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# SOLUBILITY ENHANCEMENT OF ROSUVASTATIN VIA NANOSPONGES: PREPARATION AND *IN-VITRO* CHARACTERIZATION

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## Abstract

Rosuvastatin is regarded as super statin and being a member of BCS class II exhibit low aqueous solubility and dissolution rate with poor oral bioavailability of less than 20%. The current study involved rosuvastatin loaded nano-sponges preparation utilizing polyvinyl alcohol (surfactant), ethyl cellulose (polymer) while dichloromethane was used as cross linker. Nanosponge represents a scaffold structure of size less than 100 um demonstrating an excellent approach to deliver drugs that are poorly absorbed or show low solubility in GIT. The formulations were crafted by emulsion solvent evaporation method, initially screened via pre-formulation studies and finally were characterized by various physico-chemical tests. FTIR analysis showed no interaction among pure drug and formulation excipients. Zeta experiment revealed the stable formulations having droplet sizes in the range of 270-343 nm further confirmed by SEM indicating the porous sponge like appearance. There were high percent yield values of 80-87% with 62-74% entrapment efficiency of prepared formulations. Additionally dissolution and kinetic model analysis was established on the formulated nano-sponges to further examine their sustained release property, highlighting initial burst release of surface adhered drug followed by controlled release with anomalous non-Fickian diffusion mechanism. The findings of this study advocated successful fabrications of rosuvastatin loaded nanosponges that could improve low solubility and oral bioavailability of the drug.

**Keywords:** Rosuvastatin, solubility enhancement, oral bioavailability, nanosponges, *in-vitro* drug release

## 1. Introduction

One of the most crucial factors for attaining the systemic drug concentration required for the intended pharmacological response is water solubility (1). The partition coefficient i.e. Log P, of a pharmaceutical drug substance is a critical factor that influences bioavailability, especially for drugs administered orally (2). Greater value of Log P might indicate a slow rate of dissolution in biological fluids, which is related to reduced drug bioavailability. Recently, advancements in enhancing the physicochemical characteristics of pharmaceutical drug substances with low solubilities have been devised through techniques, like particle size reduction, surface area enhancement, coupling or coating with other substances etc. (3). Through these processes, the capacity of drug substances to dissolve in water is increased, enabling them to reach the necessary therapeutic concentrations (4).
Nanotechnology employed in formulations improves the solubility as well as permeability of drugs that are characterized by reduced solubility and permeability. Additionally, it enhances therapeutic efficacy through active or passive targeting mechanisms, while safeguarding the drug against physical and chemical deterioration (5). From the past few decades, researchers are keenly utilizing the potential of nano-technology to launch enormous advancements in drug delivery to minimize toxicity and augment efficacy The use of nanosponges has expanded globally as a means of improving the solubility of hydrophobic drugs (6). Initially nano sponges were used to soak up the harmful materials to be eliminated from body, later they became protein carriers because of their high molecule carrying capacity. Now a days, drug molecules are being infused in their sponge like structure to achieve targeted delivery for prolonged duration (7). Nano sponges are tiny structures of 200-500 um and contain cavities. They are stable up to 300°C temperature, and under wide range of pH i.e. 1-11. The outer hydrophilic surface is usually porous suspended in a dispersion medium, while the inner hydrophobic core is loaded with medicament which makes them an excellent choice of delivery for low water-soluble drugs and allows for sustained drug release to targeted areas while also preventing drug and protein decomposition (8).

Nano sponge contains hyper cross-linked polymers which help improve drug stability, lessen adverse effects, and alter drug dissolution. Cross linkers enable the polymers to get converted into spherical shaped voids of smaller size. Later these voids are used to incorporate drug molecules via encapsulation, complex formation and conjugation (9).

Hypercholesterolemia is a health state in which the plasma cholesterol level (especially low- density lipoproteins) gets elevated beyond normal levels, which gets deposited on the walls of arteries, hardens the walls and forms a plaque. This plaque hinders the supply of oxygen rich blood flow toward heart leading to vascular complications (10). Use of statins is widely used therapy to control high cholesterol levels and maintain them between optimal ranges. These drugs inhibit the cholesterol synthesis by liver and also help to remove already formed plaques in arteries. Rosuvastatin is considered as super statin among the statins. It is a member of BCS class II, with compromised aqueous solubility and diminished dissolution rate. It inhibits the enzyme named HMG-COA reductase involved in the synthesis of cholesterol (11). Taken into account all the afore-mentioned factors, this study was carried out to encapsulate rosuvastatin into nanosponges through emulsion solvent diffusion method by using polymers like polyvinyl alcohol and ethyl cellulose to devise a novel dosage form for increasing its solubility and bioavailability.

