International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.10, pp 298-310, 2017

Formulation, development and optimization of polymeric micelles of Telmisartan for enhancement of solubility using 3² factorial design.

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Abstract : This study describes a 3² full factorial experimental design to optimize the formulation of telmisartan (TEL) loaded polymeric micelles (PM's) by the direct dissolution method. The variables polymer concentration and sonication time were studied at three levels and arranged in a 3²factorial design to study the influence on the response variables particle size and entrapment efficiency (%EE). From the statistical analysis of data polynomial equations were generated. The particle size and %E.E for the 9 batches (F1 to F9) showed a wide variation of 111.5-219.1 nm and 91.32 – 98.23 %, respectively. The physical characteristics of TEL-loaded polymeric micelles were evaluated using a particle size analyzer, differential scanning calorimetry and X-ray diffraction. The results of the optimized formulation showed an average particle size of 111.5 nm and entrapment efficiency of 97.07 %.

Keywords : Telmisartan, Polymeric micelles, 3² factorial design, Solubility enhancement.

Introduction

Polymeric micelles (PMs) as drug delivery system (DDSs) were introduced in the early 1990s by Kataoka's group through the development of doxorubicin-conjugated block copolymer micelles.[1]Delivery of poorly water-soluble drugs with clinically useful bioavailability is one of the most important problems in formulations.[2] Great efforts have been made to resolve solubility problems associated with poorly water-soluble compounds, namely, drug nanocrystals, use of solubilizing excipients, pH adjustment, co-solvent, self-emulsifying drug delivery system, micelles, liposomes and chemical conjugates.[3]

Polymeric micelles have attracted increased attention as a promising vehicle for poorly soluble drugs.[4]Polymeric micelles are known to have high drug loading capacity, high water-solubility and appropriate size for long circulation in blood.[5]AlsoPolymeric micelles provide long circulation in systemic fluid, passive targeting into specific tissues and simple sterilization.[6]

Telmisartan is an angiotensin II receptor antagonist, is used as an antihypertensive drug for arterial hypertension.[7] The solubility of Telmisartan in aqueous solutions is strongly pH-dependent. Telmisartan is manufactured and supplied in the free acid form and is has a very poor solubility, and so low bioavailability.[8]

In this study, a mixed polymeric micellar formulation of telmisartan (TEL-PM) was prepared using pluronic F127. The objective of this study was to develop a new polymer micelles system to overcome the limitations like poor water-solubility and low bioavailability. Also this study was to develop a mathematical model using 3² experimental design in order to deduce the adequate condition for preparation of polymeric

micelles of telmisartan. Also localization of optimized formulation with maximum drug entrapment efficiency and minimum particle size were examined.

Material and Methods

Material

Telmisartan was obtained as a gift sample from Aarti drug Pvt. Ltd. Mumbai. Pluronic F127 and Pluronic F68 were provided byBASF, Mumbai. Polyacrylic acid was provided by Corel Pharma Chemicals, Ahemadabad. All other chemicals were of reagent grade and used without further purification.

Preparation of polymeric micelles

Polymeric micelles were prepared by direct dissolution method followed by probe sonication. 150 mg polymer (pluronic F127) and 10 mg drug (telmisartan) was dissolved in water in beaker and stirred at room temperature using high speed homogenizer. The solution was sonicated using probe sonicator with sonication time 15 min. the obtained solution was centrifuged at 13000 rpm for 40 min. and supernatant was collected.[9]

Experimental Design and Statistical Analysis

In this study, a 3^2full factorial experimental design was introduced to optimize the formulation of polymeric micelles. Initial studies were undertaken to decide on the excipients and their levels in the experimental design.[10]

In order to optimize the preparation of formulation, the polymer conc. (X1) and probe sonication time (X2), were chosen as independent variables. These two factors that might affect the polymeric micelles formulation and three levels of each factor were selected (Table-1) and arranged according to a 3^2 full factorial experimental design table.[11] (Table-2)

Table-1: Independent variables and their selected levels for polymeric micelles formulation.

Factors	**Coded levels**		
	-1	**0**	**+1**
Polymer conc. (mg) (X1)	100	150	200
Probe sonication time (min.) (X2)	10	15	20

Table-2: A 3^2 full factorial experimental design layout.

Formulation code	**Coded factor levels**	
	Polymer conc.(X1)	**Probe sonication time (X2)**
F1	-1	-1
F2	0	-1
F3	1	-1
F4	-1	0
F5	0	0
F6	1	0
F7	-1	1
F8	0	1
F9	1	1

Evaluation of Polymeric micelles

Particle Size Analysis

The mean particle size and particle size distribution of drug loaded polymeric micelles were determined by Horiba SZ-100 nanoparticle analyzer, at 28°C. Each sample was measured in triplicate.

Zeta Potential Measurement

The zeta potential of TEL loaded polymeric micelles was measured by nano particle analyzer. Laser Doppler Micro-electrophoresis was used to measure zeta potential. An electric field was applied to a solution of molecules or a dispersion of particles, which then move with a velocity related to their zeta potential. This velocity was measured using laser interferometry technique which enables the calculation of electrophoretic mobility, and from this, the zeta potential and zeta potential distribution.

Entrapment Efficiency

The entrapment efficiency of prepared polymeric micelles was calculated by centrifugation method. About 5 mL of dispersion of polymeric micelles was taken in centrifuge tube and further it was centrifuged in cooling centrifuge (REMI-C24 BL. Remi Elektrotechnik ltd. Vasai, India) at 13,000 rpm for 40 mins. After centrifugation the supernatant was removed and diluted with appropriate solvent. The concentration of drug (free drug) in supernatant layer was determined by using UV-VIS Spectrophotometry.

