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# DESIGN, FABRICATION AND EVALUATION OF ROSUVASTATIN PHARMACOSOME - A NOVEL SUSTAINED RELEASE DRUG DELIVERY SYSTEM

**Pal Tapas Kumar*[1], Jayita Mishra[2] and Abhishekh Podder[2]**

[1]NSHM College of Pharmaceutical Technology, NSHM Knowledge Campus, Kolkata Group of Institutions, 120, B L Shah Road, Kolkata 700053, West Bengal.

[2]Research Scholar (M. Pharm), Department of Pharmaceutics, NSHM Knowledge Campus, Kolkata – Group of Institutions, 120, B L Shah Road, Kolkata 700053, West Bengal.

***Correspondence for Author: Pal Tapas Kumar**
NSHM College of Pharmaceutical Technology, NSHM Knowledge Campus, Kolkata Group of Institutions, 120, B L Shah Road, Kolkata 700053, West Bengal.,

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## ABSTRACT

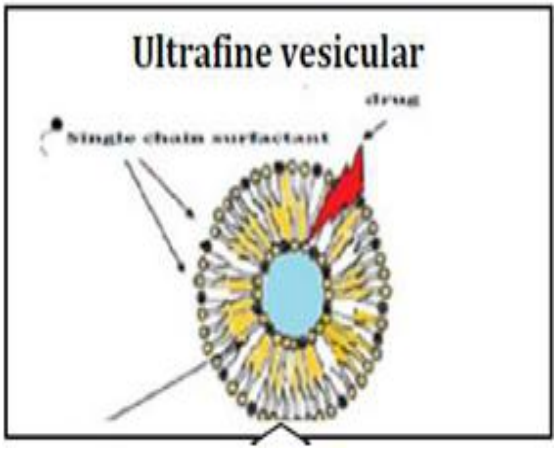
Pharmacosomes are amphiphilic lipid vesicular systems containing phospholipid complexes with characteristic potential to improve solubility, absorptivity and bioavailability of both poorly water soluble as well as poorly lipophilic drugs. With an objective to improve the aqueous solubility and subsequent bioavailability of a model BCS class III drug, Rosuvastatin Calcium, its Pharmacosomes were developed and subjected to evaluation of physicochemical characteristics. The cumulative release profile and permeation studies had been also done by *in-vitro* dissolution test, *in-vitro* diffusion study by modified Franz diffusion cell using egg membrane and *in-vivo* study for antihyperlipidemic effect by Tritron induced hyperlipidemia animal model. Solubility of prepared Pharmacosomes was found to be higher than pure Rosuvastatin Calcium. Drug content was found to be in the range of 90.4±0.52% to 94.4±0.61% in all the batches of Pharmacosomes. FTIR data also demonstrated superimposed curves to confirm the stability of Pharmacosome complex after 2 months stability study at 40 deg C temp and 75% RH. After 24 hours, maximum drug released from formulation F1 was found as 66.93% in the dissolution study and maximum drug permeated by diffusion through egg membrane from formulation F1 was 49.50% in the diffusion study. *In-vitro* and *in-vivo* experiments justify and confirm Rosuvastatin Pharmacosomes formulations as significantly better than Rosuvastatin Calcium as a unique sustained release delivery system with simultaneous reduction of Dosage and improved bioavailability.

**KEYWORDS:** Pharmacosome, Hand shaking method, Hyperlipidemic, Statins, Log P value, Vesicular systems.

## INTRODUCTION

**Rosuvastatin** is recommended to be used for the treatment of dyslipidemia only after other measures such as diet, exercise, and weight reduction have not improved cholesterol levels. Rosuvastatin is a competitive inhibitor of the enzyme HMG-CoA reductase, with mechanism of action similar to that of other statins. It has approximate elimination half-life of 19 hrs & time to reach peak plasma concentration within 3–5 hrs following oral administration. Dose: Start with 5 mg OD, increase if needed up to 20 mg/day, (max 40 mg/ day)

**Pharmacosomes**, bearing unique advantages over liposome and niosome vesicles have come up as potential alternative to conventional vesicular drug delivery. They are colloidal dispersions of drugs covalently bound to lipids. Depending upon the chemical structure of the drug–lipid complex they may exist as ultrafine vesicular, micellar, or hexagonal aggregates. As the system is formed by linking a drug (pharmakon) to a carrier (soma), they are termed as "Pharmacosomes". They are an effective tool to achieve desired therapeutic goals such as drug targeting and controlled release.



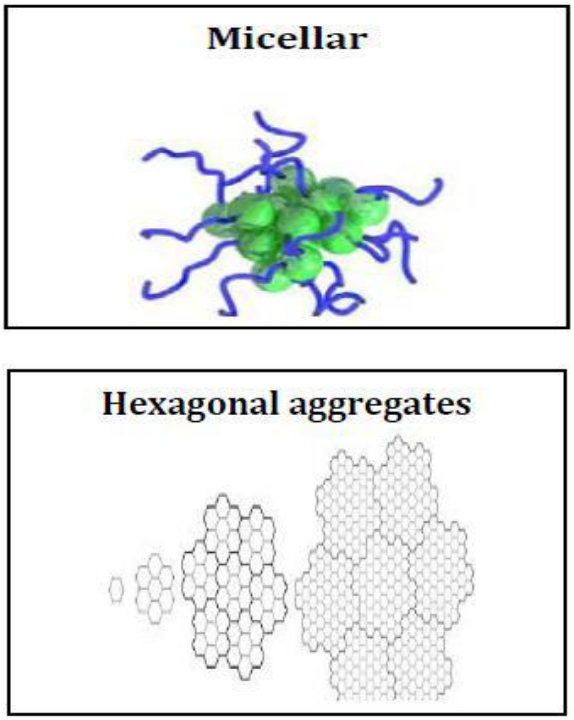



**Figure 1: Depending upon the chemical structure of the drug–lipid complex they may exist as ultrafine vesicular, micellar, or hexagonal aggregates.**

The criterion for the development of the vesicular Pharmacosomes is dependent on surface and bulk interactions of lipids with drug. Any drug possessing an active hydrogen atom (-COOH, -OH, -NH2, etc.) can be esterified to the lipid, with or without spacer chain that strongly result in an amphiphilic compound, which will facilitate membrane, tissue, or biological cell wall transfer. The prodrug conjoins hydrophilic and lipophilic properties, thus acquires amphiphilic characters, and therefore found to reduce interfacial tension, and at higher concentrations exhibits mesomorphic behavior.[17, 27-30]

## MATERIALS FOR PHARMACOSOMES

There are three essential components for Pharmacosomes preparation.

**1) Drugs:** Drugs containing active hydrogen atom (-COOH, OH, NH2) can be esterified to the lipid, with or without spacer chain and they forms amphiphilic complex which in turn facilitate membrane, tissue, cell wall transfer in the organisms.

**2) Solvents:** For the preparation of Pharmacosmes, the solvents should have high purity and volatile in nature. A solvent with intermediate polarity is selected for Pharmacosomes preparations.

**3) Lipid:** Phospholipids are the major structural component of biological membranes, where two types of phospholipids generally used- phosphoglycerides and spingolipids. The most common phospholipid is phosphatidylcholine molecule. Phosphatidylcholine is an amphipathic molecule in which a glycerol bridges links a pair of hydrophobic acetyl hydrocarbon chains, with a hydrophilic polar head group, Phosphatidylcholine.[28-30]


**Figure 2: Molecular structure of Phosphatidylcholine – a Phospholipid**

## PREPARATION OF PHARMACOSOMES

In general two methods have been employed to prepare Pharmacosomes.

- **Hand-shaking method:** In the hand-shaking method, the dried film of the drug–lipid complex (with or without egg lecithin) is deposited in a round-bottom flask and upon hydration with aqueous medium, readily gives a vesicular suspension.
- **Ether-injection method:** In the ether-injection method, an organic solution of the drug– lipid complex is injected slowly into the hot aqueous medium, wherein the vesicles are readily formed. At low concentration the amphiphiles exists in the monomer state. Further increase in monomers may lead to variety of structures i.e., micelles of spherical or rod like or disc shaped type or cubic or hexagonal shape. Mantelli et al., compared the effect of diglyceride prodrug on interfacial tension, with the effect produced by a standard detergent dodecylamine hydrochloride, and found similar effect on lowering of surface tension. Above the critical micelle concentration (CMC), the prodrug exhibits mesomorphic lyotropic behavior, and assembles in supramolecular structures.
- **Other Approaches:** Another approach for producing Pharmacosomes was recently developed in which a biodegradable micelle forming drug conjunct was synthesized from the hydrophobic drug driamycin and a polymer composed of polyoxyethylene glycol and polyaspartic acid. This method has the benefit that although it may be possible to dilute out the micelle, the drug will probably not precipitate because of the water solubility of the monomeric drug conjunct.[17, 31] Muller-Goymann and Hamann produced fenoprofen Pharmacosomes using a modified technique that involved diluting lyotropic liquid crystals of amphiphilic drugs.[17, 32] Approaches have been done to attach drugs to various glyceride-like groups, and the resulting amphiphilic molecules have been

spontaneously dispersed. They were labeled Pharmacosomes because of their tendencies to form unilamellar vesicles. It was suggested that these molecules should enhance lymph transport[17,33] Zhang et al. optimized the preparation of 3', 5'-dioctanoyl-5-fluoro-2'-deoxyuridine Pharmacosomes and found that the drug Phosphatidylcholine ratio, glycerol tristearate concentration and pluronic F-68 concentration, have an influence on the mean particle size, entrapment ratio, and drug loading.[34] Singh et al. formulated "vesicular constructs" by encapsulating antibiotic amoxicillin in aqueous domain by using phosphatidylethanolamine with various molar ratios of Phosphatidylcholine and cholesterol which significantly enhanced cytoprotection.[35]

