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Formulation and Evaluation of Rosuvastatin Calcium Polymeric Micelles

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ABSTRACT

The objective of the study was to formulate and evaluate polymeric micelles (PMs) containing rosuvastatin calcium (RC), an antilipidemic agent by thin-film hydration technique. PMs were prepared by thin-film hydration technique. Historical data design suggested the optimum amounts of 3 mg of RC and 60 mg of Pluronic F127, and predicted particle size (nm), drug loading (DL) (%), and encapsulation efficiency (EE) (%) were 14.11 nm, 19.98%, and 95.5%, respectively. Particle size, DL, and EE obtained for the optimized formulation was 15.01 nm, 20.23%, and 94.03%, respectively. Scanning electron microscopy image showed smooth surfaced spherical micelles. Percentage cumulative drug release from the optimized formulation (F7) was 97.24% for 12 h. The release kinetics for most of the formulations indicated that drug release followed Korsmeyer–Peppas model and non-Fickian diffusion mechanism. F7 indicated that it was stable for 3 months. It can be concluded that RC PMs formulation has significantly prolonged the release of the drug up to 12 h. Drug was successfully formulated into sustained release PMs by thin-film hydration technique.

Key words: *Polymeric micelles, Rosuvastatin calcium, Pluronic F127, Historical data design, Thin film hydration.*

1. INTRODUCTION

One of the most challenging aspects of drug development is the enhancement of drug solubility and its oral bioavailability. Various approaches are available and reported in literature to enhance the solubility of poorly water-soluble drugs [1]. Apart from those, there are several other nanotechnology approaches available to enhance the solubility of hydrophobic drugs and one among them is development of polymeric micelles (PMs). PMs are nanoscopic carriers (10-100 nm) with novel core–shell structure possessing a hydrophobic core which acts as a reservoir by entrapping hydrophobic drug, and thus protects the drug payload and a hydrophilic shell which reduces the interaction of hydrophobic drug with aqueous environment and mainly increases the aqueous solubility and stability [2,3]. The main aim of PMs is to enhance the solubility of the poorly water-soluble drugs, especially Class II and IV drugs of the Biopharmaceutical Classification System of drugs [4].

PMs are of great interest in recent days for their exclusive properties such as size in nanoscale, excellent biocompatibility and biodegradability, low toxicity, stability in plasma, enhanced circulation *in vivo*, high drug loading (DL) capacity, controlled-drug release, and ability to solubilize large number of hydrophobic drugs in the micellar core. PMs for anticancer drugs have been proven to be useful. Most successful attempts for anticancer drugs have been made. In the current research work, an attempt has been made to incorporate a drug other than anticancer drug; rosuvastatin calcium (RC), an antilipidemic agent, and study the influence of PMs on the dissolution of RC. Thin-film hydration technique is effective, fastest, and simplest method to prepare PMs. This method is most often used due to its simplicity, practicability, and its ability to yield small and uniform particles.

2. MATERIALS AND METHODS

2.1. Materials

RC was obtained from Melody Healthcare Pvt. Ltd., Tarapur, and PF127 (Poloxamer 407) was procured

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from Sigma-Aldrich, Bengaluru, and were used as received. All other chemicals used were of laboratory grade.

2.2. Preparation of RC Loaded PMs

RC loaded PMs were prepared by thin-film hydration method. Drug (RC) and the block copolymer (PF127) in the ratio 1:20, 1:10, and 1:5 were dissolved in acetone. It was rotated for about 30 min in a rotary evaporator at 50°C. Vacuum was applied for a specific period until a thin film of drug-impregnated in polymer was formed. Required volume of pH 7.4 phosphate buffer was added to the film to form drug-loaded micelle solution at 60°C for 30 min. Unincorporated drug was removed by filtering through 0.2 µm cellulose nitrate membrane, followed by lyophilization [5,6]. Formulation chart of RC PMs is shown in Table 1.

2.2.1. Evaluation of RC PMs

2.2.1.1. Particle size determination

The particle size, polydispersity index (PDI), and zeta potential of diluted formulation were determined using a Zetasizer 3000 (Malvern Instruments Ltd., Japan). The samples were analyzed by elsewhere method [7].

2.2.1.2. Determination of DL and encapsulation efficiency (EE)

Lyophilized micelles were taken and diluted with 10 ml of methanol:water (1:1) and sonicated for 30 min to promote swelling and breakup of the cross-linked structure, which in turn facilitated the encapsulated drug to get dissolved. The solution was filtered through 0.22 µm filter, and absorbance of the solution was measured at 242 nm.