The entrapment efficiency (EE) is calculated by using Eq^{n} (i)

$$EE = \frac{\boldsymbol{W}initial\ drug - \boldsymbol{W}free\ drug}{\boldsymbol{W}initial\ drug} \times 10 \quad \text{..... (i)}$$

Where,

$\mathbf{W}_{initial}$ drug= Weight of initial drug added into the formulation.

$\mathbf{W}_{free}$ drug= Weight of free drug into the formulation.

FTIR Spectroscopy

Previously dried sample of TEL in one part was mixed with 100 parts of KBr. The mixture was triturated to form fine powder. Thus formed fine powder mixture was compressed in a hydraulic press under pressure of 10 tons to form thin pellet. Same procedure was done for Pluronic F127. The pellets was scanned over a wave number range of 4000 to 400 cm^{-1} in FTIR instrument (Perkin Elmer, Spectrum Bx) and spectral analysis was done.

Scanning Electron Microscopy

SEM analysis of the optimized polymeric micelles formulation was carried out to understand the morphology of polymeric micelles.

Differential Scanning Calorimetric (DSC) study

The DSC thermogram of polymeric micelles was recorded by using a differential scanning calorimeter (PerkinElmer 4000, UK) equipped with a computerized data station. The sample (approx. 1mg) was weighed and heated in a closed pierced aluminum pan at a scanning rate of 10°C/min between 30- 300°C and 20 mL/min of nitrogen flow.

X-ray Diffraction Study

Polymeric micelles were studied for X-ray diffraction. The powder X ray diffraction patterns was recorded using an X-ray diffractometer (Bruker D8 advance) with 2.2 KW copper as an anode material and dermic X-ray tube as a source. The sample was analyzed using the 2θ angle of 3-30° using lynux eye detector and filtered using Ni filter.

Saturation Solubility

Saturation solubility study of pure drug and polymeric micelles was carried in water to find out the solubility of drug. This is done by determining the solubility of drug and polymeric micelles at 24 hrs and 48 hrs.

***In vitro* Drug Release Study**

The in vitro drug release from TEL loaded polymeric micelles in PBS pH 1.2, 6.8, 7.4 and 7.5 was examined by the dialysis bag method (D 5 8000, Lab India. dissolution apparatus). In brief, polymeric micelles dispersion (containing 10 mg of TEL) was added to the dialysis bag (molecular weight cut off 12000) and the dialysis bag was tied to place into 900 mL each dissolution medium (PBS pH 1.2, 6.8, 7.4 and 7.5) with stirring rate of 50 rpm at 37°C. Then 10 mL of dissolution medium was withdrawn at the different time points for 24 hours (1, 2, 3, 4, 6, 8, 12, and 24 hour) and fresh release medium to equal volume was added quickly to maintain the sink condition. The samples were analyzed by UV-VIS Spectrophotometry. Each experiment was performed in triplicate.[12]

Result and Discussion

Experimental design and statistical analysis

The objective of study was to prepare polymeric micelles of telmisartan by direct dissolution along with probe sonication method and to optimize the effect of formulation variables on responses. Based on preliminary studies, pluronic F127 were selected as polymer in formulation. Polymer conc. And probe sonication time was selected as variables and entrapment efficiency and particle size as response parameter. A 3^2 full factorial design was selected as it helps to study the effect on response parameters by changing both variables simultaneously with minimum number of experimental runs.

The particle size and entrapment efficiency for 9 batches (F1-F9) showed a wide variation 111.5-219.1 nm and 91.32-98.23%, respectively (Table III). The data clearly indicated strong dependence on response variables on the selected independent variables.

Table 3- Values of particle size and entrapment efficiency of TEL-loaded polymeric micelles (F1-F9) as per full factorial.

Formulation code	Particle size (nm)	%E.E.
F1	219.1	91.55
F2	211.3	91.32
F3	209.5	93.78
F4	128.8	93.31
F5	135.0	94.81
F6	146.8	95.61
F7	111.5	97.07
F8	142.3	97.23
F9	136.0	98.23

In order to quantify the effect of formulation variables on the response parameters, it was important to construct a mathematical model which would help in predicting values of response parameters at any selected values of formulation variables within the boundaries of the design. Design Expert 10 software was used to generate a mathematical model for each response parameter and the subsequent statistical analysis.

The coefficients of the polynomial equations generated using MLRA (Design expert 10) for particle size and %EE of Telmisartan-loaded polymeric micelles dispersion studied are listed in (Table IV) along with the values of r^2. Five coefficients (a to e) were calculated with k as the intercept.

$$Y = k + aA + bB + cAB + dA^2 + eB^2 \quad - (2)$$

$$P.S. = 139.78 + 5.48*A - 41.72*B + 8.52*AB - 4.32*A^2 + 34.78*B^2 \quad - (3)$$

$$E.E = 94.26 + 0.95*A + 2.65*B - 0.27*AB + 0.47*A^2 + 0.29*B^2 \quad - (4)$$

The equation was used to obtain estimates of the responses at various factor combinations, where the optimum combination was found to be similar to that corresponding to F7 and hence F7 was treated as the optimized batch.

Table 4- Values of coefficient for polynomial equations and r^2 for various response variables of TEL-loaded polymeric micelles.