## ADVANTAGES OF PHARMACOSOME

- As drug is covalently bound, membrane fluidity has no effect on release rate, but in turn
- Depends upon the phase-transition temperature of the drug-lipid complex.
- No leakage of drug take place as the drug is covalently linked to the carrier.
- Drug can be delivered directly to the site of infection.
- Their degradation velocity into active drug molecule, after absorption depends very much on the size and functional groups of the drug molecule, the chain length of the lipids, and the spacer.
- Reduced cost of therapy
- Suitable for both hydrophilic and lipophilic drugs. The aqueous solution of these amphiphiles exhibits concentration dependent aggregation.
- High and predetermined entrapment efficiency as drug and carrier are covalently linked together.
- Volume of inclusion doesn't influence entrapment efficiency
- No need of removing the free un-entrapped drug from the formulation which is required in case of liposomes.
- Improves bioavailability especially in case of poorly soluble drugs.
- Reduction in adverse effects and toxicity.
- Their degradation velocity into active drug molecule, after absorption depends very much on the size and functional groups of the drug molecule, the chain length of lipids and the spacer.[4, 17, 36]

**Table no. 1: Pharmacosomes differs from Liposome, Niosome and Transferosome.**

| VESICULAR SYSTEM | ISSUES ENCOUNTERED | ADVANTAGE OF PHARMACOSOME |
|---|---|---|
| **LIPOSOME** | **1.**Expensive<br>**2.**Degradation by oxidation<br>**3.**Lack of purity of natural phospholipids<br>**4.**Chances of leaching of drug | **1.**Cheaper<br>**2.**Oxidation resistant<br>**3.**Pure natural phospholipid not needed<br>**4.** Covalent linkage prevents drug leakage. |
| **NIOSOME** | **1**. Time consuming prepn.<br>**2.** Comparatively less efficient<br>**3.** Instability | **1.** Less time consuming prepn.<br>**2.**More efficient<br>**3**.More stable |
| **TRANSFEROSOME** | **1**.Expensive,<br>**2**.Chemical instability | **1**.Cheap<br>**2**.Chemically stable |

## LITERATURE REVIEW

In the study of **Akbari B.V et *al***[50] by using inclusion complexation with β-cyclodextrin (β-CD); the solubility of Rosuvastatin Ca was found significantly increased in the phase solubility profile and apparent stability constant ($K_C$) was found as 42.003$M^{-1}$. The inclusion complex prepared with β-CD by kneading method exhibited greatest enhancement in solubility and fastest dissolution (98.96% RST release in 30 min) of RST.

**Salih et al**[49] studied formulation and in vitro evaluation of Rosuvastatin calcium niosomes using non-ionic surfactants (Span 20, Span 60, span 80), cholesterol and lecithin in different ratios by film hydration method and evaluated 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. Fourier transform infrared (FTIR) was used for study of drug – excipients compatibilities.

**Anup Kumar Chakraborty et al**[51] studied formulation of dosage form of Rosuvastatin calcium and development of a new, simple, precise, rapid, and accurate validated reverse phase liquid chromatographic RP-HPLC method for its estimation in the routine analysis. Another simple, specific and economic spectroscopic method has been developed for the estimation of Rosuvastatin calcium in bulk and tablet dosage form by **Rekha rajeevkumar et al.**[53] In the developed method water was used as the solvent. The absorption maximum of the drug was found to be 241nm. The drug follows a linear Lambert-Beer law relationship with respect to the drug concentration in the range of 5-30µg/mL, with linearity coefficient of 0.9998. The proposed method can be applied for the routine estimation of Rosuvastatin Calcium in the laboratory.

**J. Dwivedi et al**[55] studied formulation & evaluation of Sustained Release multi-particulate pellets of Rosuvastatin Calcium, prepared by using fluidized bed

coating method. Different pellet formulations were made by using sustained release rate controlling polymer like Eudragit NE30D. The release of Rosuvastatin calcium from the tablet for a period up to 16 hrs was recorded in controlled manner.

## MATERIALS

**Table 2: List of Materials.**

| Sl. No. | Materials | Source |
|---|---|---|
| 1. | Rosuvastatin Calcium | Hygeia Pharmaceuticals Kolkata, India. |
| 2. | Soya Lecithin (30%) | Himedia, India |
| 3. | Cholesterol | Merck Specialties Pvt. ltd, India |
| 4. | Hydrochloric Acid | Merck Specialties Pvt. ltd, India |
| 5. | Methanol | Merck Specialties Pvt. ltd, India |
| 6. | Chloroform | Merck Specialties Pvt. ltd, India |
| 7. | Dichloromethane | Merck Specialties Pvt. ltd, India |
| 8. | Potassium dihydrogen phosphate | Merck Specialties Pvt. ltd, India |
| 9. | Disodium hydrogen phosphate | Merck Specialties Pvt. ltd, India |
| 10. | Tritron X 100 | Loba chemie pvt ltd, India |
| 11. | Cholesterol test kit | Span diagnostics ltd, India |
| 12. | HDL- Cholesterol test kit | Span diagnostics ltd, India |
| 13. | Triglycerides test kit | Span diagnostics ltd, India |

**Table 3: List of Instruments.**

| SL No. | Instrument | Manufacturer |
|---|---|---|
| 1 | Digital weighing balance | Metler |
| 2 | pH meter | Model no. -LI- 615, Elico |
| 3 | Ultrasonic bath with digital timer, temp. control | Model no.- UD200SH-6L, Takashi, Japan |
| 4 | Vacuum drying oven | Indian Instrument pvt. ltd. |
| 5 | Hot air oven | Labquip |
| 6 | Magnetic stirrer with thermostat | Remi-make |
| 7 | Water bath (thermostatically control) | Labquip |
| 8 | Double distillation apparatus (Borosil glass) | Testing instrument mfg. Co. Pvt. ltd |
| 9 | Heating Mantle | Sinha Scientific |
| 10 | UV/visible Spectrophotometer | Model no. UV 1800, Shimadzu,Japan |
| 11 | FTIR Spectrophotometer | Model no. Alpha- T, Bruker, Germany |
| 12 | Micro centrifuge Spinwin | Remi-make |
| 13 | Projection microscope (Trinocular) | Model no. PRM- 18T, Scientific equipment pvt .ltd. |
| 14 | Scanning electron microscopy | Model no. JSM6360, Jeol make, UK |
| 15 | X-Ray Diffraction | Model no. ULTIMA-III, Rigaku make, Japan |

## METHODS

### (A) ANALYTICAL: Development of UV Spectroscopic Methods

**(a) Determination of absorption maxima:** Absorption maxima or the wavelength at which absorption takes place. For accurate analytical work it is important to determine the absorption maxima of the substance under study. Double beam UV-VIS Spectrophotometer (Shimazdu model no. uv-1800-240v) in the range of 200-800 nm is used.

**(b) Preparation of standard calibration curve in water and in Ph 6.8 Phosphate buffer:** It is done using double beam UV-VIS Spectrophotometer (Shimazdu model no. uv-1800-240v) at 242 nm spectrophotometrically.

### (B) EXPERIMENTAL

**1. Preparation of Pharmacosomes by Hand shaking method:** Pharmacosomes of Rosuvastatin Calcium were prepared with an equimolar ratio (1:1) of Rosuvastatin Calcium and Phosphatidylcholine. Pharmacosomes were prepared in two steps.

**(a) Acidification of Rosuvastatin Calcium:** Rosuvastatin Calcium(1mole) dissolved in water then acidify with 1(N) HCL, which results Rosuvastatin Calcium converts into Rosuvastatin that contain free carboxyl group or an active hydrogen atom can be esterified with or without spacer chain to the hydroxyl group of a lipid molecule.

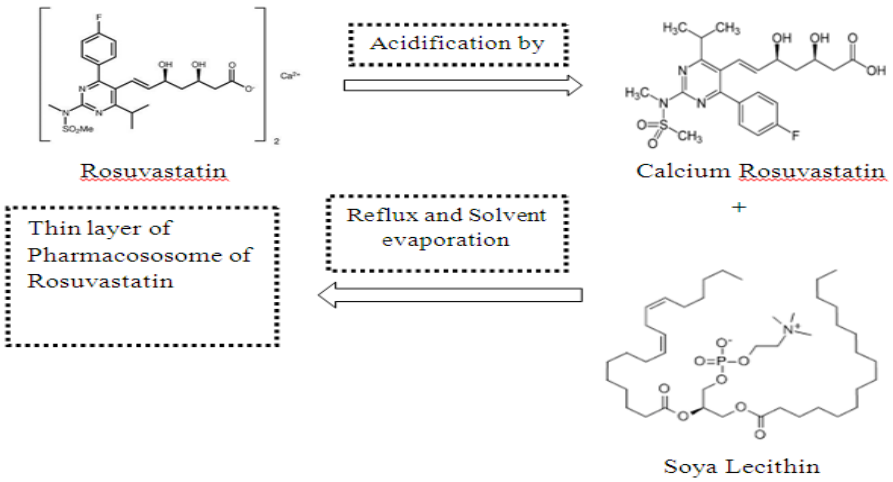



**Figure 3: Preparation of Rosuvastatin Pharmacosomes.**

After acidification, these aqueous solutions of Rosuvastatin extracted into chloroform by shake flask method. This aqueous solution (50 ml) was then transferred to a 100 ml of separating funnel with 25 ml of chloroform in two times and was shaken well for 30 minutes. Then the separating funnel was kept still for about 24 hours. Then Separate 50 ml chloroform layer and measured the concentration of drug from the aqueous layer spectrophotometrically at 242 nm (Double beam UV Spectrophotometer).

