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Formulation and Evaluation of Polymeric Nanoparticles as Carriers of Rosuvastatin Calcium for Oral Administration

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Abstract

Aim of this research was to formulate and evaluate the polymeric nanoparticle as carriers of rosuvastatin calcium for oral administration. Rosuvastatin calcium-loaded nanoparticles were formulated by nanoprecipitation method using different ratios of polymers (Eudragit L100 and Eudragit S100) and different concentrations of stabilizers (Pluronic F68 and PVA) with constant drug concentration. The formulations were evaluated for particle size, zeta potential, drug content, entrapment efficiency, *in vitro* release, kinetics, solubility, *ex vivo* intestinal permeability and Transmission Electron Microscopy (TEM). Fourier Transform-Infrared (FT-IR) spectroscopy and Differential Scanning Calorimetry (DSC) studies were carried out to check compatibility between the drug and polymers. No significant drug-polymer interactions were found. To enhance drug entrapment particle size range from 100-250 nm were prepared and entrapment efficiencies were found be 28-79 %. *In vitro* release studies showed a biphasic release pattern of rosuvastatin calcium from nanosuspensions: One initial burst release in the first 2 hours which could be helpful to improve the penetration of drug followed by a second slow release phase consistent with a Higuchi diffusion mechanism. The solubility of rosuvastatin calcium loaded polymeric nanoparticles compared to pure drug form was increased to about two-fold. Intestinal permeability of rosuvastatin calcium entrapped in Eudragit L100 an Eudragit S100 nanoparticles across rat small intestinal segments was significantly improved compared with rosuvastatin calcium in solution. Nanoparticles observed by TEM showed extremely spherical shapes. Results indicated that nanoparticle formulations could be a promising delivery system for oral administration of rosuvastatin calcium with enhanced solubility, intestinal permeability and improved oral bioavailability.

Keywords: Eudragit L100, Eudragit S100, Entrapment Efficiency, *Ex vivo* Intestinal Permeability, Nanoparticles, Solubility

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1. Introduction

Nanoparticles are defined as solid, submicron-sized drug carriers that may or may not be biodegradable[1,2]. The term nanoparticle is a collective name for both nanospheres and nanocapsules. Nanospheres have a matrix type of structure. Drugs may be absorbed at the sphere surface or encapsulated within the particle. Nanocapsules are vesicular systems in which the drug is confined to a cavity consisting of an inner liquid core surrounded by a polymeric membrane. In this case, the active substances are usually dissolved in the inner core but may also be adsorbed to the capsule surface[3]. Nanoparticles are receiving considerable attention for the delivery of therapeutic drugs. The literature emphasizes the advantages of nanoparticles over microparticles[4] and liposomes[5,6]. The submicron size of nanoparticles offers a number of distinct advantages over microparticles, including relatively higher intracellular uptake compared with microparticles. In terms of intestinal uptake, apart from their particle size, nanoparticle nature and charge properties seem to influence the uptake by intestinal epithelia. Uptake of nanoparticles prepared from hydrophobic polymers seems to be higher than that of particles with more hydrophilic surfaces[7], thus more hydrophilic particles may be rapidly eliminated.

Thus the polymeric composition (hydrophobicity, surface charge, and biodegradation profile) of the nanoparticles, any adjuvant substances, and the associated drug (molecular weight, charge, localization in the nanospheres by adsorption or incorporation) have a great influence on the drug absorption, biodistribution pattern, and elimination. There are now numerous preparation methods available for producing nanoparticles.

The oral delivery of drugs is regarded as the optimal means for achieving therapeutic effects for a number of conditions. For drug delivery the enteric route has the advantages of increased patient compliance, relieves the need for injection and does not require the presence of trained personnel. Unfortunately, the oral delivery route is beset with problems such as: Poor solubility, poor permeability.

Poor solubility of a drug is in most cases associated with poor bioavailability. The contents of the gastrointestinal tract are aqueous and hence a drug having poor aqueous solubility has a low saturation solubility which is typically correlated with a low dissolution velocity, resulting in poor oral bioavailability. About 10% of the present drugs are poorly soluble, about 40% of the drugs in the pipeline possess poor solubility, and even 60% of drugs coming directly from synthesis have a solubility below 0.1mg/ml. Out of the research around 40% of lipophilic drug candidates fail to reach the market although exhibiting potential pharmacodynamic activities[8]. No matter how active or potentially active a New Molecular Entity (NME) is against a particular molecular target, if it is not available in a solution form at the site of action, it is not a viable development candidate. As a result, the development of many exciting NMEs has stopped before their potential is realized. Low bioavailability is the most common with oral dosage forms of poorly water-soluble, slowly absorbed drugs.

Too hydrophilic drugs would not be able to permeate through the gastrointestinal mucosa and too lipophilic drug will not dissolve in the aqueous gastrointestinal contents. For optimum absorption, the drug should have sufficient aqueous solubility to dissolve in the gastrointestinal contents and also adequate lipid solubility to facilitate its partitioning into the lipoidal membrane and then into systemic circulation. Drugs having partition coefficient (log P) value in the range of 1 to 3 shows good passive absorption across lipid membranes, and those having log Ps greater than 3 or less than 1 have often poor transport characteristics[9].

Management of hypercholesterolemia continues to be challenging with the currently available drugs due to poor bioavailability by the oral route and due to toxicity which occur at higher doses. Since, the preferred route of oral administration is limited to those drug molecules due to its poor solubility and/or poor permeability across the gastric mucosa.

Rosuvastatin calcium is a potent inhibitor of HMG CoA reductase, which is widely used in the treatment of hypercholesterolemia and may decrease the relative risk of heart attack and stroke. Currently this drug is administered orally as tablets. Since the drug is poorly soluble and poorly permeable (partition coefficient (octanol/water) – 0.13 at pH 7.0) across the gastric mucosa, the drug displays oral bioavailability (absolute bioavailability-20%) problems in conventional dosage forms. Reported side effects are myopathy, rhabdomyolysis which occurs at higher doses. Thus it could be a promising candidate in nanoparticulate drug delivery taking into account its poor solubility and poor permeability.