## 2. Methodology

### 2.1. Materials

Rosuvastatin was received as a generous gift by Highnoon Laboratories, Lahore, Pakistan. Polyvinyl alcohol, ethyl cellulose, sodium hydroxide (Sigma Aldrich, USA). Distilled water and phosphate buffer were provided by the Research Loaboratory of College of Pharmacy, University of the Punjab, Lahore. All the chemicals and reagents used in this study were of analytical grade and used without further purification.

## 2.2. Pre-formulation Studies

### 2.2.1. Physical Appearance

The organoleptic characteristics of the drug rosuvastatin powder, such as color, taste, and odor, were studied.

### 2.2.2. Solubility study

Shake flask method was used to determine the solubility of drug molecules in suitable solvents. Saturated solution of drug was prepared with different solvents including water, dichloromethane, DMSO, acetone at room temperature and followed by stirring to achieve equilibrium. About 5ml of each solvent was taken separately in 5 different stoppered vials and excess amount of drug was added in them, these vials were placed on vortex for 10 minutes, the vials were centrifuged at rpm of 9000 for 5minutes and later analyzed on UV- spectrophotometer to find maximum solubility of rosuvastatin in each solvent (12).

### 2.2.3. Melting Point Determination

Melting point determination was done by capillary tube method using a digital melting point apparatus. The capillary tube was fused by gently pressing the open end into a sample of pure drug. As a result, drug was packed into the bottom of the capillary tube by tapping its bottom against a hard surface. The temperature at which the drug melts was documented and regarded as melting point (13).

### 2.2.4. Standard Calibration Curve

Standard calibration curve of rosuvastatin was crafted by forming a stock solution of (1 mg/1ml) in dimethyl sulfoxide (DMSO). From the stock solution, various dilutions were made by taking appropriate volume of stock solution and diluting with the above mentioned solvent. The dilutions were ranged from 2 µg/ml to 10 µg/ml. Later the absorbance of these serial dilutions was taken at 246nm by using UV visible spectroscopy to plot calibration curve between concentration and absorbance.

## 2.3. Formulation of Rosuvastatin Loaded Nanosponges

Rosuvastatin loaded nanosponges were prepared by utilizing solvent evaporation method. Ethyl cellulose and polyvinyl alcohol were used as release retarding polymer and stabilizing agent, respectively. Dichloromethane played the role of cross linker between hydrophilic and hydrophobic phases to form solvent based emulsion. In order to form dispersion phase, accurately weighed amount of drug (10mg) and ethyl cellulose were dissolved in dichloromethane.

On the other hand, the aqueous phase of emulsion was formed by dissolving varying quantities of polyvinyl alcohol in distilled water (Table 1). This aqueous phase was added in dispersion phase in a drop wise manner along continuous stirring on magnetic stirrer at a speed of 1000 rpm and left overnight at room temperature. The obtained nano-sponges were collected in Eppendorf tubes, centrifuged and washed with distilled water to remove any residual organic solvent (14).

**Table 1.** Composition of different formulations of rosuvastatin laden nanosponges

| Formulation Codes | Drug (mg) | Ethyl Cellulose (mg) | Polyvinyl Alcohol (%) | Dichloromethane (ml) | Distilled Water (ml) |
|---|---|---|---|---|---|
| **F1** | 10 | 40 | 2 | 6 | 50 |
| **F2** | 10 | 60 | 2 | 6 | 50 |
| **F3** | 10 | 75 | 1 | 6 | 50 |
| **F4** | 10 | 80 | 1 | 6 | 50 |
| **F5** | 10 | 90 | 1 | 6 | 50 |

## 2.4. Physico-chemical Characterization

### 2.4.1. Particle Size, Size Distribution and Zeta Potential

Using a Malvern Zeta Sizer, the particle size, PDI and zeta potential of rosuvastatin nano-sponges

was determined at 25±2°C. For each measurement, the samples were properly diluted with distilled water [NS: water (1:200)]. All samples were analyzed using the same fixed angle of 90°. Aqueous dispersion was diluted up to specified scattering at 25°C (15).