**(b) Preparation of Pharmacosomes of Rosuvastatin:** Rosuvastatin extracted into chloroform (50ml) mix with 50ml dichloromethane in which lecithin soya (30%) dissolved in a 250 ml round bottom flask and reflux for 3hours at 45$^{o}$C. After 3 hrs this mixed solution transferred into beaker for solvent evaporation. After solvent evaporation, a thin film of solid mixture is deposited on the wall of beaker. Then this beaker was kept in a vacuum dryer for vacuum drying for 24 hours at 45$^{o}$C. The Pharmacosomes were collected and placed in a vacuum desiccator overnight and then subjected to characterization.

**Table 4: List of different codes with different ratio for preparation of Pharmacosomes**

| CODE | DRUG: LECITHIN SOYA | WEIGHT TAKEN (MG) | |
|---|---|---|---|
| | | DRUG | LECITHIN SOYA |
| $F_1$ | 1:1 | 50 | 127 |
| $F_2$ | 1:1 | 100 | 254 |
| $F_3$ | 1:1 | 200 | 508 |
| $F_4$ | 1:1 | 300 | 762 |
| $F_5$ | 1:1 | 400 | 1016 |
| $F_6$ | 1:1 | 500 | 1270 |

**2. Preparation of Liposome of Rosuvastatin by Hand Shaking method:** Liposome of Rosuvastatin Calcium were prepared with a ratio (1:1:0.66) of Rosuvastatin Calcium, lecithin soya and cholesterol. Liposomes were prepared in two steps, these are:

**(a) Acidification of Rosuvastatin Calcium:** Rosuvastatin Calcium(1 mole) dissolved in water then acidify with 1(N) HCL, which results Rosuvastatin Calcium converts into Rosuvastatin that contain free carboxyl group or an active hydrogen atom can be esterified with or without spacer chain to the hydroxyl group of a lipid molecule.

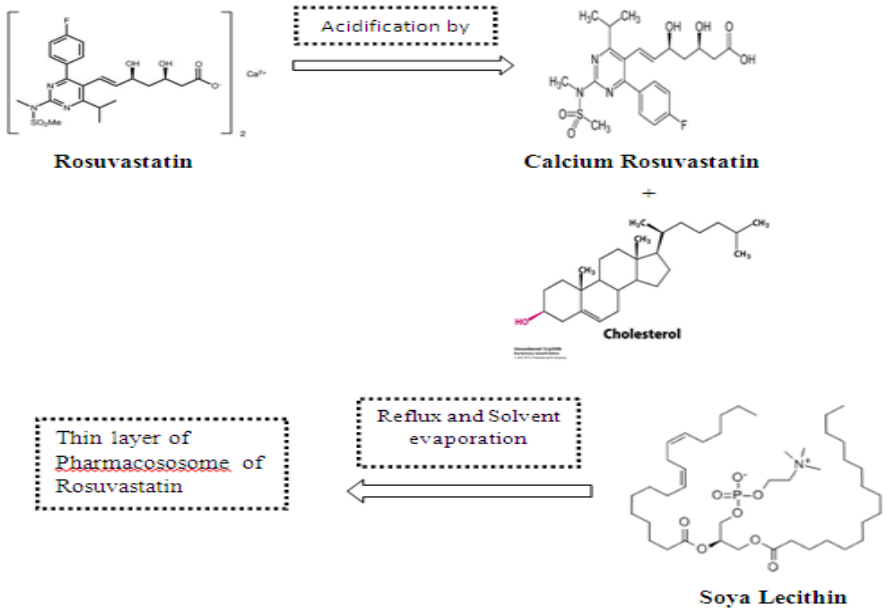



**Figure 4: Preparation of Liposome of Rosuvastatin.**

After acidification, this aqueous solution of Rosuvastatin extracted into chloroform by shake flask method. This aqueous solution (50 ml) was then transferred to a 100 ml of separating funnel with 25 ml of chloroform in two times and was shaken well for 30 minutes. Then the separating funnel was kept still for about 24 hrs. Then separate 50 ml chloroform layer and measured the concentration of drug from the aqueous layer spectrophotometrically at 242 nm

**(b) Preparation of Liposome of Rosuvastatin (L1):** Rosuvastatin extracted into chloroform(50ml) mix with 50ml dichloromethane in which lecithin soya (1mole) and cholesterol (0.66mole) dissolved in a 250 ml round bottom flask and reflux for 3hours at 45$^{o}$C. After 3 hrs this mixed solution transferred into beaker for solvent evaporation. After solvent evaporation, a thin film of solid mixture is deposited on the wall of beaker. Then this beaker was kept in a vacuum dryer for vacuum drying for 24 hours at 45$^{o}$C. The liposomes were collected and placed in vacuum desiccators overnight and then subjected to characterization.

## EVALUATION OF PHARMACOSOMES OF ROSUVASTATIN

- **Drug content study**

36 mg Rosuvastatin (RSV) Pharmacosomes complex equivalent to 10 mg Rosuvastatin Calcium was weighed and taken with pH 6.8 phosphate buffer. At the end of 24 hours, after membrane filtration (0.45µm membrane filter) of this colloidal suspension, 50ml of this solution was diluted with 50ml of pH 6.8 phosphate buffer. Then suitable dilutions measured for drug content at 242 nm spectrophotometrically in a double beam UV Spectrophotometer.

- **Solubility study**

From the rest of the 50ml RSV- Pharmacosomes solution in buffer, 40 ml transferred in a 100ml separating funnel with 40ml octanol and was shaken well for 30 minutes. Then the separating funnel was kept still for about 1 hour. Concentration of the drug was determined from the aqueous layer as well as octanol layer spectrophotometrically at 242 nm in a double beam UV Spectrophotometer.

- **Vesicle Shape Determination**

The surface morphology (roundness, smoothness, and formation of aggregates) of Pharmacosomes were studied by Scanning Electron Microscopy (SEM).

- **Drug Excipient Interaction Studies (Compatibility Studies)**

IR spectra for Rosuvastatin Calcium, Phospholipid (soya-lecithin) and Rosuvastatin-phospholipid complex were obtained on an IR spectrometer in the transmission mode with the wave number region 3500- 1000 cm-1.

- **X-Ray Powder Diffraction Analysis (XRPD)**

The crystalline state of Rosuvastatin Calcium was evaluated with X-ray powder diffraction. The X-ray generator was operated at 40 KV tube voltages and 40 mA of tube current, using the Ka lines of copper as the radiation source. The scanning angle ranged from 1 to 600 of 2θ in step scan mode.

- **In vitro dissolution studies**[44]

In vitro dissolution studies of all Pharmacosomes of Rosuvastatin formulations (F1 to F6) as well as pure Rosuvastatin Calcium were performed in triplicate in a USP eight station dissolution test apparatus, type II at 100 rpm and at 37 °C and then analyzed at 242 nm in Double beam UV VIS spectrophotometer.

- **In vitro diffusion studies**[48,63]

The release of Rosuvastatin from the Pharmacosomes formulations was determined by using modified membrane diffusion technique using egg membrane. The

collected samples were analyzed at 242 nm in Double beam UV VIS spectrophotometer.

- **Comparison of *in vivo* efficiency of Pharmacosomes with standard drug Rosuvastatin calcium as lipid lowering agent to reduce blood cholesterol level**

**1. Experimental animals and housing**

Wistar male rats were purchased from local distributor, Kolkata. They were housed, three per polypropylene cage under standard laboratory conditions at room temperature (25°C ± 2°C) with 12 hr light /dark cycle. The animals were provided with standard food and water, except during experimentation. Ethical clearance was obtained from Institutional Animal Ethical committee (IAEC) of NSHM College of Pharmaceutical Technology, Kolkata.

**2. Experimental Design**[73]

**Triton X 100 induced hyperlipidemia:** Twenty four male Wistar rates weighing 190 to 200 gm were randomly divided into 4 groups of 6 each and kept in their cages for 5 days prior dosing to allow for acclimatization to the laboratory conditions. The animals received the drugs as per the table 5.

**Table 5: Different groups with animal model:**

| Group | Model |
|---|---|
| Group1 | Administered vehicle(0.1% Na CMC suspension) and served as **Normal control** |
| Group2 | Administered Triton X 100 and served as **Positive control**(200mg/kg) |
| Group3 | Administered daily dose of **Rosuvastatin calcium**(10mg/kg) B.Wt + Tritron (200mg/kg) |
| Group4 | Administered single dose of **Pharmacosomes** of Rosuvastatin (36mg/kg) B.Wt + Tritron (200mg/kg) |

After 24 hours and 48 hours, blood was collected by retro orbital puncture in the ear under localized ether anesthesia and subject to centrifugation to obtain serum. Serum was analyzed for serum TGs, serum TC, serum HDL-C.

**3. Measurement of Lipid profile:** Total Cholesterol (TC), High Density Lipoprotein (HDL), Triglycerides (TG) have been measured by the test kit of Span Diagnostic ltd, India.

**I. Total Cholesterol (TC)**[64-70]

**(a) Clinical significance:** Serum cholesterol serves as an indicator of propensity towards Coronary Heart Disease (CHD), Liver function, Biliary function, Intestinal absorption, Thyroid Function and Adrenal disease.

**Increased concentration:** Increased cholesterol concentration is found in Idiopathic Hypercholesterolemia, Hyperlipoproteinemia, Nephrotic syndrome, Hypothyroidism, Nephrosis and Diabetic Mellitus. Hypercholesterolemia is known to be associated with an increased risk of coronary Heart Disease (CHD).