2. Materials and Methods

2.1 Materials

Rosuvastatin calcium was obtained from Safe tab life science (Pondicherry, India) as a gift sample. Eudragit L100 (EL 100), Eudragit S100 (ES 100), Pluronic F68, Polyvinyl alcohol (PVA) were kindly provided by Orchid pharmaceuticals (Chennai, India). Dialysis membrane was purchased from Hi Media Laboratories Ltd (Mumbai, India). All other reagents and chemicals were of analytical grade and used as received.

2.2 Drug-Polymer Interaction Studies

2.2.1 Fourier Transform- Infra Red Spectroscopy (FT-IR)

FT-IR spectroscopy was carried out to check the compatibility between the drug (Rosuvastatin calcium) and the polymers (Eudragit L 100, Eudragit S 100). 10mg of the sample and 400mg of KBr were taken in a mortar and triturated[10]. A small amount of the triturated sample was taken into a pellet maker and it was compressed at 10 kg/cm^2 using a hydraulic press. The pellet was kept onto the sample holder and scanned from 4000 cm^{-1} to 400 cm^{-1} in FT-IR spectrophotometer, Shimadzu, Japan. Samples were prepared for drugs, polymers and a physical mixture of drug and polymers. The spectra obtained were compared and interpreted for the shifting of functional peaks and the disappearance or appearance of new functional peaks[11].

2.2.2 Differential Scanning Calorimetry (DSC)

DSC is a useful tool to Monitor the effect of additives on the thermal behavior of material[12]. An accurately weighed sample, equivalent to 5mg of the drug (Rosuvastatin Calcium) was placed into the sealed standard aluminum pans with lids. Subsequently, the polymers (Eudragit L 100, Eudragit S 100) and physical mixtures of drug and polymers were ascertained using the differential scanning calorimetry thermogram analysis, DSC Q200, Shimadzu, Japan. The heating rate was 20°C/min and the heat flow was recorded from 20°C to 180°C. The aluminum oxide and indium powders were employed as reference and standard, respectively[13].

2.3 Preparation of Rosuvastatin Calcium Loaded Polymeric Nanoparticles

The rosuvastatin calcium loaded polymeric nanoparticles were prepared by nanoprecipitation according to the method developed by Fessi *et al*[14]. Briefly, 20mg of rosuvastatin calcium and 200mg of Eudragit L100/ Eudragit S 100 were dissolved in 20 mL of acetone[15]. This organic phase was quickly injected into the aqueous phase containing 40 mL of either 1% or 2% w/v of Pluronic F68/ PVA solution with moderate magnetic stirring at room temperature. The organic phase to aqueous phase ratio is 1:2[16]. Nanoparticles were spontaneously formed and turned the solution slightly turbid. Then, acetone was removed by continuous stirring for 3-4 hrs. Drug free nanoparticles were prepared by the same procedure omitting the drug[17]. In this study, the effects of various process parameters on nanoparticles size and drug entrapment efficiencies were assessed, including different drug-to-polymer ratios (1:10,1:20,1:30,1:40,1:50), Pluronic F68 concentration (1%, 2%) in the aqueous phase, PVA concentration (1%, 2%) in the aqueous phase[18].

2.4 Evaluation of Rosuvastatin Calcium Loaded Polymeric Nanoparticles

2.4.1 Particle Size and Polydispersity Index

Particle size and size distribution are the most important characteristics to be evaluated for nanoparticles systems[19]. The particle size distribution was reported as Polydispersity Index (PDI). The particle size and Polydispersity Index (PDI) of rosuvastatin calcium loaded polymeric nanosuspension were measured using a Zeta sizer nano ZS (Malvern Instruments Ltd., Malvern, UK)[20].

2.4.2 Zeta Potential

The zeta potential of nanoparticles was commonly used to characterize the surface charge property of nanoparticles[19]. The zeta potential of rosuvastatin calcium-loaded polymeric nanosuspension were measured using a Zeta sizer Nano ZS (Malvern Instruments Ltd., Malvern, UK) at 25 ± 0.5°C[18,21]. A potential of ±150 mV was set in the instrument. A disposable cuvette of 0.75ml capacity was used for all measurements[10].

2.4.3 Drug Content

The total drug content in nanosuspension was quantified by spectrophotometric analysis[20,22]. 1mg equivalent of rosuvastatin calcium polymeric nanosuspension was dissolved in 1 ml of acetone and the volume was made up to 100ml to make 10µg/ml concentration and the absorbance was measured at 241nm (λ_{max}) using UV spectrophotometer.

The calculation was performed as follows:

$$\text{Total drug content} = \frac{\text{Vol. Total}}{\text{Vol. Aliquot}} \times \text{Drug amount in aliquot} \quad (1)$$

2.4.4 Entrapment Efficiency

The amount of rosuvastatin calcium embedded in the NPs was calculated from the difference between the total amount incorporated in the NP formation medium and the amount of non-embedded rosuvastatin calcium remaining in the aqueous suspending medium[20]. The latter amount was determined by the separation of rosuvastatin calcium-loaded NPs from the aqueous medium by centrifugation using a refrigerated centrifuge (Eppendorf, 5417R, Germany) at 14,000 rpm for 2hours at 4°C. After centrifugation, the supernatant solution was made up to the desired volume with buffer and the amount of free drug was determined by measuring the absorbance of samples at 241 nm using UV spectrophotometer.

The % entrapment efficiency was calculated by the following formula:

$$\% \text{ drug entrapment} = \frac{(\text{Total drug} - \text{Drug in supernatant liquid})}{\text{Total drug}} \times 100 \quad (2)$$

2.4.5 In Vitro Release Studies

Dialysis bag diffusion technique was used to study *in vitro* release of drug from the prepared nanosuspension[23]. Dialysis membrane having pore size 2.4 nm, molecular weight cut off 14,000 was used. The nanosuspension equivalent to 1 mg of rosuvastatin calcium (2 ml) was placed in the dialysis bag, hermetically sealed and immersed into a 250ml beaker containing 100ml of the release media maintained at 37±0.5°C. Aliquots of samples (5ml) were withdrawn at pre determined intervals and immediately restored with the same volume of fresh media maintained at the same temperature. The study was carried out by buffer change method using acidic buffer (pH 1.2) for the first 2 hours and phosphate buffer (pH 6.8) for the rest of the study period, i. e. 10 hrs[20,24]. The amount of rosuvastatin calcium dissolved was determined with UV spectrophotometer at 241nm.

