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ORAL DELIVERY OF ROSUVASTATIN LIPID NANOCARRIERS: INVESTIGATION OF *IN VITRO* AND*IN VIVO* PROFILE

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ABSTRACT: Present study is a systematic approach to provide 'proof of concept' for effective oral delivery of Rosuvastatin lipid nanocarriers for treatment of hyperlipidemia. Rosuvastatin is a 'Superstatin' belonging to BCS class II having low bioavailability (20%). Development of lipid nanocarriers for oral delivery of rosuvastatin can be beneficial in enhancing bioavailability and providing sustained release. In the current work, Rosuvastatin lipid nanocarriers were developed using Precirol® ATO 5 as lipid carrier and Tween® 80as surfactant, employing solvent emulsification-evaporation method. Investigation of effect of shear rate, solution temperature and concentration of organic solvent as process parameters were studied. Three-level two-factor(3^2) experimental design was applied to study the effect of lipidand surfactant on percent entrapment efficiency and particle size. Complete characterization of optimized formulation such as particle size, zeta potential, entrapment efficiency, TEM and *in vitro* release profile was carried out. Lipid nanocarriers exhibited mean particle size 79.31±4.82 nm, entrapment efficiency 81.29±1.11% and zeta potential -6.89±3.29 mV. *In vitro* diffusion of ROS from lipid nanocarriers using dialysis bag diffusion method provided pH-independent drug release. *Ex vivo* intestinal permeability through chick ileum revealed improvement in apparent permeability coefficient due to lipid coated nano-sized particles. *In vivo* absorption of ROS from lipid nanocarriers resulted in about 4.41-fold increase in $AUC_{0\text{-last}}$ and 8.14-fold increase in mean residence time compared to plain drug. In gist, Rosuvastatin lipid nanocarriers are advantageous in reducing dose and drug consumption, by effectively providing sustained release, which will ultimately help in minimizing dose-dependent adverse effects.

INTRODUCTION: Rosuvastatinis a "super-statin" offering high potency and enhanced cholesterol reduction. It belongs to BCS class II exhibiting elimination half-life of 19 hand is a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, exhibiting dose-dependent pharmacokinetics.

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ROS has been approved by US-FDA in 2003 for the treatment of dyslipidemia [1]. It has also been explored for the treatment of osteoporosis, benign prostatic hyperplasia and Alzheimer's disease [2]. Lipid nanocarriers are basically colloidal carriers having the capability of incorporating both hydrophilic and lipophilic drugs. They can be employed in improving bioavailability and obtaining sustained release of the drug. They incorporate drug in lipid carrier that can be composed of long chain fatty acids which are usually absorbed via chylomicron formation. This improves lymphatic transport of drugs and simultaneously the bioavailability, bypassing the portal circulation [3].

The objective of the current research work was to develop rosuvastatin loaded lipid nanocarriers by solvent emulsification-evaporation technique. Preliminary screening allowed determination of influence of various process parameters on development of nanocarriers. A two-factor, three-level full (3^2) factorial design enabled to study the effect of formulation components on development of nanocarriers. Hypothesis of the work involved improvement of oral bioavailability of rosuvastatin using long chain fatty acids like Precirol® ATO 5(Glycerylpalmitostearate) in conjunction with Tween® 80 (Polysorbate 80) as emulsifier-stabilizer to prepare drug loaded lipid nanocarriers.

MATERIAL AND METHODS:

Materials:

Precirol® ATO 5, was provided as gift sample by Gattefosse India Pvt. Ltd. (Mumbai, India). Rosuvastatin Calcium (ROS) was obtained as gift sample from Astra Zeneca (Gujarat, India). Tween® 80 (polyoxyethylene sorbitan monooleate) and Acetonitrile, HPLC grade were purchased from s.d. Fine chemicals Ltd. (Mumbai, India). Deionized water was obtained using Milli-Q purification system from Millipore. All other chemicals used were of analytical grade.

Preparation of ROS lipid nanocarriers:

A previously reported method by Alshamsan et al., (2014) [4] was employed for the development of ROS lipid nanocarriers, albeit with minor modifications. Briefly, ROS(0.25% w/v) and Precirol® ATO 5 (quantity varied as per experimental design) were dissolved in chloroform forming the lipid phase of emulsion. About 40%v/v of the aqueous phase was prepared by dissolving Tween® 80 (quantity varied as per experimental design) into de-ionized water. Lipid phase and aqueous phase were cyclomixed for 2 min to obtain a white milky emulsion.

