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Research Article

ROSUVASTATIN CALCIUM PRONIOSOME POWDER: A NOVEL APPROACH TO IMPROVE INTESTINAL ABSORPTION AND BIOAVAILABILITY

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ABSTRACT

Objective: The main objective of the present study was to develop proniosomal formulations to enhance the oral bioavailability of rosuvastatin calcium by improving solubility, dissolution, and/or intestinal permeability.

Methods: Proniosomal powder formulations were prepared with rosuvastatin calcium drug varying the Span 40 and cholesterol ratio in the range of 0.8:0.2–0.2:0.8 using maltodextrin as carrier by slurry method. The prepared proniosomal powder was filled into capsules. The bioavailability enhancement of proniosomes loaded with drug was studied focusing on non-ionic surfactants composition and drug:Span 40 ratio. Prepared proniosomes were characterized for their particle size distribution, zeta potential, entrapment efficiency, *in vitro* dissolution study, and thermal characteristics to understand the phase transition behavior. Further, the formulated proniosomes were subjected to stability behavior, *ex vivo* permeation studies using rat intestine followed by *in vivo* studies.

Results: Physicochemical studies help in optimization of formulations. Enhancement in dissolution is due to incorporation of rosuvastatin calcium into the non-ionic surfactant and change in the physical state from crystalline to amorphous, thus improving oral bioavailability. *Ex vivo* studies show significant permeation enhancement across gastrointestinal membrane compared to control.

Conclusion: Proniosomes provide a powerful and functional way of distribution of inadequately soluble rosuvastatin calcium drug which is proved from *in vivo* studies based on the enhanced oral delivery.

Keywords: Proniosomes, Niosomes, Permeability, Pharmacokinetics, Bioavailability.

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INTRODUCTION

The conventional techniques of improving the bioavailability act by enhancing the dissolution behavior [1] such as reducing the particle size by cogrinding technique [2], *in situ* crystallization [3], spray drying technique [4] but cannot preclude or alter the gastrointestinal tract barrier function and pre-systemic metabolism along with particulate aggregates formation, and toxicity from vehicles [5,6].

Hence to overcome all such difficulties encapsulation of the drug in vesicular structures [7] in the form of pharmacosomes, liposomes [8], niosomes [9], and cubosomes which can be expected to prolong the duration of the drug in systemic circulation, and reduce the toxicity by selective uptake along with lymphatic transport thus leading to improved permeation [10] along with rate and extent of absorption by avoidance of first pass metabolism [11]. Niosomes [12] which are comparatively stable chemically and cheap in price face physicostability problems, namely, sedimentation of particles, leading to fusion and aggregation and also leakage of drug on storage.

To improve the stability of vesicular systems [13], provesicular system [14] was introduced without altering or affecting intrinsic properties of the drugs entrapped. Proniosomes [15-17] are defined as vesicles composed of actively drug with readily aqueous soluble carrier that is coated with surfactant (non-ionic) to form dry, free-flowing product. Thus, on contact with sterilized water, niosomes are generated. With benefits of firmness during sterilization, measuring, transfer, distribution, and storage proniosomes became a versatile system for delivery of numerous drugs [18,19].

Rosuvastatin calcium and hydroxy-3-methylglutaryl coenzyme A reductase are a dyslipidemic agent used in the treatment of hypercholesterolemia [20] and for the management of coronary artery disease [21-23]. Rosuvastatin peak plasma concentrations are reached in 3–5 h, the absolute bioavailability is approximately 20% with poor water solubility and a mean plasma elimination half-life of 19 h [24]. To the best of our knowledge, no articles are available in literature on proniosomes of rosuvastatin calcium and henceforth an attempt was made to formulate and evaluate the same.

The rationale and novelty of the study are about developing a more stable proniosome dosage form then delivering it into body thorough oral delivery which leads to the generation of niosomes after coming in contact with biological fluids. Subsequently, the existing difficulty of poor absorption and bioavailability related to low solubility for the drug rosuvastatin calcium is overcome by formulating a novel drug delivery system in the form of proniosomes.

METHODS

Animal studies for both *ex vivo* and *in vivo* experiments were conducted at Albino research center (Registration No. 1722/RO/Ere/S/13/CPCSEA) with prior approval of the Institutional Animal Ethical Committee. Euthanasia and disposal of carcass were in accordance with the guidelines.

Rosuvastatin calcium was a kind gift sample from MSN Labs, Hyderabad, India. Span and cholesterol (>99%) procured from Sigma, St. Louis, Missouri, United States of America. Maltodextrin was a generous gift sample from Dr. Reddy's Laboratories, Hyderabad. All other chemicals were of analytical grade and solvents were of high-performance liquid chromatography (HPLC) grade. Freshly collected double-distilled water was used all through the experiment.

Formulation of proniosomes

Essential components of proniosomes used in the formulation are non-ionic surfactants acting as permeability enhancers, carrier or coating material, and membrane stabilizers. Among the carriers, maltodextrin and sorbitol are available in the preparation of proniosomes. Maltodextrin usage as carrier in preparation of proniosomes permits stretchability in the incorporation of surfactant and other ingredients at various ratios. Sorbitol usage results in the formation of mass that is like solid cake [25]. Non-hygroscopicity and compatibility with drugs are added advantages with maltodextrin. Finally, efficient rehydration is the outcome when the surfactant is coated as thin coat which is made possible by maltodextrin because of its high surface area and preserved morphology [26].

The maximum benefit of proniosomes systems can be achieved when it forms stable niosomes with high entrapment efficiency after hydration in the gastric fluids. Cholesterol and structural lipid help in accentuating the entrapment of drugs preventing it leakage by stabilizing the bilayered membrane. Although lecithin is other alternative for cholesterol as membrane stabilizer, lecithin is not widely used as it imparts lesser stability when measured against cholesterol [25].