Coefficient code	Polynomial coefficient values for response variables	
	Particle size	**E.E.**
k	+139.78	+94.26
a	+5.48	+0.95
b	-41.72	+2.65
c	+8.52	-0.27
d	-4.32	+0.47
e	+34.78	+0.29
r^2	0.9824	0.9781

For particle size response, the Model F-value of 33.47 implies the model is significant. There is only a 0.78% chance that a "Model F-Value" this large could occur due to noise. P values were found to be 0.0078, with a value less than 0.0500 indicating model terms are significant.

The "Predicted R-Squared" of 0.8030 is in reasonable agreement with the "Adjusted R-Squared" of 0.9530. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 13.943 indicates an adequate signal thus the proposed model can be used to navigate the design space.

For **%**EEresponse, the Model F-value of 26.79 implies the model is significant. There is only a 1.08% chance that a "Model F-Value" this large could occur due to noise. P value was found to be 0.0108, with a value less than 0.0500 indicating model terms are significant.

The "Predicted R-Squared" of 0.7979is in reasonable agreement with the "Adjusted R-Squared" of 0.9416."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 14.661indicates an adequate signal. Thus the proposed model can be used to navigate the design space.

Since the values of $r^2$are relatively high for both the responses, i.e., 0.9824 for particle size and 0.9781 for %EE, the polynomial equations form an excellent fit to the experimental data and are highly statistically valid.

Three-dimensional response surface plots for each response parameter were constructed to study the effects of both formulation variables simultaneously along with the behavior of the system.

Figure 1 shows response surface plot for particle size. It can be observed from the figure that polymer conc. had a positive effect on particle size i.e. particle size increased with increase in polymer conc. Least particle size was observed at the lowest level of polymer conc. A greater amount of polymer may have resulted in increasing the size of polymeric micelles.

Sonication time had the opposite effect on particle size. It can be observed from Figure 1 that particle size decreased with increased sonication time. Figure 1 indicates that the sonication time had a greater impact on particle size compared to polymer content, moreover the effect of polymer content was more pronounced at a high level of sonication which might be due to less effort required to disperse the small polymer agglomerates. At low sonication time, polymer concentration had little influence.

Figure 2 shows effect of probe sonication time and polymer conc. on entrapment efficiency. It can be observed that EEincreased with increased sonication time. Increased sonication time resulted in decreased particle size thereby increasing the total surface area and favoring entrapment. Polymer conc. also had a similareffect. The value of EE was maximal when both formulation variables were employed at their highest levels. The reasons can be attributed to the maximum amount of polymer present for entrapment of the drug. The effect of sonication on entrapment was not evident at high polymer levels. At low polymer conc. however, the decrease in sonication reduced entrapment which may be accounted for by less availability of polymer and decrease in polymer solubility of drug due to reduced sonication.

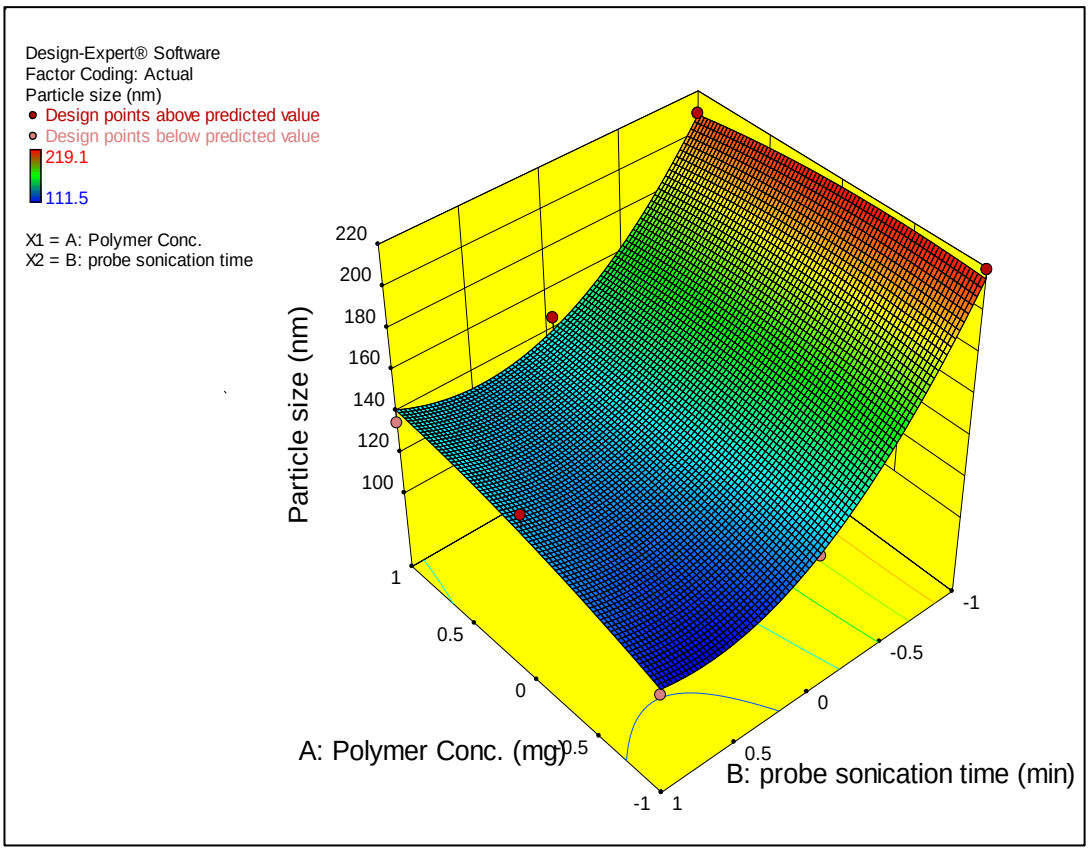

Figure 1- Three-dimensional response surface plots for particle size.