**Decreased concentration:** Decreased cholesterol concentration is found in Hepatocellular disease, Hyperthyroidism, Chronic Anemia, Starvation and Hypobetalipoproteinemia. Serum cholesterol concentration is very low in rare genetic disease like Abetalipoproteinemia.

**(b) Assay principle**: The principle is being showed in figure 5. Absorbance of colored dye is measured at 505 nm and is proportional to amount of Total Cholesterol concentration in the sample.

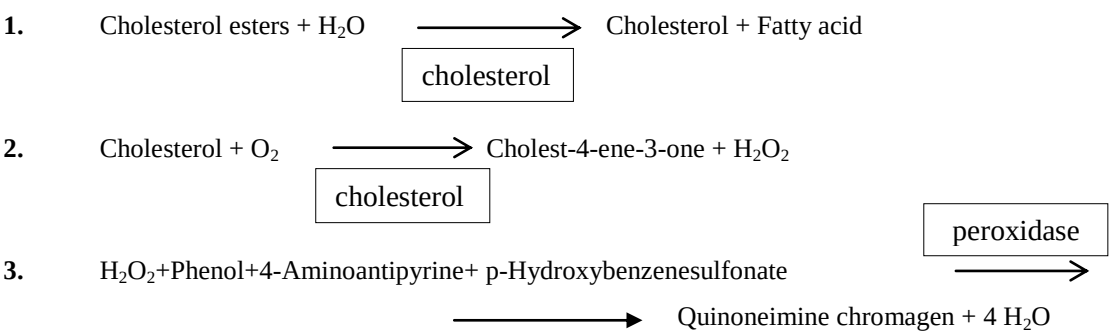



**Figure 5: Coupled assay steps used to determine the concentration of cholesterol.**

**(c) Assay procedure**

Mix the reagents well as directed. Incubate at 37°C for 10minutes or at room Temperature (15-30°C) for 30 minutes.

**Table 6: Assay procedure for Total Cholesterol.**

| Pipette into tubes marked | Blank | Standard | Test |
|---|---|---|---|
| Serum/Plasma | - | - | 10 µL |
| Reagent 2 | - | 10 µL | - |
| Reagent 1 | 1000µL | 1000 µL | 1000 µL |

**(d) Calculation**

*Cholesterol concentration (mg/dL) = [(Absorbance of test)/ (Absorbance of standard)]*200*

**II. High Density Lipoprotein (HDL)[64-70]**

**(a) Clinical Significance:** HDL transports Cholesterol from tissues to the liver for catabolism while LDL transports cholesterol from sites of origin to deposition in tissues.

**Increased concentration:** Increased HDL Cholesterol concentration reduces the risk of cardiovascular disease. Moderate to vigorous exercise, estrogens and moderate consumption of alcohol may increase serum HDL – Cholesterol.

**Decreased concentration:** Decreased HDL-Cholesterol concentration increased the risk of cardiovascular disease. It is lowered in Tangier disease, heavy cigarette smoking, obesity, very high carbohydrate diets, uncontrolled Diabetes Mellitus and in male sex hormone therapy.

**(b) Assay Principle:** Low Density Lipoprotein, Very Low Density Lipoproteins and chylomicron fractions are precipitated by addition of polyethylene Glycol 6000 (PEG). After centrifugation, the High Density Lipoprotein (HDL) fraction remains in the supernatant and is determined with CHOD-PAP method.

**(c) Assay procedure**

**Table 7: Step 1: HDL Cholesterol separation.**

| Pipette into tubes marked | Test |
|---|---|
| Serum/Plasma | 200 µL |
| Reagent 3 | 200µL |

Mix the reagents well as directed and keep at room temperature (15-30$^{o}$C) for 10 minutes. Centrifuge for 15 minutes at 2000 rpm and separate clear supernatant. Use the supernatant for HDL –Cholesterol estimation.

**Table 8: Step 2: HDL Cholesterol estimation**

| Pipette into tubes marked | Blank | Standard | Test |
|---|---|---|---|
| Supernatant from step 1 | - | - | 100 µL |
| Reagent 4 | - | 100 µL | - |
| Reagent 1 | 1000µL | 1000 µL | 1000 µL |

Mix above ingredients well as directed. Incubate at 37$^{o}$C for 10minutes or at room Temperature (15-30$^{0}$C) for 30 minutes.

**(d) Calculation**

*HDL-Cholesterol concentration (mg/dL)*
*= [(Absorbance of test)/ (Absorbance of standard)]*50*2#*
*# (2= Dilution factor, as sample is diluted 1:1 in step 1)*

**III. Triglycerides[64-72]**

**(a) Clinical Significance:** Triglycerides are family of lipids produced endogenously from carbohydrates and absorbed from the diet and are found in all plasma lipoproteins. Triglycerides measurement is an important tool in the diagnosis of Hyperlipidemias.

**Increased concentration:** Increased concentration of triglycerides is found in hypertriglyceridemia, Ischemic Heart Disease, Hyperlipoproteinemia types 1 and 5, Nephrotic syndrome, Hypothyroidism, Diabetes Mellitus, acute pancreatitis, Glycogen storage disease and tangier disease.

**Decreased Concentration:** Decreased concentration of triglycerides is found in rare disease like Abetalipoproteinemia.

**(b) Assay Principle:** The assay is carried out as per steps in figure 6 and the absorbance of colored dye, measured at 505nm, is proportional to Triglycerides concentration in the sample.

1. Triglycerides $\xrightarrow{\text{LPL}}$ Glycerol+ FFA

2. Glycerol+ ATP $\xrightarrow{GK}$ Glycerol 3-Phosphate+ADP

3. Glycerol 3-Phosphate + $O_2$ $\xrightarrow{GPO}$ DAP+ $H_2O_2$

4. $2H_2O_2$ +4-AAp+ 4-chlorophenol $\xrightarrow{POD}$ quinoneimine dye+ 4 $H_2O$

**Figure 6: Coupled assay used to determine the concentration of Triglycerides.**

**(c) Assay procedure**

**Table 9: Assay procedure for estimation for Triglycerides:**

| Pipette into tubes marked | Blank | Standard | Test |
|---|---|---|---|
| Serum/Plasma | - | - | 10 µL |
| Reagent 2 | - | 10 µL | - |
| Reagent 1 | 1000µL | 1000 µL | 1000 µL |

Mix above ingredients well as directed .Incubate at 37$^{o}$C for 10 minutes.

**(d) Calculation**

*Triglycerides (mg/dl) = [(Absorbance of test)/ (Absorbance of standard)]*200*

*Atherogenic Index= TC/HDL-C*

- **STABILITY STUDY**

Optimized formulation F3 RSV-Pharmacosomes and another vesicular formulation L1 liposome were selected for comparison as well as the stability study. F3 RSV-Pharmacosomes and L1 liposome were covered by aluminum foil and stored in an incubator at 40$^{0}$ C and 75% Relative humidity. After 2 months, Pharmacosomes and liposome were examined for Drug content and FTIR spectral evaluation and the compatibility study.

**RESULT AND DISCUSSION**

**(A) Analytical: Development of UV Spectroscopic Method**

**(a) Determination of absorption maxima:** The absorption maxima obtained at 242 nm with a characteristic peak. The obtained results confirm the identification of Rosuvastatin Calcium.

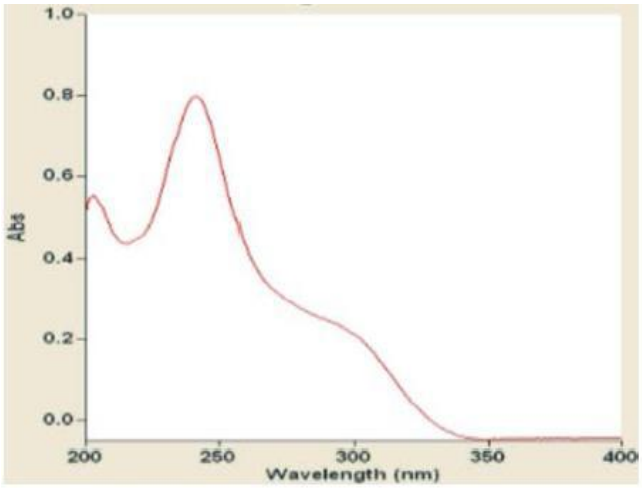



**Fig. 7: Determination of absorption maxima of Rosuvastatin Calcium.**

**(b)Preparation of standard calibration curve in water**

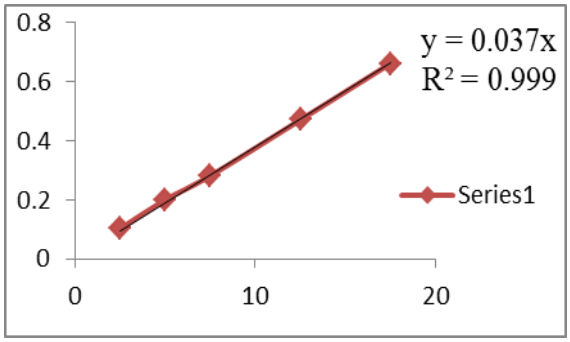
The standard calibration curve of Rosuvastatin Calcium in water was obtained by plotting absorbance Vs Concentration [Table 10].The standard curve is shown in Figure 8(a) with the slope of 0.037 and correlation coefficient of 0.999. The curve was found to be linear in the concentration range of 2-18 µg/ml (Beer's range) at 242 nm.