Proniosomes were prepared varying the non-ionic surfactant and cholesterol ratios in the range of 0.8:0.2–0.2:0.8 using maltodextrin as carrier assigning the codes for various formulations. All surfactants used were in excess of their critical micelle concentrations. In the present invention among spraying method, coacervation phase separation method, and slurry method for the preparation of proniosomes; slurry technique or process [25] is adapted and the composition is represented (Table 1).

Evaluation/characterization of proniosomes

Hydration of proniosomal systems

Hydration of proniosomal systems is judged by studying the form, shape and structure of the niosomes formed from the proniosomes by optical microscopy (Olympus-CH20i). Procedure involves addition of small or little quantities of water onto a cavity glass slide where the proniosome powder was taken. Under an optical microscope, the formation of niosomes was monitored and photomicrograph was taken.

Measurement of micromeritic properties of proniosomal systems

Micromeritic properties are assessed by flow properties of powder which are vital in handling and processing operations and for further dosage form development. Flow properties are reviewed from angle of repose [27] studied by fixed funnel technique [28]. Briefly, proniosome powder was poured into a funnel which was fixed at a position so that the 13 mm outlet orifice of the funnel is 10 cm above a level black surface. The powder flowed down from the funnel to form a cone on the surface, and the angle of repose was then calculated by measuring the height of the cone and the diameter of its base. And also, Carr's index and Hausner's ratio are obtained from bulk and tapped density calculations [29].

Determination of size of niosomes, zeta potential, entrapment efficiency, and number of niosomes

For quantifying these parameters, proniosome powders are to be converted into niosome dispersions, which are obtained by hydration followed by 3 min bath sonication (Soltec 2200 MH, India). For the formed niosomes, average size and its distribution are checked by Nanoparticle Analyzer (Horiba SZ-100, Japan). The principal behind which is spectroscopic technique by photon correlation approach. Smoluchowski equation method is used to check zeta potential [30].

$$\zeta = UE\eta/\varepsilon$$

Where, "ζ" is zeta potential, "UE" is electrophoretic mobility, "η" is viscosity of the medium, and "ε" is dielectric constant.

After suitable dilution of proniosomes with freshly collected double-distilled water, the samples are proceeded for further analysis. Niosome size and polydispersity index (PI) of formed niosomes were obtained directly from the nanoparticle analyzer and Zetasizer equipment and entrapment efficiency from earlier mentioned and reported ultrafiltration technique which was carried out using Centrisart (Sartorius AG, Gottingen, Germany) equipment [31]. %Entrapment efficiency is obtained by subtracting unentrapped drug from total amount of drug.

Provesicular formulations are optimized based on the number of vesicles formed after the hydration. The niosomes formed after hydration of proniosome powder were counted by optical microscope (Olympus CH20i, India) using a hemocytometer, and the number of niosomes per cubic mm was calculated using the following formula [31].

$$\text{Total number of niosomes per mm}^3 = \frac{\text{Total number of niosomes counted} \times \text{dilution factor} \times 4000}{\text{Total number squares counted}}$$

***In vitro* dissolution study**

In vitro dissolution studies (Lab India DS 8000, India) for proniosome powders (filled in capsule dosage form) compared against control (filled in capsule dosage form) are performed in USP type-I (basket) apparatus containing 900 ml of dissolution medium (pH 6.8 phosphate buffer for rosuvastatin calcium) kept at a controlled temperature of 37±0.5°C for which the basket speed is set at 50 rpm. Five milliliters sample was removed maintaining the sink conditions at definite time points, filtered through 0.45 µm Millipore membrane filter before analysis and drug release was analyzed by UV–Visible Spectrophotometer (Lab India UV 3000+, India), determining the absorbance at 241 nm for rosuvastatin calcium [32].

Scanning electron microscopy (SEM)

By SEM (Hitachi S-3700N, Japan) technique, morphology of surface for both the pure drugs and developed optimized proniosomes was identified [33]. Scanning electron microscopic images or pictures are taken at an accelerating voltage of 15 keV. On brass, stub samples are made to adhere with the help of adhesive tape (double sided), followed by slender layer gold coating on sample makes it electrically conductive which is prerequisite for observing and taking images.

Transmission electron microscopy (TEM)

TEM (JEOL-100CX-II, Tokyo, Japan) technique also helps in knowing the form, shape and structure of samples as in SEM but in more detail.

Table 1: Composition for rosuvastatin calcium loaded proniosome powder using maltodextrin

Formulation code	API (mg)	Maltodextrin (mg)	Molar ratio (SPAN 40:CHOL)	SPAN 40 (mg)	CHOL (mg)
$RPNS_1$	10	250	0.8:0.2	80.4	19.3
$RPNS_2$	10	250	0.7:0.3	70.4	28.9
$RPNS_3$	10	250	0.6:0.4	60.3	38.6
$RPNS_4$	10	250	0.5:0.5	50.3	48.2
$RPNS_5$	10	250	0.4:0.6	40.2	57.9
$RPNS_6$	10	250	0.3:0.7	30.2	67.5
$RPNS_7$	10	250	0.2:0.8	20.1	77.2

API: Active Pharmaceutical Ingredient: Rosuvastatin calcium; CHOL: Cholesterol

Before observation of samples, on a copper grid (coated with carbon) sample, lean film is to be negatively stained using 0.2% w/v solution of sodium phosphotungstate left for air drying followed by imaging [34].

Differential scanning calorimetry (DSC)
DSC (Shimadzu 60H, Tokyo) analysis of optimized formulation and pure drug is studied for the molecular state of the compound. DSC curves help in deducing heat of fusion and melting point. Approximately 5±2 mg of sample is taken in a sealed pan (hermitically) made of aluminum and gradually exposed to temperature at 10°C min^{-1} heating rate from 20 to 300°C under gas (Nitrogen) flow at a constant rate of 30 $ml.min^{-1}$.

