptors $P2Y_1$ and $P2Y_{12}$ in the platelet membrane, $P2Y_{12}$ plays a major role in the clotting cascade. Pathological thrombosis occurs when the hemostatic pathway is so strongly activated that it exceeds the normal regulatory counterbalance. [1] There are a number of classes of drug to prevent pathological thrombosis, but anti-platelet drug plays a major role in arterial thrombosis.

Thienopyridines (Ticlopidine, Clopidogrel and Prasugrel) and Direct $P2Y_{12}$ antagonists (Ticagrelor and Cangrelor) are different drugs in the antiplatelet class. [2] Among these clopidogrel is most used, but when compared to clopidogrel, TGL has some advantages there is less inter-individuality, it is not a prodrug, doesn't require the CYP genes to show activity and noncompetitive inhibitor for receptors. But TGL belongs to BCS class IV which has low solubility and low permeability. The oral bioavailability of TGL was around 36%, and most of the drug was eliminated through feces (57.8%). [3]

Solid lipid nanoparticles (SLNs) are colloidal dispersions made of solid lipid core stabilized by surfactants, which overcome the stability problems of the traditional colloidal carriers. The SLNs are biocompatible and non-toxic as they are made of physiological and biodegradable lipids.[4] For providing long circulation time in the body, the SLNs can be surface modified by using polyethylene glycol (PEG) provides stealth SLNs or PEGylated SLNs (PSLNs) that can also be used for drug targeting and long-term drug release. The pg-p efflux can also be modified by using a suitable surfactant, tween 80, pluronic and the combination of both can overcome pg-p efflux.[5,6]

There are a number of process variables like lipid to drug ratio, the concentration of surfactant, homogenization speed, time, amount of PEG and more. Developing a suitable optimized formulation by controlling all these variables can be done by using quality by design. Response surface methodology (RSM) is an established method supported by statistical software to develop and optimize pharmaceutical formulations by obtaining the maximum information with a few well-designed experiments. Central composite design (CCD) is one of the techniques in RSM that allows the development of an optimized formulation of a suitable target with less number of experiments.[7] The present aim of our research work is to develop, optimize and characterize theticagrelor-loadedd PEGylated SLNs (T-PSLNs) using a central composite design.

MATERIALS AND METHODS:

Materials: The drug ticagrelor was obtained as a gift sample from Lee Pharma Pvt. Ltd. Glyceryl monostearate, poloxamer 407, Tween 80 and Polyethylene glycol 2000 were also obtained from the Lee Pharma. The remaining chemicals like Stearic acid, Palmitic acid, Tween 20, etc., are used in this study were purchased from an authorized chemical provider.

Methods:

Preparation of standard graph of ticagrelor:

The standard graph of TGL was studied in 1.2 and 6.8 pH buffers of 0.2%w/v tween80. 25mg of TGL was weighed accurately and transferred into a 25mL volumetric flask, to this 1.2pH buffer was added up to the mark. From this, the stock solution working standard of 100µg/mL and the working aliquots of concentrations 2,4,6,8, and 10µg/mL were prepared, and the absorbance was observed at 298nm using a UV spectrophotometer. The same procedure was followed for the 6.8 phosphate buffer. This was done in triplicates.

Selection of solid lipid:

Glyceryl monostearate, Stearic acid and Palmitic acid are the three types of lipids selected for this study. For the selection of suitable lipids among these solubility studies are conducted. 10 mg of the drug was accurately weighed and transferred into a test tube. With continuous stirring, 0.1 g of solid lipid was added incrementally. The test tube was heated in a temperature-controlled water bath that was kept at $80\pm5^{o}C$ above the melting point of the respective lipids. The amount of solid lipid absorbed by the drug in order to form a clear

solution was measured. The experiments were carried out in triplicate.

Selection of surfactant:

In the present study Tween 80, Tween 20 and Poloxamer 407 were selected. An excess amount of the TGL was added to 10 ml of 1%w/v of surfactant solution. The mixture was stirred for 4 h and the sample was centrifuged at 10,000rpm for 15 minutes. The supernatant was diluted with methanol and analyzed by using a UV spectrophotometer. The experiment was performed in triplicates.[8]

Fourier Transmission infrared (FT-IR) analysis:

The physicochemical compatibility between TGL and other excipients used in the research was carried out by using an alpha Fourier Transform Infrared Spectrophotometer, Bruker. In this method, 10 mg of drug sample/sample mixture and 200 mg of KBr were taken in a mortar and triturated. A little amount of triturated sample was taken into a pellet maker and was compressed at 6 to 8 tons. The pellete scanned from 4000 cm^{-1} to 400 cm^{-1}. Initially, FTIR spectra of pure TGL were taken and then 1:2 ratios of the drug and each excipient and physical mixture were studied.

Preparation of Ticagrelor-loaded PSLNs:

The SLN s was prepared by using the hot homogenization method followed by sonication method. Accurately weigh the required amount of lipid and it was heated above its melting point in the water bath. To the melted lipid required amount of PEG and TGL was added. This was heated until a clear organic phase was formed. Simultaneously the aqueous phase of 50mL (1:1 of Tween 80 and Poloxamer 407) was prepared with the required amount of surfactant and heated up to the temperature of the organic phase. Now the aqueous phase was slowly transferred into the organic phase at the same temperature under a magnetic stirrer at 500rpm, this resulted in microemulsion. Now the microemulsion was homogenized by using ultra turrax 25 homogenizers at 10000 rpm for 15 min. The formed dispersion was cooled at room temperature. Then the dispersion was sonicated for 5 min at 50% amplitude with 2 sec run time and 3 sec pause time.[9]

Optimization of T-PSLNs:

The quality of the optimized formulation depends on different factors, so initially some preliminary trials were done and the parameters affecting were identified. To investigate the parameters statistically of the selected responses a response surface methodology (RSM) was used. Design-Expert 13.0.1.0 software (Stat-Ease Inc.), a central composite design (CCD) of three factors and two levels was used to optimize the independent variables and evaluate both the main and interaction effects on the dependent variables. Lipid to drug ratio (X_1), surfactant concentration (X_2), and amount of PEG (X_3) were used as independent variables, and their effects on particle size (Y_1), zeta potential (Y_2), %encapsulation efficiency (Y_3), and PDI (Y_4) were investigated. Table 1 shows the dependent and independent variables chosen for SLN formulation.