2.2.3. In vitro drug release studies

The release behavior of RC from the formulations was carried out by dialysis method [8,9]. Withdrawn sample after dialysis was filtered and analyzed for RC using ultraviolet spectrophotometer at 242 nm.

2.2.3.1. Kinetic analysis of *in vitro* drug release data

To determine the release mechanism that provides the best description to the pattern of drug release *in vitro* release data were fitted to zero order, first order, Higuchi matrix model, and Korsmeyer–Peppas model using the software, PCP Disso v2.08. The model with the highest correlation coefficient values or determination coefficient (R^2) was considered as the best-fit model. The release data were also kinetically analyzed using the Korsmeyer–Peppas model, and the release exponent (n) describing the mechanism of drug release from the matrices was calculated by regression analysis using the following equation (1).

$$M_t/M_\infty=k_t^n \quad (1)$$

Where, M_t/M_∞ is the fraction of drug released at time t and k is a constant incorporating the structural and geometric characteristics of the release device. When n=0.5, Case I or Fickian diffusion is indicated, 0.5<n<1 for anomalous (non-Fickian) diffusion, n=1 for Case II transport (zero-order release), and n>1 indicates super Case II transport.

3. RESULTS AND DISCUSSION

3.1. Fourier Transform Infrared (FT-IR) Spectroscopy Studies

The FT-IR spectra obtained for RC pure drug and the physical mixture of RC with Pluronic F127 are shown in Figure 1. Both the drug and drug with polymer showed characteristic peaks of O-H, C-H, C=O, C-N, C-O, and C-F stretching. It was observed that there was no appearance of new peaks or any disappearance of existing peaks in the spectra of formulation, which indicates that the drug and polymer used for the study are compatible.

3.2. Differential Scanning Calorimetric (DSC) Studies

From the phase transition study, it was observed that DSC thermogram of pure drug showed a sharp endothermic peak at 149°C, which corresponds to its melting point. DSC thermogram of formulation containing RC and

Table 1: Formulation chart of RC PMs.

Formulation	RC (mg)	Pluronic F127 (mg)	Acetone (ml)	pH 7.4 PBS (ml)
F1	2	40	5	10
F2	4	40	5	10
F3	8	40	5	10
F4	2.5	50	5	10
F5	5	50	5	10
F6	10	50	5	10
F7	3	60	5	10
F8	6	60	5	10
F9	12	60	5	10

RC=Rosuvastatin calcium, PM=Polymeric micelles, PBS=Phosphate buffer saline

PF127 showed two endothermic peaks at 147.2°C and 53.9°C corresponding to their melting point, respectively. The thermograms revealed no interaction between the drug and the polymer (Figure 2).

3.3. Evaluation of RC PMs

3.3.1. Particle size, PDI, and zeta potential determination

The particle size, PDI, and zeta potential were determined by Zetasizer 3000 and the results obtained are tabulated in Table 2. Among the 9 formulations, F7 showed lowest particle size of 15.01 nm (Figure 3) and highest zeta potential of about −25.69 mV (Figure 4), which accounts to its higher stability when compared with other formulations. Moreover, as the concentration of polymer increased, particle size decreased. It was shown that formulations of RC PMs prepared using PF127, have negative surface charges. The polydispersity value was <1.0 in all formulations indicating narrow size distribution of particles. Therefore, it can be concluded that RC PMs prepared by thin-film hydration method exhibited a homogeneous size distribution.

3.3.2. Determination of DL and EE

Loading efficiency decreased with increasing drug concentration. This may be due to precipitation or aggregate formation in the media caused by increasing drug concentration, which leads to a decrease in loading efficiency. Loading of drug increased with increase in the concentration of polymer. The EE of micelles decreased with increase in concentration of drug, whereas increased with increase in the concentration of the polymer. The DL and EE results are summarized in Table 2.

3.3.3. Scanning electron microscopy (SEM)

The SEM photograph (Figure 5) showed that PMs formed were roughly spherical without deformations. The smooth surface of PMs indicates that RC was well-dispersed inside the PMs.

3.3.4. In vitro drug release studies

Drug release from all the formulations was extended up to 12 h (Figures 6 and 7); the release of drug was dependent on the particle size. A smaller particle size improves drug release and provides larger interfacial area across which drug can diffuse into the gastrointestinal fluids. Results showed that the micellar carrier sustained the drug release. Optimized formulation (F7) showed highest drug release (97.24%) at the end of 12 h when compared to other formulations due to smaller particle size. Results showed RC PMs exhibited a sustained drug release (12 h) when compared to pure drug (1 h), shows that RC incorporated into the hydrophobic core of Pluronic F127 stayed securely by the micelles.