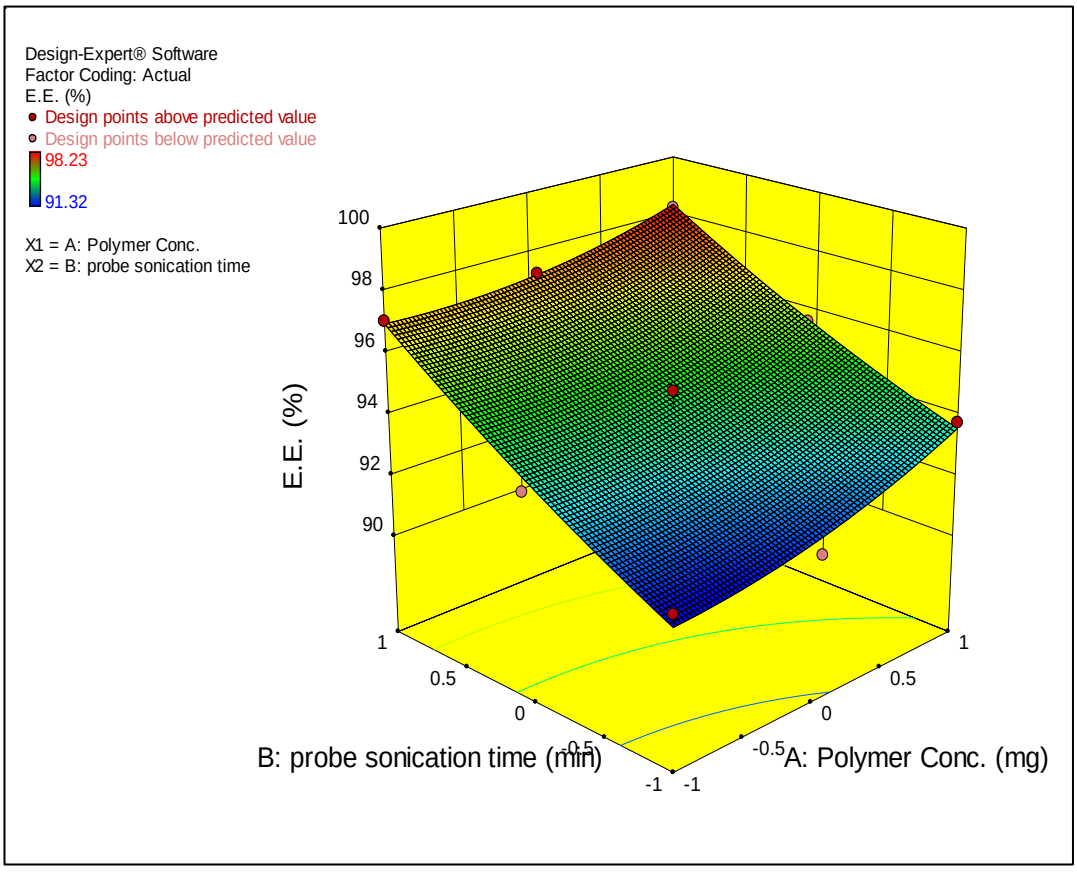

Figure 2- Three-dimensional response surface plots for entrapment efficiency

Evaluation of polymeric micelles

Particle size analysis

The average particle size of the preparedpolymeric micelles was found to be in the range of 111.5-219.1nm which was summarized in Table III. BatchF_7 shows the lowest particle size of 111.5 nm and preparation F_1shows highest particle size of 219.1nm. Polydispersity Index (PDI) of all the formulations was found to be less than 0.4.Particle size distribution of the optimized batch is shown in Figure 3.

