FORMULATION AND IN VITRO EVALUATION OF ROSUVASTATIN CALCIUM NIOSOMES

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ABSTRACT
Objective: Poor solubility and permeability of slightly soluble drug "rosuvastatin calcium", face a problem of low bioavailability (absolute bioavailability 20%) as its dissolution and permeation are the rate limiting factors, so it becomes a requirement to improve dissolution and permeability of Rosuvastatin calcium by formulating it as a niosomal dosage form.

Methods: using non-ionic surfactants (Span 20, Span 60, span 80), cholesterol and lecithin in different ratios by film hydration method and evaluate the formulas in terms of assay of drug in each formula (entrapment efficiency) by HPLC, particle size, morphology, in-vitro drug release and ex-vivo permeation study. (SEM) and transmission electron microscope (TEM) were used for characterization of the selected formula. Fourier transform infrared (FTIR) was used for study of drug – excipients compatibilities.

Results: Niosomal formulations were prepared and all formulas gave obvious morphology in the presence of cholesterol as a stabilizing agent, formula with span 60 had more entrapment efficiency than all other formulas, with slower release after 7 hours in vitro dissolution media, TEM results show vesicle size of F6 niosomal vesicle was 150nm in diameter. The polymers span 60 and lecithin have numerous polar groups (C=O,OH,NH₂) in each that may be involved in intra H bonding, thus no chance for inter H bonding with drug was observed. Characteristic peaks of rosuvastatin calcium were 2968.55 cm⁻¹ for N-H stretching and C=O stretching of aacid at 1732.13 cm⁻¹.

Conclusion: Niosomes were promising dosage form for enhance dissolution and permeability of slightly soluble drugs prepared by film hydration method.

Keywords: Rosuvastatin calcium; Film hydration; Niosome; Release; Entrapment efficiency.

INTRODUCTION
Over the decades, oral route is preferred route of administration for most of the drugs. Majority of the discovered and existing drugs administered via oral route may encounter bioavailability problems because of many reasons such as poor dissolution, unpredictable absorption and inter - intra subject variability [1]. Various approaches were developed for enhancing solubility, dissolution rate, and oral bioavailability of poorly water soluble drugs, so for complete development works within a limited amount of time, establishment of a suitable formulation strategy for the poorly water-soluble drugs were invented.

The basic approaches for poorly water-soluble drugs for enhancing solubility are crystal modification, amorphization, cyclodextrin complexation, and pH modification[1].

Niosomes as drug carriers have shown advantages such as being chemically stable; overcomes the physicochemical issues exhibited by liposomes [2], it is composed of a bilayer of non-ionic surface active agents and hence the name niosomes[3].

Non-ionic surfactant vesicles may be formulated with different ionic amphiphiles such as stearylamine and dicyethylphosphate to achieve higher protection against flocculation in vesicle suspensions [4].

Unlike other statins, Rosuvastatin is hydrophilic[5]. It is white crystalline powder that is slightly soluble in water and methanol, and slightly soluble in ethanol[6].

Low bioavailability of rosuvastatin calcium when taken as a tablet make the researcher to develop a new dosage for to enhance permeability and hence bioavailability of the drug when taken orally, so niosomal approach is one of the methods to enhance bioavailability of rosuvastatin calcium.

MATERIALS AND METHOD
Materials

The following materials were used: Rosuvastatin calcium (Atra Pharmaceuticals, India), Cholesterol (HIMEDIA Laboratories, India), Egg lecithin (Provizer Pharma, India), Span20 (Fluka, Chemi, USA), Span60 (HIMEDIA Laboratories, India), Span 80 (SIGMA - ADRICH CO., USA), Maltodextrin (Provizer Pharma, India), Chloroform (Scharlau, Spain), Methanol (Scharlau, Spain), Acetonitrile HPLC grade (HIMEDIA Laboratories, India), Disodium hydrogen orthophosphate anhydrous (Sodium-Chem limited, Mumbai, India), Potassium dihydrogen orthophosphate anhydrous (Thomas Baker, Mumbai, India), Phosphoric Acid (SIGMA-ADDRICH CO., USA). All reagents used were of analytical grade.

Methods
Preparation of niosomes

Rosuvastatin calcium niosomes were prepared using Thin Film Hydration Technique by Rotary flash Evaporator (as shown in table 1). Weighed quantity of drug, cholesterol, surfactant and lecithin were dissolved in chloroform and bathsonicated for 30 minutes then taken in a round bottom flask. The flask was rotated by using rotary flash evaporator at 100 rpm for 20 minutes in a thermostatically controlled water bath at 60°C ± 2°C. The flask was rotated under reduced pressure (10-15 mm mercury) until all the organic phase evaporated and a slimy layer was deposited on the wall of a round bottom flask[7], 10ml of phosphate buffer saline pH 6.8 used to hydrate the lipid film and the flask was rotated at the same speed and temperature but without vacuum for 30 minutes for lipid film removal and dispersion[7].