### 2.4.2. Percent yield

The actual yield of nano-sponges was determined by weighing dried nano-sponges (NS). The weight of each solid ingredient utilized to formulate the nano-sponges, including the drug, ethyl cellulose NS, was taken as the theoretical yield. Percent yield is calculated by the following formula (16):
Percent Yield = Actual Yield / Theoretical Yield * 100

### 2.4.3. Percent Drug Loading

Accurately weighed NS (10mg) and 0.1 N NaOH (5 ml) was taken in vortex tube (15ml) and shaken for 1 minute. With further addition of 0.1 N NAOH, volume was made up to 10ml after shaking. About 1 ml of filtered solution was taken in volumetric flask after filtration and volume was made up to 10ml with 0.1 N NAOH. In order to calculate the concentration of rosuvastatin, absorbance was determined at lambda max 246nm (17).
Drug Loading (%) = Drug content of NS / Weight of NS recovered * 100

### 2.4.4. Entrapment Efficiency

The prepared nanosponges were centrifuged to obtain supernatant. This resultant supernatant was diluted with distilled water and analyzed by using spectrophotometer at the wavelength of 246nm in order to calculate entrapment efficacy by following formula (18):
Entrapment Efficiency (%) = Drug content of NS / Initial weight of drug * 100

### 2.4.5. FTIR Analysis

During the formulation of nano-sponges, drug excipient compatibility studies were performed to assess any physical or chemical instability between drug and excipients by using FTIR which involved pure drug, polymer, stabilizer and drug loaded formulation of nano-sponges. This technique is used to determine the presence of functional groups and a vibrational spectrum is acquired for crystalline substances. The spectra were obtained in the wavenumber between 4000 and 650 cm-1. FTIR also enables in figuring out the hydrophobic and hydrophilic components of the developed system (19).

### 2.4.6. SEM Analysis

The morphology of prepared nanosponge formulations was determined using scanning electron microscopy. A slab was covered with a concentrated aqueous suspension, which was then vacuum-dried. The sample was covered in a 20 nm thick coating of gold in a cathodic evaporator. An image processing tool was used to enhance the photographs, and the individual nanosponge diameters were measured to get the mean particle size (20).

### 2.4.7. *In-vitro* drug release & Kinetic Studies

Dialysis membrane method was used to investigate *in-vitro* drug release from the optimized nanosponge formulation. Briefly, an aqueous dispersion of nano-sponges (1 mL) containing the drug was placed in the donor compartment, while the receptor compartment, separated with the aid of a hydrophilic dialysis membrane (mol. weight 14 kDa), and dipped in 150 ml beaker full of phosphate buffer at pH 7.4 at 37°± 2°C. A section of dialysis membrane weighing 12,000–14,000 Da was cut and activated by dipping in 2% sulfuric acid for 3-5 minutes, washing with distilled water, and then soaking in phosphate buffer solution for overnight. At fixed intervals, the receptor buffer was completely withdrawn and changed with fresh buffer. The amount of drug inside the medium was determined by suitable analytical method and drug release kinetics was determined by fitting the release data in different kinetic models like zero order, first order, Higuchi and Korsemeyer Peppas models (21).

### 2.5. Statistical Analysis

All measurements were performed multiple times, and the results are presented as the mean ± standard deviation. A paired t-test was used to statistically analyze the data from the different groups. Probability values (p-values) less than 0.05 were regarded as statistically significant. The calculations were carried out using GraphPad Prism (version 8.0.2).

## 3. Results

### 3.1. Pre-formulation Studies

Pre-formulation study was carried out to investigate the confirmation as well as purity of the active ingredient by noting the melting point, organoleptic properties and solubility assessment.

### 3.1.1. Organoleptic Attributes of Rosuvastatin Powder

Organoleptic assessment of rosuvastatin showed an amorphous white colored powder with bitter to unpleasant taste and odor. The melting point determination revealed 158 ºC which is within the official compendial limit (155 ºC-160 ºC), confirming the active ingredient as rosuvastatin (22).