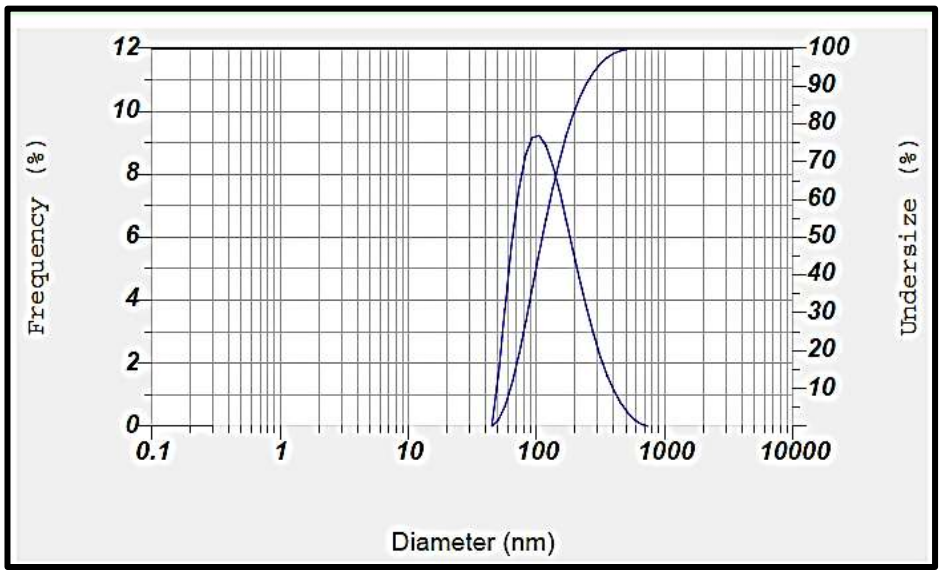

Figure 3 – Particle size distribution curve of optimized batch

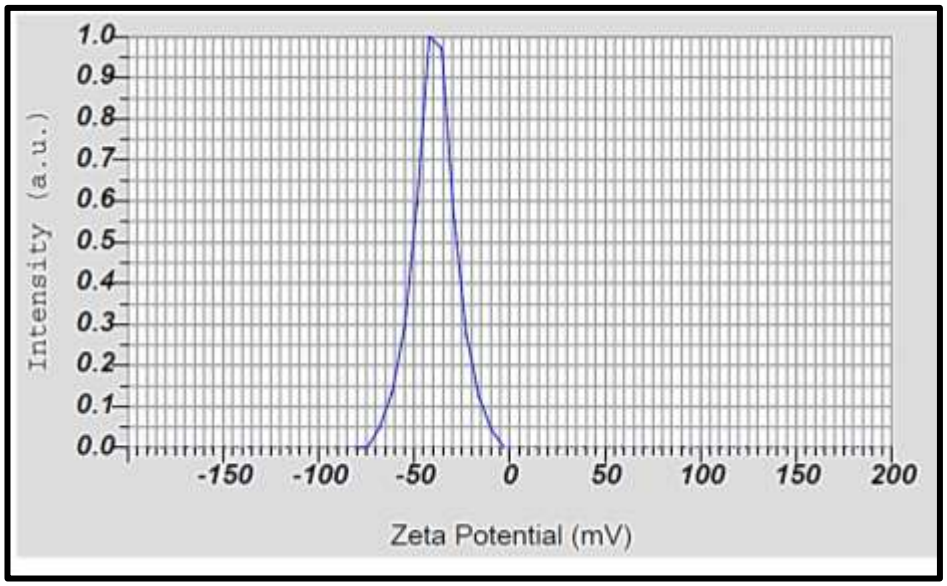

Figure 4 – Zeta potential curve of optimized batch

The second population of particles, as shown in the above figure, may be due to inadequate sonic energy at the periphery of the dispersion or to particle growth during the time period between sonication and size analysis.

Zeta Potential

The zeta potential of telmisartan loaded polymeric micelleswas measured. Zeta potential value of optimized polymeric micelles batch is found to be -39.1 mV(Fig.4).Optimized polymeric micelle batch having the negative charge on its surface.

It refers to the surface charge of the particles and indicates the degree of repulsion between close and similarly charged particles in the dispersion. The repulsion force prevents aggregation of the particles. Result of zeta potential measurement showed that surface charge of optimized polymeric micelle batch consistently negative (-31.9) which indicates that the polymeric micelles dispersion was stable.

Entrapment efficiency

The average entrapment efficiency of the prepared polymeric micelles was found to be in the range of 91.55-98.23% which was summarized in Table III. BatchF1 shows the lowest entrapment efficiency of 91.55% and preparation F9 shows highest entrapment efficiency of 98.23%.

FTIR Spectroscopy

From FTIR study, the characteristic peak of drug such as of the aromatic C=C (2468.20 cm-1), aliphatic C=O (1692.22 cm-1), aromatic C-N(1112.55 cm–1) disappeared and were replaced by the peak Pluronic F127 where remaining peaks also either shifted or were replaced in the IR spectrum of the formulation shown in Figure 5.

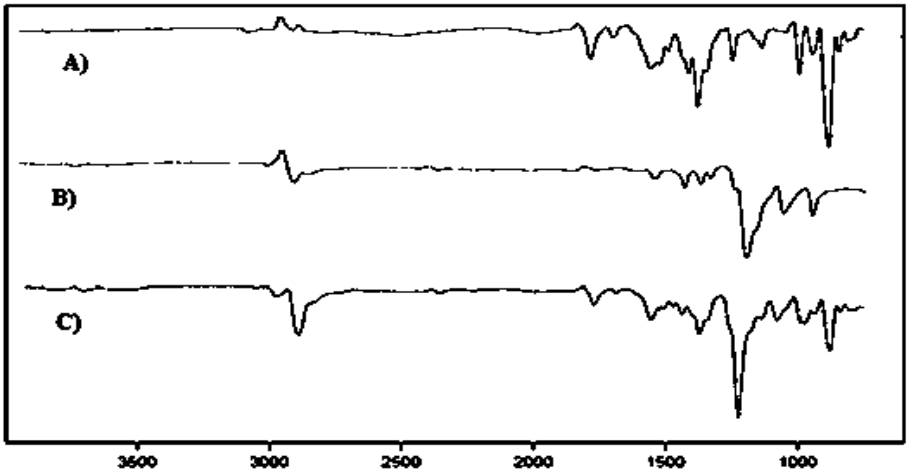

Figure.5-FTIR spectrum of TEL (A), PF127 (B), TEL + PF127(C)