Powder X-ray diffractometry (PXRD)
The PXRD (Shimadzu 7000, Tokyo) repeated format for both drugs and most effective powder formulations is received at a voltage of 45 kV; when a beam of Cu Kα radiation, generated from 40 mA current moves forward through Ni filtered monochromator made up of graphite onto detector. All samples were run at 1° (2 θ) min^{-1} from 3° to 45° (2 θ).

Fourier-transform infrared (FT-IR) spectroscopy
IR spectra of drug, best powder formulation, and excipients such as maltodextrin are acquired from FT-IR spectrophotometer (Shimadzu 8400S, Japan) at a scanning range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} to study the drug-excipient interactions. The sample for which is prepared following potassium bromide (KBr pellet) procedure.

Stability studies
Stability for the best formulations is studied for a period of 180 days for various parameters such as particle size of niosomes formed from proniosomes, percentage retention of drug, and *ex vivo* permeation across rat intestine. At predetermined time points, that is, 0, 30, 60, 90, 120, and 180 days, samples kept at 24±2°C and 4±2°C in aluminum foil covered glass vials are taken out hydrated, looked for crystal formation of drug under microscope (optical) and also for above-mentioned parameters.

***Ex vivo* absorption study using rat intestine**
The study was conducted at albino research center (Registration No. 1722/RO/Ere/S/13/CPCSEA) with prior approval of the Institutional Animal Ethical Committee. Euthanasia and disposal of carcass were in accordance with the guidelines. Albino Wistar rats (male) approximately weighing 200 g are taken. The animals were housed in separate cages in a clean room and maintained under controlled condition of temperature and the rats had free access to food and water. Before *ex vivo* absorption study experiments, the rats fasted overnight with free access to water were sacrificed by ether inhalation technique. An incision was made through a midline to expose the abdominal content. Ten centimeters of small intestine (ileum) segment used for study were collected and flushed with freshly prepared Krebs Ringer solution to clean intestine so that it is free from mucus and all adhered contents. Into the ileum which was tightly closed at one end, proniosome dispersion (0.5% w/v PEG200) equivalent to 2 mg of drug was introduced and further closed tightly on another end. The tissue was immersed into a continuously aerated organ bath further into receptor compartment consisting of 250 ml of phosphate buffer (pH 6.8) maintained at 37±0.5°C. Five milliliters sample was withdrawn at definite time intervals and sink conditions are maintained and are further analyzed. Control (drug dispersion equivalent to 2 mg) and proniosomal systems are compared [35]. The cumulative amount of drug permeated was plotted against time. The steady-state flux was calculated from the slope of linear portion of the cumulative amount permeated per unit area versus time plot. The enhancement ratio was calculated by dividing flux of formulation with flux of control.

***In vivo* bioavailability studies**
HPLC (Waters separation module – Model No: 2690; Detector: PDA – Model No: 2996, USA) method is developed and validated for the estimation of respective sample drug in serum [36].

Sample analysis
Rosuvastatin calcium was quantitatively determined in serum by HPLC using 45:55 (v/v) orthophosphoric acid and acetonitrile, respectively, as mobile phase at a flow rate of 1.0 ml/min equipped with LC-10 AT solvent delivery unit. A C8 reverse-phase stainless steel analytical column (250 mm×4.6 mm) with 5 μm particle size was employed for chromatographic separation (Kromasil). The column eluent was monitored at a wavelength of 241 nm using a PDA detector, and the sensitivity was set at 0.005 AUFS at ambient temperature. The serum samples were processed by protein precipitation method. Briefly, 100 μl of serum sample was treated with 100 μl of internal standard (1 μg/ml of atorvastatin in methanol) and vortexed (Remi Equipments, India) for 1 min. The drug was extracted with 0.2 ml of acetonitrile, vortexed for 10 min followed by centrifugation at 4000 rpm for 15 min and the separated organic layer was injected onto the HPLC (20 μl). The limit of detection and quantification were 10 and 30 ng/ml, respectively. The concentration versus peak area ratio plot was linear (r^2>0.9922) over the concentration range of interest (standard preparation), and the rosuvastatin calcium content in samples was quantified using this plot.

Pharmacokinetic study
The study was conducted at albino research center (Registration No. 1722/RO/Ere/S/13/CPCSEA) with prior approval from the Institutional Animal Ethical Committee. Albino Wistar rats (male) approximately weighing 200 g are selected for these investigations which are fasted for overnight. These Wistar rats are split up into two groups with six in every group and delivered with each treatment at a random basis. Two groups include one for control (pure drug – oral suspension) and one for optimized proniosome formulation. Accurately weighed dose of 10 mg/kg body weight the drug or optimized formulation is administered. Two hundred and fifty microliters of blood sample are taken or withdrawn into microcentrifuge tubes retro-orbital plexus at definite time points. Serum was obtained from the collected blood which was left to clot by centrifugation process for about 10 min at 10,000 rpm using centrifuge (Remi R-24, India). Thus, obtained drug containing serum is stored at a temperature of −20°C until further analysis.

Calculation of pharmacokinetic parameters
C_{max} and T_{max} are picked up from the graph plotted between concentrations of drug in serum at various time points. Trapezoidal rule technique is followed to determine area under the curve $(AUC)_{0-t}$. In the same way, $AUC_{t-\infty}$ was calculated by dividing concentration of drug at last time point in the serum with Ke. The relative bioavailability (RA) was estimated by dividing the AUC0–∞ of proniosome formulation with control oral suspension.

Statistical analysis
The data obtained were subjected to Student's "t"-test and one-way analysis of variance (ANOVA), and the significance of difference between formulations was calculated by Student–Newman–Keuls (compare all pairs) with InStat GraphPad Prism software (version 4.00; GraphPad Software, San Diego, California). The level of statistical significance was chosen as $p<0.05$.

***Ex vivo/in vivo* correlation**
Ex vivo/*in vivo* correlation was performed by determining the r^2 value from the graph plotted between the AUC of *in vivo* and *ex vivo* concentration at different time points [37].