### 3.1.2. Solubility Study of Rosuvastatin

The solubility of rosuvastatin was estimated by a saturated solubility experiment by using a variety of solvents like distilled water, aqueous buffers of pH 1.2, 6.8, 7.4, ethanol, organic solvents like DMSO, dimethyl formamide and dichloromethane at room temperature. The results of solubility analysis revealed that it is sparingly soluble in water and aqueous buffers. The maximum solubility of the drug was exhibited in the DMSO. There was a statistically significant difference in the solubility of drug in DMSO than other solvents ($P<0.05$). The solubility of rosuvastatin in different solvents is expressed in Figure 1.

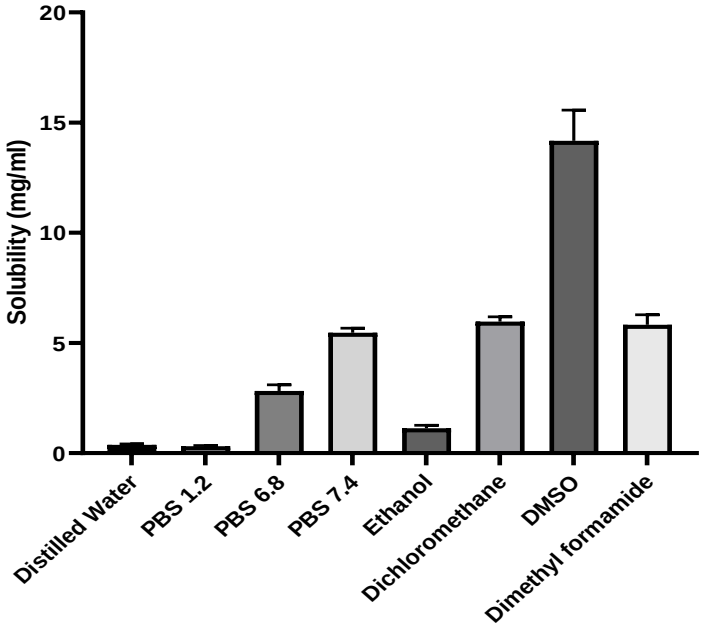



**Figure 1.** Solubility of rosuvastatin in various solvents (mean ± SD, n=3)

### 3.1.3. Calibration Curve of Rosuvastatin

The absorbance values of various known dilutions (2 to 10 µg/ml) were measured at 246nm and calibration curve was obtained by plotting them against concentration values. The average of triplicate readings was recorded to construct the standard curve. A linear relationship between the known concentration studied and resultant absorbance was shown by a straight line with a regression equation $y = 0.1152x – 0.03167$ and $R^2 = 0.9952$ (Figure 2).

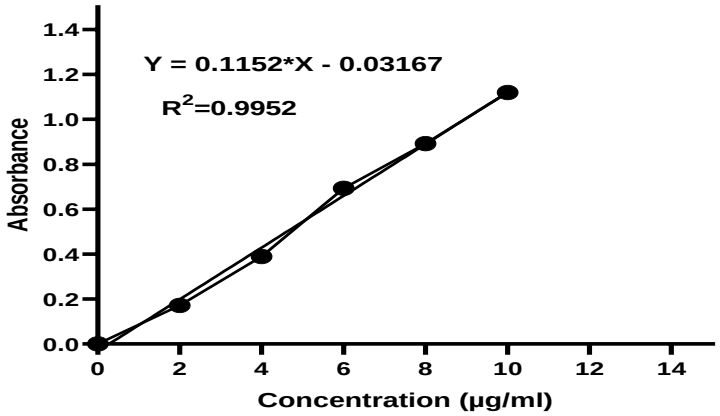



**Figure 2.** Standard calibration curve of rosuvastatin

### 3.2. Drug Loaded Nanosponge Formulation

A total of five nanosponge formulations were prepared. Although all the formulations were stable but F4 and F5 were chosen for further characterization. These formulations contained greater amount of ethyl cellulose whereas stabilized by lower concentration of polyvinyl alcohol.

### 3.3. Zeta Analysis

Among all formulations, F4 and F5 were selected for particle size analysis by Malvern zeta sizer. During analysis, it was found that the change in the concentration of polymer affected the particle size of nano-sponges. The average particle size of F4 and F5 formulations of nano-sponges were found to be 270 nm and 343 nm respectively, showing statistically significant difference ($p<0.05$), as shown in Figure 3. The nanosponge formulations were homogenously distributed with polydispersity index of 0.291 ± 0.04 and 0.312 ± 0.02 for F4 and F5, respectively. The zeta potential of F4 and F5 formulations of nano-sponges were found to be -18.1 mV and -14.3mV respectively, indicating stability of nanosponge formulations without any statistical significance ($p>0.05$).