**(c)Preparation of standard calibration curve in pH6.8 Phosphate buffer**

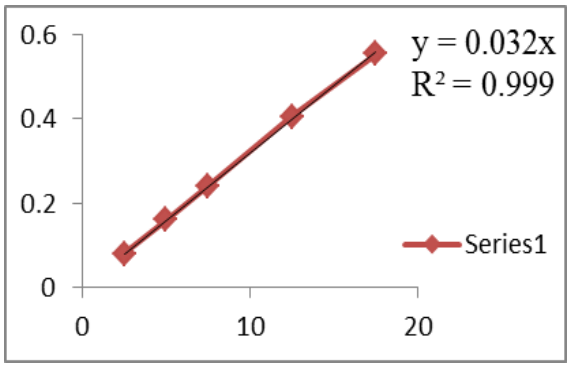
The standard calibration curve of Rosuvastatin Calcium in water was obtained by plotting absorbance Vs Concentration [Table 10].The standard curve is shown in Figure 8(b) with the slope of 0.032 and correlation coefficient of 0.999. The curve was found to be linear in the concentration range of 2-18µg/ml (Beer's range) at 242 nm.

**Table 10: Calibration curve data of Rosuvastatin calcium in water and pH 6.8 Phosphate buffer.**

| Conc. (µg/ml) | Absorbance* ± S.D (in water) | Absorbance* ± S.D (in pH 6.8) |
|---|---|---|
| 2.5 | 0.104± 0.0012 | 0.079± 0.0007 |
| 5 | 0.195± 0.0064 | 0.164± 0.0044 |
| 7.5 | 0.284± 0.0040 | 0.245± 0.0042 |
| 12.5 | 0.468± 0.0029 | 0.404±0.00212 |
| 17.5 | 0.660± 0.0053 | 0.557±0.00424 |




**(a)**




**(b)**

**Fig 8: Calibration curve of (a) Rosuvastatin calcium in water and (b) Rosuvastatin calcium in pH6.8 Phosphate buffer.**

**(B) EXPERIMENTAL**

**1. Preparation of Pharmacosomes of Rosuvastatin by Hand Shaking method**

Pharmacosomes of Rosuvastatin were prepared with an equimolar ratio (1:1) of Rosuvastatin Calcium and Soya lecithin (partially hydrolysed - 30%). Pharmacosomes were prepared in two steps, at first acidification of Rosuvastatin Calcium then reflux and evaporation. Table 11 shows that very negligible amount of drug (in salt form) ranging from 0.06 mg to 0.63 mg left in the aqueous medium after the extraction with chloroform in various (F1-F6) formulation. So, maximum amount of drug (in acid form) in different batches F1 to F6 formulation comes into chloroform after the extraction.

**2. Preparation of Liposome of Rosuvastatin by Hand Shaking method**

Liposomes of Rosuvastatin were prepared with an equimolar ratio (1:1:0.66) of Rosuvastatin Calcium, Soya lecithin (30%) and Cholesterol. Liposomes were prepared in two steps, at first acidification of Rosuvastatin Calcium, then refluxed and evaporated. Table 11 shows that very negligible amount of drug [0.71mg] left in the aqueous medium after the extraction in L1 formulation. So, maximum amount of drug in L1 formulation comes into chloroform after the extraction.

**Table 11: After extraction in chloroform, qty. of Rosuvastatin Calcium present in aqueous solution.**

| Code | Amount taken drug extracted with chloroform (mg) | Residual drug remaining in aqueous solution (mg) |
|---|---|---|
| F1 | 50 | 0.06 |
| F2 | 100 | 0.22 |
| F3 | 200 | 0.27 |
| F4 | 300 | 0.35 |
| F5 | 400 | 0.49 |
| F6 | 500 | 0.63 |
| L1 | 100 | 0.31 |

**3. Evaluation of Pharmacosomes of Rosuvastatin**

**(a) Drug content study**

The drug content of Pharmacosomes was found to be in the range of 90.4± 0.52% to 94.4± 0.61% and drug loading was found to be in the range of 25.1±0.14% to 26.2± 0.13%. On the other hand, the drug content of liposome was found to be 81.5±0.60% and drug loading was 22.3± 0.19%. Pharmacosomes provides good percentage of drug content and drug loading which is comparatively more than the drug content and drug loading of the liposomes. In case of Liposomes, special methods such as coating were needed to improve the drug loading. But in case of Pharmacosomes, these special methods are not required because the drug is reversibly bonded chemically with the phospholipids.

**Table 12: Comparative drug content, drug loading of Pharmacosomes & Liposomes.**

| Code | Drug content (%)* | Drug loading (%)* |
|---|---|---|
| F1 | 91.1±0.64 | 25.3± 0.19 |
| F2 | 94.4± 0.61 | 26.2± 0.13 |
| F3 | 92.7± 0.62 | 25.8± 0.15 |
| F4 | 90.4± 0.82 | 25.1±0.14 |
| F5 | 91.9± 0.49 | 25.5± 0.14 |
| F6 | 92.2± 0.63 | 25.6±0.11 |
| L1 | 81.5±0.60 | 22.3± 0.19 |

**(b) Solubility study**

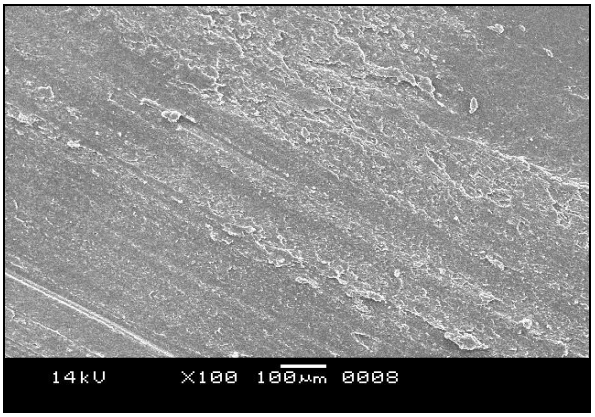
Water solubility of all the Pharmacosomes of Rosuvastatin (F1to F6) was found to be much higher than that of pure Rosuvastatin Calcium (D1) and Liposome of Rosuvastatin (L1) (Table 13). The log P value of all the Pharmacosomes of Rosuvastatin (F1 to F6) in the range of -0.25 to -0.34 which is much lower than the log P value of pure drug Rosuvastatin Calcium was 1.2459 and Liposome of Rosuvastatin was 0.14. These log P value results indicates the improvement in water solubility of Rosuvastatin in the form of Pharmacosomes. These can be explained by the solubilization theory resulted from the formation of micelle in the medium and also by the amorphous nature of the complex. The amphiphilic nature of complex, which may prove to be responsible for the improvement of bioavailability and increase in the solubility of the drug.

**Table 13: Comparative Solubility between Pharmacosomes, Liposome & RSV-Ca.**

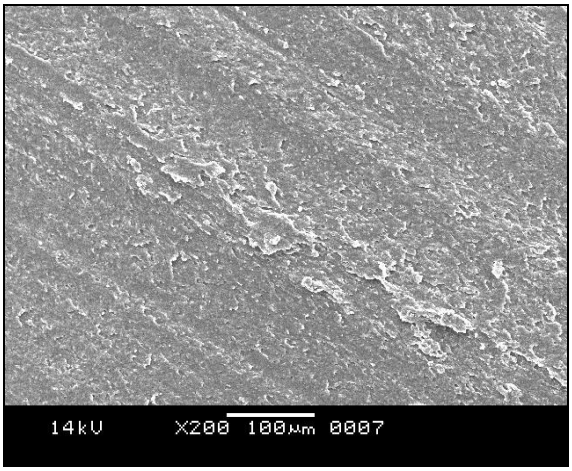
| Code | Solubility in aqueous layer(µg/ml)* | Solubility in octanol layer(µg/ml)* | Log P |
|---|---|---|---|
| F1 | 91.12± 1.24 | 42.01±0.62 | -0.33 |
| F2 | 92.37± 1.94 | 41.70±1.13 | -0.34 |
| F3 | 90.79±0.78 | 44.36±0.71 | -0.31 |
| F4 | 83.06±1.33 | 46.56±1.68 | -0.25 |
| F5 | 86.42±1.58 | 45.25±0.87 | -0.28 |
| F6 | 87.89±1.77 | 44.66±1.12 | -0.30 |
| L1 | 67.4±1.41 | 93.6±1.13 | 0.14 |
| D1 | 11.0± 0.35 | 192.62± 1.59 | 1.24 |

**(c) Vesicle Shape Determination**

The surface morphology of Pharmacosomes were studied by Scanning Electron Microscopy (SEM). Pharmacosomes of Rosuvastatin were found to be of disc shaped or irregular shaped with rough surface morphology. The surface was found to be sticky in the Pharmacosomes complexes prepared with low purity grades (30 %) of phospholipids (Figure 9). As the phospholipids are natural component their different purity grades may have different effects in shape and surface morphology. On the other hand, those Pharmacosomes complexes prepared with the high purity grades of phospholipids (80%) their surface show rough, non-sticky and free flowing nature.[29]




**(a) 100X Magnification**




**(b) 200X Magnification**

**Figure 9: Scanning Electron Microscopy of Pharmacosomes of Rosuvastatin.**

**(d) Drug Excipients Interaction Studies (Compatibility Studies)**

**(i) FTIR spectra of Rosuvastatin Calcium and acidified Rosuvastatin Calcium**

Conversion of Rosuvastatin Calcium to acidified Rosuvastatin Calcium was done by 1(N) HCL. This conversion is evaluated by FTIR Spectroscopy (Figure 10). The FTIR Spectra of Pure Rosuvastatin Calcium showed there characteristics absorption peak 3439.07 cm-1 of OH stretching functional group that shifted to 3452.80 cm-1 of the acidified Rosuvastatin Calcium. In acidified Rosuvastatin Calcium[3] additional characteristics absorption peaks seen in the region of 2500 to 3000 cm-1 these are 2966.58, 2926.79, 2862.33cm-1. In Pure Rosuvastatin calcium 2360.33cm-1 a characteristics absorption peak was seen but after acidify in acidified Rosuvastatin Calcium this peak is missing. So, this result justify the conversion of Rosuvastatin calcium to acidified Rosuvastatin calcium.

