International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X; Impact Factor: RJIF 5.22
Received: 17-01-2019; Accepted: 21-02-2019
www.pharmacyjournal.in
Volume 4; Issue 2; March 2019; Page No. 30-33

Formulation and evaluation of atorvastatin calcium polymeric micelles

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Abstract
The objective of the study was to formulate and evaluate Atorvastatin Calcium (AC), polymeric micelles (PMs), an antilipidemic agent by the use of thin film hydration technique. 3 mg of AC and 60 mg of PEG 6000 was taken as optimum amounts as suggested by Historical data design, and predicted particle size (nm), drug loading (%) and encapsulation efficiency (%) were 14.11 nm, 19.98% and 95.5% respectively. Optimized formulation showed a particle size of 15.09 nm, drug loading 20.37% and encapsulation efficiency 94.45%. Smooth surfaced spherical micelles were observed by SEM image. Percentage cumulative drug release from the optimized formulation (F7) was 97.12% at the end of 12 h. The release kinetics for most of the formulations indicated that drug release followed Korsmeyer-Peppas model and Non-Fickian diffusion mechanism. F7 was found to be stable for 3 months. It can be concluded that AC PMs formulation has significantly prolonged the release of the drug up to 12 h. By thin film hydration technique, the drug Atorvastatin calcium was successfully prepared into sustained release PMs.

Keywords: polymeric micelles, atorvastatin calcium, PEG 6000, historical data design, thin film hydration

Introduction
The enhancement of drug solubility and its oral bioavailability is one of the most challenging aspects of drug development. Many approaches are available and reported in literature to enhance the solubility of poorly water-soluble drugs [1]. Apart from those, there are several other nano technology 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
. The main aim of PMs is to enhance the solubility of the poorly water-soluble drugs, especially Class II and IV drugs of Biopharmaceutical Classification System (BCS) of drugs [4].

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

Materials and Methods
Materials
Atorvastatin Calcium was a gift sample from Dr. Reddy's lab, Hyderabad, India. Poly ethylene glycol 6000 was purchased from Merk, Mumbai, Potassium dihydrogen orthophosphate, Tarapur and PEG 6000 was procured from Sigma-Aldrich, Bangalore and was used as received. All other chemicals used were of laboratory grade.

Preparation of Atorvastatin Calcium loaded PMs
AC loaded PMs were prepared by thin film hydration method. Drug (AC) and PEG 6000 in the ratio 1:20, 1:10 and 1:5 were dissolved in acetone. It was rotated for about 30 minutes in a rotary evaporator at 50ºC. Vacuum was applied for a specific period of time 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. Un-incorporated drug was removed by filtering through 0.2 µm cellulose nitrate membrane, followed by lyophilization [5, 6]. Formulation chart of AC PMs is shown in Table 1.

Table 1: Formulation chart of Atorvastatin Calcium polymeric micelles.

Formulation	Atorvastatin Calcium (mg)	PEG 6000 (mg)	Acetone (ml)	pH PBS (ml)	7.4
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

Evaluation of Atorvastatin Calcium polymeric micelles

Particle size determination

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

Determination of drug loading (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 the absorbance of the solution was measured at 242 nm. The drug content was calculated from the calibration curve [8]. The formulae used for calculation:

$$DL\% = \frac{Wm}{Wi} \times 100 \quad \ldots\ldots\ldots\ldots \quad (1)$$

$$EE\% = \frac{Wm}{Wf} \times 100 \quad \ldots\ldots\ldots\ldots \quad (2)$$

Wm: Weight of drug in micelles, Wi: Weight of feeding copolymer and drug, Wf: Weight of feeding drug.

***In vitro* drug release studies**

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

Kinetic analysis of *in vitro* drug release data

In order 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 (3).

$$Mt/M\infty = ktn\ldots\ldots\ldots \quad (3)$$

Where $Mt/M\infty$ 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.

Results and discussion

Fourier Transform Infrared (FT-IR) spectroscopy studies

The FT-IR spectra obtained for RC pure drug and the physical mixture of AC 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.