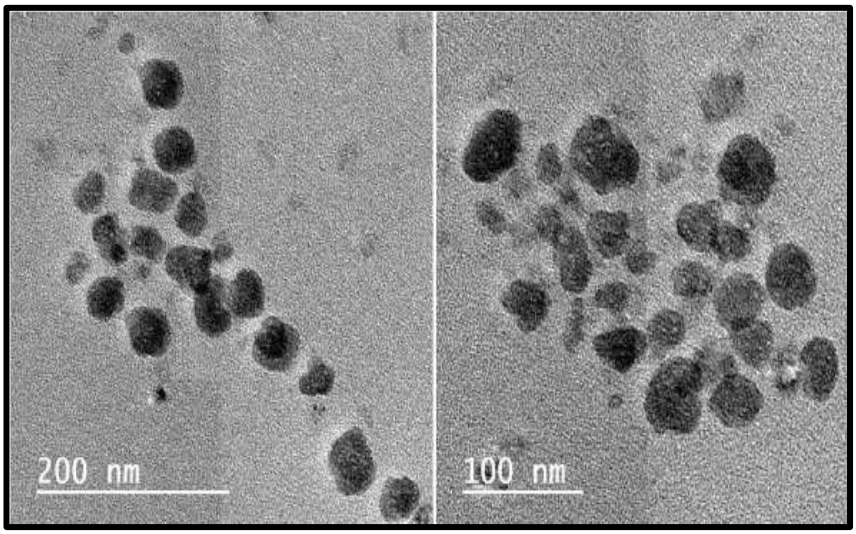

Figure 6- SEM images of optimized batch of polymeric micelles

Scanning Electron Microscopy

SEM analysis of the optimized polymeric micelle formulation was carried out to understand the morphology of polymeric micelles. Fig. 6shows the SEM image of optimized batch of polymeric micelles.

Optimized polymeric micelles batch shown spherical shaped and smooth surfaced particles. The scanning electron micrographs of the lyophilized optimized formula are shown in Fig.6. The micrograph demonstrates the porous nature of the investigated matrices. These results could support the observed rapid reconstitution due to the rapid water penetration through the lyophilized porous matrices.

Differential Scanning Calorimetric (DSC) study

The DSC thermogram of TEL, TEL+PF127 physical mixture, polymeric micells is shown in Fig. 7. The peak of TEL is completely absent in lyophilized polymeric micelles batch, while it is clearly evident in physical mixture of TEL and PF127. It has been reported that when the TEL does not show its endothermic peak in the polymeric micelles, it is said to be in the amorphous state. Hence, it could be concluded that the drug is present in the amorphous phase and may have been homogeneously dispersed in the lipid nanoparticles

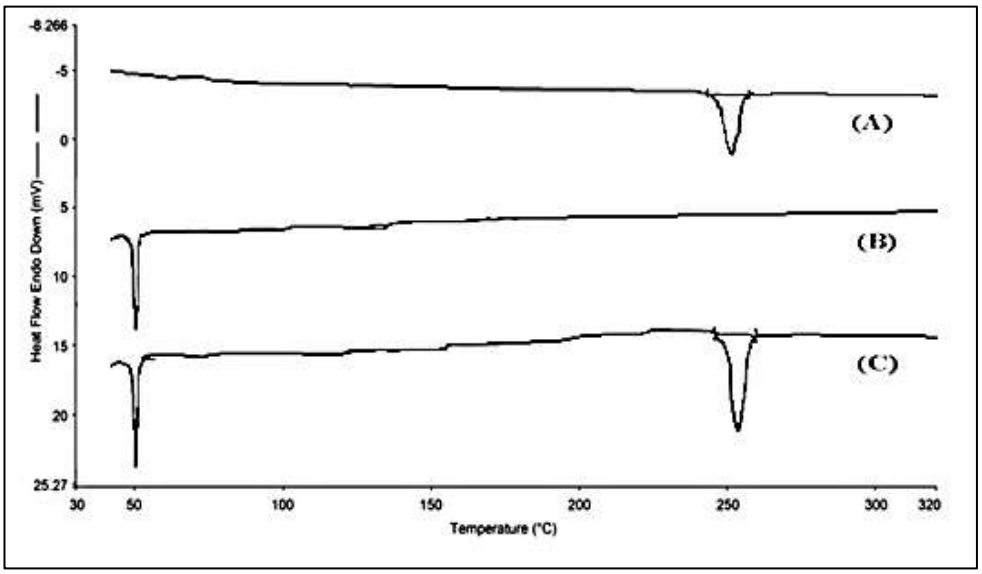

Figure.7. DSC thermogram of (A) TEL, (B) TEL- loaded polymeric micelles and (C) TEL+PF127