2.4.6 Kinetics of Drug Release

Several mathematical models can be tested to determine which best describes the kinetics and mechanism of drug release from polymeric nanoparticles. In the present study, the *in vitro* drug release data was fitted to the following mathematical models:

$$\text{Zero order } Q_t = Q_0 + k_0 t \quad (3)$$

$$\text{First order } \log C = \log C_0 - k_1 t / 2.303 \quad (4)$$

$$\text{Higuchi } Q_t = k_2 t^{1/2} \quad (5)$$

$$\text{Hixon-Crowell cube root } (W_0^{1/3} - W_t^{1/3}) = k_h t \quad (6)$$

$$\text{Korsmeyer – Peppas } Qt / Q\infty = kp\ tn \quad (7)$$

where Q_0, Q_t, and Q are the amounts of drug dissolved initially, at time t and at time, (in most cases, $Q_0 = 0$), C_0 and C are the concentrations of drug initially and at time t, W_0 and W_t are the amounts of drug in the pharmaceutical dosage form initially and at time t, and k_0, k_1, k_2, k_h and kp refer to the rate constants obtained from the linear curves of the respective models[25,26].

2.5 Evaluation of Selected Best Formulation

2.5.1 Solubility Studies

The solubility of the best formulation was compared with the solubility of the pure drug solution. For this purpose, saturation solubility measurement was carried out. Accurately weighed amounts of pure drug and rosuvastatin calcium-loaded polymeric nanoparticles were introduced into separate 25 ml stoppered conical flasks containing 10 ml of distilled water. The sealed flask was agitated on a rotary shaker for 24 hr. An aliquot was filtered and the filtrate was suitably diluted and analyzed on a UV spectrophotometer[27-29].

2.5.2 Ex Vivo Intestinal Permeability Studies

The *ex vivo* intestinal permeability studies were approved by the institutional ethical committee (Ref. No: 14024/ E1/ 4/ 2011) using 9 albino rats. Albino rats weighing 150-200 g were used for the study. After overnight fasting, rats were anesthetized by some ether sprinkled to a piece of cotton wool in a glass container equipped with a lid. After making a midline incision in the abdomen, the intestinal segments were isolated as follows: duodenum segment of 8cm was isolated starting from the pylorus; jejunum segment of 10-15 cm was isolated 25 cm from the pylorus; an ileum segment of 10-15 cm was isolated 20 cm upward from caecum[30]. The isolated segments were washed with pH 7.4 phosphate buffer saline (USP 30) to remove any mucous and lumen contents. These segments were tied at one end with

suture thread and the selected formulations of rosuvastatin calcium nanosuspension (equivalent to 1mg of drug) and pure drug solution (drug in PBS pH 7.4 equivalent to 1 mg) were injected separately into the different parts of intestine using a syringe and the other end of intestine were tied with the help of suture thread. Then the tied segments were placed separately in a beaker containing 100ml of PBS (pH7.4) continuously bubbled with 95% O_2 and 5% CO_2 with constant stirring at 37°C. The studies were completed in triplicates and aliquots of samples (5ml) were withdrawn at 15, 30, 60, 90 and 120 min. The samples were measured using a UV-Visible spectrophotometer at a wavelength of 241nm. The cumulative amount of drug permeated was plotted against time to calculate the apparent permeability coefficient (P_{app})[31-33].

The apparent permeability coefficient (P_{app}) (cm/s) is calculated as follows:

$$Papp = \frac{\frac{dQ}{dT}}{AC}$$

where dQ/dt is the rate of drug appearance in the receptor (µg/s), A is the surface area of the intestinal sac (cm^2) and C is the initial concentration of the drug in the sac (µg/ml)[34-36].

2.5.3 Statistical Analysis

Statistical analysis for the determination of differences in permeability profiles of rosuvastatin calcium-loaded polymeric nanosuspensions and rosuvastatin calcium pure drug solution was assessed by the use of Student's t-test (Graph pad Instat Version 3.0 software). Statistical probability (p) values less than 0.05 were considered significantly different[37].

2.5.4 Transmission Electron Microscopy (TEM)

The morphology of the prepared nanosuspension formulations was determined by TEM: (Hitachi, Japan). Before analysis, the samples were diluted 1:5 and stained with 2% (w/v) phosphotungstic acid for 30 s and placed on copper grids with films for observation[18].

3. Results and Discussion

3.1 Drug-Polymer Interaction Studies

3.1.1 Fourier Transform-Infra Red Spectroscopy (FT-IR)

FT-IR spectroscopy was carried out separately to check the compatibility between drug (rosuvastatin calcium) and the polymers (Eudragit L 100, Eudragit S 100) used for the preparation of nanoparticles. The FT- IR performed for pure drug, polymers and physical mixture of drug and polymers are shown in Figure 1. It was found from the spectra that there was no major shifting as well as any loss of functional peaks in the spectra of drug, polymers and physical mixture of drug and polymers. The results indicate that the selected polymers (Eudragit L 100, Eudragit S 100) were found to be compatible with the chosen drug rosuvastatin calcium.

3.1.2 Differential Scanning Calorimetry (DSC)

DSC thermograms of pure drug (rosuvastatin calcium), polymers (Eudragit L100 and Eudragit S100) and the physical mixtures of drug and polymers are shown in Figure 2. Pure rosuvastatin calcium showed a sharp endothermic peak at 88.12°C, Eudragit L 100 showed a melting endothermic peak at 83.02°C, Eudragit S 100 at 91.50°C. The physical mixture of drug (rosuvastatin calcium) and polymer (Eudragit L100) exhibited an endothermic peak at 85.36°C and the physical mixture of drug (rosuvastatin calcium) and polymer (Eudragit S 100) exhibited an endothermic peak at 88.93°C. Thus, an endothermic peak corresponding to the melting point of pure drug (88°C) was prominent in all the drug-polymer mixtures, which suggested clearly that there was no interaction between the drug and the polymers and thus the drug found to have existed in its unchanged form.