Solubility studies

Saturated solutions were prepared by adding excess rosuvastatin calcium to each of buffer solutions (PH 1.2 and PH 6.8) and shaking on the shaker (J Lab Tech, Korea) for 48h at 25 ± 0.5°C under constant vibrations[8]. The solubility was determined in phosphate buffer 1.2 and PH 6.8. Flask were sealed and shaken for 48 hr. In thermostatically controlled water bath maintained at 37°C. Samples withdrawn, filtered and then the solubility determined by RP-HPLC, the solubility was measured in a triplicate.

Fourier transform infrared spectroscopy (FTIR)[9]

Sample of pure rosuvastatin calcium powder was grinded, mixed with potassium bromide and pressed in the form of disc (13mm in
diameter). The disc was analyzed by Shimadzu FTIR spectroscopy from 4000-400 cm^{-1}. Figure 1 showed the FTIR of pure rosvastatin calcium.

FTIR compatibility with cholesterol and span also studied as shown in figure 2 and figure 3.

Microscopic evaluation of niosomes

Optical microscope used for visualizing niosomes (F3, F6 and F9) by placing drops of the niosomal dispersion on slide and covered with cover slide, photographs were taken for each sample[10] as shown in figure 4, as well as size distribution of 100 vesicles for formula F3, F6 and F9 were determined using a calibrated ocular and stage micrometer fitted in the microscope at 100x, the average diameter of the vesicles and standard deviation was determined as shown in table 2. Furthermore, Polydispersity index (PI) of the prepared formulas was determined, which indicates the diversity of the particle size. PI was calculated from the square of the standard deviation divided by the mean diameter of the vesicles as shown in the following equation[11].

\[
(PI) = \frac{\sigma^2}{m^2} \quad \text{Equation 1}
\]

In which, \(\sigma\): standard deviation and \(m\): mean particle size in the dispersion.

Encapsulation efficiency (Entrapment)

Free rosvastatin calcium was separated from niosomes–entrapped rosvastatin by re-precipitation centrifugation. A 10 ml aliquot of niosomal dispersion was centrifuged at 9,000 rpm at 4°C for 30 min by using cooling centrifuge. The supernatant was separated, and the niosomal lipid layer was resuspended in buffer 7.4 and centrifuged again. This washing procedure was repeated two times to ensure that the free drug was no longer present in the voids between the niosomes. The collected niosomal residue was lyzed with acetonitrile each formula with specific amount of solvent depends on the nature of the niosomal layer separated from each formula, then determine the entrapment state of rosvastatin calcium in the solution obtained via HPLC. The percent of Entrapment efficiency of rosvastatin calcium was obtained by dividing the amount of entrapped drug from the total drug incorporated[12]:

\[
\text{Encapsulation Efficiency}\% = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100 \quad \text{Eq 2}
\]

Transmission electron microscope (TEM)[13]

The size and morphology of the selected formula (F6) was examined by TEM (PHILIPS CM 10), with an accelerating voltage of 100 kV, drop of sample was placed on a carbon coated copper grid and allowed to stand at room temperature for 90 sec to form a thin film. Excess of the solution was drained off with a filter paper. The grid was allowed to thoroughly dry in air, samples were viewed and photomicrographs were taken at suitable magnification.

Scanning electron microscope (SEM)

Formulation of Proniosomes powder from niosomes of rosuvastatin calcium of the selected formula (F6) is used in SEM (The VEGA3 SBU - EasyProbe, TESCAN). This done for visualize the surface morphology of the Proniosomes powder samples.

Proniosome powder was prepared by slurry method. Where accurate amounts of lipid mixture consist of span 60(400mg), cholesterol (100mg), lecithin (100mg) and rosuvastatin calcium (10.4 mg) were dissolved in 20 mL of solvent mixture consist of chloroform and methanol (2:1).

The final mixture was poured into a round bottomed flask (250 ml) and maltodextrin powder weighing (250 mg) was added as a carrier to form slurry. The flask was attached to a rotary flash evaporator and the organic solvent was evaporated under reduced pressure at a temperature of 60 ± 2°C. After ensuring the complete removal of solvent, the resultant powders were further dried overnight in an oven at 40°C so as to obtain dry, free-flowing product. Proniosome powder was stored in a closed container at 4°C for further study.[14]

In-vitro drug release

The in-vitro drug release of niosomal formulations was performed by using dialysis method[15]. Dialysis bag which was fitted in a USP Drug Dissolution Apparatus II (paddle type). Niosomal Formulation was added in to the dialysis tube and aliquots (5ml) were withdrawn each hour and replaced by the same amount of fresh buffer to maintain sink condition.