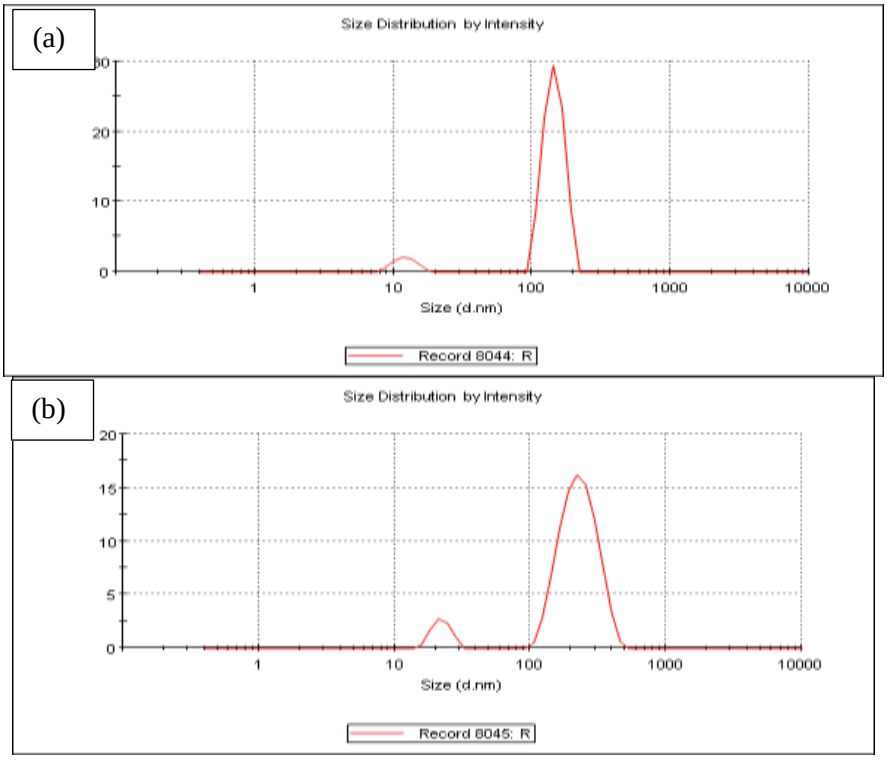



**Figure 3.** Particle size of (a) F4 and (b) F5 nanosponge formulations

### 3.4. Percent Yield and Drug Entrapment Efficiency

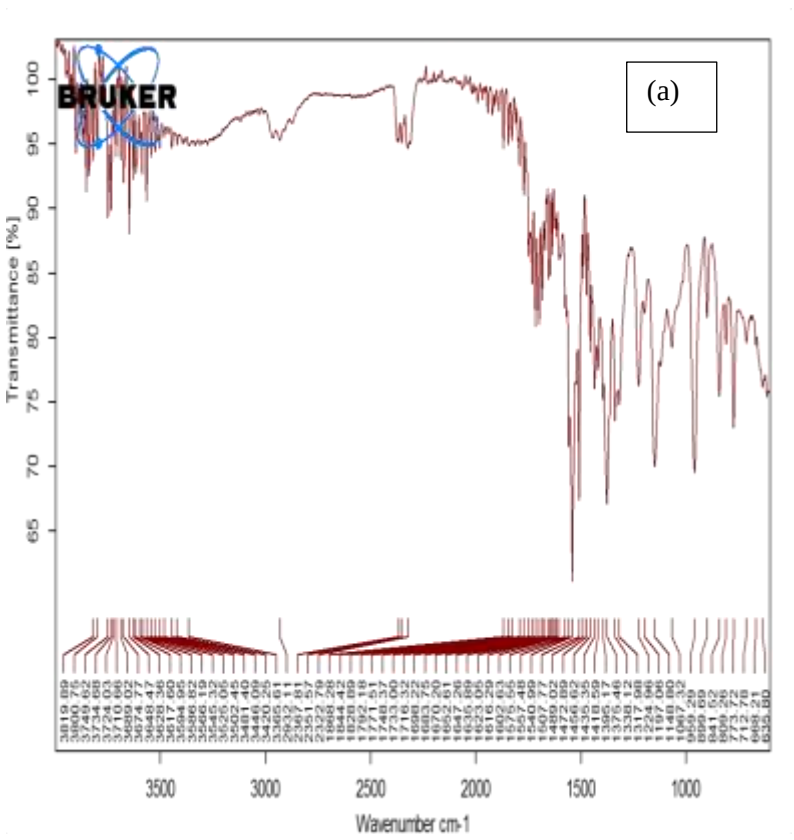
The percent yield of several formulations was found to range from 80 % to 87 %. The drug entrapment efficiency was found in the range of 62 % to 74 % (Table 2) The increase in concentration of polymer, the drug entrapment efficiency also increases in a direct relation (23). Entrapment efficiency was calculated to evaluate the percentage of drug being successfully placed inside the voids of nanosponges.