3.2 Evaluation of Rosuvastatin Calcium-Loaded Polymeric Nanoparticles

3.2.1 Particle Size and Polydispersity Index

Particle size plays a critical role in influencing the physicochemical and biological characteristics of the nanoparticles. Nanoparticles were characterized by mean particle diameter and their distribution. The particle size of nanoparticles ranged between 125.9nm - 191.9nm with respect to nanoparticles prepared with EL100 containing Pluronic F68 (1%) as stabilizer (F1-F5), 110.5nm-182.8nm with respect to nanoparticles prepared with EL100 containing Pluronic F68 (2%) as stabilizer (F6-F10), 135.9nm-209.3nm with respect to nanoparticles prepared with EL 100 containing PVA (1%) as stabilizer (F11-F15), 127.0nm-190.1nm with respect to nanoparticles prepared with EL100 containing PVA (2%) as stabilizer (F16-F20), 139.2nm-229.3nm with respect to nanoparticles prepared with ES100 containing Pluronic F68 (1%) as

stabilizer (F21-F25), 131.0nm-210.1nm with respect to nanoparticles prepared with ES100 containing Pluronic F68 (2%) as stabilizer (F26-F30), 149nm-231.0nm with respect to nanoparticles prepared with ES100 containing PVA (1%) as stabilizer (F31-F35), 137.0nm-229.5nm with respect to nanoparticles prepared with ES100 containing PVA (2%) as stabilizer (F36-F40).

3.2.1.1 *Effect of Drug-Polymer Ratio*

The influence of different drug-polymer ratios of rosuvastatin calcium-loaded EL100 and ES100 nanoparticles on the particle size was investigated. It could be seen that the particle size was affected by increasing the polymer concentration. The particle size of rosuvastatin calcium-loaded polymeric nanoparticles showed a positive relationship with polymer (EL100/ES100) concentration. This is because increasing EL100 and ES100 concentrations led to an increase in the viscosity of the organic phase. A more viscous organic phase provides a higher mass transfer resistance, the diffusion of polymer-solvent phase into the external aqueous phase is reduced and larger nanoparticles are formed[18,37]. A decrease in the viscosity of the organic phase increases the distribution effect of the polymer–solvent phase into the external phase leading to the formation of smaller nanoparticles[38].

3.2.1.2 *Effect of Stabilizers*

The effect of stabilizers (Pluronic F68 and PVA) on the particle size of rosuvastatin calcium loaded EL 100 and ES 100 nanoparticles was investigated. Rosuvastatin calcium loaded polymeric nanoparticles prepared with Pluronic F68 were smaller than those prepared with PVA. This result is in accord with the earlier studies of Gershon Golomb *et al*[39].

3.2.1.3 *Effect of Stabilizer Concentrations in the Aqueous Phase*

The influence of stabilizers (Pluronic F68/PVA) concentration (1%, 2%) in the aqueous phase of rosuvastatin calcium-loaded EL 100/ES 100 nanoparticles on the particle size was investigated. It could be seen that the particle size of rosuvastatin calcium-loaded polymeric nanoparticles showed a negative relationship with stabilizer concentrations. The mean diameter of polymeric nanoparticles decreased with the increase in stabilizer concentrations. At high concentrations, more Pluronic F68/PVA could be oriented at the organic solvent/water interface to reduce efficiently the interfacial tension, which resulted in a significant increase in the net shear stress at a constant energy density during emulsification and promoted the formation of smaller emulsion droplets[18].

3.2.1.4 *Polydispersity Index (PDI)*

PDI is another factor that represents the dispersion homogeneity, the range for the PDI is from 0 to 1. Values close to 0 indicate homogeneous dispersion, and those greater than 0.5 indicate high homogeneity. The PDI for all the formulations as shown in is smaller than 0.5, which indicates a relative homogenous dispersion[40].

3.2.2 *Zeta Potential*

The rosuvastatin calcium-loaded polymeric nanoparticles were characterized to evaluate the effect of different polymers and stabilizers at different concentrations on the surface charge of nanoparticles. Zeta potential of formulations F1-F20 prepared with EL 100 showed negative zeta potential (-25mV to -29mV). Zeta potential of formulations F21-F40 prepared with ES 100 showed negative zeta potential (-26mV to -30mV). All the formulations showed negative surface charge possibly due to the presence of terminal carboxylic acid groups in the polymers (EL 100 and ES 100)[40]. Commonly, zeta potential is an index of the stability of the nanoparticles. Muller considered that a zeta potential of about -25mV allows an ideal stabilization of nanoparticles because the repulsive forces prevent aggregation upon ageing[24]. However, zeta potential values between -25mV and -30mV allow predicting good colloidal stability due to high energy barrier between particles[39].

3.2.3 *Drug Content*

The drug content was found to be in the range of 90.75% to 96.86%, indicating uniform distribution of drug for all formulations from F1-F40

3.2.4 *Entrapment Efficiency*

The entrapment efficiencies ranged between 42.1%-78.59% with respect to nanoparticles prepared with EL 100 containing Pluronic F68 (1%) as stabilizer (F1-F5), 38.35%-74.25% with respect to nanoparticles prepared with EL 100 containing Pluronic F68 (2%) as stabilizer (F6-F10), 47.5%-75.25% with respect to nanoparticles

prepared with EL 100 containing PVA (1%) as stabilizer (F11-F15), 36.06%-70.35% with respect to nanoparticles prepared with EL 100 containing PVA (2%) as stabilizer (F16-F20), 33%-70.5% with respect to nanoparticles prepared with ES 100 containing Pluronic F68 (1%) as stabilizer (F21-F25), 28%-62% with respect to nanoparticles prepared with ES 100 containing Pluronic F68 (2%) as stabilizer (F26-F30), 29.5%-60.4% with respect to nanoparticles prepared with ES 100 containing PVA (1%) as stabilizer (F31-F35), 28%-55.5% with respect to nanoparticles prepared with ES 100 containing PVA (2%) as stabilizer (F36-F40). The fabrication parameters such as different drug-polymer ratios and different stabilizers at different concentrations were used to achieve the highest entrapment of rosuvastatin calcium.