Table No 1: Composition of TGL loaded SLNs dispersion and their respective responses

Run	Factors				Responses		
	A: X1	B: X2 (%)	C: X3 (mg)	Particle size(nm)	Zeta potential (mV)	%EE	PDI
SLN-1	3.5	325	2	448.3	-30.13	88.12	0.42
SLN-2	3.5	619.31	2	593.8	-28.17	87.98	0.44
SLN-3	5	500	3	587.4	-33.31	90.37	0.52
SLN-4	5	150	3	533.1	-37.63	90.83	0.49
SLN-5	2	500	3	448.2	-27.68	83.28	0.44
SLN-6	2	500	1	587.7	-21.45	85.73	0.40
SLN-7	6.02	325	2	633.5	-30.45	91.77	0.48
SLN-8	0.98	325	2	323.6	-25.17	84.12	0.43
SLN-9	5	500	1	648.6	-26.54	92.00	0.48
SLN-10	3.5	325	0.32	465.9	-25.37	87.98	0.40
SLN-11	5	150	1	608.3	-29.18	91.67	0.46
SLN-12	2	150	1	447.9	-23.61	86.13	0.44
SLN-13	2	150	3	395.9	-29.73	83.75	0.46
SLN-14	3.5	325	3.68	420.5	-31.40	86.97	0.48
SLN-15	3.5	30.69	2	383.2	-31.31	88.01	0.48
SLN-16	3.5	325	2	439.4	-30.29	87.76	0.43

A total of 16 runs are provided by the design, out of these factorial points are 8, axial points are 6 and 2 central points. The responses were evaluated statistically using variance analysis (ANOVA). Further, the optimized formulation was selected by using numerical points.

***Characterization**:*

Particle Size, PDI and Zeta Potential:

The particle size distribution, PDI and zeta potential were measured by using a Horiba Nanoparticle size analyzer. The prepared dispersion was diluted twice using double distilled water. The dynamic strength of light dispersion was set by the instrument on the basis of the medium viscosity, i.e. 90^o light dispersion. SLNs PDI < 0.7, suggested the distribution of the unimodal or uniform monodisperse size. In an electrophoretic cell with an electric field of 80 mV, the diluted SLN dispersions were loaded into the probe at 25^oC and Zeta potential was measured.

%Encapsulation Efficiency: *[10]*

The % encapsulation efficiency was calculated by using an indirect method known as the ultracentrifugation method. For this, the SLN formulation was centrifuged in a cooling centrifuge at 15000 rpm for 1h. The amount of TGL-free drug in the supernatant was analyzed by using UV spectroscopy at 298nm. The below equation was used to calculate the %EE.

$$\%\ \text{Encapsulation efficiency} = \frac{\text{Total amount of ticagrelor} - \text{Amount of free ticagrelor}}{\text{Total amount of ticagrelor}} \times 100$$

In-vitro drug release and kinetics: *[11]*

The drug release was performed by the modified dialysis bag diffusion method. The dialysis membrane bag retains nanoparticles and allows the free drug into the dissolution media. Before the 12h use of the dialysis bag was soaked in double distilled water. Take the required amount of lyophilized T-PSLNs equivalent to 10mg of TGL and 10 ml of distilled water was transferred into the bag. The bag was clamped at both ends. The bag was displaced in 250 ml of 0.2%w/v polysorbate 80 pH 1.2 phosphate buffer for an initial 2h and then the dissolution media was replaced with the 0.2%w/v polysorbate 80 pH 6.8 phosphate buffer. At 37 °C and 250 rpm, the beaker was put into a thermostatic magnetic stirrer. A syringe equipped with a 0.22 m filter was used to remove 5 mL samples from the dissolution media at predetermined intervals up to 24 h for analysis. By backwashing the same volume of drug-free media through the syringe during sampling, the T-PSLNs that were drawn into the syringe filter were transferred back into the dissolution media. The samples were examined with a UV spectrophotometer to determine their drug content.

The optimized T-PSLNs' *in vitro* drug release data were fitted to a number of kinetics equations, including the zero order, first order, Higuchi model, and Korsmeyer Peppas model. For figuring out the mechanism and kinetics of drug release, the correlation coefficient (R^2) values were obtained.

Ex vivo studies: *[12]*

The study was performed by using a Sprague Dawley rat of 250g. A non-everted gut sac method was used for permeation of TGL from pure drug suspension and lyophilized optimized T-PSLNs formulation of an equivalent amount of drug. The test procedure was approved by the animal ethics committee of Andhra University. According to the procedure the animals were sacrificed and the small intestine was removed and a length of 7cm of jejunum region was carefully separated and washed with cold Krebs ringer phosphate buffer and oxygenated with O_2 /CO_2 (95%/5%) at 37°C. TGL suspension and T-PSLNs of the appropriate amount of 2mg were transferred into the intestine and sealed at both ends with silk thread. The intestine was suspended in 200ml of 0.2%polysorbate 80 of 6.8 phosphate buffer which was stirred at 200rpm at 37 ± 0.5°C. At certain time intervals, 5ml of the sample was removed and 5ml of methanol was added and the sample was analyzed using UV spectroscopy method at 298nm. The apparent permeability and study state flux were calculated by using below equation.

$$P\text{app} = \frac{dQ}{dt} \times \frac{1}{A.Co}$$

Where dQ/dt is drug permeation rate, C_o is the initial concentration of TGL, A is the area of cross section of intestine.

Differential Scanning Calorimetry:

The nature of the SLNs whether crystalline or amorphous can be determined by using Differential Scanning Calorimetry (DSC) analysis using a DSC 6000 (Hitachi, Japan). Indium was used to calibrate the parameters of melting point. In the temperature range of 30°C-300°C, a heating rate of 10°C/min was used. Standard aluminum pans were used to weigh the samples, with an empty pan used as a control. Pure drugs, physical mixtures, and T-PSLNs were evaluated using DSC thermograms.

Surface Morphology:

Scanning Electron Microscopy:

The surface morphologies of the PEGylated and non-PEGylated TGL-loaded SLNs were characterized by SEM (Zeiss, Jena, Germany). Deionized water was used to dilute the samples (1:50). A thin liquid film made of 20µl of the diluted suspensions was placed onto a copper grid that had been coated with carbon before being dried with filter paper. The grids were examined by SEM after drying.

Transmission Electron Microscopy:

Transmission electron microscopy was used to study the morphology of T-PSLNs. With freshly prepared phosphor tungstic acid solution (1% w/v), the samples were negatively stained. After drying, these samples were examined using a TEM Jeol 1010 (Tokyo, Japan).