RESULTS AND DISCUSSION

Preparation of proniosome powders
In the present study, proniosomes are formulated, developed, and assessed for their scope in raising the quality of the delivery of rosuvastatin calcium drugs by oral route. Accurately weighed amounts using weighing balance (sartorius TE 124S, Germany) for mixture (250 μmol) containing different types of Spans and cholesterol at diversified molar ratios (0.8:0.2, 0.7:0.3, 0.6:0.4, 0.5:0.5, 0.4:0.6, 0.3:0.7, and 0.2:0.8) and active component or ingredient (10 mg) were solubilized in 20 ml of chloroform and methanol (2:1). The formed

mixture was shifted into 250 ml flask with round bottom and upon addition of maltodextrin (250 mg) slurry was formed. The round bottomed flask was connected to a rotary vacuum evaporator (Rotavap PBU-6, India) to remove organic solvent by evaporation process at low pressure and 45±2°C temperature with 60–70 rpm. Complete removal of organic solvent, results in dry product and leaving it in a vacuum oven at 24°C for a night further make the product free flowing. Thus, acquired proniosome powders are kept in glass vial at 4°C which can be delivered in the form of capsules. For comparison, control formulation devoid of Spans and cholesterol was processed.

Niosomes are formed from proniosomes immediately on contact with water at *in vitro* conditions or with body fluids at *in vivo* conditions which is considered to be achieved without great effort when compared with traditional technique of preparation of niosomes by dry-film procedure as plan of work involves tedious shaking process to absorb water by surfactants. Thus, the production from proniosomes is considered as the greatest advantage, that is, eases of use.

Evaluation/characterization of proniosomes
Evaluation of proniosomes after preparation aids in characterizing the formulation and identifying the optimized formulation [38-40].

Hydration of proniosome systems
On hydration of proniosomes, niosomes were derived and were formed immediately on contact with water. The photomicrographic images are shown (Fig. 1) at different magnifications.

Measurement of micromeritic properties of proniosome systems
The measurement of flow properties of the proniosome powders plays a key role as values help in various operations during manufacturing of a dosage form such as ease with which the powders are filled into the container and uniformity of the dose. Angle of repose, Carr's compressibility index, and Hausner's ratio results help in evaluating flow measurements. Cohesion or internal friction between the particles is dependent on the angle of repose values which are directly proportional to each other. Therefore, smaller the angle of repose values (<26°) better will be the flow properties. This is strengthened by Carr's compressibility index values which are <17 and Hausner's ratio which are <1.23 [27] and the results are indicated (Table 2).

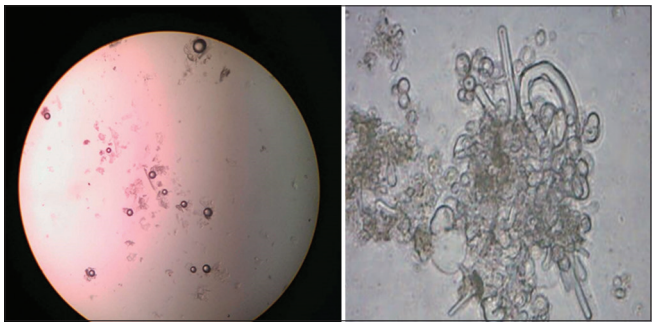

Fig. 1: Formation of niosomes from proniosomes under ×10 and ×450

Table 2: Flow properties of various proniosomal formulations

Formulation code	Angle of repose*	Compressibility index*	Hausner's ratio*
$RPNS_1$	17.90±0.07	10.40±0.09	1.18±0.01
$RPNS_2$	18.20±0.11	11.30±0.08	1.22±0.26
$RPNS_3$	21.30±0.14	16.20±0.05	1.20±0.06
$RPNS_4$	24.20±0.22	16.40±0.13	1.17±0.13
$RPNS_5$	26.20±0.21	16.80±0.16	1.23±0.16
$RPNS_6$	21.30±0.10	15.50±0.12	1.19±0.18
$RPNS_7$	20.10±0.13	11.30±0.22	1.20±0.13

*Results indicate mean±SD, n=6

Determination of Size of niosomes, zeta potential, entrapment efficiency, and number of niosomes
Determination of size of the niosomes and size distribution of niosomes formed is significant [41]. The average niosomes size was in between 180 and 220 nm. Concentration of the cholesterol and length of the surfactant alkyl chain influences the size of the niosomes. Longer the chain length, smaller the size of niosomes.

The particle size increased because of increase in the width of lipid bilayers, when cholesterol concentration increases to have positive effect on the bilayer characteristics such as rigidness and hydrophobicity.

The PI values are inside the allowable limits for all the proniosomal formulations, indicating enhanced homogeneity which is to judge unimodal size distribution. The lower the polydispersity index value, the more monodispersed the dispersion which is <1 for all the proniosomal formulations.

A zeta potential value for all proniosomal formulations was between −48.2 and +54.4 mV.

Ultrafiltration procedure is employed to determine the amount of drug entrapped as the procedure does not involve any dilution levels as in chromatographic techniques, that is, column and dialysis. Very high encapsulation efficiency results were exhibited for niosomes prepared from Spans which is based on the following explanation, that is, the more the lipophilic part of drug molecule, the better the drug accommodation would be.

Entrapment efficiency for all proniosomal formulations is in the range of 77% and 89% whose values are directly proportional to cholesterol concentration. Better the values, lesser the drug leakage from the rigid and compact stable bilayers due to increased hydrophobic interactions. However, extremely high content of cholesterol leads to decrease in entrapment of drug within niosomes which is because of disruption of rigid hydrophobic bilayers.