3.2.4.1 *Effect of Drug-Polymer Ratio*

The effect of different drug-polymer ratios of rosuvastatin calcium-loaded EL100/ES100 nanoparticles on the entrapment was investigated. The results are shown in Figure 3. It could be seen that the entrapment was affected by increasing the polymer concentration. The entrapment efficiency of rosuvastatin calcium-loaded polymeric nanoparticles showed a positive relationship with polymer (EL100/ES100) concentration. This is because, an increase in polymer concentration the in organic phase increases drug entrapment due to an increase in organic phase viscosity, which increases the diffusional resistance to drug molecules from the organic phase to aqueous phase, thereby entrapping drugs in the polymeric nanoparticles[15,18].

3.2.4.2 *Effect of Stabilizers*

The effect of stabilizers (Pluronic F68 and PVA) on the entrapment efficiency of rosuvastatin calcium-loaded EL 100 and ES 100 nanoparticles was investigated. The results are shown in Figure 3. Rosuvastatin calcium-loaded polymeric nanoparticles prepared with Pluronic F68 showed better entrapment than those prepared with PVA. This result was in accord with the earlier studies of Gershon Golomb *et al*[39].

3.2.4.3 *Effect of Stabilizer Concentration in the Aqueous Phase*

The influence of stabilizer (Pluronic F68/PVA) concentrations (1%, 2%) in the aqueous phase on the entrapment efficiency of rosuvastatin calcium-loaded polymeric nanoparticles was investigated. The results are shown in Figure 3. It could be seen that the entrapment efficiencies of rosuvastatin calcium-loaded polymeric nanoparticles showed a negative relationship with stabilizer concentration. The entrapment efficiencies of polymeric nanoparticles decreased with the increase of stabilizer concentration. This was probably caused by the decrease in particle size. Moreover, with the increase of stabilizer concentration, more molecules of the drug may partition out rapidly in to the aqueous phase during the emulsification procedure and fewer drug molecules remained in emulsion droplets to interact with polymers (EL100/ES100), hence decreasing the entrapment efficiencies[18].

3.2.5 *In vitro Release Studies*

The suitability of EL 100/ES 100 nanoparticles for the release of rosuvastatin calcium was studied in vitro at pH 1.2 and 6.8 to mimic the in vivo condition eg: gastric pH and intestinal pH, since the aim of this study was to administer the nanoparticles by the oral route. Rosuvastatin calcium loaded polymeric nanoparticles containing two different polymers (EL 100 and ES 100) displayed a similar biphasic drug release pattern with a burst release within 2 hours followed by sustained release (Figures 4 and 5). The reason for burst release is possibly due to the unentrapped drug adsorbed on the surface of nanoparticles[15]. The Burst phase was, however, followed by hydration and swelling of the nano-matrix which eventually led to a controlled release profile. Hydration brings about an increment in the diffusional path length of molecules and consequently, the rate of their diffusion becomes lower. Therefore, the gaining of a controlled release profile and its maintenance could be assumed to be dependent upon the relative hydration rate of the polymer and integrity of hydrated matrix[23]. Therefore, superiority of one formulation over the other could be established on the basis of avoidance of burst release, achievement of a controlled release profile and its maintenance in a time dependent manner. The influence of particle size and entrapment efficiency on rosuvastatin calcium release was studied.

3.2.5.1 *Influence of Particle Size on In vitro Drug Release*

Among all formulations, F6 (EL 100 1:10, Pluronic F68 2%) showing lower particle size (110.5nm) provided a higher burst effect when compared to formulation F35

(ES 100 1:50, Pluronic 1%) showing a larger particle size (231nm). This is because decrease in the mean particle size in nanoparticles leads to an increase in the release rate which could be explained on the basis of surface area relationship[42,43].

3.2.5.2 *Influence of Entrapment Efficiency on In vitro Drug Release*

Among all formulations, F5 (EL 100 1:50, Pluronic F68 1%) showing higher entrapment (78.59%) provided a better controlled release when compared to formulation F26 (ES 100 1:10, Pluronic 2%) showing lower entrapment (28.0%). This is because increase in entrapment efficiency leads to a better controlled release which could be explained on the basis of free drug concentration on the surface of nanoparticles.

3.2.6 *Kinetics of Drug Release*

The results obtained from the in vitro release studies were attempted to fit into various mathematical models as follows:

Cumulative percentage drug release Vs time (zero order rate kinetics)

Log cumulative percentage drug remaining Vs time (first order rate kinetics)

Cumulative percentage drug release Vs square root of time (Higuchi classical diffusion model)

Cube root of percentage drug remaining Vs time (Hixon Crowell erosion equation).

Log cumulative percentage drug release Vs log time (Korsmeyer Peppas exponential equation)

Among the models tested, the drug release profile of all formulations F1-F40 were best fitted with first order with r values ranging from 0.943-0.994 and Higuchi model with r values ranging from 0.966-0.999. Thus Higuchi model describes drug release as purely diffusion controlled. Fickian and non Fickian anomalous behaviors have been used for determining the mechanism of drug release of polymeric nanoparticles. Korsmeyer Peppas model was used, which plots the log cumulative percentage of drug release up to 60% Vs log time and the release exponent n which indicated the release mechanism was determined. Different values of n for cylindrical, spherical and slab of geometrices are available in the literature. For spheres, values of 0.5, 0.5 < n < 1.0, 1.0 and higher than 1.0 are related to Fickian diffusion, anomalous, case II transport and super case II transport respectively[25,26]. For *in vitro* release kinetics of rosuvastatin calcium loaded Eudragit S100 nanoparticles and Eudragit L100 nanoparticles, the values of exponent n were within 0.5 which indicated that the drug release mechanism followed pure Fickian diffusion. This report was in accord with the earlier studies of Annick Ludwig *et al*[37].