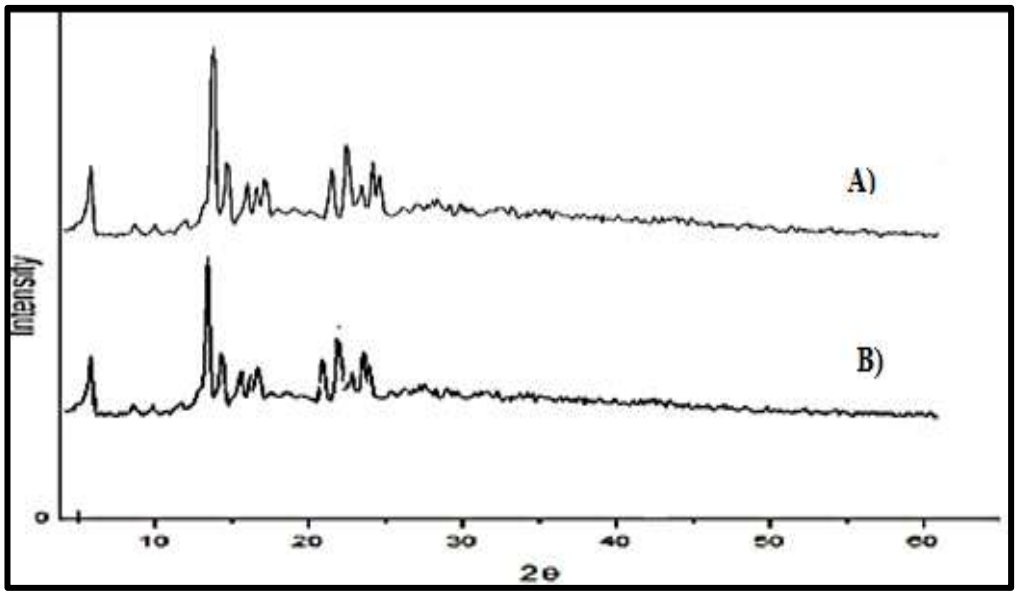

Figure 8 XRD of (A) Telmisartan and (B) TEL-loaded polymeric micelles.

X-ray Diffraction Study

From the X-ray diffraction data (Fig. 8), it is clear that pure TEL showed highly crystalline nature with principal peak at 14value, whereas the polymeric micelles formulation showed deformed peak for TEL, indicating its presence in amorphous or molecular dispersion state.

Saturation solubility study

From saturation solubility data pure drug showed less solubility after 24 and 48 hrs. and polymeric micelles formulation showed 100 folds increase in solubility in water as compare to pure drug after 24 and 48 hrs.

***In vitro*Drug Release Studies**

The in vitro drug release from plain drug suspension and polymeric micelles is plotted against different time points for 24 hours.The drug release was studied in PBS pH 1.2, 6.8, 7.4 and 7.5. The total % release from polymeric micelles shown in Table 6, 7 resp. and Fig. 9, 10, 11, 12 respectively for two medium (PBS pH 1.2, 6.8, 7.4 and 7.5)

Table no.5 Values of saturation solubility study

Sampling Time(Hrs.)	Saturation Solubility	
	Pure Telmisartan	Optimized batch of polymeric micelles
After 24 hrs.	0.06672 mg/100ml	6.8536 mg/100ml
After 48 hrs.	0.1126 mg/100ml	11.0665 mg/100ml

Table 6.% cumulative release

Time (Hr.)	% CR in PBS pH 1.2		% CR in PBS pH 6.8	
	TEL Suspension	Polymeric micelles	TEL Suspension	Polymeric micelles
1	35.21±0.783	22.03±0.02	41.34±0.12	13.15±0.79
2	47.2±0.346	34.92±0.03	48.12±0.03	24.77±0.416
3	59.32±0.665	42.72±0.025	53.48±0.125	34.61±0.695
4	64.8±1.52	61.80±0.317	61.36±0.317	37.86±0.52
5	69.07±0.138	66.091±0.594	69.38±0.294	42.57±0.0138
6	73.9±1.399	70.60±0.744	73.48±0.07	50.32±1.399
7	78.41±0.362	74.54±0.829	78.49±1.29	53.59±0.862
8	86.28±0.812	78.20±0.368	84.52±0.368	60.82±0.012
12	91.36±0.312	83.81±1.92	92.95±1.01	75.43±0.57
24	95.36±0.356	90.89±1.55	95.61±0.55	88.50±0.95

Table 7.% cumulative release

Time (Hr)	% CR in PBS pH 7.4		% CR in PBS pH 7.5	
	TEL Suspension	Polymeric micelles	TEL Suspension	Polymeric micelles
1	18.48±0.802	22.34±0.079	37.43±	25.16±0.02
2	27.18±0.23	32.26±0.04	47.41±	28.63±0.14
3	34.49±0.325	44.03±0.06	63.05±	37.33±0.061
4	42.68±0.03	52.50±0.32	71.36±	43.72±0.432
5	51.69±0.051	59.89±0.01	83.35±	53.98±0.019
6	61.8±0.07	63.11±0.399	89.36±	60.55±0.599
7	70.24±0.81	66.24±0.862	92.4±	73.49±0.062
8	75.41±0.318	69.38±0.012	94.34±	83.14±0.022
12	83.91±0.92	78.58±0.157	99.87±	91.81±0.017
24	90.4±1.13	86.31±0.095	---	99.70±0.084