Spans 20 and 80 have low-phase transition temperature, thus leading to bilayers which are low in permeation compared to Span 60 [42]. Phase transition temperature and availability of lipophilic ambience also play major role in entrapment efficiency. Span 40 and Span 60 which are solids in normal room temperature have relatively elevated phase transition temperatures hence highest drug entrapment. The lower entrapment of drug when Span 80 with unsaturated alkyl chain is used though it has same head group as Span 60 is because of the presence of double bond in the chemical structure unlike Span 60. Same kind of pattern is followed by Tween 80 among other tweens (20, 40, and 60) which fortifies the fact that tendency toward aqueous and non-aqueous phase plays an important part in release of the drug. Proniosome formulations prepared using Tween 20, 40, 60, and 80 showed relatively low drug encapsulation against prepared using Spans, which is due to insufficient amount of cholesterol, that is, vesicles are obtained only when the cholesterol content is above 33.33 mol% [43]. Amount of cholesterol directly influences the encapsulation of the drug with reverse action on vesicle size.

The more the number of niosomes formed from proniosomes in the GI tract on hydration, the better the dosage form can be speculated. Among various formulations, the formulation with following code $RPNS_3$ demonstrates good number of niosomes. The results of all above-mentioned physicochemical parameters are represented (Table 3) for various proniosomal formulations.

***In vitro* dissolution study**
The percentage drug release was outstandingly more for almost all proniosomal powder formulations when measured against control for all proniosomal formulations in the respective dissolution medium. The amount of rosuvastatin calcium released from proniosomes was between 81% and 94% in 10 min and was remarkably higher compared to control (6%).

Dissolution efficiency of drugs has drastically increased when the drug is delivered in the form of proniosomal systems, which might be attributed to the improved solubility of drug by non-ionic surfactant molecules or transformation of the crystalline state of the drug to amorphous state [44]. The data is shown in Table 4.

SEM

SEM images aid in studying the surface morphology of the proniosome powders along with drug and carriers. Non-existence of drug crystalline structure in proniosome preparations specified the conversion of drug to molecular or amorphous state from crystalline form. And also, maltodextrin porous structure was not visible in formulations because of settling of Spans on the surface of carrier. The images are shown in Fig. 2.

TEM

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 1000 times better than that can be achieved with a light microscope [45].

TEM analysis confirms the spherical shape of niosomes formed after hydration of proniosomal systems. The image analysis showed (Fig. 3) that the niosomes were surrounded by homogeneous shading, this suggests that formed niosomes resembled the drug-enriched core model.

DSC

In DSC thermograph of proniosome formulations, endotherm peak fades away or vanishes around melting point which is clearly visible in pure drug DSC thermograph, indicating the metamorphosis of crystalline state in pure drug to amorphous state in formulations. The DSC images of pure drug rosuvastatin calcium and optimized formulation images are shown, respectively (Fig. 4a and b).

PXRD

Reduction in the intensity or absence of characteristic drug peaks in the proniosome optimized formulations compared to pure drug rosuvastatin calcium further confirms the amorphization of the drug by PXRD analysis. The PXRD images of pure drug rosuvastatin calcium and optimized formulation images are shown, respectively (Fig. 5a and b).

FT-IR analysis

FTIR spectrum of proniosomal formulation without any extra peaks when compared to pure drug spectrum extrapolates the lack of chemical interaction. IR spectrum of drug, optimized formulation, and maltodextrin are shown, respectively (Fig. 6a-c).

Based on the various evaluation parameter results, the optimized formulation further proceeded for stability, *ex vivo,* and *in vivo* studies.

Stability studies

Shelf life for drug understated conditions is dictated by the amount of drug retained within the niosomes. Physical appearance, size of the niosomes, percentage retention, and *ex vivo* permeation across rat intestine of respective drugs after storing at 24°C and 4°C temperature for 6 months are investigated in the stability studies for optimized formulation. Samples were pulled out at specified time points and

Table 3: Physicochemical characteristics of different proniosomal preparations

Formulation code	Particle size*	Polydispersity index	Zeta potential*	% drug entrapped*	Number of niosomes/mm^3×10^3
$RPNS_1$	219±12	0.342	49.2±2.6	82.6±1.7	3.09
$RPNS_2$	192±24	0.234	50.9±3.2	77.5±2.4	3.21
$RPNS_3$	180±17	0.136	52.3±3.9	89.5±2.9	3.90
$RPNS_4$	190±19	0.140	48.2±1.9	82.6±1.9	3.76
$RPNS_5$	187±14	0.249	52.1±4.2	83.1±3.4	4.10
$RPNS_6$	214±13	0.256	48.8±4.0	82.2±4.5	3.34
$RPNS_7$	220±23	0.333	54.4±3.9	80.1±3.6	3.51

*Results indicate mean±SD, n=3

Table 4: Dissolution data of rosuvastatin calcium in pure form, and various proniosomal formulations using Span 40

Time (min)	Cumulative percentage drug released*							
	Control	$RPNS_1$	$RPNS_2$	$RPNS_3$	$RPNS_4$	$RPNS_5$	$RPNS_6$	$RPNS_7$
5	2.13±0.13	75.32±0.99	76.97±0.93	82.89±0.79	76.74±0.44	75.32±0.19	76.03±0.74	77.68±0.30
10	5.93±0.29	83.79±0.84	87.59±0.24	94.49±0.67	86.64±0.57	81.42±0.57	81.66±0.82	81.19±0.38
20	9.99±0.51	91.83±0.49	89.96±0.79	96.19±0.27	92.33±0.90	85.18±0.39	85.19±0.35	85.67±0.14
30	14.55±0.5	92.81±0.82	93.54±0.64	97.67±0.71	95.20±0.42	91.81±0.44	89.45±0.90	88.27±0.98
45	19.13±0.6	94.74±0.59	97.13±0.41	99.39±0.81	97.38±0.29	94.92±0.64	92.54±0.18	91.12±0.26
60	25.39±0.5	98.09±0.43	99.32±0.30	100.40±0.64	98.15±0.17	96.15±0.24	96.13±0.77	96.12±0.29