3.3 Selection of Best Formulation

From the above results of characterization, F5 and F25 were selected as the best formulations showing,

F5 (Eudragit L100 1:50, Pluronic F68 1%):

Particle size	:	191.9nm
Entrapment efficiency	:	78.59%
In vitro drug release	:	60.5% in 12 hours.
Release kinetics	:	Closest linearity to first order kinetics (R2 - 0.994) and Higuchi model (R2- 0.999)

F25 (Eudragit S100 1:50, Pluronic F68 1%):

Particle size	:	229.3nm
Entrapment efficiency	:	70.5%
In vitro drug release	:	62.9% in 12 hours.
Release kinetics	:	Closest linearity to first order kinetics (R2 - 0.992) and Higuchi model (R2- 0.997)

3.4 Evaluation of Selected Best Formulation

3.4.1 *Solubility Studies*

The solubility of pure drug solution was about 357.60µg/ml and the selected formulation of rosuvastatin calcium loaded polymeric nanoparticles had a solubility of about 743.94µg/ml (F5- EL100 1:50, Pluronic F68 1%) and 624.60 µg/ml (F25-ES100 1:50, Pluronic F68 1%). The results are shown in Figure 6. It has been reported that a progressive reduction in particle size increases the solubility. According to Thomson – Freundlich equation, solubility of low soluble drugs is increased by decrease in particle size. So, reduction of particle size of poorly soluble drug rosuvastatin calcium had an influent effect on drug solubility[28]. In conclusion solubility of selected

formulation of rosuvastatin calcium loaded polymeric nanoparticles compared to pure drug form was increased to about two-fold.

3.4.2 Ex vivo Intestinal Permeability Studies

The results are shown in Figure 7. In the duodenum region, the cumulative amount of drug permeated for pure drug solution was about 0.32 mg and the nanoparticle formulations had the permeability of about 1.00mg (F5-EL100 1:50, Pluronic F68 1%) and 0.91mg (F25- ES100 1:50, Pluronic F68 1%) at the end of 2 hours. Similarly in the jejunum region also, the cumulative amount of drug permeated for pure drug solution was 0.35mg and for the nanoparticle formulations had the permeability of 1.00mg (F5- EL100 1:50, Pluronic F68 1%) and 0.89mg (F25- ES100 1:50, Pluronic F68 1%) at the end of 2 hours. The same type of results was also obtained from the ileum region of rat intestine. The pure drug solution had the cumulative amount of drug permeability of 0.327 mg and the formulations had the permeability of 0.99mg (F5- EL100 1:50, Pluronic F68 1%) and 0.87mg (F25- ES100 1:50, Pluronic F68 1%) at the end of 2 hours.

The intestinal permeability was found to be increase in the order of

F5 (EL 100 1:50, Pluronic F 68 1%) > F25 (ES 100 1:50, Pluronic F 68 1%) > Pure drug solution.

From the results, it was observed that the nanoparticle formulations showed better permeability than the pure drug solution.

3.4.2.1 Effect of Particle Size on Intestinal Permeation

Polymeric nanoparticles showed particle size dependent permeation through intestinal segments. Maximum permeation was observed for F5- EL100 1:50, Pluronic F68 1% (191.9nm size). Increase in particle size of polymeric nanoparticles F25- ES100 1:50, Pluronic F68 1% (229.3nm size) revealed decrease in permeation through intestinal segments[44].

The Anova analysis of apparent permeability (Papp) values in the duodenum region indicated that the best formulations F5 and F25 exhibited significant increase in rosuvastatin calcium permeability compared to pure drug ($p<0.001$), similarly in the jejunum and ileum region also, the apparent permeability values showed significant increase when compared to pure drug ($p<0.001$). Hence, from these results it was inferred that the nanoparticle formulations showed significant improvement in the rosuvastatin calcium permeability compared to pure drug.

3.4.3 Transmission Electron Microscopy (TEM)

TEM was used to investigate the morphology of nanoparticles. The TEM micrograph of the best formulation F5 is presented in Figure 8. TEM shows nanoparticles with a mean diameter of 101nm, spherical shape and smooth surface. The size of the formulation F5 (Rosuvastatin calcium: EL 100 (1:50) containing Pluronic F68 (1%) as stabilizer) determined by Photon Correlation Spectroscopy (PCS) was not consistent with that determined by TEM, which was probably caused by the different mechanisms of the two methods. PCS and TEM are based on scattering (Hydrodynamic radius) and diffraction technique in particle size measurement, respectively. The diameters detected by PCS were 'hydrated diameters', which are usually larger than their genuine diameters. In the case of TEM sample preparation, the formulation F5 were stained with 2% w/v phosphotungstic acid and all the free water and even some hydrated water were stained. This implied that the size of formulation F5 derived from TEM might be considerably smaller than their real diameters[18].

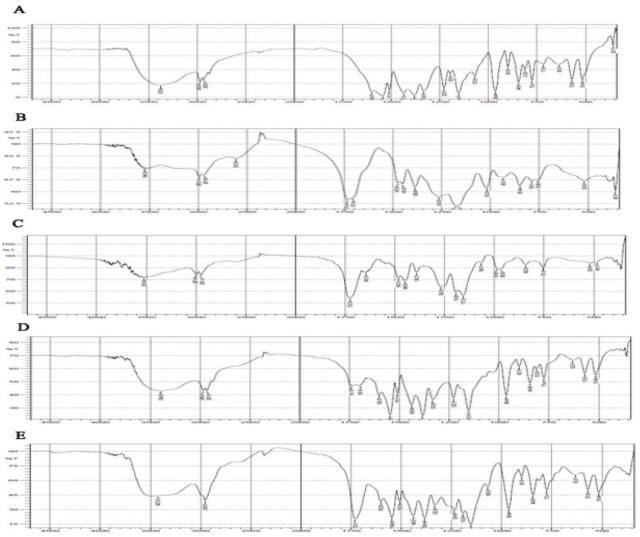


Figure 1. FT-IR spectra **(A)** Rosuvastatin calcium **(B)** Eudragit L100 **(C)** Eudragit S100 **(D)** Rosuvastatin calcium and Eudragit L100 and **(E)** Rosuvastatin calcium and Eudragit S100.