This emulsion was added drop-wise to 60%v/v aqueous phase and kept under stirring at 1200 rpm using a magnetic stirrer. This resulted in o/w emulsion which was heated at 55±5°C under stirring until a clear dispersion was obtained. On evaporation of organic phase, the solution was immediately cooled in an ice-bath, with constant stirring, to form ROS lipid nanocarriers. Final volume was adjusted with cold deionized water.

Pre screening study:

Effect of process parameters:

Effect of three process parameters i.e. temperature of solution (35±5, 45±5 and 55±5 °C), concentration of organic phase (5, 10, 15 and 20 %v/v) and speed of stirring (600, 900 and 1200 rpm) were studied to determine their effect on formation of lipid nanocarrier dispersion.

Effect of formulation parameters:

Experimental design:

3^2 experimental design (**Table 1**) describes the proportion in which independent variables Precirol® ATO 5 (X_1) and Tween® 80 (X_2) were used in the formulation of lipid nanocarriers. Entrapment efficiency and particle size were selected as dependent variables. Nine formulations were prepared in three sets in triplicate. Linear regression model was derived from Enter Method using SPSS version 11.5, statistical software. Significance terms were chosen at 95% confidence interval ($p<0.05$) for final equations. In addition, surface plots were obtained by Statistica-6, to represent the effect of independent variables graphically.

The effect of independent variables upon responses were modelled using the second order polynomial equation (1):

$$Y=b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad (1)$$

Where Y indicates predicted response; $b_0$is the arithmetic mean response of 9 runs; b_1 and b_2 are the estimated coefficients for factors X_1 and X_2, respectively. Main effects (X_1 and X_2) represent average result of changing one factor at a time from its low to high value. Interaction (X_1X_2) shows how values of entrapment efficiency and particle size change when two factors are changed simultaneously. The polynomial terms (X_1^2 and X_2^2) are included to investigate non-linearity [5, 6].

TABLE 1: DESIGN LAYOUT OF 3^2 FACTORIAL DESIGN AND SUMMARY OF EXPERIMENTAL RESULTS

Code	ROS %w/v	Precirol®%w/v	Tween® 80 %w/v	Entrapment efficiency (%)Y_1		Particle size (nm) Y_2	
				OV*	PV**	OV*	PV**
F-1	0.25	1	4	25.75±1.52	20.56	2316±79.27	2206.773
F-2		1	6	20.66±1.19	19.31	631.03±308	834.455
F-3		1	8	9.95±1.16	16.51	96.45±12.17	86.105
F-4		2	4	47.56±2.70	58.42	1468±56.24	1569.89
F-5		2	6	62.56±1.77	64.33	771.73±39.90	591.208
F-6		2	8	81.29±1.11	68.68	74.01±11.31	150.494
F-7		3	4	57.26±1.69	51.59	723.53±64.87	728.539
F-8		3	6	65.05±1.66	64.66	168.53±3.21	143.493
F-9		3	8	70.08±1.93	76.17	78.53±1.37	96.415

*OV = Observed value; **PV = Predicted value

Conversion of ROS lipid nanocarrier dispersion to freeze-dried powder:

Dispersion of ROS lipid nanocarriers with 5-8% w/v of mannitol as cryoprotectant was freeze-driedin a lyophilizer (Virtis Benchtoplyophilizer, SP Industry, USA; Model: 2K XL) [7].

Characterization of formulations:

Particle size and zeta potential analysis:

Mean particle size and zeta potential of undiluted ROS lipid nanocarriers and suitably diluted freeze-dried powder (5 mg in 10 mL de-ionized water) were determined using Zeta Sizer Nano Series ZS90 (Malvern Instruments, UK; Model: ZEN3690) [8, 9].

Drug content estimation:

To 10 mL volumetric flask, 1 mL of ROS lipid nanocarrier dispersion was added and volume was made up with acetonitrile. The mixture was sonicated for 10 min to achieve complete extraction of drug, followed by centrifugation at 3000 rpm for 10 min[10]. Solution was filtered using 0.2 µ membrane filter and 1 mL of filtrate was suitably diluted to 50 mL using mobile phase. The content of ROS was assayed by validated RP-HPLC method at λ_{max} 241 nm.