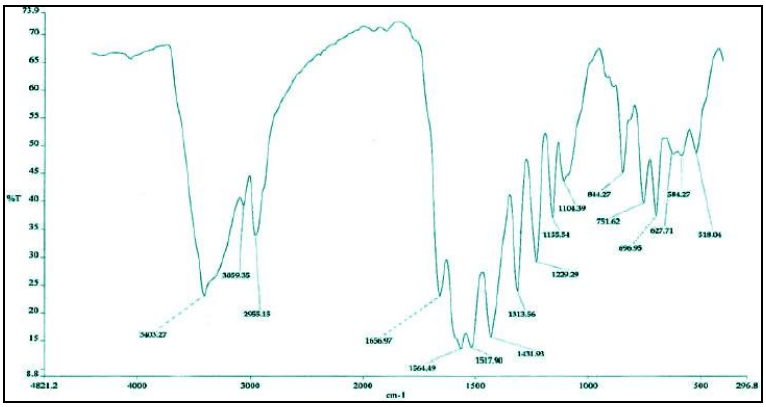

Fig 1: FTIR spectrum of Atorvastatin Calcium pure drug

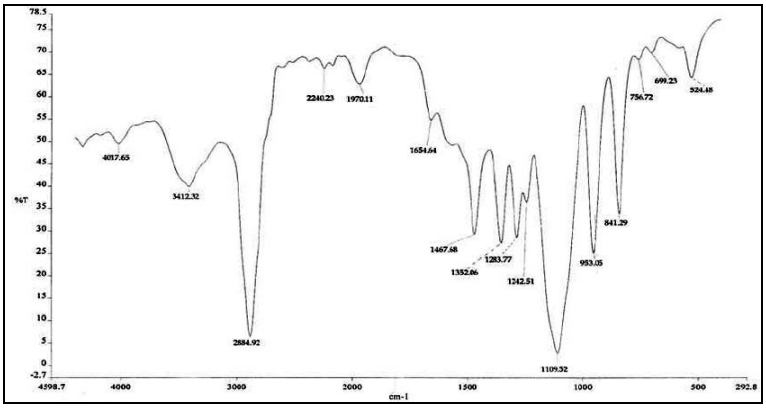

Fig 2: FTIR spectrum of Atorvastatin Calcium with PEG 6000.

Evaluation of Atorvastatin Calcium polymeric micelles

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 15.09 nm (Figure 3) and highest zeta potential of about -25.53 mV 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 AC PMs prepared using PEG 6000, have negative surface charges. The polydispersity value was less than 1.0 in all formulations indicating narrow size distribution of particles. Therefore, it can be concluded that AC PMs prepared by thin-film hydration method exhibited a homogeneous size distribution.

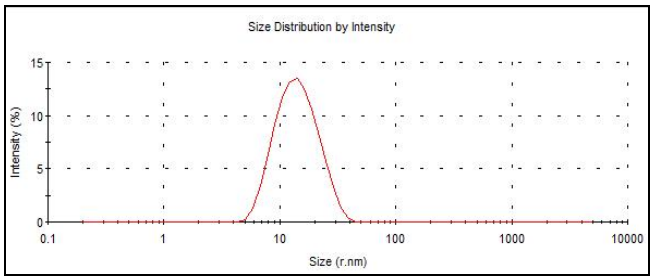

Fig 3: Particle size distribution curve of optimized formulation F7.

Determination of drug loading and encapsulation efficiency

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.

Table 2: Particle size, polydispersity index and zeta potential of Atorvastatin Calcium polymeric micelles.

Formulation code	Particle size (nm)	Zeta potential (mV)	PDI	Drug loading (%) ±SD*	Encapsulation efficiency (%) ±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± S.D: n=3

Scanning Electron Microscopy (SEM)

The SEM photograph (Figure 3) showed that PMs formed were roughly spherical without deformations. The smooth surface of PMs indicates that AC was well dispersed inside the PMs.

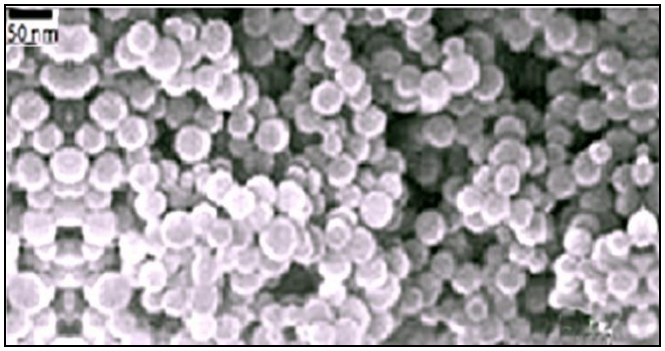

Fig 4: SEM image of the optimized formulation.

***In vitro* drug release studies**

Drug release from all the formulations was extended up to 12 hrs; 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 AC PMs exhibited a sustained drug release (12 h) when compared to pure drug (1 h), shows that AC incorporated into the hydrophobic core of Pluronic F127 stayed securely by the micelles.