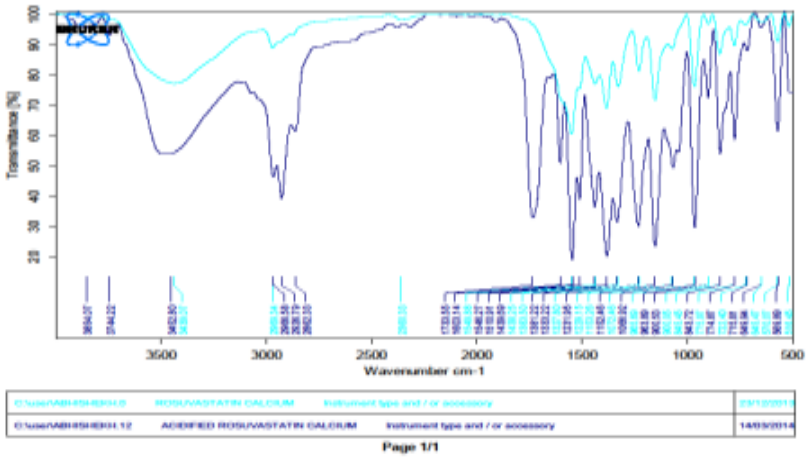



**Figure 10: FTIR Overlaid spectra of Rosuvastatin Calcium and acidified Rosuvastatin Calcium.**

**ii) FTIR spectra of acidified Rosuvastatin Calcium, Soya Lecithin (PDC), Pharmacosomes and Physical mixture Drug: Phosphatidylcholine.**

The formation of complex can be confirmed by the FTIR Spectroscopy. FTIR Spectra of Pharmacosomes of Rosuvastatin were significantly different from all the individual component and that of physical mixture (Figure 11). FTIR Spectra of acidified Rosuvastatin Calcium shows OH stretching at 3452.80 cm-1, C=O stretching at 1733.55(PDC) 1737.63 cm-1 to 1738.62 cm-1 in the Pharmacosomes. -CN stretching of cm-1. FTIR Spectra of Soya Lecithin Shows OH stretching at 3384.64 cm-1, C=O stretching at 1737.63 cm-1, -CN stretching at 1231.94 cm-1. The FTIR Spectra of Pharmacosomes of Rosuvastatin shows OH stretching at 3393.11 cm-1. C=O stretching shifted from acidified Rosuvastatin Calcium1733.55 cm-1 and soya lecithin Soya Lecithin (PDC) at 1231.94 cm-1 shifted to 1232.35 cm-1. Thus the FTIR Spectra indicate the interaction of Soya lecithin with the acidified Rosuvastatin Calcium COOH group and formation of Pharmacosomes.

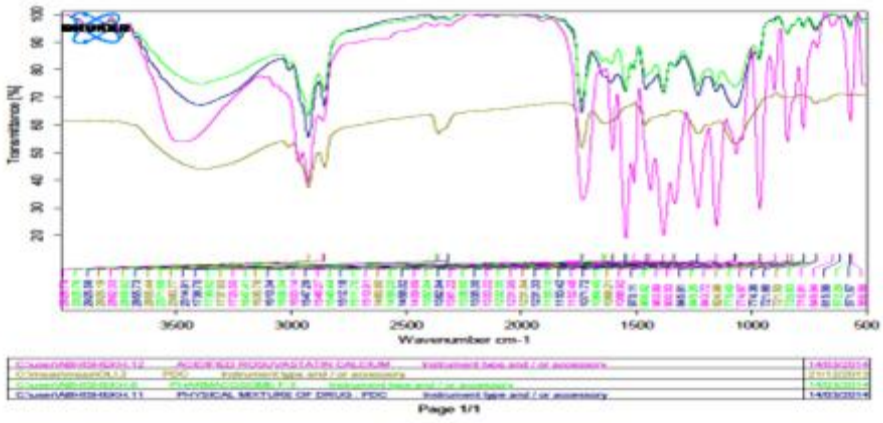



**Figure 11: FTIR Overlaid spectra of acidified Rosuvastatin Calcium, Phosphatidylcholine (PDC), Pharmacosomes and Physical mixture.**

**Drug: Phosphatidylcholine**

**(iii) FTIR Spectra of all Pharmacosomes formulations**

FTIR Spectra of all F1 to F6 Pharmacosomes formulation indicates the functional group such as -CN stretching, C=O stretching, OH stretching present in or within the range in the region. Moreover, No additional peaks does not seen in comparison of all the Pharmacosomes formulations (Figure 16).

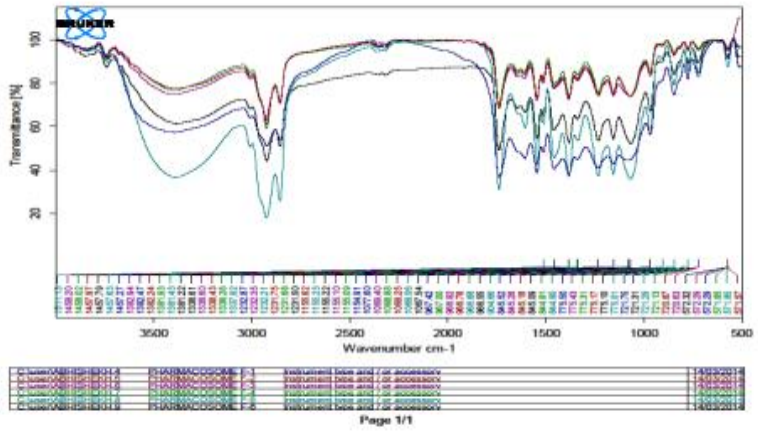



**Figure 12: FTIR Overlaid spectra of all Pharmacosomes formulations.**

**(iv) FTIR spectra of Pharmacosomes, Liposome and Physical mixture Drug: Phosphatidylcholine.**

The FTIR Spectra of Pharmacosomes of Rosuvastatin shows OH stretching at 3393.11 cm-1, C=O stretching at 1738.62 cm-1, -CN stretching 1232.35 cm-1. The FTIR Spectra Liposome of Rosuvastatin shows OH stretching at 3366.58 cm-1, C=O stretching at c 1739.03 cm-1, -CN stretching at 1230.86 cm-1. In Pharmacosomes a characteristics absorption peak 3009.10 cm-1 was seen but in Liposome this peak is missing. Thus the FTIR Spectra indicate characteristics difference between Pharmacosomes and Liposome (Figure 13).

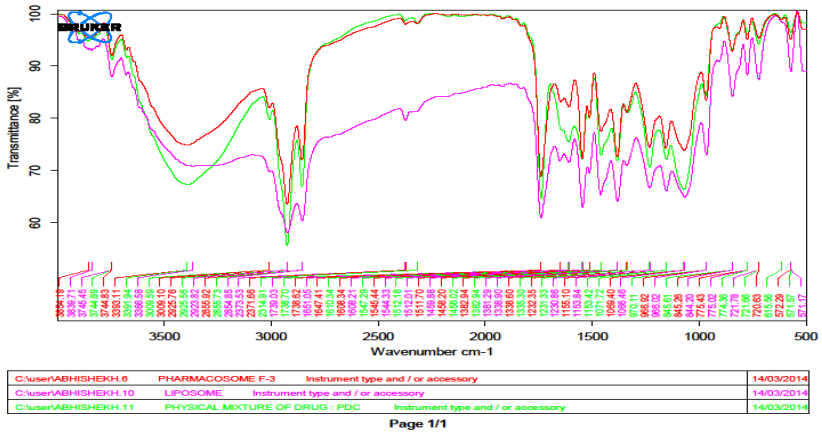



**Figure 13: FTIR Overlaid spectra of Pharmacosomes, Liposome and Physical mixture Drug: Phosphatidylcholine.**

**(e) X-Ray Powder Diffraction Analysis (XRD)**

The XRD of Pharmacosomes of Rosuvastatin (Figure 16) revealed a broad peak which is similar to the peak of soya Lecithin (PDC)(Figure 15) It suggested that the Rosuvastatin Calcium in the phospholipid complex was either in amorphous form or molecularly dispersed. The disappearance of Rosuvastatin Calcium crystalline diffraction peaks (figure 14) confirmed the formation of the phospholipid complex. Unlike liposomes, chemical bonding between the drug and phospholipids in the development of Pharmacosomes might have resulted into a significant change of its X-ray diffraction. These results are well supported by previous studies done with the Pharmacosomes of Diclofenac.[44]