Entrapment efficiency (EE) determination:

For determination of EE, ROS lipid nanocarriers were precipitated by adding saturated sodium chloride solution. Clear liquid (I) and solid residue were collected after centrifugation (CS120GXL, Hitachi, Japan) at 10,000 rpm for 15 min at 4 °C. Solid residue was dispersed in 20 mL of de-ionized water to separate free drug from lipid nanocarriers and then separated by centrifugation. The clear liquid (II) was added to the initial liquid (I) and the free drug content (W_{free}) was determined by validated RP-HPLC at λ_{max} 241 nm. Percentage EE was calculated according to the equation (2) [11].

$$\% EE = (W_{total} - W_{free})/ W_{total} \times 100 \quad (2)$$

Where, W_{total} = Drug content of the system.

Transmission electron microscopy and negative staining:

Morphological examination ROS lipid nanocarrier was performed with TEM (model Philips "CM 200 super twin STEM") using negative staining method[12-14].

***In vitro* drug release studies:**

In vitro release of drug from ROS lipid nanocarrier and plain drug suspension was determined by dialysis membrane method. For *in vitro* release studies, samples containing drug equivalent to 5 mg, re-dispersed in pH 1.2 buffer, were charged into cellulose acetate dialysis bags (Hi media, Dialysis Membrane – 150, molecular weight cut off, 12,000-14,000 Da). Sealed bags were suspended in glass beakers containing 100 mL of buffer pH 1.2.

The medium was continuously stirred on a shaking incubator maintained at 75 rpm and 37±0.5 °C. At specified time intervals, aliquots of 2 mL were withdrawn, filtered through membrane filter and analyzed using validated RP-HPLC at λ_{max} 241 nm. Subsequently withdrawn aliquots were replaced with equal volume of fresh buffer to compensate for loss due to sampling and to maintain sink condition. *In vitro* release of drug was similarly performed in other media (i.e. buffer pH 4.6 and

6.8) to observe the effect of pH on drug release. The measurements were carried out in triplicate [12, 15, 16].

***Ex vivo* intestinal permeability study:**

Intestinal permeability of drug from ROS lipid nanocarrier and plain drug suspension was performed as per procedure mentioned by Ibrahim et al., 2013 [17]. Briefly, samples containing drug equivalent to 2.5 mg were re-dispersed in Krebs ringer solution (pH 6.5) and charged into freshly isolated chick ileum segments and sealed at both ends. Sealed segments were immersed in 100 mL beakers containing oxygenated Krebs solution maintained at 37±0.5 °C. Aliquots of 2 mL were withdrawn at 15, 30, 45, 60 and 90 min and replaced with equal volume of fresh buffer. Amount of drug transported through intestinal segments was determined using validated RP-HPLC at λ_{max} 241 nm. Likewise, permeability of drug through everted gut was also carried out [17].

Pharmacokinetic study:

For pharmacokinetic study, male Wistar rats (180-250g) were purchased from Bharat serum, Mumbai, India. Study protocol was approved by Institutional Animal Ethical Committee (IAEC Protocol Approval no. CPCSEA/SPTM/P-63/2014) and the experiments were conducted as per the norms of Committee for the Purpose of Supervision of Experiments on Animals, India.

Male Wistar rats were divided into three groups as follows: Group I - Control (distilled water 10 mL/ kg, p.o.); Group II: Plain drug suspension in CMC-Na (0.5%w/v) (700 ng/ kg, p.o.) and Group III: ROS lipid nanocarriers (700 ng/ kg, p.o.), with six animals in each group. The animals were kept on fasting for 12 h before study. Under light ether anesthesia, blood samples (about 0.3 mL) were withdrawn into heparinized tubes at 0, 0.083, 0.25, 0.5, 1, 1.5, 2, 6, 8, 24 h and 48 h through retro-orbital route. Plasma was separated by centrifugation at 3000 rpm, 15 min at 4 °C.