RESULTS AND DISCUSSION:

Standard graph of TGL:

The standard graph of TGL in 1.2 and 6.8pH buffer was shown below in the Table 2 and Fig 1 and the R^2 values are shown. The absorbance was increased linearly with increasing in the concentration of TGL.

Table No 2: Absorbance of TGL in 1.2pH and 6.8pH buffer

Concentration	Absorbance	
	1.2Ph	**6.8pH**
2	0.2065 ± 0.0003	0.1133 ± 0.0003
4	0.3901 ± 0.0004	0.1836 ± 0.0003
6	0.5224 ± 0.0004	0.2699 ± 0.0003
8	0.6242 ± 0.0025	0.3503 ± 0.0002
10	0.7734 ± 0.0004	0.4395 ± 0.0004

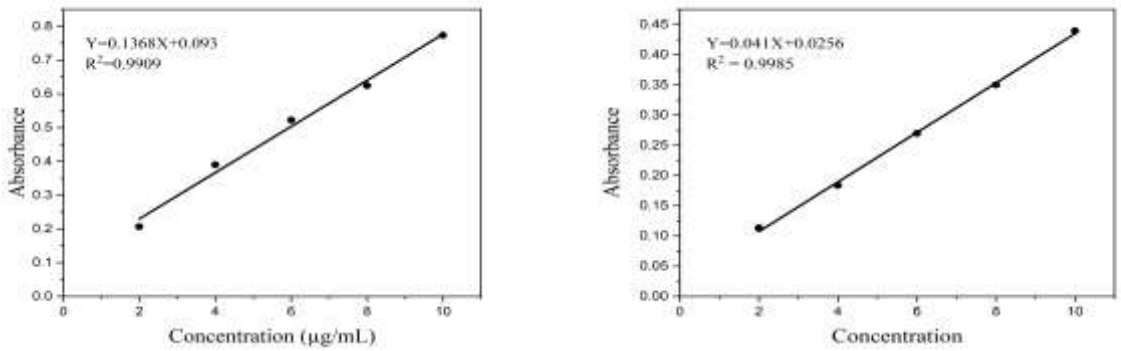


Fig 1: Calibration curve of TGL in 1.2pH and 6.8pH buffer

Selection of lipids:

It is assumed that high lipid solubility can result in high encapsulation efficiency. The experiment data showed in Table 3 the solubility of drug in the lipids follow this order glyceryl monostearate > palmitic acid > stearic acid. The drug solubility in the glyceryl mono stearate is high when compared to the saturated fatty acids (Palmitic acid and Stearic acid) is due to the crystalline nature of the lipids. Generally, compounds with high crystallinity incorporate low amounts of drug. So, GMS was selected as the solid lipid due to its high solubility of drug in it.

Table No 3: solubility of TGL in solid lipids

S.No	Solid lipid	Solubility of drug (mg/g of lipid)
1	Glyceryl monostearate	75.50
2	Palmitic acid	29.30
3	Stearic acid	5.50

Selection of the surfactant:

The screening of the surfactants was done by the solubility of the drug in the 1% w/v of surfactant solution. The solubility of TGL was followed this order Tween 80 > Tween 20 > Poloxamer 407 as shown in Table 4. Generally, the surfactant which

has low solubility of the drug can stabilize the SLNs. So Poloxamer 407 was selected as the surfactant. But the mixture of surfactant can decrease the Pg efflux of the particle. So, the mixture of Tween 80 and Poloxamer 407 was selected and used in 1:1 proportion.

Table No 4: solubility of TGL in surfactants

S.No	Solid lipid	Solubility of drug (mg/g of lipid)
1	Glyceryl monostearate	75.50
2	Palmitic acid	29.30
3	Stearic acid	5.50

Drug excipient compatibility study:

The compatibility studies were done by using FTIR of the pure drug and mixture of pure drug with each excipient and the final physical mixture and the peaks of the TGL pure drug were compared to all the mixtures and the FTIR peaks were given in the figure. The FTIR spectra revealed that TGL exhibited characteristic absorption peaks at 3400 to 3700 cm^{-1}(O-H Str), 3293.92cm^{-1}(N-H Str), 2700 to 3000 cm^{-1} (C-H str) 2025.81 cm^{-1}(N=C=S stretch) 1625.41 cm^{-1}(C=C Str) 1588.03 cm^{-1}(C=N Str)1517.98 cm^{-1} (N=N Str),1273.85 cm $^{-1}$ (C-F Str). The drug with each excipient and the physical mixture also showed these characteristic peaks as shown in Fig 2.