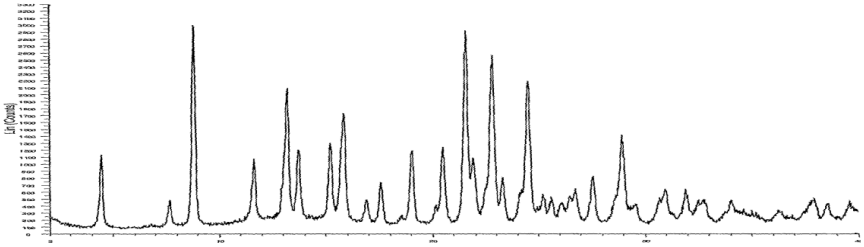


**Figure 14: X Ray Diffraction(XRD) of Rosuvastatin Calcium.**

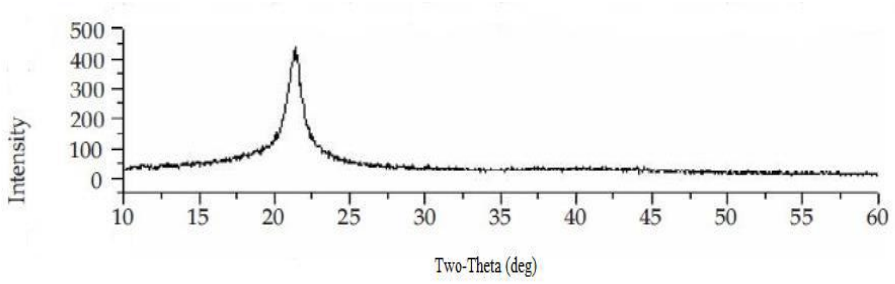



**Figure 15: X Ray Diffraction(XRD) of Soya Lecithin(30%).**

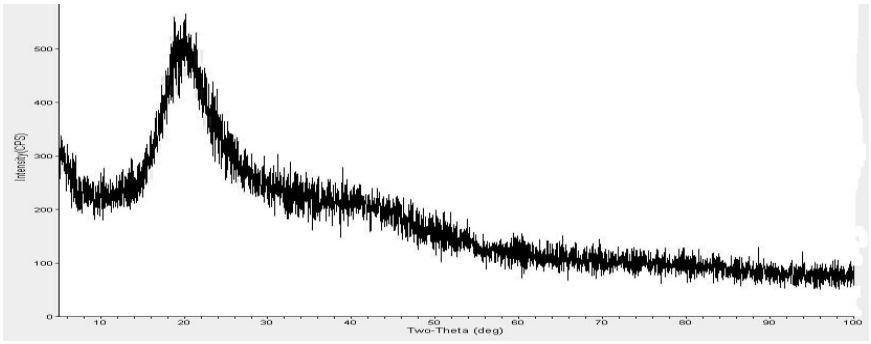



**Figure 16: X Ray Diffraction(XRD) of Pharmacosomes of Rosuvastatin.**

**(f)In vitro dissolution studies**

In vitro release studies of Pharmacosomes of Rosuvastatin (F1 to F6) and pure Rosuvastatin Calcium were carried out in phosphate buffer pH 6.8, as showed in Figure 23. At the end of 24 hours, % cumulative release of drug from Pharmacosomes of Rosuvastatin was found to be in the range of 66.93% to 56.81% (Table 14). In case of pure Rosuvastatin Calcium, the % cumulative release of drug amount at the end of 90 minutes is 100% (Table 14). Phospholipids being an amphiphilic surfactant increased the solubility of the drug by the action of wetting and dispersion. And that's why the dissolution profile of the Pharmacosomes of Rosuvastatin was found to be improved. The release experiments clearly indicated sustained release of Rosuvastatin from all Pharmacosomes formulations. On the other hand, immediate release pattern shows on the dissolution study of pure Rosuvastatin Calcium.

**Table 14: Comparative % Cumulative dissolution release data of Pharmacosomes formulations at different time with Pure RSV Calcium.**

| Time (mins) | F1 (% Release) | F2 (%Release) | F3 (%Release) | F4 (%Release) | F5 (%Release) | F6 (%Release) | Pure RSV Ca (% Release) |
|---|---|---|---|---|---|---|---|
| 15 | 1.4 | 2.25 | 1.69 | 2.53 | 3.09 | 3.78 | 30.1 |
| 30 | 2.53 | 4.78 | 4.22 | 4.5 | 5.34 | 5.31 | 55.5 |
| 45 | 3.93 | 7.03 | 5.34 | 7.59 | 7.03 | 7.87 | 85.7 |
| 60 | 4.78 | 8.72 | 5.91 | 11.25 | 10.96 | 10.12 | 98.5 |
| 90 | 12.65 | 12.38 | 9.56 | 12.65 | 12.09 | 11.53 | 100.0 |
| 120 | 19.96 | 23.91 | 22.50 | 18.56 | 14.9 | 11.81 | 100.0 |
| 240 | 23.9 | 26.16 | 25.31 | 22.5 | 20.53 | 18.28 | 100.0 |
| 360 | 29.25 | 31.50 | 30.09 | 27.56 | 27 | 24.46 | 100.0 |
| 1440 | 66.93 | 65.53 | 62.72 | 64.4 | 59.34 | 56.81 | 100.0 |

**(g) In vitro diffusion studies**

In vitro diffusion studies Pharmacosomes of Rosuvastatin (F1 to F6) and pure Rosuvastatin Calcium were carried out in phosphate buffer pH 6.8, (Figure 17) by modified franz diffusion cell using egg membrane. At the end of 24 hours, % cumulative release of drug from Pharmacosomes of Rosuvastatin was found to be in the range of 49.50% to 41.40 (Table 15). In case of pure Rosuvastatin Calcium, the % cumulative release of drug amount at the end of 24 hours minutes is 96.88% (Table 15). The release experiments also clearly indicated sustained release of Rosuvastatin from all Pharmacosomes formulations comparative with release pattern shows on the diffusion study of pure Rosuvastatin Calcium.

**Table 15: Comparative % Cumulative diffusion data of Pharmacosomes formulations at different time with Pure Rosuvastatin Calcium.**

| Time (mins) | F1 (% Release) | F2 (%Release) | F3 (%Release) | F4 (%Release) | F5 (%Release) | F6 (%Release) | Pure RSV Ca (% Release) |
|---|---|---|---|---|---|---|---|
| 5 | 4.12 | 3.00 | 2.10 | 2.70 | 1.70 | 1.40 | 9.30 |
| 15 | 5.00 | 4.70 | 3.20 | 4.20 | 2.70 | 2.50 | 15.00 |
| 30 | 6.50 | 7.00 | 5.70 | 6.10 | 4.30 | 4.10 | 21.60 |
| 45 | 8.00 | 8.10 | 6.50 | 7.40 | 5.40 | 5.00 | 24.00 |
| 60 | 8.60 | 8.20 | 7.10 | 8.00 | 6.50 | 6.20 | 26.50 |
| 90 | 10.80 | 10.50 | 8.70 | 9.50 | 8.10 | 7.50 | 29.70 |
| 120 | 12.60 | 12.20 | 9.60 | 11.40 | 8.90 | 8.40 | 32.70 |
| 240 | 29.10 | 22.00 | 19.40 | 21.30 | 18.10 | 17.50 | 42.37 |
| 360 | 33.13 | 27.50 | 23.30 | 25.70 | 22.40 | 21.70 | 55.88 |
| 1440 | 49.50 | 48.00 | 45.10 | 47.20 | 43.30 | 41.40 | 96.88 |

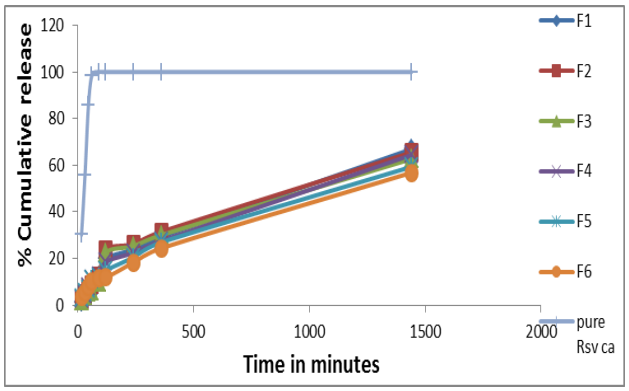



**Figure 17: Comparative % Cumulative diffusion release study of Pharmacosomes formulations at different time with Pure Rosuvastatin Calcium.**

**(h)Comparison of *in vivo* efficiency of Pharmacosomes with standard drug Rosuvastatin calcium as lipid lowering agent to reduce blood cholesterol level**

Animal study data (Table 16) showed that total cholesterol, triglycerides and HDL level in standard drug treated group (RSV-Ca) and RSV- Pharmacosomes treated group had been returned back to normal level (24hr) and even below (48hr) when compared total cholesterol, triglycerides and HDL level normal control group. However, the difference existed in the dosage schedule (Table 5). When compared to the positive control group evident from( Figure 18) the reduction in total cholesterol level in 24 hours and 48 hours are quiet significant and comparable which justified total dose reduction sustained release profile as well as improved bioavailability of Pharmacosomes formulation.

**Table 16: Data for the Effect of Pharmacosomes of Rosuvastatin and standard drug Rosuvastatin Calcium in blood Triglycerides levels, Total Cholesterol levels, HDL Cholesterol levels.**

| Treatment Group | Total Cholesterol (mg/dl) | | Triglycerides (mg/dl) | | HDL-Cholesterol (mg/dl) | |
|---|---|---|---|---|---|---|
| | 24 hr | 48 hr | 24 hr | 48 hr | 24 hr | 48 hr |
| Normal Control (Gr 1) | 66.87± 0.7745 | 66.87 ± 0.6927 | 57.40± 0.6154 | 57.73± 0.4279 | 38.67± 0.6667 | 38.92± 0.7569 |
| Positive (Tritron) Control (Gr 2) | 133.3± 0.9545* | 126.7± 1.022* | 173.3± 0.7282* | 177.0± 1.143* | 13.50± 0.7638* | 12.45± 0.533* |
| Standard drug (Rave-Ca) treated (Gr 3) | 47.67± 0.7719*# | 25.42± 0.8352*# | 47.11± 0.7514*# | 46.17± 1.067*# | 19.11± 0.6641*# | 32.54± 0.3257*# |
| Formulation (Pharmacosomes) Treated (Gr 4) | 59.26± 0.5863*# | 23.69± 0.337*# | 49.21± 0.5163*# | 45.93± 1.291# | 15.82± 0.7821*# | 34.69± 0.60*# |

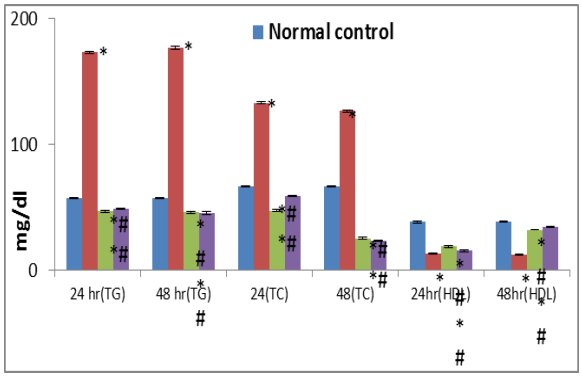



**Figure 18: Comparative effect of Pharmacosomes of Rosuvastatin and standard drug Rosuvastatin Calcium in Triglycerides(TG) , Total cholesterol (TC), HDL- Cholesterol.**

The biological parameter Atherogenic Index (Table 17) in standard drug treated group (RSV-Ca) and RSV-Pharmacosomes treated group had been returned back to the normal level (24hr) and even below (48hrs) when compare to the Atherogenic Index of a normal control group(figure 19).