3.3.4.1. Kinetic analysis of dissolution data for RC PMs

The drug release mechanism followed Korsmeyer–Peppas model for most of the formulations. The n value of more than 0.45 indicated that the drug release mechanism from the formulation is by non-Fickian diffusion process for most of the formulations.

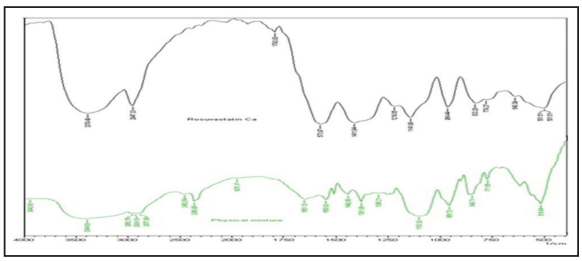

Figure 1: Fourier transform infrared spectrum of rosuvastatin calcium (RC) pure drug and RC with Pluronic F127.

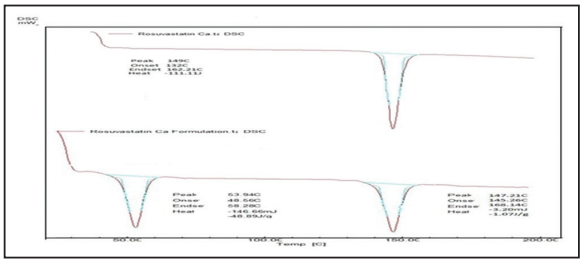

Figure 2: Differential scanning calorimetric thermograms of rosuvastatin calcium (RC) and RC polymeric micelles.

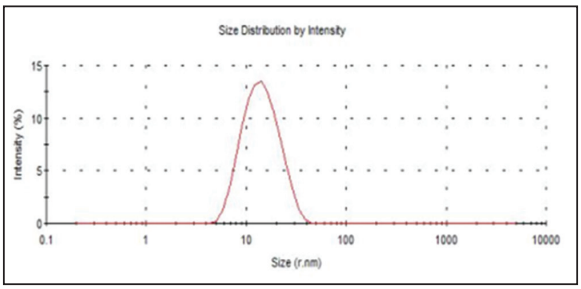

Figure 3: Particle size distribution curve of optimized formulation F7.

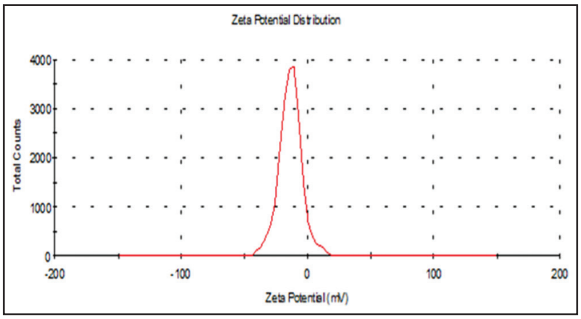

Figure 4: Zeta potential distribution curve of optimized formulation F7.

Table 2: Particle size, PDI, and zeta potential of RC PMs.

Formulation code	Particle size (nm)	Zeta potential (mV)	PDI	DL (%)±SD*	EE (%)±SD*
F1	23.26	−15.75	0.527	13.62±0.22	81.21±2.17
F2	24.03	−14.23	0.639	12.54±0.26	74.53±1.15
F3	24.31	−13.21	0.692	10.31±0.24	66.08±0.93
F4	16.30	−16.52	0.314	15.98±0.19	85.97±2.25
F5	16.56	−18.86	0.389	14.04±0.15	79.65±1.24
F6	16.82	−19.17	0.413	13.51±0.25	73.24±1.12
F7	15.01	−25.69	0.171	20.23±0.20	94.03±3.87
F8	15.69	−23.14	0.192	19.17±0.18	91.28±3.22
F9	16.08	−20.03	0.213	16.72±0.19	88.34±2.98

*Mean±SD. n=3. SD=Standard deviation, RC=Rosuvastatin calcium, PM=Polymeric micelles, PDI=Polydispersity index, DL=Drug loading