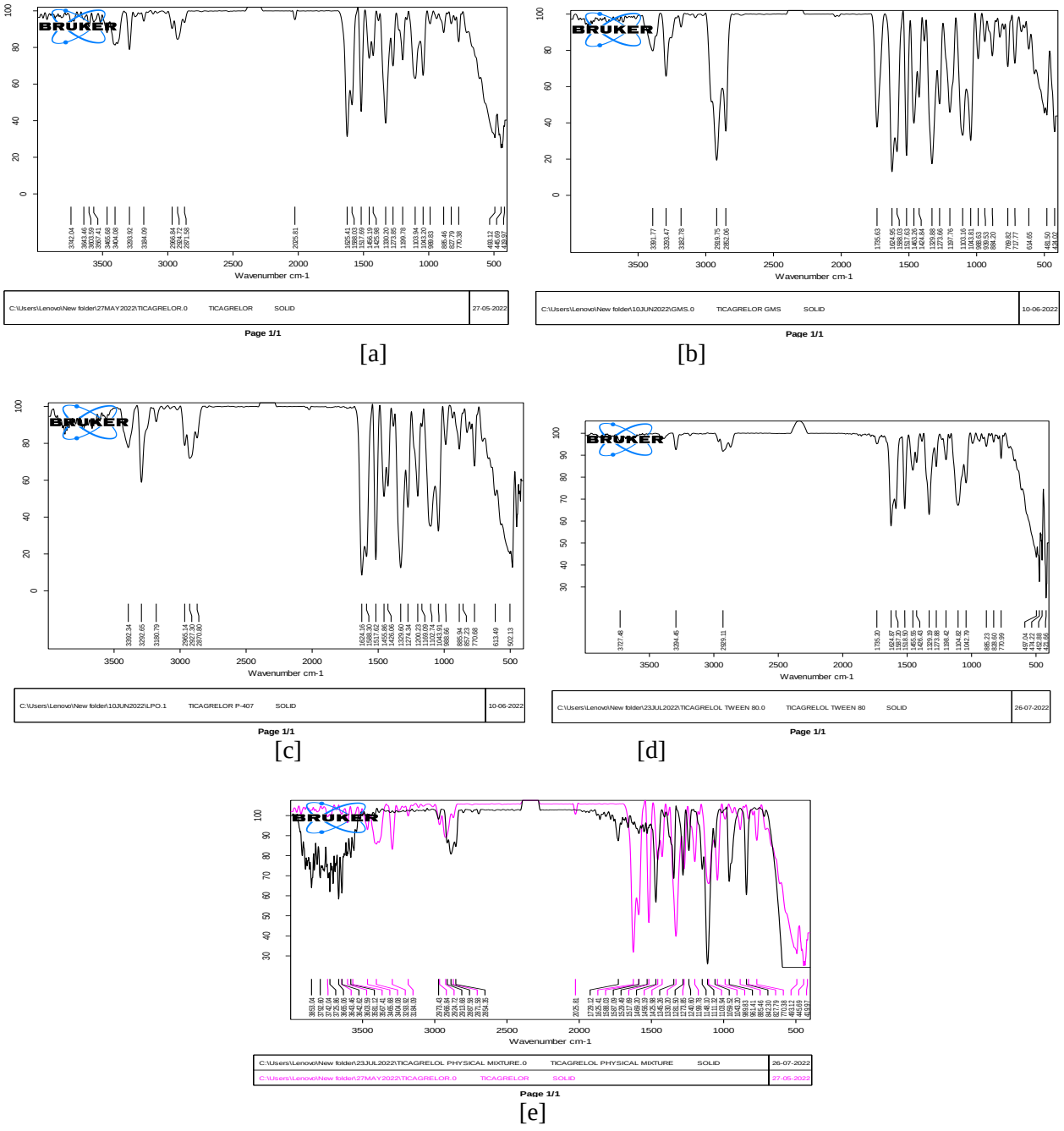


Fig 2: FTIR data of [a] TGL pure drug; [b] TGL and GMS; [c] TGL and P-407; [d] TGL and Tween 80; [e] TGL and Physical mixture

Optimization of T-PSLNs:

A central composite design was used for optimizing the T-PSLNs by fitting the response values obtained from the experiments developed by the DoE. Optimizing the formulation variables was done by the response surface methodology of the design. By giving the lower limit and upper limit of the independent variables the design provided the 16 trail runs. All the runs are prepared by using the abovementioned method. The particle size(Y_1), zeta potential (Y_2), % encapsulation efficiency(Y_3) and PDI(Y_4) are considered as the responses that evaluate the stability and bioavailability of the formulated T-PSLNs. The smaller Y_1 and Y_4 mean the smaller particle size and particle size distribution can enhance the intestine permeability through paracellular and intracellular and the formulation was homogenous. The lower or optimum Y_2 can result in the production of stable SLNs dispersion. The higher Y_3 means more amount of drug encapsulation in the SLNs resulting in more drug permeation. The statistical model of Y_1, Y_2, Y_3 and Y_4 responses all fitted in the linear model. Statistical values such as sequential *p*-value, lack of fit, adjusted R^2 and predicted R^2 were evaluated to determine the suitability of the model. The sequential *p*-value in all the models suggested by the design was less than 0.05, that indicating the statistical hypothesis was significant at the 95% confidence interval. The design was interpreted as a good fit as the difference between the adjusted R^2 and predicted R^2 was less than 0.2.

Effect of independent variables on particle size:

The particle size of all the 16 runs ranged from 323.6(SLN-8) to 633.5nm (SLN-7). The independent variable X_1 and X_3 were statistically significant parameters from the ANOVA test as the *p* value is less than 0.05 and whereas the concentration of surfactant does not show that significant effect. The ANOVA test for particle size shows that the linear model was significant and fit the data. In terms of coded value, the generated equation was as follows:

Particle size = $472.95 + 99.51X_1 – 29.62X_2 + 46.93X_3$

This means the increase in the lipid to drug ratio and amount of PEG can increase the viscosity of the organic phase and result in a larger particle size, as the PEG was directly added to the lipid phase the interaction of PEG with the lipid increases as the amount of PEG increased and resulting in larger particle size. As the amount of surfactant increased the particle size was found to be decreased, this is due to the decrease in the interfacial tension between the lipid and aqueous phase and thus leading to the formulation of stable and small nanoparticles. The plots in Fig 3a and 3b show the effect of the independent variables on the particle size.

Effect of independent variables on zeta potential:

The zeta potential ranged from -21.45(SLN-6) to -37.63mV (SLN-4) for all the runs provided by the design. The independent variables X_1, X_2 and X_3 were statistically significant parameters from the ANOVA test as the *p-value* is less than 0.05. The ANOVA test for zeta potetial shows that the linear model was significant and fit the data. In terms of coded value, the generated equation was as follows:

Zeta potential = $-28.03 - 3.23X_1 – 2.76X_2 + 1.20X_3$

The lipid to drug ratio (X_1), concentration of surfactant (X_2) showed a negative effect while the amount of PEG(X_3) showed positive effect on the zetapotential. The lipid and the surfactant have some negative charges. As the concentration of lipid and surfactant were increasing there is an increase in the negative charge, that leads to the lesser zeta potential of the SLNs. The increase in amount of PEG reduced the charge around the SLNs thus showed the lesser negative charge The plots in Fig 3c and 3d show the effect of the independent variables zeta potential.