**Table 2.** Percent yield and entrapment efficiency values of different batches of nanosponges.

| Formulation Codes | Percent Yield | Percent Entrapment Efficiency |
|---|---|---|
| **F1** | 86 | 66 |
| **F2** | 81 | 62 |
| **F3** | 80 | 73 |
| **F4** | 85 | 71 |
| **F5** | 87 | 74 |

### 3.5. FTIR Analysis

FTIR analysis was carried out for pure drug, polymer, stabilizing agent and drug loaded nano-sponge and depicted in Figure 4. The presence of characteristic peaks of rosuvastatin in the prepared formulation of nano sponges showed no significant change in their positions which ruled out the chances of any unwanted chemical interaction between rosuvastatin and polymer (24). All these compatibility analysis showed fair stability of nano-sponges in the presence of acidic environment which is necessary to provide maximum absorption by drug during acidic medium for better performance.



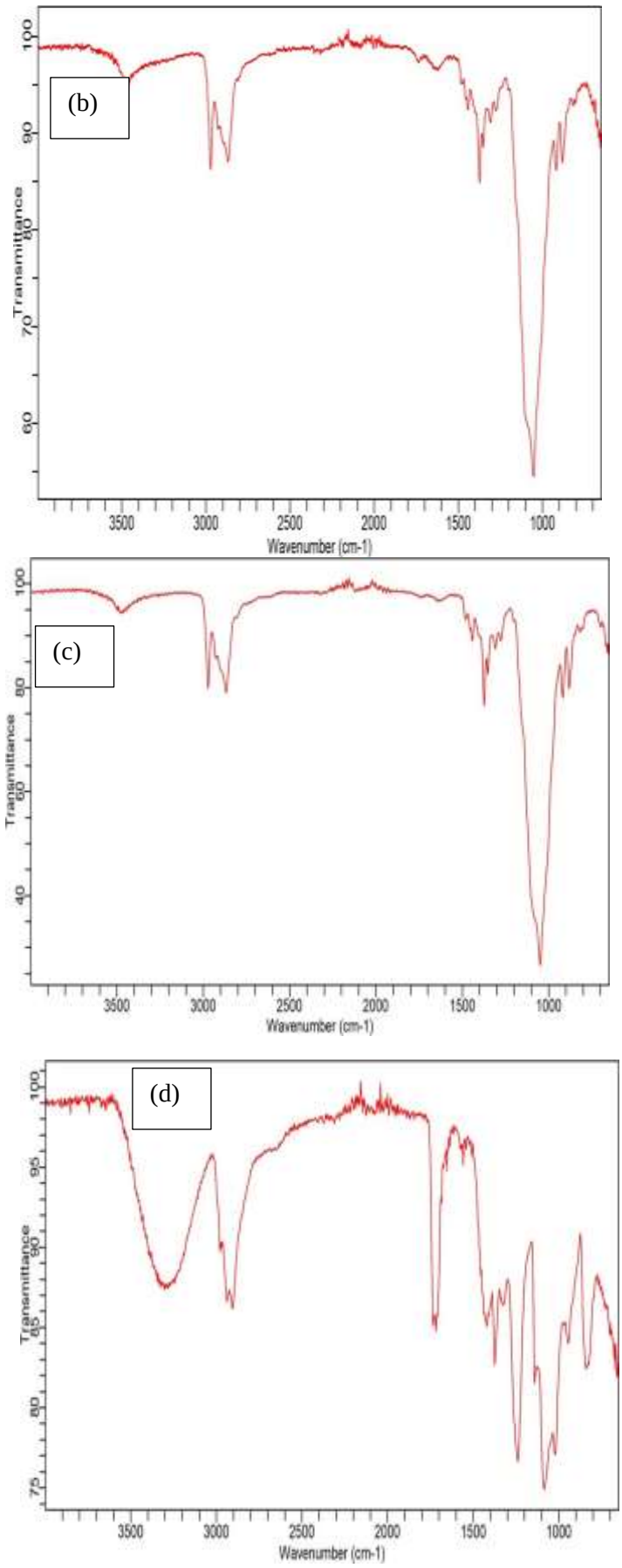



**Figure 4.** FTIR spectra of (a) rosuvastatin (b) ethyl cellulose (c) PVA and (d) rosuvastatin loaded nanosponge formulation (F4)

### 3.6. Morphology of Nanosponges

The scanning electron microscopy (SEM) was carried out to determine the morphology of ethyl cellulose and PVA based rosuvastatin loaded nanosponge formulation. Surface morphology of formulations (F4 and F5) confirmed the formation of orange peel like shape with porous and spongy nature. SEM images show that the polymer based nanosponges had a porous three-dimensional surface with some variations (Figures 5 and 6).

**Figure 5. SEM image of F4 nanosponge formulation**

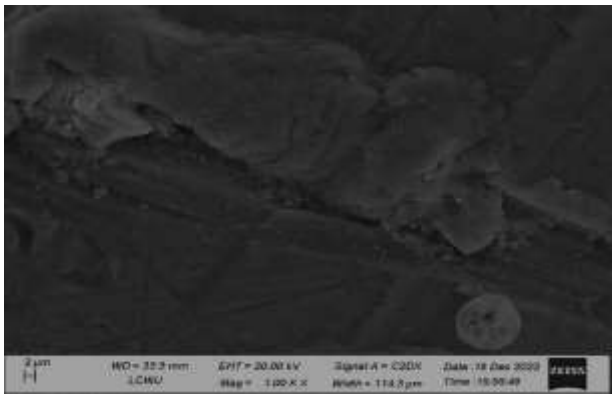


**Figure 6. SEM image of F5 nanosponge formulation**

### 3.7. *In-vitro* Drug Release & Kinetics

During *in-vitro* studies, two patterns of release were observed (Figure 7). Initially it was comparatively fast and burst release i.e. 30% release, which was later followed by sustained release for at least 24 hours. The designed formulation retarded the release of rosuvastatin in a controlled manner over span of time and kinetic models were put to application to study release behavior of rosuvastatin. The kinetics data best fitted Korsemeyer Peppas equation and revealed anomalous non-Fickian diffusion mechanism (n=0.624).