About 100 µL of plasma sample along with 100 ng/mL of atorvastatin, as internal standard, was mixed with 1 mL ethyl acetate as extracting solvent (liquid-liquid extraction technique). The samples were vortexed for 5 min and then centrifuged at 4000 rpm for 10 min. Resulting supernatant (900 µL) was evaporated under a gentle stream of nitrogen at 40 °C using Nitrogen evaporator (Biotage Turbovap ® LV Workstation). The dried samples were reconstituted with sufficient amount of mobile phase, vortexed, centrifuged and injected on LC-MS/MS[18]. Pharmacokinetic parameters of all plasma samples for each group was calculated using Win Nonlin software®.

Statistical analysis:

The results were expressed as mean ± standard deviation. Statistical analysis was performed using Graph Pad Prism 6 (Graph Pad software Inc.) by analysis of variance (ANOVA). The significance between treatment pairs in pharmacokinetic study was assessed using Bonferronimultiple comparison and differences were considered significant for p-values <0.05[19].

RESULTS:

Preparation of ROS lipid nanocarriers:

Rosuvastatin loaded lipid nanocarriers were prepared by using solvent emulsification-evaporation method. Chloroform was used as organic solvent, Precirol® ATO 5 as lipid phase and Tween® 80 as surfactant.

Effect of process parameters:

Prescreening was carried out to determine the effect of various process parameters on development of ROS loaded lipid nanocarriers. From **Fig. 1** it is evident that 10%v/v of chloroform was inadequate in dissolving the required amount of components; while 15%v/v of chloroform ensured complete solubility of components. Lower concentration of organic solvent resulted in precipitation of components during emulsification stage leading to slow entrapment of drug.

Additionally, lower stirring speed lead to formation of larger globules at emulsification stage; while stirring speed of 1200 rpm influenced the formation of small spherical globules of uniform size. This also contributed towards faster evaporation of chloroform from the mixture, reducing time required for completion of formulation development. During evaporation stage, solution temperature was considered important, where lower temperature increased the processing time to more

than 24 h and reduced the evaporation capacity of the organic phase. Solution temperature closer to boiling point of organic phase was desirable to ensure complete evaporation of organic phase with less processing time. Thus, solution temperature of 55±5° C was desired to expedite the process of formation of lipid nanocarriers, as higher temperature influenced evaporation of chloroform from the solution.

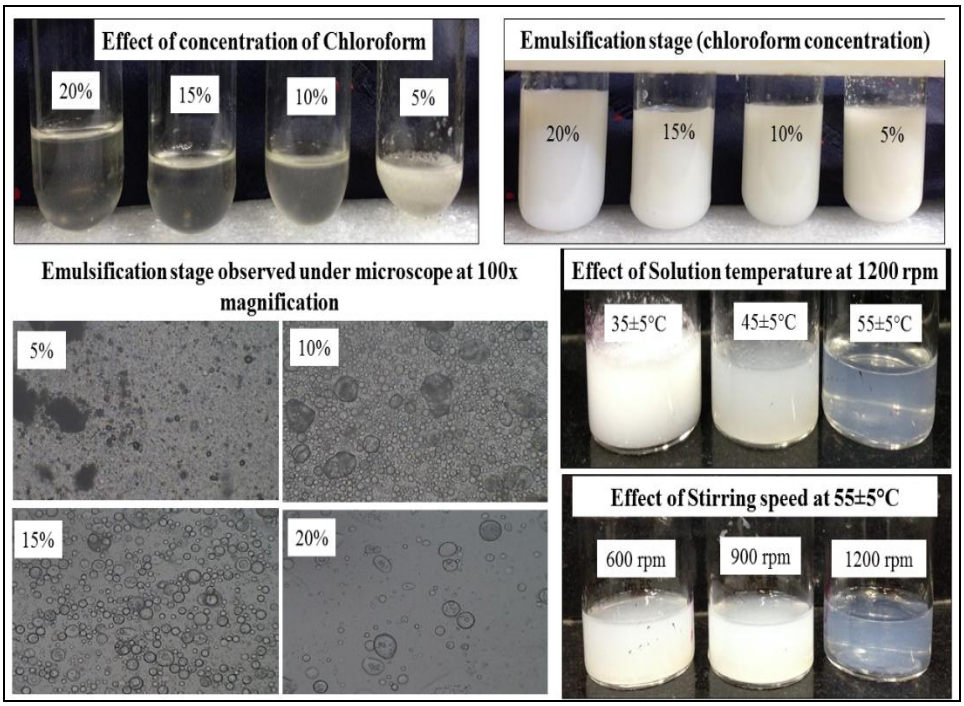

FIG. 1: IMAGES SHOWING EFFECT OF VARIOUS PROCESS PARAMETERS ON FORMULATION OF LIPID NANOCARRIERS

Effect of formulation parameters:

Optimization of ROS loaded lipid nanocarriers was carried out by employing 3^2 factorial design and the experimental runs with observed and predicted responses are depicted in **Table 1**. Least-square second order polynomial equations for entrapment efficiency and mean particle size are shown in equation (3) and (4), respectively:

$$Y_{EE} = (-49.839) + 90.586*X_1 + (-2.263)*X_2 + 3.578*X_1X_2 + (-22.345)*X_1^2 + (-0.194)*X_2^2 \quad (3)$$

$$Y_{PS} = 7785.914 + (-1117.453)*X_1 + (-1555.473)*X_2 + 196.818*X_1X_2 + (-102.234)*X_1^2 + 67.246*X_2^2 \quad (4)$$

Model was found to be significant ($p < 0.0001$) with F-value 42.62 and 102.17 for EE and PS, respectively. The regression co-efficient value (r^2) was 0.91-0.96 indicating good correlation between response and selected factors. Analysis of Variance (ANOVA) of dependent variables obtained are shown in **Table 2** and surface plots in **Fig. 2**.

TABLE 2: ANALYSIS OF VARIANCE (ANOVA) OF DEPENDENT VARIABLES

Dependent Variables	Source of variation	Sum of Squares	Degree of freedom	Mean square	F-ratio	P-value
Y_{EE}	Regression	13340.66	5	2668.13	42.62	0.0001
	Residuals	1314.77	21	62.61		
	Total	14655.43	26			
	r^2	0.910				
Y_{PS}	Regression	13572296.38	5	2714459.28	102.17	0.0001
	Residuals	557938.42	21	26568.50		
	Total	14130234.80	26			
	r^2	0.961				

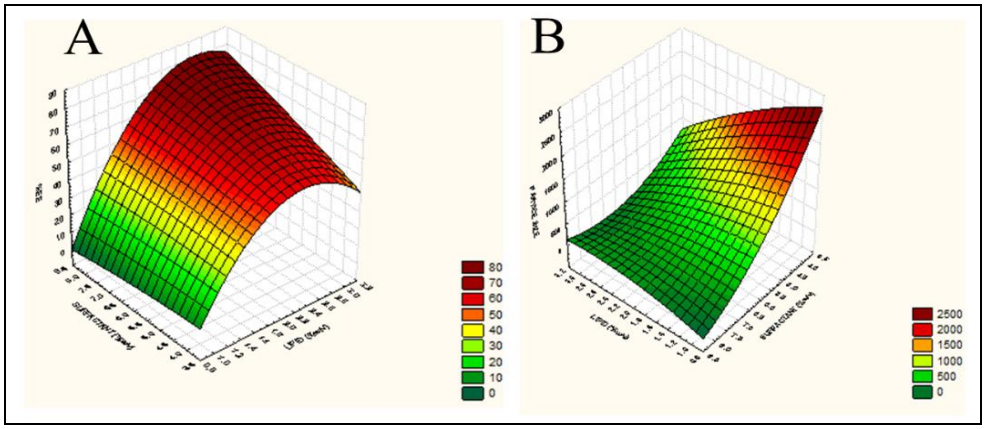

FIG. 2: SURFACE RESPONSE CHARTS OF EXPERIMENTAL DESIGN SHOWING (A) % ENTRAPMENT EFFICIENCY AND (B) PARTICLE SIZE FOR ROS LIPID NANOCARRIERS

Particle size and zeta potential analysis:

ROS lipid nanocarrier provided nanocarriers of mean particle size 79.31±4.82 nm, while the re-constituted freeze-dried powder demonstrated mean particle size of 282.4±12.77 nm. Further, the placebo lipid nanocarrier, ROS lipid nanocarrier and re-constituted freeze-dried powder exhibited zeta potential of -4.39±1.04, -6.89±3.29 and -19.6±4.58 mV, respectively.

Drug content estimation and entrapment efficiency:

Drug content and percent entrapment efficiency of ROS lipid nanocarriers was found to be 100.03±0.89 % and 81.29±1.11%, respectively.