**Table 17: Data for the effect of Pharmacosomes of Rosuvastatin and Standard drug Rosuvastatin Calcium in Atherogenic Index(TC/HDL-C).**

| Treatment Group | Atherogenic Index | |
|---|---|---|
| | 24 hr | 48 hr |
| Normal Control (Gr 1) | 1.72 | 1.71 |
| Positive (Tritron) Control (Gr 2) | 9.54 | 10.17 |
| Standard drug (Rsv-Ca) Treated (Gr 3) | 2.49 | 0.78 |
| Formulation (Pharmacosomes) Treated (Gr 4) | 3.74 | 0.68 |

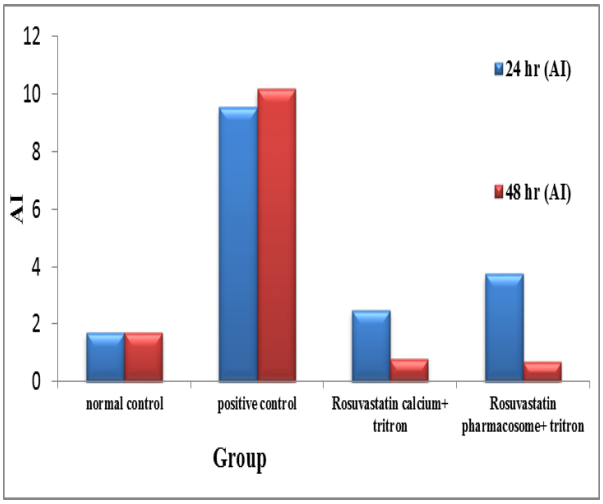
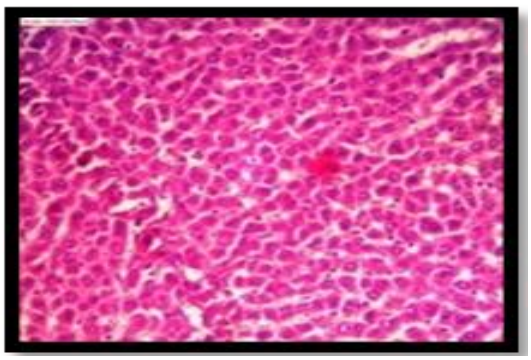


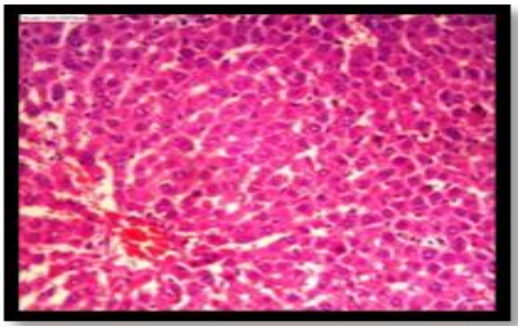

**Figure 19 : Comparative effect of Pharmacosomes of Rosuvastatin and standard drug Rosuvastatin Calcium in Atherogenic Index (TC/HDL-C)**

**(i) Histopathology study of liver**

In the histopathological study, Tritron induced positive control group (Figure 29B) shows fatty infiltration and granular degeneration as compared to normal control (Figure 29A). Standard drug Rosuvastatin Calcium shows reduction of cytoplasmic fatty infiltration and granular degeneration (Figure 29C). Pharmacosomes of Rosuvastatin also shows cytoplasmic fatty infiltration and granular degeneration (Figure 29D) as shown in standard drug Rosuvastatin Calcium treated group.



**(A)**



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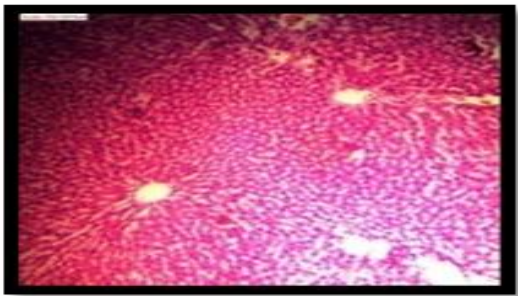
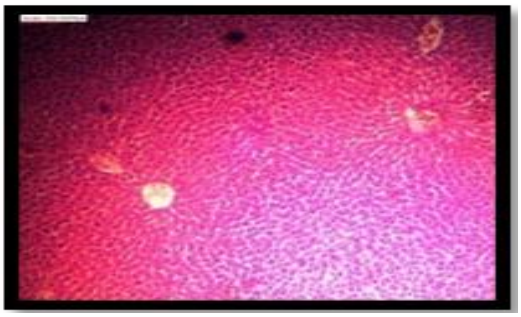


**(C)**



**(D)**

**Figure 20: (A) Normal control , B) Positive control, C) Standard drug treated, D) Pharmacosomes treated.**

**(j) Stability study of optimized formulation**

Pharmacosomes of Rosuvastatin was compatible at 40$^{o}$C and 75% RH and the stability of Pharmacosomes was confirmed by FTIR. No additional peak was observed after 2 month. Drug content and loading was almost same after 2 months and in initial condition in Pharmacosomes of Rosuvastatin. (Figure 22, Table 18) On the other hand, the stability of Liposome was also confirmed by FTIR and a new peak was observed in 2320.48 cm-1. So, this stability results indicate Liposome was not compatible at 40$^{o}$C and 75% RH; whereas Pharmacosomes was compatible.

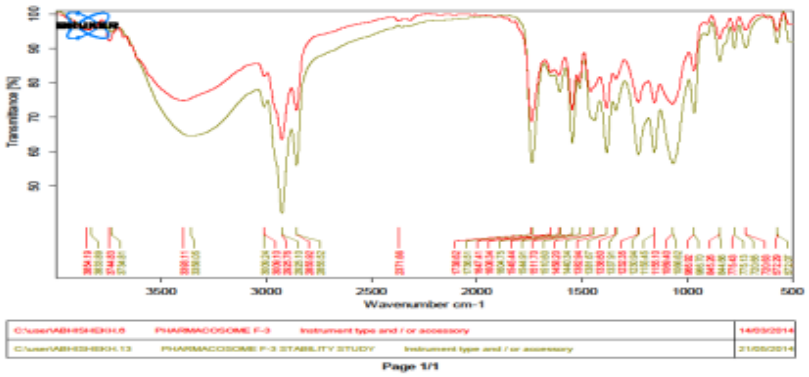



**Figure 22: FTIR Overlaid spectra of Pharmacosomes for stability study (examined after 2month of storage on 40$^0$C and 75% RH).**

**Table 18: Stability study of optimized Pharmacosomes formulation**.

| Code | Drug Content (%) | Drug loading (%) |
|---|---|---|
| **F3(Initial)** | 92.7± 0.62 | 25.80± 0.15 |
| **F3 (After 2 month)** | 91.11±0.47 | 25.29±0.10 |

## CONCLUSION

In the present study, a Pharmacosomes of Rosuvastatin was prepared by a simple and reproducible Hand Shaking method and evaluated for various Physicochemical, *in-vitro* and *in-vivo* experiments. Physicochemical investigations showed that Pharmacosomes of Rosuvastatin improved solubility comparative with the solubility of Pure Rosuvastatin Calcium. The dissolution profile of Pharmacosomes of Rosuvastatin showed a sustained release drug delivery comparative with the dissolution profile of pure Rosuvastatin Calcium. The diffusion profile of Pharmacosomes of Rosuvastatin Calcium also showed a sustained release drug delivery comparative with the diffusion profile of pure Rosuvastatin Calcium. The FTIR and XRPD studies confirmed the formation of the complex. SEM study shows irregular shape and rough surface morphology of Rosuvastatin Pharmacosomes. From the statistical analysis, it is evident that Pharmacosomes formulation really provide a sustaining effect on TC, TG and HDL cholesterol results. However comparing the difference in reduction level in TC and TG in 24 hrs and 48 hrs and increase level in HDL-C in 24 and 48 hrs, it may be concluded that an ideal novel sustained release delivery system should include and immediate loading dose of parent drug of Rosuvastatin Calcium along with a maintenance dose of Rosuvastatin Pharmacosomes. Pharmacosomes of Rosuvastatin Calcium was compatible at 37$^O$C and 75% RH and the stability of Pharmacosomes was confirmed by FTIR. No additional peak was observed after 2 month and at initial condition and after 2 month the drug loading was almost same. This Pharmacosomes of Rosuvastatin Calcium may be of potential use for improving bioavailability. Thus, the formulated Pharmacosomes seem to be potential candidate as an oral sustained drug delivery system in this era of novel and sustained drug delivery systems. The developed formulations are expected to improve the patient compliance, form better dosage regimen, dose reduction and provide optimum maintenance therapy to Hyperlipedimic patients.

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