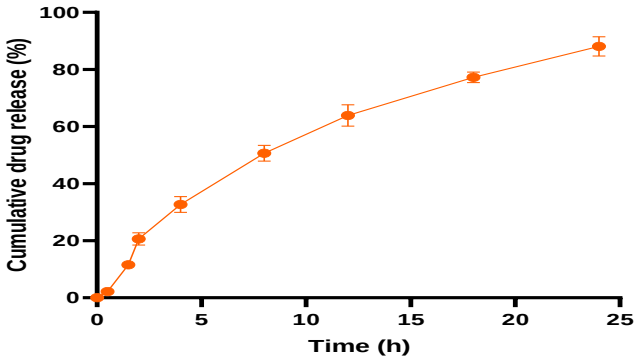



**Figure 7.** Cumulative percent drug release of rosuvastatin from optimized nanosponge formulation F4 (mean ± SD, n=3)

## 4. Discussion

This study aimed to address the obstacles that cause low bioavailability of rosuvastatin when taken orally by using nanotechnology. Rosuvastatin is regarded as a super statin due to its clinical developmental claim of higher potency and enhanced cholesterol lowering as compared to its rivals in the same class. It is basically HMG-CoA reductase inhibitor and results in cholesterol lowering. It is a member of BCS class II having lower water solubility and reduced dissolution rate (25). The reduced aqueous solubility of rosuvastatin hinders the oral bioavailability and subsequently the drug effect (26). Various nanotechnological systems were reported to enhance its solubility (27–29). This study involved that rosuvastatin loaded nanosponges were created using the emulsion-solvent diffusion method with EC as the polymer matrix. These nanosponges form spontaneously when the polymer phase is added to the surfactant solution under constant stirring. This method involves the evaporation of the organic solvent, allowing the polymer blend to create spherical porous particles that encapsulate the drug molecules. Surfactant molecules in the external phase act as pore-forming agents. In this study, we used PVA to investigate the surfactant's impact on various characteristics of the nanosponges. Our findings indicated that using EC as the polymer matrix and PVA as the pore-forming agent resulted in nanosponges with desirable properties to control the release of rosuvastatin (30).