The dialysis bag (cut off of membrane 70 nm) could retain niosomal dispersion and allow the diffusion of free drug into dissolution medium. The bags were soaked in distilled water for 24 h before being used. The two ends fixed by strings and 50 rpm rotation speed.

The drug content was determined by HPLC method every one hour for a total period of 7 h. All the operations were carried out in triplicate.

The in-vitro drug release study was conducted in pH progression medium at 37°C ± 0.5°C[16]. The steps of using dissolution media at different pH was as follows:

- 1st 2hours: 900 ml of hydrochloric acid aqueous solution at pH 1.2.
- 3rd – 7th hours: 900 ml of phosphate buffer solution at pH 6.8.

Kinetics of drug release profile[17]

The cumulative amount of rosvastatin calcium release from niosome was fitted to different models like zero order kinetics, first order kinetics, Higuchi model, Koresmeyer-Peppas model and Hixson-crowell model to characterize the kinetics of drug release.

In-vitro (Ex-vivo) permeation studies[18]

Preparation of animal skin

Male rat (Animal’s house, college of pharmacy, Baghdad university), weighing 450 grams was sacrificed using ether inhalation with ethics control system by expert person, the skin was sloughed, intestine was taken and stored in 7.4 buffer, for study of permeation to the selected formula (F6) and to the commercial tablet.

Intestine was cut longitudinally using sharp blade and surgical scissors.

Drug Permeation studies[18]

Formulation which possessed the best result (F6) was exposed to permeation testing of the drug through rat intestinal membrane which was kept in buffer 7.4. The apparatus used to test the permeation consisted of a glass tube (1.33 cm² area) closed from one end using the intestinal mucosa and the loaded membrane was stretched over an open end of the glass tube by rubber band, forming donor chamber. The niosomal formula placed inside the donor compartment, 2 ml phosphate buffer pH 6.8 was transferred to donor chamber which simulate the conditions inside the intestine. The tube was attached from the other end to shaft of the USP dissolution apparatus. The tube was then immersed in 250 ml of phosphate buffer pH 7.4 contained in the USP dissolution apparatus flask in which the membrane was just below the surface of the recipient solution. The temperature was maintained at 37±0.5°C, and the apparatus was run at 50 rpm for 7 h. 5mlsample was withdrawn every 1 hour and was compensated by equal volume of fresh buffer. The samples withdrawn then analysed by RP-HPLC to calculate the concentration.

The % cumulative amount of permeated drug per square centimeter was plotted versus time (h) and steady-state flux was measured from the slope of the linear portion of the plot using the following equation (Eq. 3):

\[
\text{Flux} = \frac{dc}{dt} = \frac{\Delta C}{A} \quad \text{Equation 3}
\]

Where \(J_o\) is the steady-state flux \(dc/dt\) is the permeation rate; \(A\) is the active diffusion area (1.33 cm²).

The permeability coefficient \(P\) was calculated as follows:
RESULTS AND DISCUSSION

Solubility Studies

According to the result obtained, rosuvastatin calcium can be considered as slightly soluble drug, since 13.5 mg of rosuvastatin calcium was soluble in 10 ml at PH 1.2 and 26 mg of rosuvastatin calcium was soluble in 10 ml at PH 6.8[19,20]

Rosuvastatin calcium is a hydrophilic molecule, PKa of 4.6 (carboxylic acid group)[21]. So, the ionization and hence the solubility was increased in the higher pH medium, while started to decrease as the pH became closer to the pk,a[22].

From the solubility data, sink condition could be achieved. (10.4mg of drug in 900ml of buffer in dissolution apparatus).

FT-IR Characteristic of rosuvastatin calcium

The FT-IR spectrum of rosuvastatin calcium powder was shown in figure 1. The results reveal identical spectra for the tested powder and the reference[23]. Where, Characteristics peaks of 2968.55 cm⁻¹ for N-H stretching and C=O stretching of acid at 1732.13 cm⁻¹.

The other principle peaks are at 1546.96 cm⁻¹ for C=C stretching, 2922.25 cm⁻¹ for =C-H stretching, 1512.24 cm⁻¹ for N-H bending, 3387.11 cm⁻¹ strong and broad band for O-H stretching 1483.94 cm⁻¹ and 1383.01 cm⁻¹ for asymmetric and symmetric bending vibration of CH₂ group respectively also 1330.95 cm⁻¹ represents the asymmetric vibration for SeO₂ while 777.34 cm⁻¹, 570.95 cm⁻¹ and 515.01 cm⁻¹ are the absorption bands of out of plane for C=C of benzene ring,1287.6 cm⁻¹ bending vibration for C-H1534.7 cm⁻¹ for C-F stretching vibrations.