Effect of independent variables on %encapsulation efficiency:

The encapsulation efficiency (EE) ranged from 92 (SLN-9) to 83.28% (SLN-5) for all 16 runs provided by the design. The independent variable X_1 and X_2 were statistically significant parameters from the ANOVA test as the *p-value* is less than 0.05 and X_3 does not show that significant effect. The ANOVA test for %EE shows that the linear model was significant and fit the data. In terms of coded value, the generated equation was as follows:

%encapsulation efficiency = $86.96 + 3.79X_1 – 0.6594X_2 – 0.0769X_3$

The lipid to drug ratio(X_1) has shown a positive effect, the concentration of surfactant(X_2) showed a negative effect and the amount of PEG(X_3) does not show that significant effect on the %EE. The

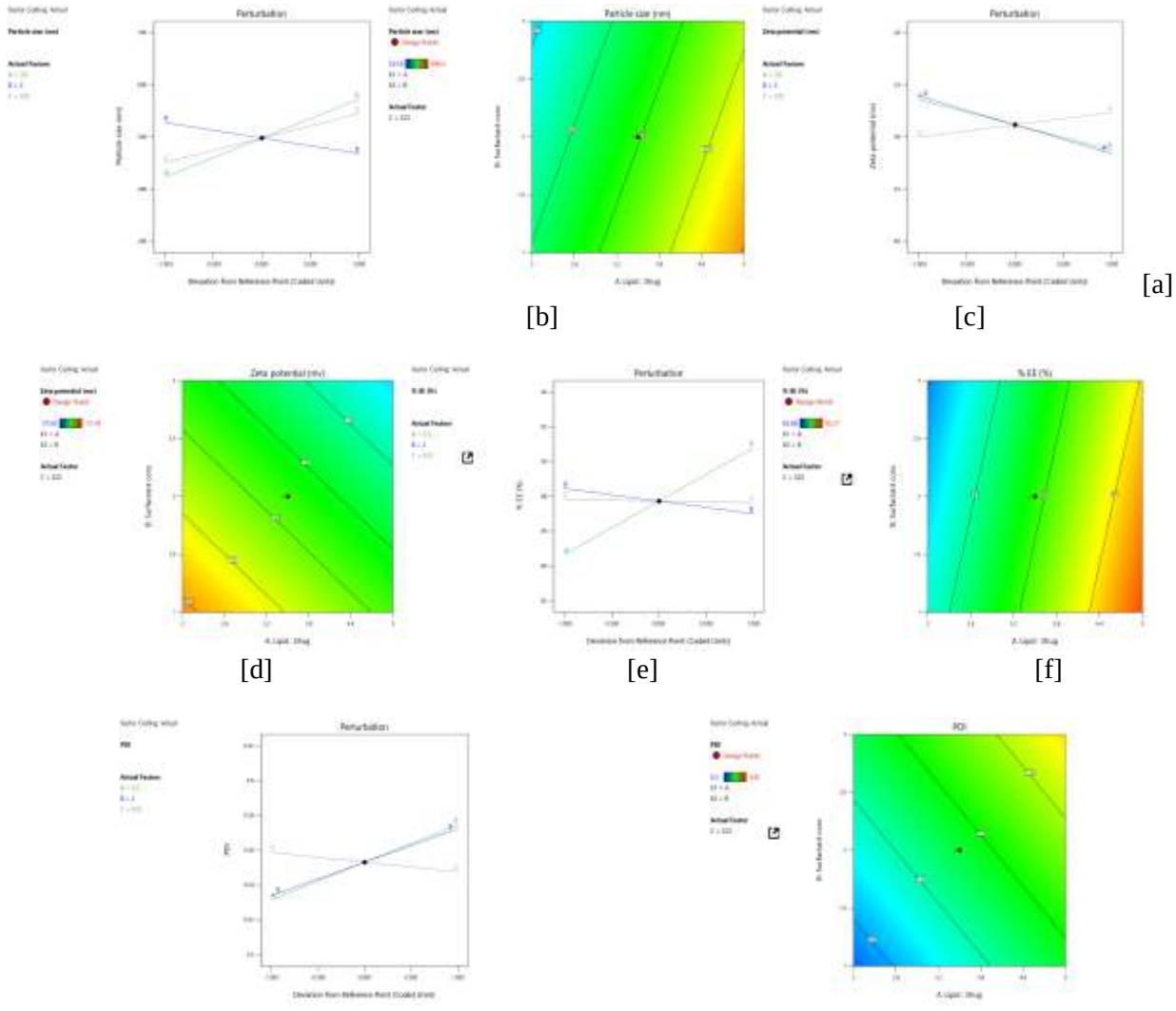
increase in the lipid amount in the formulation has encapsulated the more drug into the particle as the drug is lipophilic. The negative effect of surfactant is the drug can be portioned into the surfactant solution as that increase in surfactant solution the amount of drug can be portioned more into the aqueous surfactant solution. The plots in Fig 3e and 3f show the effect of the independent variables on the %EE.

Effect of independent variables on poly dispersity index:

The Poly dispersity index (PDI) ranged from 0.40 (SLN-6,10) and 0.52 (SLN-3) for all the runs. The independent variable X_1 and X_2 were statistically significant parameters from the ANOVA test as the *p* value is less than 0.05 and X_3 does not show that significant effect. The ANOVA test for PDI shows that the linear model was significant and fit the data. In terms of coded value, the generated equation was as follows:

Poly dispersity index = $0.4459 + 0.0287X_1 + 0.0194X_2 – 0.0057X_3$

The X_1 and X_2 variables showed positive effects but the X_3 variable showed a negative impact but not a significant. The increase in lipid to drug ratio can increase the viscosity of the lipid phase, as the viscosity increased it is more difficult to produce more homogeneous solution while homogenization so that showed the increase in PDI. The plots in Fig 3g and 3h show the effect of the independent variables on the PDI.



[a] [b] [c] [d] [e] [f] [g] [h]

Fig 3: Perturbation Plot and Surface Overlay Plot of independent variables on [a, b] Particle size; [c, d] Zetapotential; [e, f] %EE; and [g, h] PDI respectively

Optimization and validation:

After analyzing the dependent variables, further optimization and validation of the formulation were done by the numerical method using DoE software with desirable characteristics of SLN of minimum particle size, zeta potential, PDI and maximin %EE. The composition of the optimized formulation was determined as 3.8366 lipid to drug ratio, 1.99% w/v concentration of surfactant and 150mg of PEG2000 fulfilled the requirements of optimization. The predicted values and the observed values are given in Table 5, and the particle size, PDI and zetapotential of optimized formulation was showed in Fig 4.