Particle size and PDI are crucial parameters for evaluating nanosponges. These factors significantly influence the stability, solubility, dissolution rate, and bioavailability of an active ingredient (31). The nanosystems advocate size of the particles to be within nanoscale with uniform homogenous distribution (32). The stability of any nanosystem is highly dependent on its zeta potential which is the ability of the nano-formulation to keep its particles sufficiently apart due to steric hindrance and inhibit aggregation of the particles and hence instability (33). The particle size, PDI and zeta potential of our optimized formulation was within the appropriate dimensions. Zeta potential is a degree of the surface charge hence important to find out the stability of the system. Zeta potential size entails attention to the electric potential, i.e., diffusion coefficient and electrophoretic mobility. Those values were converted to zeta potential after calculating them from the Stokes equation (34). The pH and electrolyte concentration were taken into consideration while measuring the zeta potential. Zeta potential in water ought to be approximately ± 30 mV, which is sufficiently high to provide stable nanosponges that do not aggregate over time. A high zeta potential value, whether negative or positive, is beneficial in drug formulation as it helps maintain sufficient repulsive forces between particles, preventing aggregation and enhancing system stability (35). The highest entrapment efficiency of 74 % was observed with an increased percentage of ethyl cellulose. This is likely due to the higher viscosity resulting from greater EC concentration and its coil-like structure, which restricts drug entrapment (36).

For the analysis of structure and morphology of prepared NS formulations FTIR along with SEM were used. Higher drug loading and entrapment efficiency formulations were selected for structural analysis. FTIR spectroscopy was utilized to examine potential interactions among rosuvastatin, the matrix-forming polymer ethyl cellulose, PVA as a stabilizer, and the optimized drug-loaded nanosponge formulation. The spectra of the rosuvastatin-loaded nanosponge formulation displayed characteristic drug peaks, albeit with decreased intensity and no observable red or blue shifts, indicating that rosuvastatin did not interact with EC and PVA. The diminished intensity of rosuvastatin peaks also suggests that the drug was encapsulated within the nanosponges (37).

The optimized formulation underwent *in vitro* release testing over a 24-hour period. Incorporating rosuvastatin into the nanosponge (NS) led to prolonged drug release, with F4 showing a notably higher cumulative release percentage, likely due to enhanced solubility. Various factors, such as matrix-forming agents, gelling agents, and the presence of surfactants or stabilizers, influence drug release from nano-formulations (38). The released drug percentage was analyzed using different kinetic models, including Korsmeyer-Peppas, Higuchi, Hixson-Crowell, first-order, and zero-order

models, to determine the release mechanism. The Korsmeyer−Peppas model was selected as the best fit, with an $R^2$ value of 0.997. The drug release exponent (n) value for the optimized formulation exceeded 0.5, indicating a mechanism of anomalous non-Fickian diffusion (39).
Further investigation is required to comprehend the interactions between the matrix-forming agent EC and therapeutic agents, as well as to clarify the precise mechanism by which this nanosponge delivery system enhances the oral bioavailability of rosuvastatin. Additionally, to validate this system and demonstrate its effectiveness in orally delivering rosuvastatin, it is advisable to assess the efficacy of the formulated product on animals post-storage.

## 5. Conclusion

The current study comes to the conclusion that delivering BCS class II medications in the form of nanosponges may increase their solubility and bioavailability. The findings of the study advocated that the concentration of the polymer ethyl cellulose and stabilizer PVA (1 % w/v) are crucial to the formulation's optimization. With variable polymer and stabilizing agent concentrations, the drug's particle size and percent entrapment efficiency varied. The optimized formulation showed initial burst release followed by controlled release up to 24 h, with anomalous non-Fickian diffusion mechanism, satisfying Korsemeyer-Peppas equation.

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