The structural characterization of the polymers and blends was performed by recording FT-IR spectra of the samples. The comparison between the spectrum of pure rosuvastatin calcium powder with cholesterol shows the presence of rosuvastatin calcium and cholesterol bands similar to each one alone, 3410.26 cm⁻¹ for broad O-H stretching vibration, 2935.76 cm⁻¹ for N-H stretching band, 1057.03cm⁻¹ O=O stretching for cholesterol, 1600.97cm⁻¹ for C=O stretching of rosuvastatin calcium and 1153.47 cm⁻¹ for C-F stretching, no incompatibilities are present and no new bands were appeared. As shown in figure 2 and figure 3.

The spectra in mixture of rosuvastatin calcium with span 60 are similar to that of span 60 alone, with some bands of rosuvastatin calcium at 3448.84 cm⁻¹ & 3408.33 cm⁻¹ for O-H stretching and 2920.32 cm⁻¹ at lower frequency for N-H stretching vibration and 1546.89 cm⁻¹ for N-H bending vibration, no drug interaction was present and no new bands were appeared.

Statistical analysis

To investigate the significance of difference between the results of studied formulations, the one way analysis of variance (ANOVA) test was used. The level of significance was set at α 0.05, and (P < 0.05) was considered to be statistically significant.

Microscopic evaluation of niosomes

Most of the niosomal vesicles prepared showed a wide range of variability ranging from several nanometers to several micrometers depending on the niosomal composition and the device used. When optical microscope used only vesicles with micro size vesicles were obtained while nano size vesicles can be revealed only by more sophisticated microscope like transmission electron microscope. Optical microscope used for evaluation of the morphology of the micro size vesicles and to study some properties of the vesicles like polydispersity index while (TEM) used for measuring the nano size of the vesicle and it was studied to the selected formula F6.0. Optical microscope under 100x showed the size range of vesicles are decreased in the sequence of F3>F6>F9 that correspond to span 20, span 60 and span 80, respectively, the results obtained indicated that the mean size of the niosomes showed a regular increase with increasing the hydrophilic lipophilic balance (HLB) of the surfactant because surface free energy decreases with increasing hydrophobicity[24]. These results are in agreement with that established during determination the size of acyclovir niosomes[25].

Generally the surfactant with longer alkyl chains shows smaller vesicles because the diameter of vesicles is dependent on the length of the alkyl chains of surfactant[26].

Uniformity of vesicles size is determined by polydispersity index values in which the low value means the more uniformity in size. The lower the PI value, the more monodispersed the dispersion. Considering that the polydispersity index (PI) is calculated from the square of the (standard deviation/mean) diameter, less value of polydispersity index indicates enhanced homogeneity of the dispersion the most suitable polydispersity index of 0.2 is also reported in many works[27].

A complete hydration takes place leading to the formation of niosomes when shaken with excess aqueous phase[28].

Small amounts of the formed niosomes (F3, F6 and F9) were spread on a glass slide and examined for the vesicular structure using optical microscope with 100Xmagnification power. The morphology of hydrated niosome dispersions prepared by film hydration method was almost spherical or slightly elongated in shape. The bilayers tend to form spherical structures randomly giving rise to multilamellar structure.

Encapsulation efficiency (Entrapment)

The entrapment efficiency of the drug was defined as the ratio of the amount of niosome-associated drug to the total amount of drug initially used. It was expressed as a percentage of the total amount of rosuvastatin calcium used initially, the data are listed in table 3. Niosomal vesicles were able to efficiently entrap water-soluble substances and the membrane was in gel state. Vesicles obtained from the long alkyl chain surfactants give higher entrapment efficiency and were more stable than those obtained from the shorter alkyl chain surfactants like in span 60 which they give more entrapment(P < 0.05) than span 20.

Phase transition temperature (Tc) of surfactants also affects entrapment efficiency. Span 60 having higher Tc provides better entrapment[24]. Moreover, the balance of the hydrophilic and hydrophobic moiety of the surfactants/cholesterol mixture has been an important consideration for high entrapment of water-soluble substances.