Table No 5: Predicted and observed values of the optimized T-PSLNs

Responses	Predicted Mean	Predicted Median	Observed	Std Dev	SE Mean	95% CI low for Mean	95% CI high for Mean
Particle size	467.719	467.719	468.9	52.8812	19.7414	424.706	510.732
Zeta potential	-30.5657	-30.5657	-29.3	1.51767	0.566572	-31.8002	-29.3313
% EE	88.5137	88.5137	88.87	0.767066	0.286358	87.8898	89.1377
PDI	0.363461	0.363461	0.36	0.020913	0.0078073	0.346451	0.380472

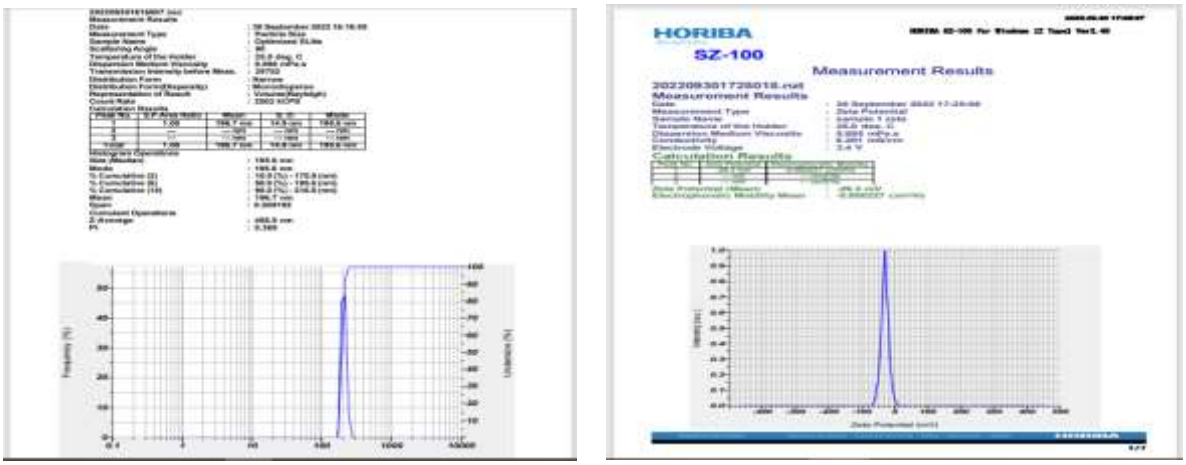


Fig 4: Particle size, PDI and zeta potential of optimized formulation.

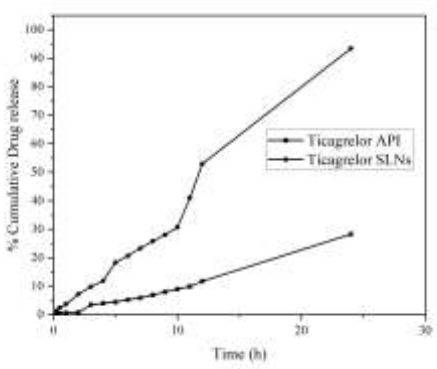
In vitro drug release and kinetics:

The *in-vitro* release studies (table) were performed for 24h for T-PSLNs and pure TGL drug shown in Fig.5. The cumulative % drug release of TGL pure drug and T-PSLNs at the end of 24h was found to be 28.2838% and 93.4232% respectively shown in Table 6, this may as pure drug has crystalline nature it has low solubility, while the T-PSLNs formulation has amorphous nature there is increased in solubility. The drug in nanoparticles was released 24h as the glyceryl monostearate can act as rate controlling lipid. The release kinetics of the T-PSLNs were studied and it showed zero order release. As the release exponent value(n) is 0.52 that indicates the release was non fickian diffusion transport of drug from the SLNs.

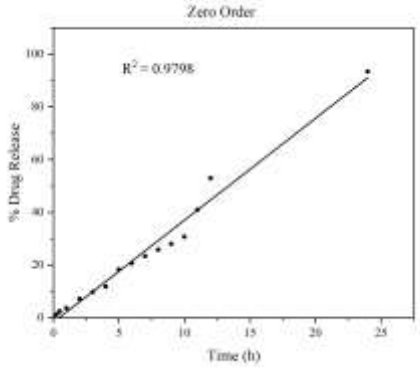
Table No 6: Cumulative % drug release of TGL pure drug and optimized SLNs formulation

Time (h)	Cumulative % Drug Release	
	Ticagrelor Pure Drug	Ticagrelor SLNs Formulation
0	0	0
0.25	0.1754	1.3020
0.5	0.4366	2.4224
1	0.6846	3.6852
2	0.8041	7.1974
3	3.4992	9.8225
4	4.0226	11.8707
5	4.4457	18.3232
6	5.2601	20.6577
7	5.9064	23.3381
8	6.9481	25.7912
9	7.9965	28.0448

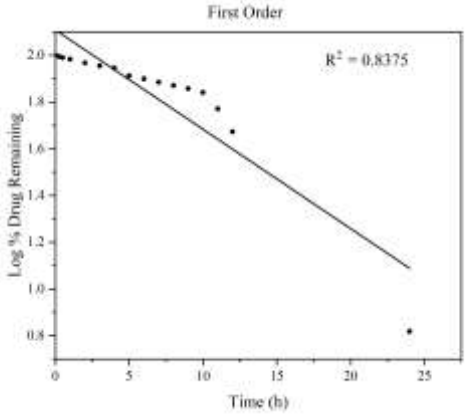
10	8.9965	30.6880
11	9.9102	40.9730
12	11.7537	52.9210
24	28.2832	93.4232



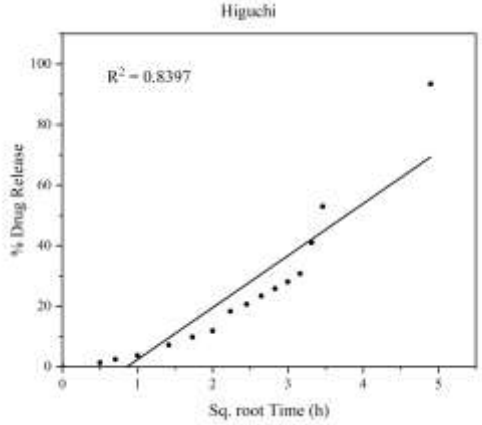
[a]



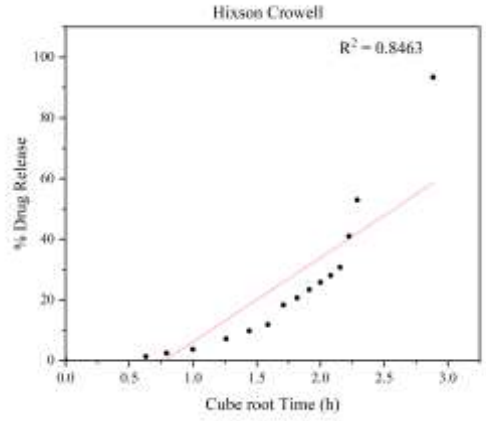
[b]