Transmission electron microscopy:

Figure 3 illustrates TEM images of ROS lipid nanocarriers revealing spherical shaped nanocarriers.

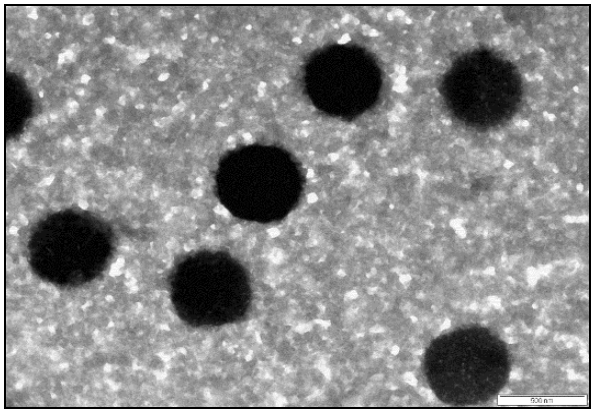

FIG. 3: TEM IMAGE OF ROS LIPID NANOCARRIER

***In vitro* drug release studies:**

Solubility of ROS was more in alkaline media showing diffusion of >90% drug at pH 6.8 while at pH 1.2 the diffusion was seen to be the least, owing to less solubility of drug in the acidic media. The release profiles of ROS lipid nanocarriers showed sustained release of the drug from the lipid matrix when compared with ROS suspension. The lipid nanocarriers showed comparable drug release pattern at all pH, with 20% release within initial 4h at pH 6.8, followed by sustained release. Comparative dissolution profiles of ROS suspension and ROS loaded lipid nanocarriers in multimedia are shown in **Fig. 4**. Since solubility of ROS was more in alkaline medium, pH 6.8 phosphate buffer is used as the diffusion medium to study the release kinetics. The ROS lipid nanocarriers followed first order release kinetics with r^2 value 0.9803.

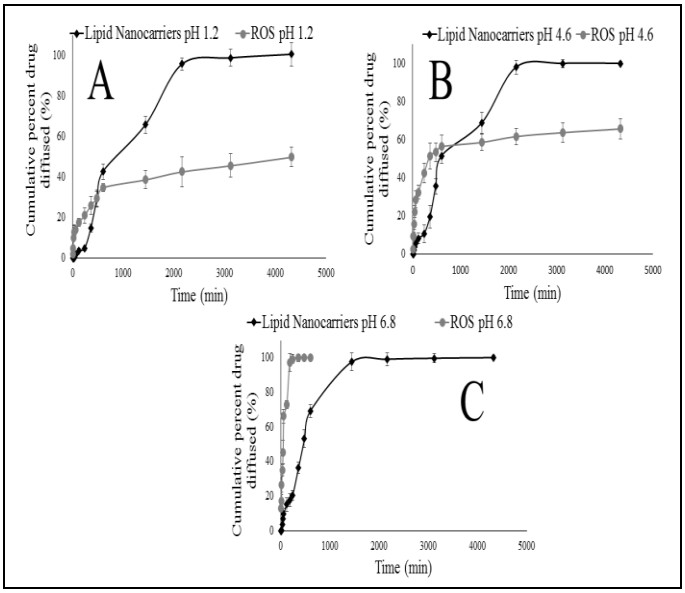

FIG. 4: *IN VITRO* DIFFUSION STUDIES OF ROS FROM PLAIN DRUG SUSPENSION AND ROS LIPID NANOCARRIERS IN (A) pH 1.2, (B) pH 4.6 AND (C) pH 6.8 BUFFER

Ex vivo intestinal permeability study:

In everted gut sac, the diffusion of drug from lipid nanocarriers was higher indicating higher permeability while drug permeability was comparatively less through non-everted gut sac. Permeability of drug from plain drug suspension showed similar trend for everted and non-everted model, however the magnitude was significantly lower (students t-test, $p < 0.05$) for non-everted gut sac than everted gut sac, reaching a value of 33.9±8 μg/cm^2 after 90 min (**Fig. 5**).