The nature of the hydrophobic alkyl chain affects the encapsulation efficiency of drug. Span 20, and span 60 have the same head group with different alkyl chain but span 60 has an unsaturated alkyl chain so the double bond made the chain bend. This means that the adjacent molecules cannot be tight when they form the membrane of niosome and might be the reason for the lower entrapment efficiency of the span 80 system[29,30].
The vesicles prepared with span 60 showed the most efficient entrainment \( F_6 = 86.53 \pm 0.018 \) when compared with those prepared by span 80 \( \left( P < 0.05 \right) \), because the size of vesicles may also responsible for high drug entrainment since rosuvastatin calcium is hydrophilic. The large or intermediate sized unilamellar vesicles are the most appropriate types to achieve as a high value as possible for entrapped volume lipid ratio. This means a large core available for entrapping a large amount of drug mass, so that the entrapment efficiency is higher for larger vesicles prepared by span 60 than those prepared by span 80[31].

Increase in the concentration of the surfactant also effects on the entrapment efficiency, it showed that increase in the concentration of the surfactant leads to enhancement in the encapsulation efficiency and decrease in the leakage of the drug[32].

### Effect of cholesterol on entrapment efficiency

Variations in cholesterol ratio have an effect on the physical and structural properties of bilayers of vesicles such as size and entrapment efficiency of vesicles as shown in table 4. As the amount of cholesterol was changed from 0 to 200 mg, an unimportant results was achieved in which the entrapment percentage increased whereas particle size decreased. The same result was observed from minoxidil niosome[30] and also no significant differences was noticed in encapsulation efficiency of estradiol in prepared proniosome with or without cholesterol for span and tween series[33]. In addition, another study reported that cholesterol free niosomes were found to show less change in size[34], due to the fact that these niosomes are more permeable to solutes.

However, the encapsulation efficiency also was increased at 400 mg of cholesterol in F13, which may be due to the effect of cholesterol that increases the stability and prevents the leakage of rosuvastatin calcium. Similar result was obtained during the study of topical application of niosomally nimesulide in which an increasing in cholesterol concentration up to 50% resulted in an increased in percent of drug entrapment and any further increase did not show any influence on encapsulation[35].

As the amount of cholesterol increased (800 mg) the encapsulation efficiency decreased and the particle size increased. McIntosh[36] found that cholesterol increases the width of lipid bilayers and consequently increases the vesicle size. Although the rigidity and hydrophobicity of the bilayer increased and improved[37], the release rate of encapsulated material will reduce[38,39]. Presence of charge tends to increase the interlamellar distance between successive bilayers in multilamellar vesicle structure and leads to greater overall entrapped volume and reduce the phase transition temperature, which responsible for escape, of drug from vesicles[40].

### Effect of lecithin

Incorporation of co surfactant egg lecithin with different ratio that ranged from zero to 800 mg (table 5), leads to an insignificant \( \left( P > 0.05 \right) \) enhancement of the percent drug entrainment \( (84.5\% \pm 0.02, 86.6\% \pm 0.03) \). There is probably formation of more dense and well-organized bilayers, which prevents the leakage of drug[41]. In addition, the encapsulation efficiency were changed slightly when the amount of lecithin increased also the size of the vesicles increased slightly and decrease in F19 due to increase the hydrophobicity of the niosomal structure[42].

### Transmission electron microscope (TEM)[13]

Transmission electron microscopy (TEM) is a microscopic technique commonly used for the analysis of materials on the nanoscale. Because it uses electrons, which have a shorter wavelength than light, it is capable of achieving resolution a thousand times better than that can be achieved with a light microscope[43].

TEM photographs of \( F_6 \), it revealed that the vesicles formed were appeared as unilamellar or multilamellar with a predominant spherical shape with sharp boundaries as seen in figure 5. Sizes of the vesicles are in nanometer and one measured was 150nm in size. The image analysis showed that the vesicles was surrounded by homogeneous shading, this suggests that formulated niosome \( (F_6) \) resembled the drug-enriched core model (the drug occupies the core of the vesicles).

### Scanning electron microscope (SEM)[14]

Surface morphological properties of formula \( F_6 \) nano size vesicles were obtained by SEM at three magnifications \((500x, 1000x \) and \( 1500x)) and shown in figure 6.

Vesicular properties of these drug carriers which formed from double layers. SEM showed the morphology of the lipids and the arrangement of the lamellar structure the encode the drug molecules. In vitro drug release

In vitro release gave precious informations to the product performance in vivo. The drug release dictates the amount of drug available for absorption. The formulations presented sustained release up to 7 hours with \( F_6 \) having sustained release of 40.29% at the end of 7 hours. On the basis of most sustained release, vesicle size and sufficient entrapment, \( F_6 \) was selected as the optimized formulation.