*Results indicate mean±SD, n=6

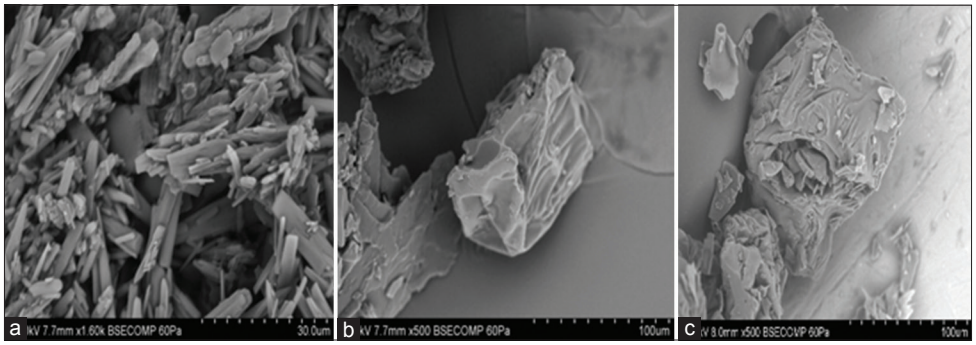

Fig. 2: Scanning electron microscope images of (a) rosuvastatin calcium. (b) Proniosome powder. (c) Maltodextrin

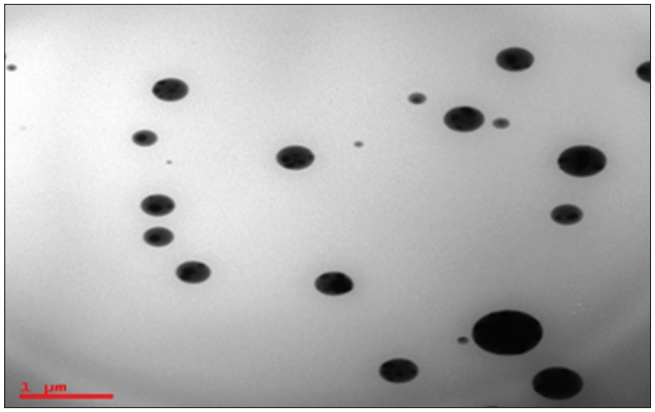

Fig. 3: Transmission electron microscopy images of rosuvastatin calcium proniosome powder

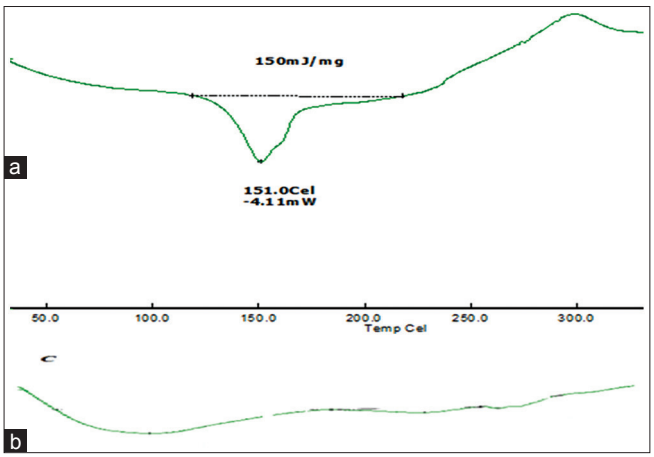

Fig. 4: (a) Differential scanning calorimetry (DSC) of pure drug. (b) DSC of optimized proniosome formulation ($RPNS_3$)

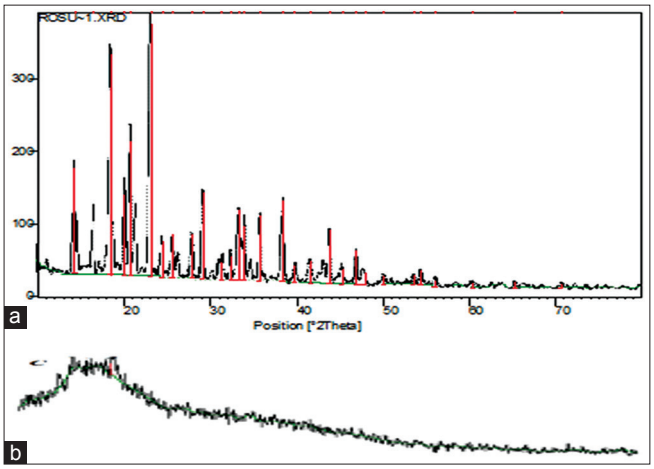

Fig. 5: (a) Powder X-ray diffractometry (PXRD) of rosuvastatin calcium. (b) PXRD of optimized formulation ($RPNS_3$)

proniosomal powders were restored to form niosomal dispersions. Initially, the powder was found to be dry, free flowing in nature without any lumps, after contact with water lead to the formation of niosomes immediately. Under optical microscope, drug crystallization was also not observed. No change in the size of the particles and size distribution represents proniosomal formulation stability. Significantly more amount of drug retention was observed at all sampling points. And also, *ex vivo* permeation study results indicated not much variation in amount of drug permeated, flux, permeability coefficient, and enhancement ratio values when compared to initial. In contrast, the formulation was destabilized at room temperature (24°C) resulting in larger niosome size, drug leakage with less entrapment efficiency with time, and chaotic *ex vivo* permeation results.

The stability studies suggest that the proniosomal powder was comparatively more stable when stored at refrigerated conditions (4°C) that at room temperature (24°C) and the results are represented (Table 5).

Ex vivo absorption study using rat intestine

Permeation across the gastrointestinal tract is also a rate-limiting step for absorption of drug other than dissolution. Therefore, *ex vivo* permeation studies were performed using the rat intestine. Since a great number of available active pharmaceutical ingredients get absorbed *in vivo* from the small intestine, it is more relevant to use the rat tissue [46]. Hence, we have used ileum portion to evaluate the potential of proniosome powder formulations for increasing the permeation of drug across the intestine barrier.