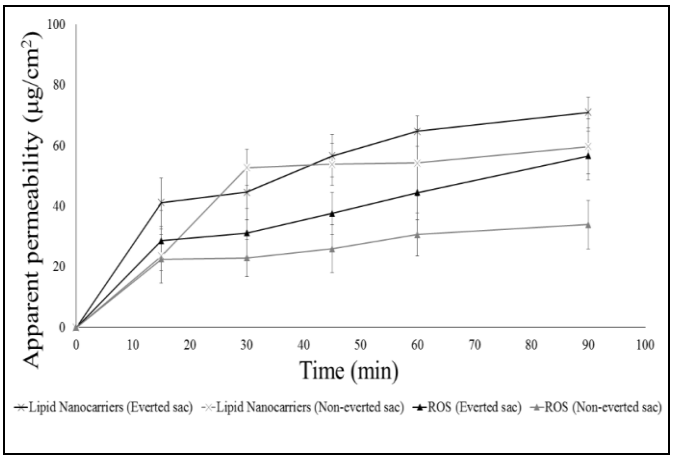

FIG. 5: *EX VIVO* PERMEATION PROFILE OF PLAIN DRUG SUSPENSION AND LIPID NANOCARRIERS THROUGH EVERTED AND NON-EVERTED GUTSAC (MEAN±SD, n = 3)

Pharmacokinetic study:

ROS lipid nanocarriers exhibited4.41-fold increase in $AUC_{0\text{-last}}$ in comparison with plain drug suspension (**Table 3**). The rate and extent of absorption of drug from lipid nanocarriers suggested superiority in the oral bioavailability of drug from the system. As per the plasma concentration-time curve (**Fig. 6**), statistically significant difference in plasma concentration was observed for lipid nanocarriers and plain drug suspension at all time-points ($p < 0.05$).

TABLE 3: PLASMA PHARMACOKINETIC PARAMETERS AFTER ORAL ADMINISTRATION OF PLAIN DRUG SUSPENSION AND LIPID NANOCARRIERS (MEAN±SD, n = 6)

Pharmacokinetic parameter	Plain drug suspension	Lipid nanocarriers
C_{max} (ng/ mL)	9.08±0.36	6.55
T_{max}(h)	1.5	1
$AUC_{0-\infty}$ (ng h/ mL)	39.44	174.05
K_{el} (h^{-1})	0.13	0.01
$t_{½}$ (h)	5.55	68.38
MRT (h)	9.80	79.79

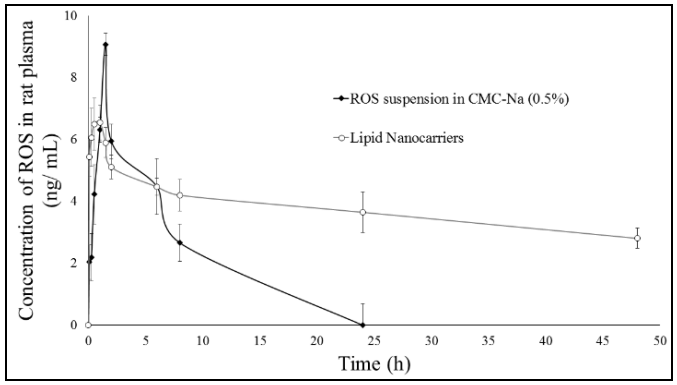

FIG. 6: PLASMA CONCENTRATION VERSUS TIME PROFILE OF ROS AFTER SINGLE DOSE ORAL ADMINISTRATION OF PLAIN DRUG SUSPENSION AND LIPID NANOCARRIERS (MEAN±SD, n = 6)

DISCUSSION: Selection of an appropriate lipid is an important criteria in development of lipid nanocarriers. The chemical nature of lipid contributes towards effective drug loading. Lipids containing fatty acids of different chain lengths have a tendency to offer more space to accommodate drugs by forming crystals with imperfections [8]. Type of lipid selected also provides information about absorption pathway. As reported by Bandyopadhyay et al., 2012 [20], long chain triglycerides are likely to enrich lymphatic transport of lipophilic drugs leading to higher oral bioavailability. In the current study, long chain fatty acid such as Precirol® ATO 5 was used as lipid.

Prescreening of process parameters showed that 10%v/v of chloroform was inadequate in dissolving the required amount of components; while 15%v/v of chloroform ensured complete solubility of components. During emulsification stage, samples containing <15% v/v chloroform revealed presence of particles confirming insolubility of components. Additionally, lower stirring speed lead to formation of larger globules at emulsification stage; while stirring speed of 1200 rpm produced smaller globules of uniform size.