Drug release from crestor® tablet also studied to compare between the release of drug from niosome systems and from crestor® tablet. (Figure 7)

### Effect of amount of surfactant on release

The amount of surfactant used in niosomal formulation was evaluated and the results showed retardation in the release of drug as the amount of surfactant increased. \((1:1)\) ratio (Surfactant: Cholesterol), \((2:1)\) and \((4:1)\) led to decrease in the amount of drug release from niosomal formula because the surfactant act as a depot for the release of the drug and so increase the molecular weight of the surfactant led to more drug entrapped and less leakage from niosome to dissolution media and more stable was the niosome[44,45]. Data are shown in figure 8, 9 and 10.

### Kinetics of drug release profile[46]

In vitro release profiles for the selected niosomal formula \( F_6 \) was applied on various kinetic models (zero order, first order, Higuchi, Korsmeyer–Peppas and Hixson crowell models). The rate constant as well as the highest correlation and the best fitted line were obtained in order to find out the mechanism of drug release, the release kinetics data of the selected niosomal formulas are given in table 6. \( F_6 \) was also chosen based upon the entrapment efficiency of the formulation. The highest correlation coefficient \( (R^2) \) was resulted with Korsmeyer–Peppas model which indicates that drug release is ruled by both diffusion of the drug and dissolution/ erosion of the lipid matrix.

### Drug Permeation studies

The results indicated that rosuvastatin calcium from niosome permeated through rat intestinal membrane better than crestor® tablet and hence could possibly permeate through the human intestinal membrane as shown in figure 11A and Figure 11B.

For Formula 6:

\[
\text{J}_{\text{ss}} = \frac{\text{dQ}/\text{dt}}{A} \\\n\text{dQ} = J_{\text{ss}} \times A \times \text{dt} = 0.0536 \text{ mg cm}^{-2} \text{ hr}^{-1} \times 1.33 \text{ cm}^2 \\
\text{P} = \frac{\text{dQ}/\text{dt}}{\text{Ac}} = 0.071288 \text{ mg hr}^{-1} \\
\text{P} = \frac{\text{0.071288 mg hr}^{-1}}{1.33 \text{ cm}^2 \times 0.036 \text{ mg cm}^{-2}} = 1.49 \text{ cm hr}^{-1}
\]
For crestor® tablet solution:

\[ J_{\text{a}} = \frac{(dQ/dt)}{A} \]

\[ (dQ/dt) = J_{\text{a}} \times A \text{ cm}^2 = 0.0267 \text{ mg cm}^{-2} \times 1.33 \text{ cm}^2 \]

\[ (dQ/dt) = 0.035511 \text{ mg hr}^{-1} \]

\[ P = \frac{(dQ/dt) \times A}{G} = \frac{0.035511 \text{ mg hr}^{-1}}{1.33 \text{ cm}^2 \times 0.0416 \text{ mg cm}^{-1}} = 0.64 \text{ cm hr}^{-1} \]

The enhancement ratio \( (E_r) \) was calculated by dividing the \( J_{\text{a}} \) of respective formulation with \( J_{\text{a}} \) of control formulation by using the equation[47]:

\[ E_r = \frac{J_{\text{a}} \text{ of formulation}}{J_{\text{a}} \text{ of control}} \]

\[ E_r = \frac{0.03556 \text{ mg cm}^{-2} \text{ hr}^{-1}}{0.0267 \text{ mg cm}^{-2} \text{ hr}^{-1}} = 2.00 \]

Cholesterol and lecithin were played a major role in enhancing the permeability of niosome beside to the action of surfactants, egg lecithin contain phosphatidylincholine, phosphatidylethanolamine, phosphatidylserine, and unsaturated fatty acid.

The presence of unsaturated fatty acids, such as oleic and linoleic acid in the phospholipids, may be responsible for the enhancer effect as well as the packing nature of unsaturated fatty acids, which may be change the fluidity of membrane lipid structure and facilitates the permeation of drug[48].

**Stability study**

Stability study was evaluated for F6 because it had the higher entrapment of drug than other formulas. Physical stability of the selected formula F6 was evaluated in terms entrapment efficiency and particle size for three months at different temperatures were considered.

No change in particle size was observed at refrigerated temperature (2-8°C) when measured under optical microscope at 100x (5.2±0.80), while the other formulas were increased in size at 37°C and 60°C.

The mean diameter of vesicles tends to increase with time, with 37°C and 60°C due to increase aggregation of the vesicles with aqueous buffer. Size of vesicles not affected greatly at refrigerated temperature. Separation of the lipid phase from aqueous phase was also seen at 37°C.

Entrapment efficiency also not affected at 4°C. Therefore, niosomes are more stable at refrigerator (75.2±0.01 of drug remaining at 12 weeks) than 37°C (24%±0.015 drug was remaining) and 60°C (19.8±0.02 drug was remaining).

Results indicate that maximum percentage drug retained was observed at refrigerated conditions due to the higher fluidity of lipid bilayers at higher temperature resulting into higher drug leakage[49].