The amount of drug permeated from proniosomal systems for rosuvastatin calcium has enhanced when compared with respective control within a period of 2 h and the results are reported (Fig. 7). The flux values also improved for proniosomal system when compared with control. The ER above 1 shows enhanced permeation of proniosome when compared with control.

Thus, the remarkable improvement in the permeation can be owed to the combination of several mechanisms such as presence of non-ionic surfactant could obviate the barrier function due to the fluidization of the intercellular lipid bilayer, better membrane contact, and permeation enhancement property of the non-ionic surfactants might have led to altered permeability characteristics of the membrane which otherwise result in improved partitioning of the drug into the bilayer and direct transfer of niosomes across the epithelial membrane.

In vivo bioavailability study

Pharmacokinetic study

The average serum concentration versus time intervals after orally administering optimized formulation of rosuvastatin calcium proniosome powder in comparison against control (pure drug at a dose of 10 mg/kg) are shown. Various pharmacokinetic parameters are calculated and represented (Table 6). Results obtained show a higher C_{max} and T_{max} for proniosomes when compared against control. Slower excretion of rosuvastatin calcium drug from proniosomes is evidently the reason for higher mean residence time when compared against control. AUC values following oral administration are remarkably more when compared against control, indicating higher systemic exposure, thus overcoming the bioavailability problem, which is raised as a result of increased hepatic metabolism. RA for drug after giving proniosomes by oral route was found to be more when compared against control

Table 5: Stability study data of optimized formulation for various parameters

Parameters*	Initial	30 days	60 days	90 days	120 days	180 days
Size (nm)	180±17	229±09	239±17	235±12	237±15	224±14
%Retention of drug	89.5±2.9	92.0±3.2	87.0±3.2	91.0±4.1	89.9±3.5	90.9±3.3
Cumulative amount drug permeated (µg/cm^2)	248.0±27	254.3±22	259.8±23	267.3±26	255.8±33	249.7±31

*Results indicate mean±SD, n=6

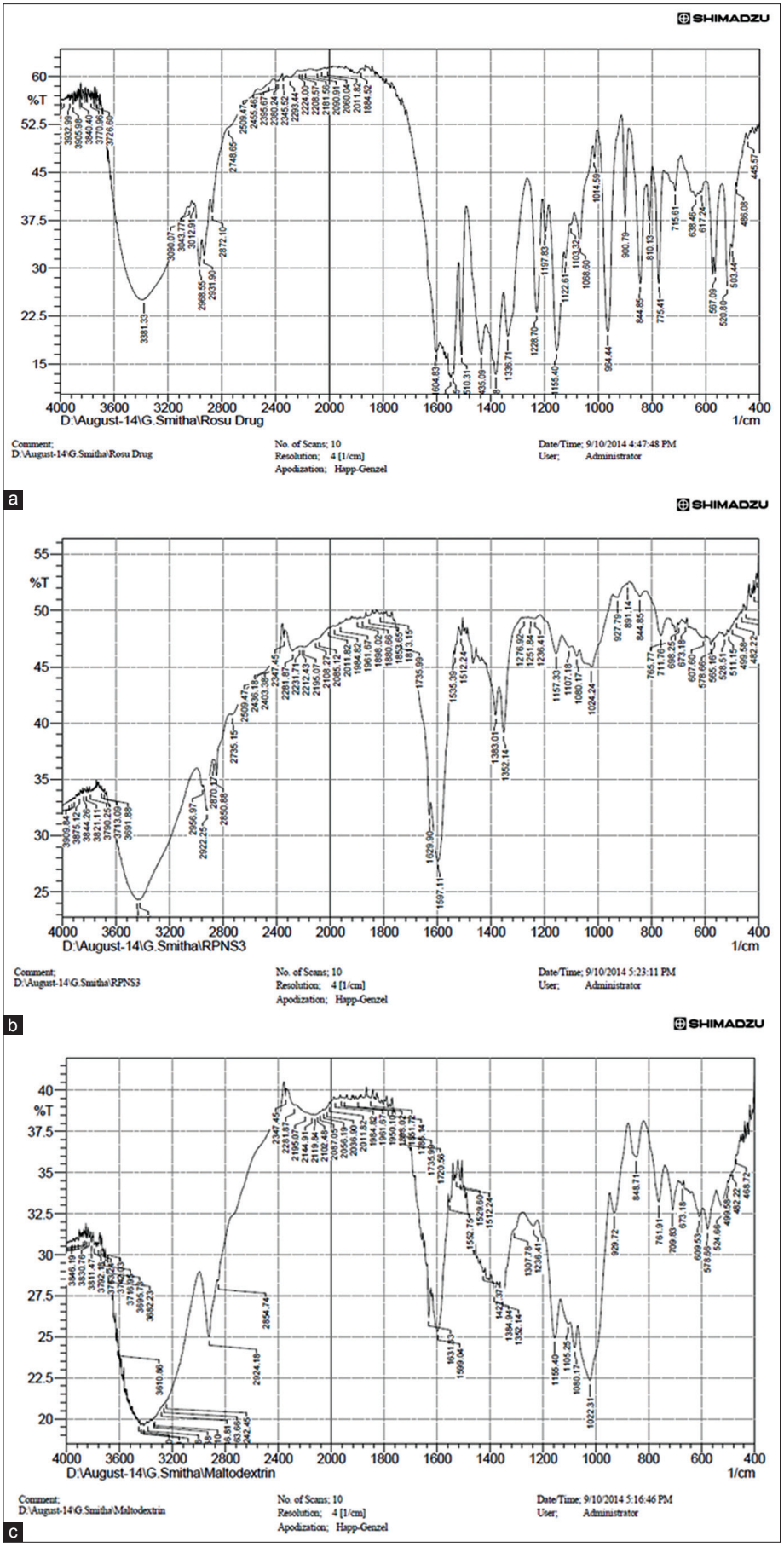

Fig. 6: (a) Infrared (IR) spectrum of rosuvastatin calcium. (b) IR spectrum of RPNS$_3$ formulation. (c) IR spectrum of maltodextrin

with a significant difference of p<0.001. Thus, it is proved from the reports that bioavailability of rosuvastatin calcium has been markedly improved from proniosomal systems when compared against control. The permeability and potential uptake of slightly soluble drugs are increased, thus enhancing the bioavailability.