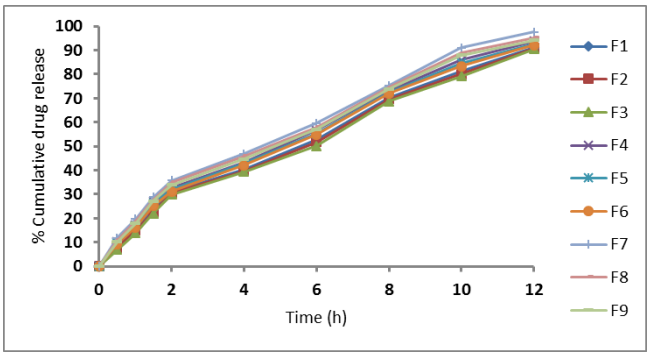

Fig 5: *In vitro* drug release profile of Rosuvastatin Calcium polymeric micelles.

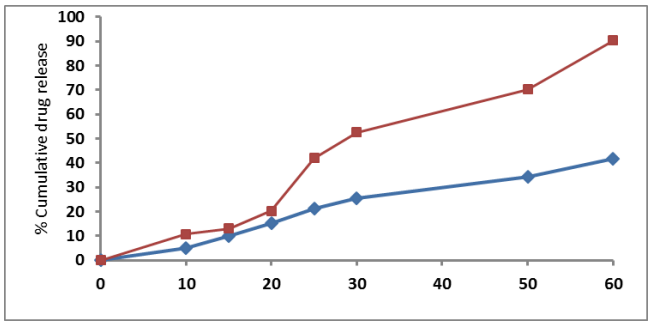

Fig 6: *In vitro* drug release profile of AC in pH 1.2 acidic buffers and pH 6.8 phosphate buffers.

Kinetic analysis of dissolution data for Atorvastatin Calcium polymeric micelles

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 (Table 3).

Table 3: Kinetic analysis of dissolution data for Atorvastatin Calcium PMs.

Formulation code	R^2					n value
	Zero order	First order	Peppas	Higuchi	Hixson	
F1	0.9763	0.9551	0.9782	0.9846	0.9062	0.7186
F2	0.9761	0.9543	0.9842	0.9775	0.9099	0.7345
F3	0.9731	0.9513	0.9796	0.9784	0.9131	0.7635
F4	0.9714	0.9506	0.9877	0.9829	0.8956	0.6759
F5	0.9723	0.9567	0.9883	0.9823	0.8974	0.6789
F6	0.9745	0.9585	0.9890	0.9815	0.9023	0.6964
F7	0.9671	0.9042	0.9897	0.9854	0.8860	0.6411
F8	0.9670	0.9411	0.9847	0.9878	0.8875	0.6579
F9	0.9686	0.9508	0.9869	0.9833	0.8918	0.6736

Stability studies: Stability studies were performed for the optimized formulation (F7). The optimized formulation was subjected to stability studies according to ICH guidelines by storing at 30ºC/65% RH, 40º C/75 % RH and 25 º C/60 % RH for 90 days. These samples were analyzed and checked 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/interaction during the study period (Table 4).

Table 4: Stability studies data for optimized formulation.

Formulation	Conditions	Physical appearance	Drug content (%)	Report
15 days	30ºC/65%RH	No change	97.36	Complies
	40ºC/75%RH	No change	97.34	Complies
	25°C/60%RH	No change	97.32	Complies
30 days	30ºC/65%RH	No change	98.40	Complies
	40ºC/75%RH	No change	97.44	Complies
	25°C/60%RH	No change	97.39	Complies
45 days	30ºC/65%RH	No change	97.28	Complies
	40ºC/75%RH	No change	97.21	Complies
	25°C/60%RH	No change	97.20	Complies
60 days	30ºC/65%RH	No change	98.95	Complies
	40ºC/75%RH	No change	98.45	Complies
	25°C/60%RH	No change	98.21	Complies
90 days	30ºC/65%RH	No change	98.00	Complies
	40ºC/75%RH	No change	97.33	Complies
	25°C/60%RH	No change	96.90	Complies

Conclusion

The FT-IR spectra and DSC thermo grams obtained for the pure drug and the formulation indicated that drug and polymer used were compatible. Thin film hydration technique was successfully used for the preparation of AC PMs. AC and 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 and zeta potential were in the range of 15.01 to 24.31 nm and -13.21 to -25.69 mV respectively. Drug loading and encapsulation efficiency of the formulations were in the range of 10.31 to 20.23% and 66.08 to 94.03% respectively. AC 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 study period, indicating that the prepared formulation was stable. From all the above results it can be concluded that PMs containing AC sustained the drug release up to 12 h.

Acknowledgments

The authors are grateful to Sarada Vilas College of Pharmacy, Mysuru, for necessary facilities to carry out this research work.

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