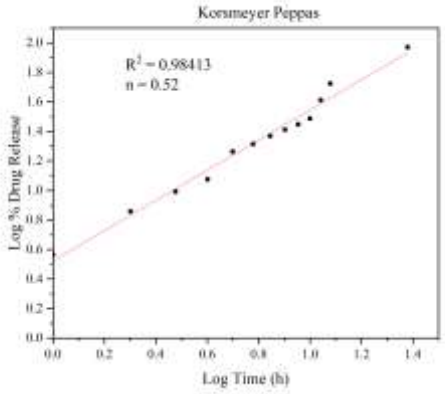
[c]



[d]



[e]



[f]

Fig 5: [a] Cumulative drug release of TGL pure drug and optimized T-PSLNs; [b-f] release kinetics of optimized T-PSLNS

Ex vivo study:

The TGL permeation from the pure drug suspension and T-PSLNs was studied for 4h using

non everted gut sac method. The permeation profile of the two were shown in fig. that clearly indicates that there is an increase in the TGL permeation through intestine from T-PSLNs formulation. At the end of 4h the apparent permeability and steady state flux of TGL suspension was 0.5067mg/cm^2 and 0.0037 mg/cm^2 min respectively and for T-PSLNs 1.6952mg/cm^2 and 0.0096 mg/cm^2 min respectively. Thus, there is an increase in around 3 folds of drug permeated from T-PSLNs when compared to pure drug. The *ex-vivo* cumulative drug release was shown in Fig 6.

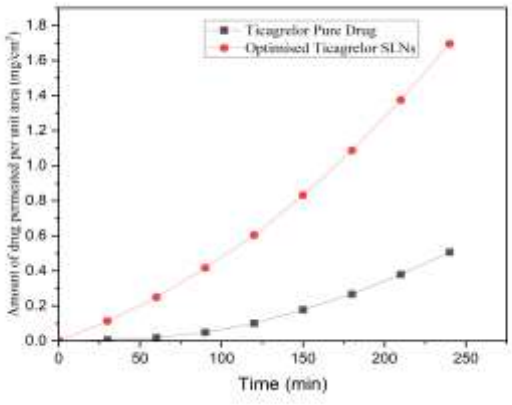


Fig 6: E*x-vivo* cumulative drug release of TGL pure drug and optimized T-PSLNs

DSC study:

The DSC study was conducted for the TGL, and T-PSLNs are shown in Fig. 7. The pure TGL showed a sharp endothermic peak at 140.1^{o}C indicating the crystalline nature of the drug. The T-PSLNs showed two peaks at 56.9^{o}C and 63.5^{o}C that are the peaks of the PEG and GMS respectively. There is no characteristic peak of the drug in the thermogram, this might be due to the drug encapsulated in the T-PSLNs.

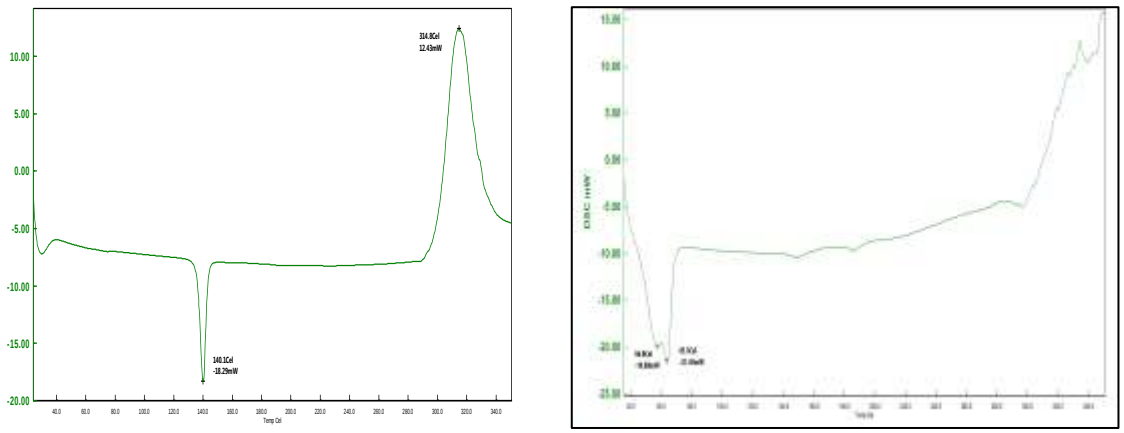


Fig 7: DSC of TGL pure drug and optimized formulation of T-PSLNs

Surface morphology:

SEM studies:

The SEM studies of the optimized PEGylated and non-Pegylated ticagrelor SLNs revealed that there is an increase in the size of the nanoparticle for PEGylated and both are spherical in shape, discrete and monodisperse. The cloudy surface around the nanoparticle revealed that the T-PSLNs were PEGylated. The SEM images were shown in the Fig 8.

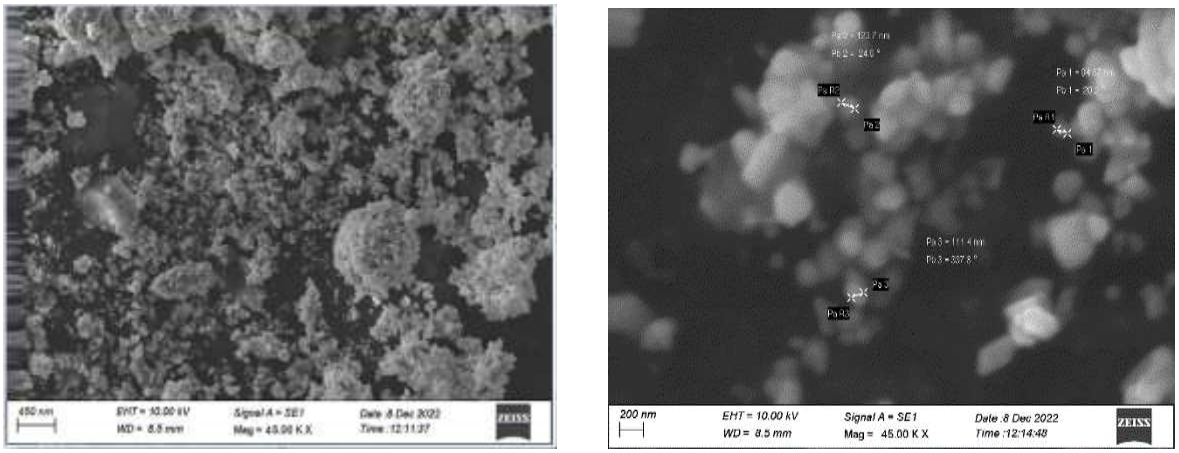


Fig 8: SEM studies optimized formulation of T-PSLNs and T-SLNs

TEM studies:

TEM images of the optimized T-PSLNs are shown in the Fig 9. that revealed that the particle was coated and the drug was loaded in the particle. The TEM image showed that particles are monodisperse, spherical in shape and discrete particles.