Overall improvement in the RA deduces the potential of proniosomes as a suitable carrier for improved oral delivery of rosuvastatin calcium.

Table 6: Pharmacokinetic parameters of rosuvastatin calcium in rats following oral administration of various formulations

Pharmacokinetic parameters*	Control	$RPNS_3$
C_{max} (µg/ml)	0.884±0.01	1.701±0.13
T_{max} (h)	3.0±0.00	3.0±0.00
$T_{1/2}$ (h)	23.409±0.6	19.463±0.8
Ke (h^{-1})	0.030±0.001	0.036±0.001
AUC_{0-t} (µg.h.ml^{-1})	27.140±0.6	39.283±0.663
$AUC_{0-\infty}$ (µg.h.ml^{-1})	29.179±0.7	43.896±1.5
$MRT_{0-\infty}$(h)	20.861±0.6	21.75±0.23
RA		1.51±0.06

*Results indicate mean±SD, n=6. $T_{1/2}$: Half-life, Ke: Elimination rate constant, AUC: Area under the curve, MRT: Mean residence time, RA: Relative bioavailability

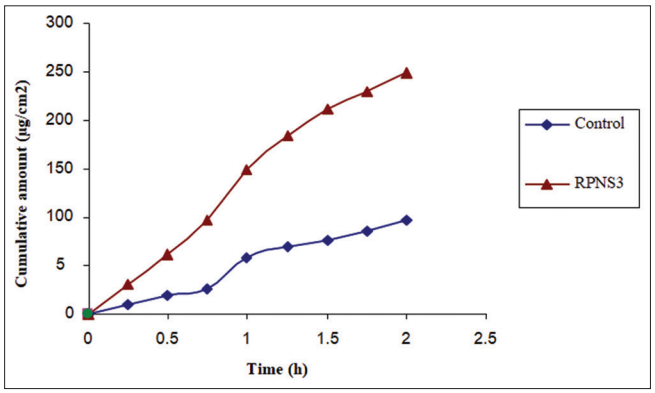

Fig. 7: *Ex vivo* permeation of rosuvastatin calcium across rat intestine from proniosomal powder (mean ± standard deviation, n=6)

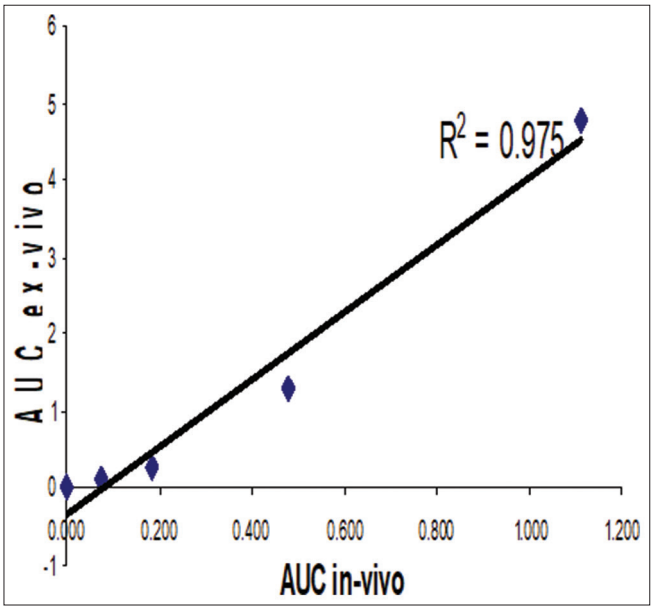

Fig. 8: *Ex vivo/in vivo* correlation graph of $RPNS_3$

***Ex vivo/in vivo* correlation**

The r^2 values are closer to linear for optimized formulation of drug when graph is plotted between the AUC of *in vivo* and *ex vivo* concentration at different time points, thus concluding a correlation and is represented (Fig. 8).

CONCLUSION

The rosuvastatin calcium proniosomes were prepared by slurry method using maltodextrin as carrier at varying ratios of Spans and cholesterol. The formulation containing 0.6:0.4 of Span and cholesterol was optimized based on the physicochemical characterization and dissolution studies. The *in vivo* pharmacokinetic studies reveal potential of proniosomes as suitable carriers for poorly soluble drugs. The results of this study clearly indicate the improvement in intestinal absorption and oral bioavailability of rosuvastatin calcium. The improved delivery of niosome entrapped drug is mediated by vesicle adsorption onto the cell surface followed by endocytosis. A particularly attractive feature of this study is that it may be applied to preparations for oral administration, that is, a proniosome composition may be placed inside a capsule which is then swallowed whole. Depending on the design of the capsule, the contents will be released somewhere in the gastrointestinal tract to form niosomes *in vivo*. The drug remains protected within the layers of the niosomes. It has been suggested that protecting a poorly absorbed, labile drug in this manner could help absorption. In this connection, it has been noted that niosomes can form, irrespective of the ionic strength of the aqueous environment, in the range of pH 3.2–8.6. It is assumed that any "free" drug remaining in the aqueous environment after spontaneous niosome formation is non-toxic and need not be removed. A further *in vivo* study in humans fortifies the improved oral delivery of rosuvastatin calcium before human use.

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AUTHORS' CONTRIBUTIONS

The author Smitha Gandra has performed the experimental work and calculations along with drafting of paper.

CONFLICTS OF INTEREST

The author reports no conflicts of interest in publishing this research article.

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