During evaporation stage, the solution temperature was considered important, where lower temperature increased the processing time to more than 24 h and reduced the evaporation capacity of organic phase. Solution temperature closer to boiling point of organic phase was desirable to ensure complete evaporation of organic phase with less processing time. Based on preliminary study results, organic

solvent concentration, stirring speed and solution temperature were fixed at 15%v/v, 1200 rpm and 55±5°C, respectively. Furthermore, 3^2 experimental design was used to determine the formulation parameters. EE of drug when viewed from lipid axis indicated increase in entrapment with increase in lipid amount, ascertaining the necessity of higher amount of lipid matrix for entrapping drug. However, increase in concentration of surfactant did not provide steady increase in the EE. At higher surfactant to lipid ratio, the entrapment was considerably reduced owing to solubility of drug in the aqueous micelles formed by surfactants.

PS of systems containing higher concentration of surfactant was found to be low (**Fig.2**). For a given concentration of surfactant, as amount of lipid increased it caused decrease in PS, owing to micellar solubilization of drug (indicated by low EE) or tendency of surfactant molecules to reduce interfacial tension between lipid and aqueous phase by forming spherical shaped particles(revealed by TEM images). Thus from experimental design results it can be concluded that maximum EE was seen at lipid: surfactant ratio of 1:4.

Mean particle size of re-constituted freeze-dried powder of ROS lipid nanocarriers was comparatively higher due to the presence of mannitol as cryoprotectant. Placebo lipid nanocarrier and ROS lipid nanocarrier showed zeta potential of -4.39±1.04, -6.89±3.29 mV, respectively, where slight negative charge may be probably due to ionization of traces of free fatty acids present in the surfactant/ lipid. The dispersion was freeze-dried to improve the physical stability of lipid nanocarriers and obtained a zeta potential -19.6±4.58 mV, respectively. Thus, freeze drying of dispersion improved the physical stability of the nanocarriers. In addition to this, negative charge on the particles also influences closeness of nanocarriers to epithelium for drug absorption and surges particle uptake via Peyer' patches [21]. Negatively charged carriers show higher drainage into the lymphatic system [22, 23].

In addition to this, anionic nanocarriers encounter electrostatic repulsive forces from negatively charged matrix leading to faster lymphatic drainage and produce longer retention period of anionic particles in lymph nodes [23].This comportment will have an impact on sustained release of the drug from lipid nanocarriers. As observed by *in vitro* dialysis bag diffusion studies, it was evident that diffusion of drug from lipid nanocarriers at all pH was statistically insignificant ($p>0.05$), reflected to be highly favorable for efficient absorption of drug throughout the GI tract and was found to be sustained for prolonged period of time. While, *in vitro* release of drug from plain drug suspension was found to be pH-dependent.

As stated earlier, permeability of drug from lipid nanocarrierin both everted gut sac (i.e. serum side) and non-everted gut sac (i.e. mucosal side) was found to be greater, credited to lipophilic nature of nanocarriers combined with nano-sized structure. Sustained release of lipid nanocarriers was also evident *in vivo* with high mean residence time values (8.14-times higher than plain drug), which can be ascribed to probable lymphatic uptake of nanocarriers.

CONCLUSION: Current work revealed the successful development of spherical shaped drug loaded lipid nanocarriers for sustaining the release of rosuvastatin calcium and enhancing its oral bioavailability. Prescreening studies enabled in deciding the suitable process parameters, while factorial design facilitated in selection of lipid to surfactant ratio for developing optimized ROS lipid nanocarriers. Spherical shaped lipid nanocarriers were formed which showed higher intestinal permeability owing to the lipid nature of the nanocarriers. Freeze-dried nanocarriers showed improved stability of developed nanocarriers with zeta potential -19.6±4.58 mV. Developed lipid nanocarriers showed significant improvement in the oral bioavailability and sustained release, which may be due to the anionic charged nanocarriers. Thus, evaluation of lipid nanocarriers through *in vitro, ex vivo* and *in vivo* studies confirmed improvement in the oral bioavailability and sustained release of rosuvastatin calcium, a BCS class II drug.

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