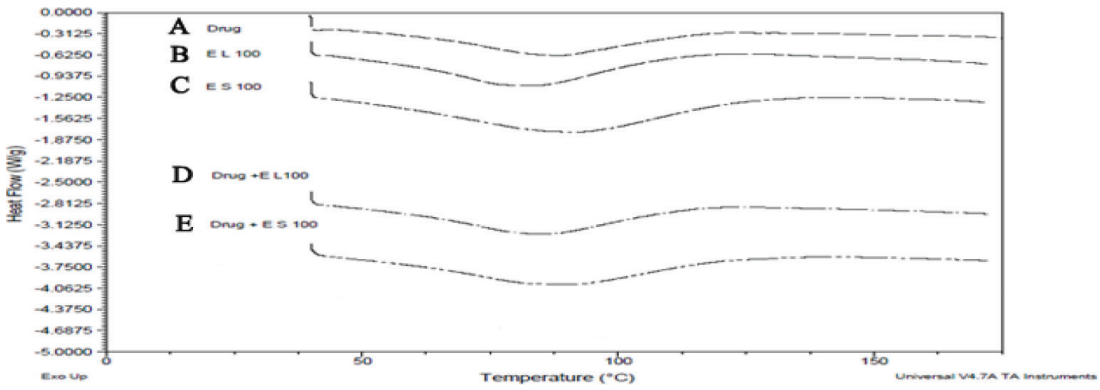


Figure 2. DSC thermogram **(A)** Rosuvastatin calcium **(B)** Eudragit L100 **(C)** Eudragit S100 **(D)** Rosuvastatin calcium and Eudragit L100 and **(E)** Rosuvastatin calcium and Eudragit S100.

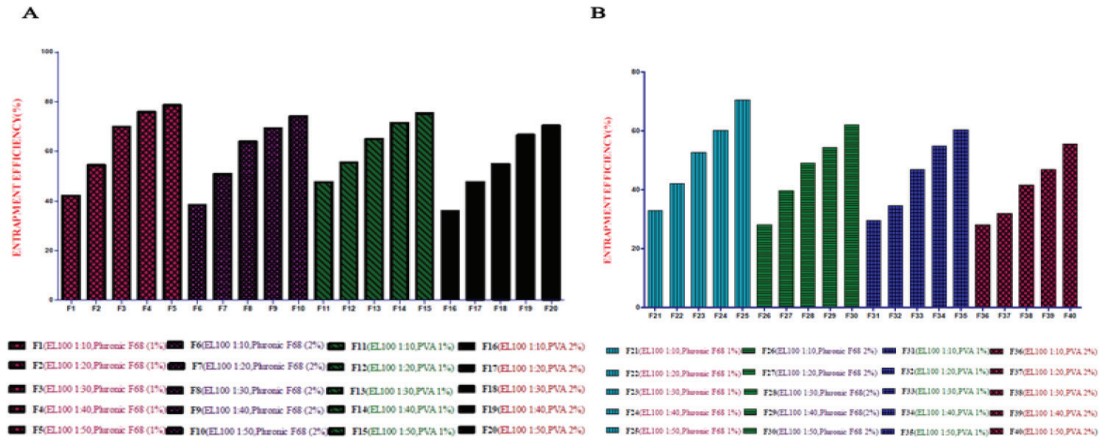


Figure 3. Entrapment efficiencies of Rosuvastatin calcium loaded polymeric nanoparticles **(A)** Eudragit L100 **(B)** Eudragit S100.

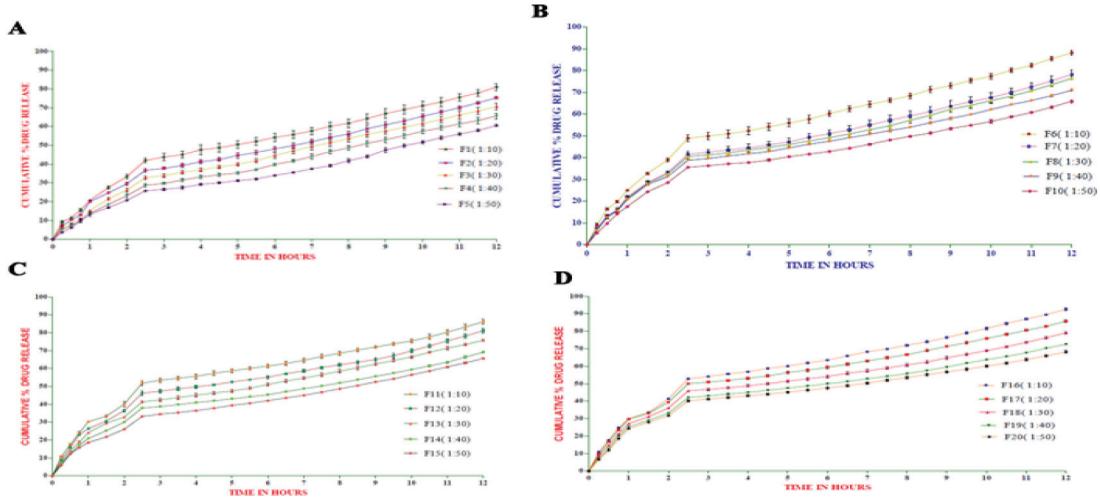


Figure 4. Comparison of in vitro release profile of Rosuvastatin calcium loaded Eudragit L100 nanoparticles **(A)** pluronic F68 1% **(B)** pluronic F68 2% **(C)** PVA 1% and **(D)** PVA 2%.