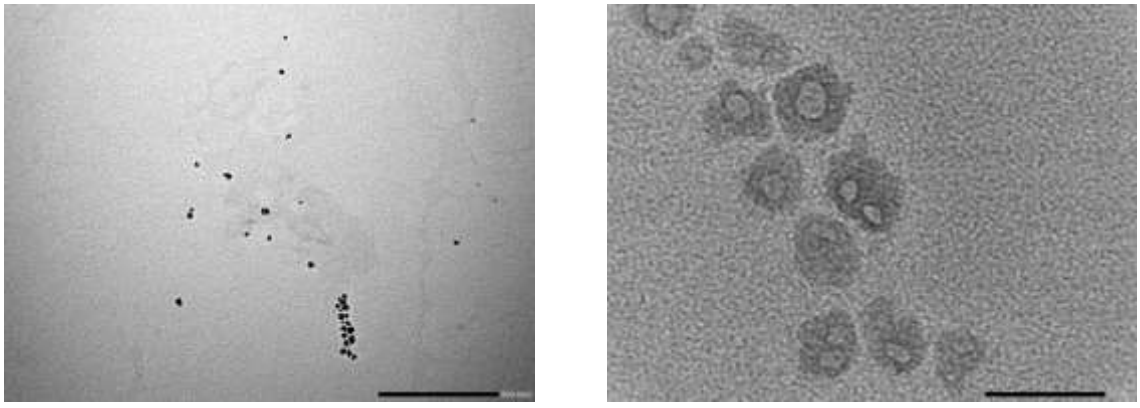


Fig 9: TEM images of the optimized T-PSLNs formulation

CONCLUSION:

T-PSLNs were successfully developed by using GMS as lipid, an equimolar ratio of Tween 80 and Poloxamer 407 as surfactants and PEG 2000 as the surface modifying agent by hot homogenization method followed by sonication. The formulation was optimized by using a central composite design. The prepared nanoparticles showed high entrapment efficiency, and optimum zeta potential and their particle size was in the nanometric range. The T-PSLNs showed a controlled release and an increase in 3 folds of apparent permeability and steady-state flux when compared to the pure drug, which might increase the bioavailability of the drug and might show long time circulation in the blood.

ACKNOWLEDGEMENTS:

We thank Dr. K. Suresh babu (CSIR -IICT) and Dr. K.T. Sunil Kumar (shri Vishnu College of Pharmacy) for providing necessity lab facilities for our research work.

REFERENCES:

1. Chang JC. Hemostasis based on a novel 'two-path unifying theory'and classification of hemostatic disorders. Blood Coagulation & Fibrinolysis. 2018 Nov 1;29(7):573-84
2. Marco Cattaneo,n51 - $P2Y_{12}$ Antagonists, Editor(s): Alan D. Michelson, Platelets (Fourth Edition), Academic Press, 2019, Pages 937-956,
3. Aparna A, Kumar YS, Bhikshapathi DV. Formulation and In Vivo evaluation of ticagrelor self-nanoemulsifying drug delivery systems. Pharmaceutical Nanotechnology. 2021 Feb 1;9(1):61-9.
4. Agrawal YO, Husain M, Patil KD, Sodgir V, Patil TS, Agnihotri VV, Mahajan HS, Sharma C, Ojha S, Goyal SN. Verapamil hydrochloride loaded solid lipid nanoparticles: Preparation, optimization, characterisation, and assessment of cardioprotective effect in experimental model of myocardial infarcted rats. Biomedicine & Pharmacotherapy. 2022 Oct 1;154:113429.

5. Fuchs H, Runge F, Held HD. Excretion of the dipeptidyl peptidase-4 inhibitor linagliptin in rats is primarily by biliary excretion and P-gp-mediated efflux. European journal of pharmaceutical sciences. 2012 Apr 11;45 (5):533-8.
6. Porfiryeva NN, Moustafine RI, Khutoryanskiy VV. PEGylated systems in pharmaceutics. Polymer Science, Series C. 2020 Jan;62:62-74.
7. Hao J, Wang F, Wang X, Zhang D, Bi Y, Gao Y, Zhao X, Zhang Q. Development and optimization of baicalin-loaded solid lipid nanoparticles prepared by coacervation method using central composite design. European Journal of Pharmaceutical Sciences. 2012 Sep 29;47(2):497-505.
8. Kim BS, Na YG, Choi JH, Kim I, Lee E, Kim SY, Lee JY, Cho CW. The improvement of skin whitening of phenylethyl resorcinol by nanostructured lipid carriers. Nanomaterials. 2017 Aug 28;7(9):241.
9. Gambhire VM, Gambhire MS, Ranpise NS. Solid lipid nanoparticles of dronedarone hydrochloride for oral delivery: optimization, in vivo pharmacokinetics and uptake studies. Pharmaceutical Nanotechnology. 2019 Dec 1;7(5):375-88.
10. Kamran M, Ahad A, Aqil M, Imam SS, Sultana Y, Ali A. Design, formulation and optimization of novel soft nano-carriers for transdermal olmesartan medoxomil delivery: In vitro characterization and in vivo pharmacokinetic assessment. International journal of pharmaceutics. 2016 May 30;505(1-2):147-58.
11. Ravi PR, Aditya N, Kathuria H, Malekar S, Vats R. Lipid nanoparticles for oral delivery of raloxifene: optimization, stability, in vivo evaluation and uptake mechanism. European journal of pharmaceutics and biopharmaceutics. 2014 May 1;87(1):114-24.
12. Varshosaz J, Taymouri S, Jahanian-Najafabadi A, Alizadeh A. Efavirenz oral delivery via lipid nanocapsules: formulation, optimisation, and ex- vivo gut permeation study. IET nanobiotechnology. 2018 Sep;12(6):795-806.