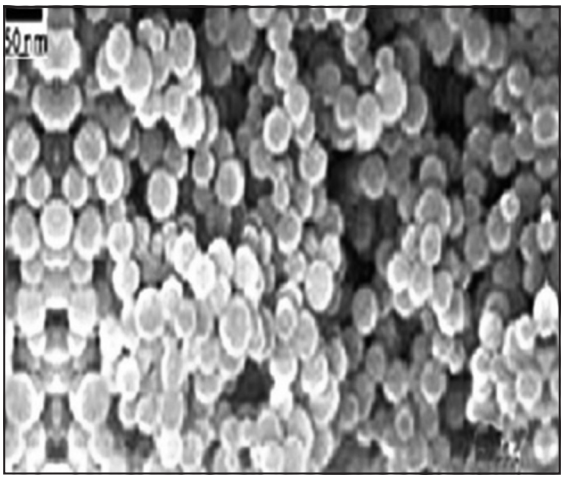

Figure 5: Scanning electron microscopy image of the optimized formulation.

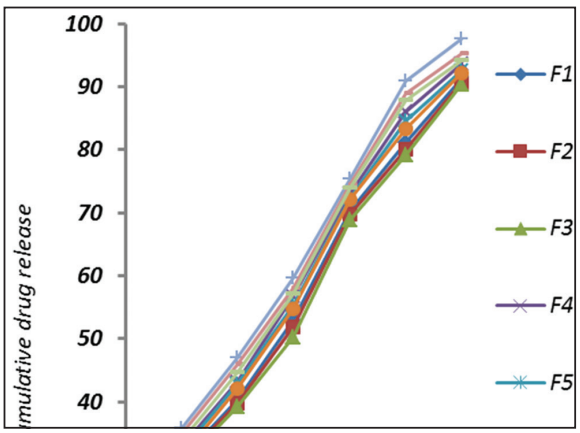

Figure 6: *In vitro* drug release profile of rosuvastatin calcium polymeric micelles.

3.3.5. Stability studies

Stability studies were performed for the optimized formulation (F7) according to the ICH guidelines by storing at 30°C/65% RH, 40°C/75% RH, and 25°C/60% RH for 90 days. These samples were analyzed for changes in physical appearance and drug content at regular intervals spectrophotometrically at 242 nm. Results showed that formulation did not undergo any chemical changes during the study period.

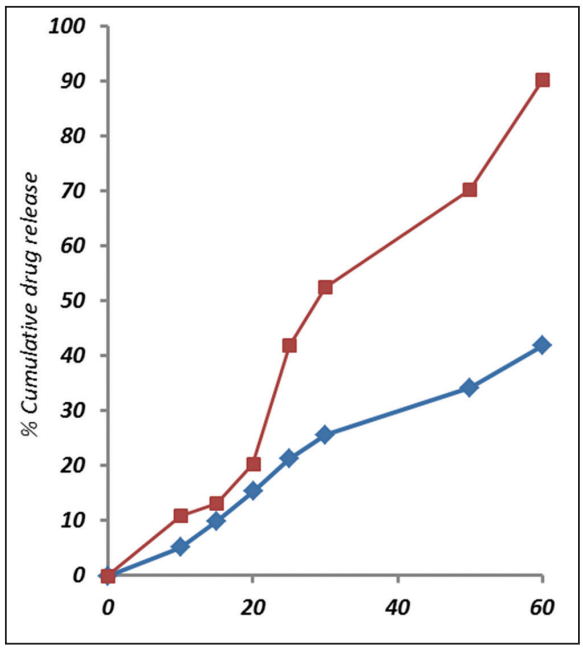

Figure 7: *In vitro* drug release profile of rosuvastatin calcium in pH 1.2 acidic buffer and pH 6.8 phosphate buffer.

4. CONCLUSION

The FT-IR spectra and DSC thermograms indicated that drug and polymer used were compatible. Thin-film hydration technique was successfully used for the preparation of RC PMs. RC, PF127, and their ratios have a great influence on particle size, DL, and EE as indicated by historical data. The experimental values were in close agreement with the predicted response, indicating adequate fitting, and validation of formula generated by constrained optimization. Particle size,

zeta potential, DL, and EE of the formulations were in the range 15.01-24.31 nm, −13.21-−25.69 mV, 10.31-20.23%, and 66.08-94.03%, respectively. RC in PMs could release the drug for 12 h. The release kinetics for most of the formulations suggests that the drug release followed Korsmeyer–Peppas model and non-Fickian diffusion mechanism. Optimized formulation (F7) showed no significant changes in the drug content after 90 days of the study period, indicating that the prepared formulation was stable. From all the above results, it can be concluded that PMs containing RC sustained the drug release up to 12 h.

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