Microscopic evaluation under optical microscope at 100x for F6 and physical product after 3 months showed homogenous dispersion for refrigerated temperature and separation of the lipid layer from the aqueous layer was shown in 37°C and 60°C respectively.

**CONCLUSION**

Vesicular systems have been recognized as extremely useful carrier systems. On the basis of the obtained results, the preparation procedure of niosomal system by film hydration method was found to be uncomplicated and does not involve more sophisticated procedures. Moreover, experiments of the niosomes showed that the surfactant nature, cholesterol and vesicular size played important role in enhancing dissolution of drugs.

### Table 1: Preparation of Different Formulas of Rosuvastatin calcium niosomes

<table>
<thead>
<tr>
<th>Formula</th>
<th>Surfactant</th>
<th>Cholesterol</th>
<th>Lecithin</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Span 20 (0.1ml)</td>
<td>100 mg</td>
<td>100 mg</td>
<td>10.4 mg</td>
</tr>
<tr>
<td>F2</td>
<td>Span 20 (0.2ml)</td>
<td>100 mg</td>
<td>100 mg</td>
<td>10.4 mg</td>
</tr>
<tr>
<td>F3</td>
<td>Span 20 (0.4ml)</td>
<td>100 mg</td>
<td>100 mg</td>
<td>10.4 mg</td>
</tr>
<tr>
<td>F4</td>
<td>Span 60 (100mg)</td>
<td>100 mg</td>
<td>100 mg</td>
<td>10.4 mg</td>
</tr>
<tr>
<td>F5</td>
<td>Span 60 (200mg)</td>
<td>100 mg</td>
<td>100 mg</td>
<td>10.4 mg</td>
</tr>
<tr>
<td>F6</td>
<td>Span 60 (400mg)</td>
<td>100 mg</td>
<td>100 mg</td>
<td>10.4 mg</td>
</tr>
<tr>
<td>F7</td>
<td>Span 80 (0.1ml)</td>
<td>100 mg</td>
<td>100 mg</td>
<td>10.4 mg</td>
</tr>
<tr>
<td>F8</td>
<td>Span 80 (0.2ml)</td>
<td>100 mg</td>
<td>100 mg</td>
<td>10.4 mg</td>
</tr>
<tr>
<td>F9</td>
<td>Span 80 (0.4ml)</td>
<td>100 mg</td>
<td>100 mg</td>
<td>10.4 mg</td>
</tr>
</tbody>
</table>

**Fig. 1:** The FT-IR spectra of pure rosuvastatin calcium powder
Table 2: Mean vesicle diameter of niosomal formulations under optical microscope at 100x (*SD: Standard deviation, n=3)

<table>
<thead>
<tr>
<th>Formula</th>
<th>Average vesicle size (µm)</th>
<th>Standard deviation</th>
<th>Poly dispersity index</th>
</tr>
</thead>
<tbody>
<tr>
<td>F3</td>
<td>6.9</td>
<td>0.4</td>
<td>0.0033</td>
</tr>
<tr>
<td>F6</td>
<td>5.44</td>
<td>0.85</td>
<td>0.024</td>
</tr>
<tr>
<td>F9</td>
<td>4.8</td>
<td>0.45</td>
<td>0.0087</td>
</tr>
</tbody>
</table>
Table 3: Effect of surfactant concentration on entrapment efficiency of niosomal formulations

<table>
<thead>
<tr>
<th>Formula</th>
<th>Surfactant type</th>
<th>Surfactant amount</th>
<th>Entrapment efficiency (%)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Span 20</td>
<td>0.1ml</td>
<td>53.9%</td>
<td>0.027</td>
</tr>
<tr>
<td>F2</td>
<td>Span 20</td>
<td>0.2ml</td>
<td>57.7%</td>
<td>0.02</td>
</tr>
<tr>
<td>F3</td>
<td>Span 20</td>
<td>0.4ml</td>
<td>67.3%</td>
<td>0.023</td>
</tr>
<tr>
<td>F4</td>
<td>Span 60</td>
<td>100mg</td>
<td>69.2%</td>
<td>0.03</td>
</tr>
<tr>
<td>F5</td>
<td>Span 60</td>
<td>200mg</td>
<td>70.1%</td>
<td>0.056</td>
</tr>
<tr>
<td>F6</td>
<td>Span 60</td>
<td>400mg</td>
<td>86.5%</td>
<td>0.018</td>
</tr>
<tr>
<td>F7</td>
<td>Span 80</td>
<td>0.1ml</td>
<td>43%</td>
<td>0.032</td>
</tr>
<tr>
<td>F8</td>
<td>Span 80</td>
<td>0.2ml</td>
<td>52.9%</td>
<td>0.03</td>
</tr>
<tr>
<td>F9</td>
<td>Span 80</td>
<td>0.4ml</td>
<td>67.3%</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 4: Effect of cholesterol concentration on entrapment efficiency