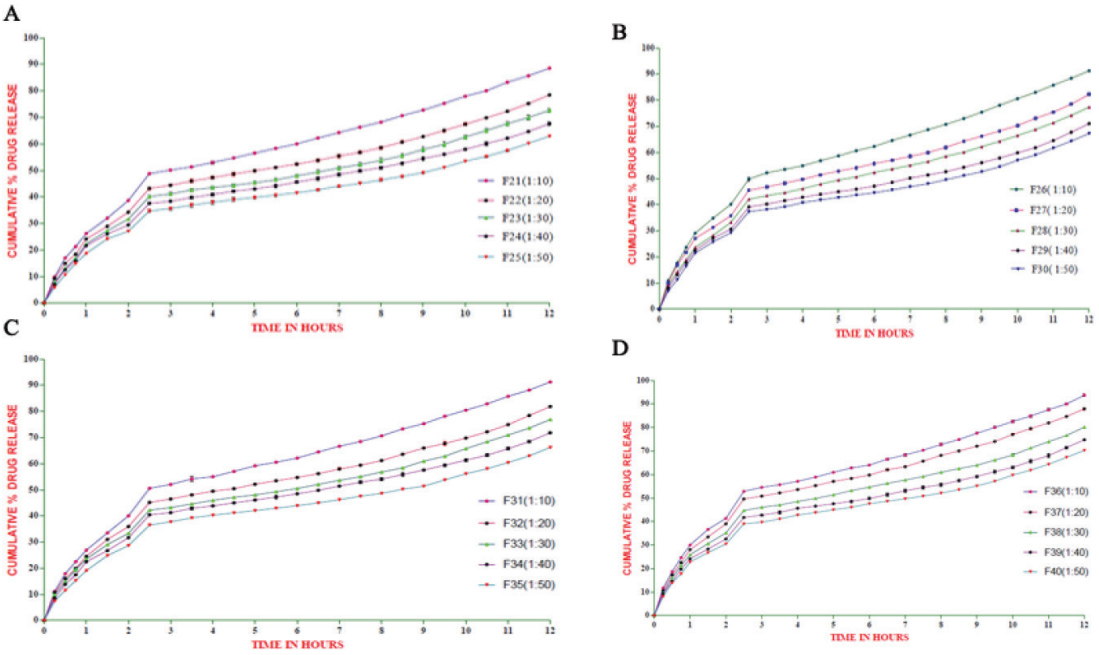


Figure 5. Comparison of in vitro release profile of Rosuvastatin calcium loaded Eudragit S100 nanoparticles **(A)** pluronic F68 1% **(B)** pluronic F68 2% **(C)** PVA 1% and **(D)** PVA 2%.

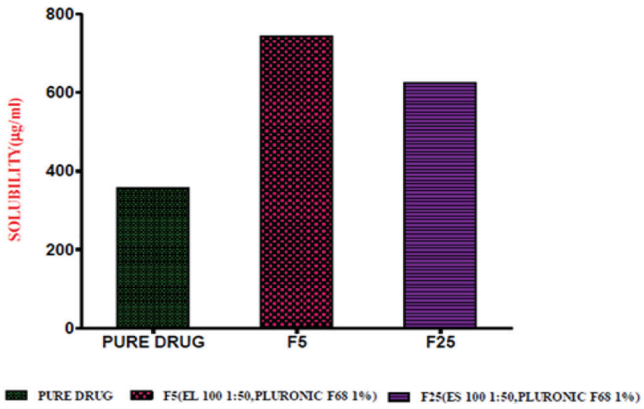


Figure 6. Comparison of solubility of best formulations (F5, F25) with pure drug solution.

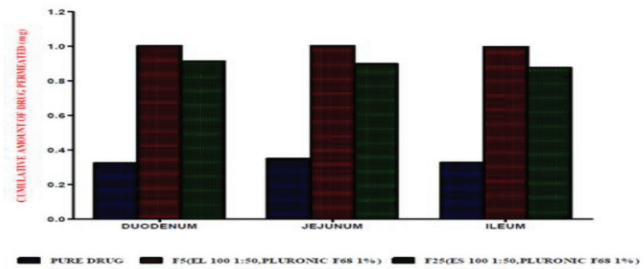


Figure 7. Comparison cumulative amount of drug permeated through the rat small intestine segments at 2nd hour.

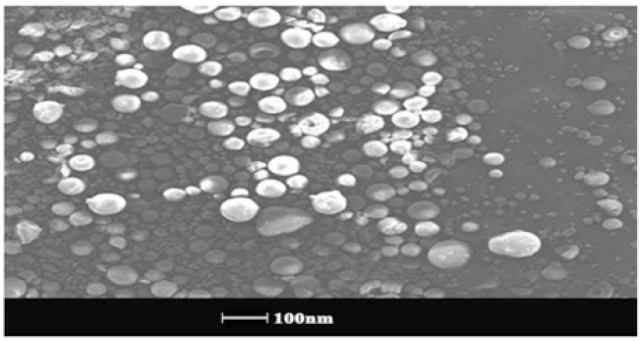


Figure 8. Transmission electron micrograph of best formulation F5 (Eudragit L100 1:50, pluronic F68 1%).

Table 1. Anova analysis *of Ex vivo* apparent permeability coefficient (P_{app}) of rosuvastatin calcium

Comparison groups	Duodenum	Jejunum	Ileum
Pure drug Vs F5	p<0.001	p<0.001	p<0.001
Pure drug Vs F25	p<0.001	p<0.001	p<0.001
F5 Vs F25	p<0.001	p<0.001	p<0.01

4. Conclusions

Hence, it was concluded that the nanoprecipitation was a useful method for the successful incorporation of rosuvastatin calcium with high entrapment efficiency. The solubility and *ex vivo* intestinal permeability studies suggested that the nanoparticle formulations can improve the bioavailability of the rosuvastatin calcium by improving its solubility and permeability across intestinal membrane. Furthermore, it could be presumed that if the nanometer range particles were obtained, the bioavailability might be increased. Hence, we can conclude that polymeric nanoparticles enhance the bioavailability of poorly water soluble and low lipophilic drug like rosuvastatin calcium as a drug delivery system.

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