<table>
<thead>
<tr>
<th>Formula</th>
<th>Cholesterol amount</th>
<th>Entrapment Efficiency (%)</th>
<th>Standard Deviation</th>
<th>Mean size(µm) under optical microscope 100x</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>F10</td>
<td>0(mg)</td>
<td>83.4%</td>
<td>0.02</td>
<td>6.12µm</td>
<td>0.72</td>
</tr>
<tr>
<td>F11</td>
<td>50(mg)</td>
<td>85.1%</td>
<td>0.016</td>
<td>5.84µm</td>
<td>0.8</td>
</tr>
<tr>
<td>F6</td>
<td>100(mg)</td>
<td>86.5%</td>
<td>0.018</td>
<td>5.44µm</td>
<td>0.85</td>
</tr>
<tr>
<td>F12</td>
<td>200(mg)</td>
<td>87.35%</td>
<td>0.03</td>
<td>5.32µm</td>
<td>0.44</td>
</tr>
<tr>
<td>F13</td>
<td>400(mg)</td>
<td>89.4%</td>
<td>0.01</td>
<td>5.1µm</td>
<td>0.4</td>
</tr>
<tr>
<td>F14</td>
<td>800(mg)</td>
<td>98.6%</td>
<td>0.013</td>
<td>6.44µm</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Table 5: Effect of lecithin concentration on entrapment efficiency

<table>
<thead>
<tr>
<th>Formula</th>
<th>Lecithin amount</th>
<th>Entrapment Efficiency (%)</th>
<th>Standard Deviation</th>
<th>Mean size(µm) under optical microscope 100x</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>F15</td>
<td>0(mg)</td>
<td>84.5%</td>
<td>0.02</td>
<td>4.12µm</td>
<td>0.23</td>
</tr>
<tr>
<td>F16</td>
<td>50(mg)</td>
<td>84.9%</td>
<td>0.015</td>
<td>4.84µm</td>
<td>0.45</td>
</tr>
<tr>
<td>F6</td>
<td>100(mg)</td>
<td>86.5%</td>
<td>0.018</td>
<td>5.44µm</td>
<td>0.85</td>
</tr>
<tr>
<td>F17</td>
<td>200(mg)</td>
<td>87.1%</td>
<td>0.01</td>
<td>5.44µm</td>
<td>0.49</td>
</tr>
<tr>
<td>F18</td>
<td>400(mg)</td>
<td>87.7%</td>
<td>0.05</td>
<td>5.44µm</td>
<td>0.5</td>
</tr>
<tr>
<td>F19</td>
<td>800(mg)</td>
<td>86.6%</td>
<td>0.03</td>
<td>5.1µm</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Fig. 5: Transmission electron microscopy micrographs of (F6). A: 13000x and B: 48000x

Fig. 6: Scanning electron micrographs of proniosomal formula F6 (A, B and C) at 3 different magnification powers
Fig. 7: Pure rosuvastatin release from dialysis bag ( ▲ at PH=1.2), ( ■ at PH=6.8)

Fig. 8: Effect of concentration of surfactant on the in vitro release of rosuvastatin calcium from niosomal formulations F1, F2 and F3

Fig. 9: Effect of concentration of surfactant on the in vitro release of rosuvastatin calcium from niosomal formulations F4, F5 and F6
Fig. 10: Effect of concentration of surfactant on the in vitro release of rosuvastatin calcium from niosomal formulations F7, F8 and F9

Table 6: Kinetic modeling of the selected formula (F6) by different kinetic equations

<table>
<thead>
<tr>
<th>Formula</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi model</th>
<th>Korsmeyer-Peppas model</th>
<th>Hixson crowell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k&lt;sub&gt;0&lt;/sub&gt;</td>
<td>R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>k&lt;sub&gt;t&lt;/sub&gt;</td>
<td>R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>k&lt;sub&gt;H&lt;/sub&gt;</td>
</tr>
<tr>
<td>F6</td>
<td>0.054</td>
<td>0.9842</td>
<td>0.001</td>
<td>0.9840</td>
<td>0.117</td>
</tr>
</tbody>
</table>

Fig. 11: A) In vitro permeation of rosuvastatin calcium from F6 using intestinal membrane of rat

y = 0.0536x + 0.006

Fig. 11: B) In vitro permeation of rosuvastatin calcium from crestor® solution using intestinal membrane of rat

y = 0.0267x - 0.0106