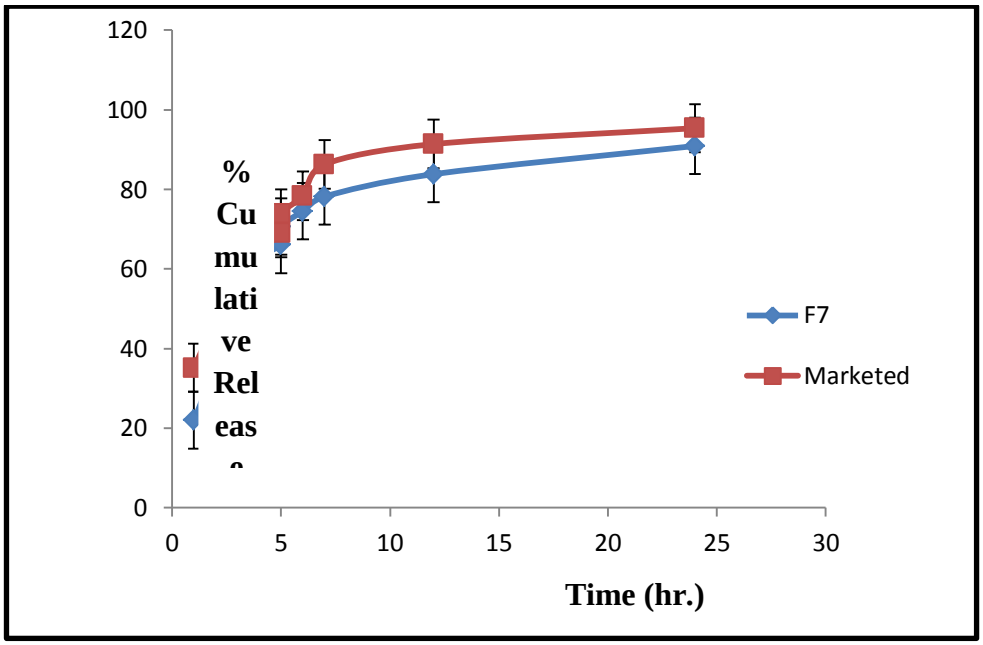

Figure 9- % Cumulative Release in PBS pH 1.2

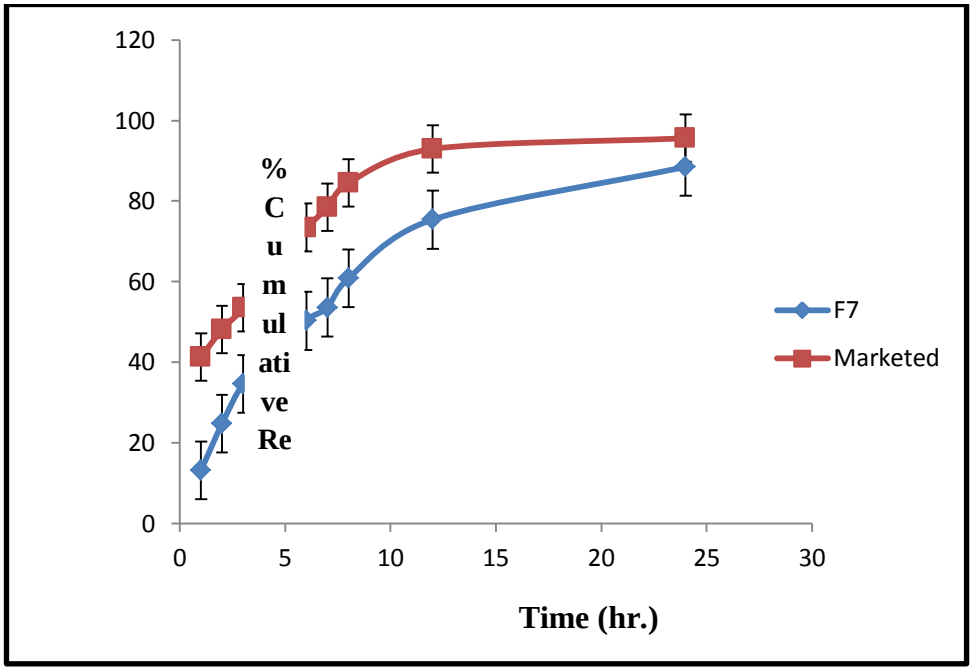

Figure 10- % Cumulative Release in PBS pH 6.8

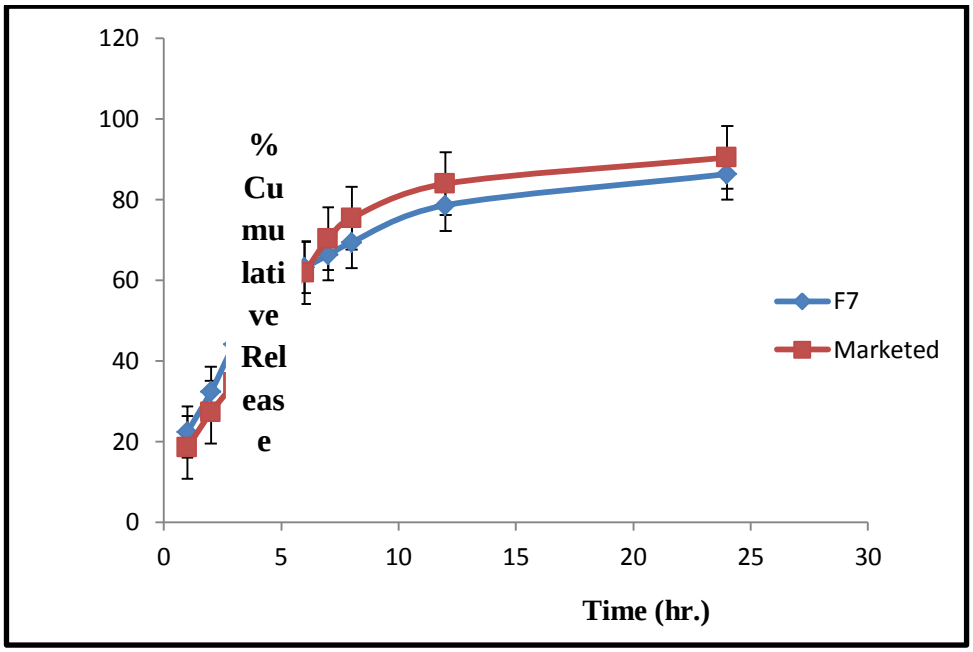

Figure-11 % cumulative release in PBS pH 7.4

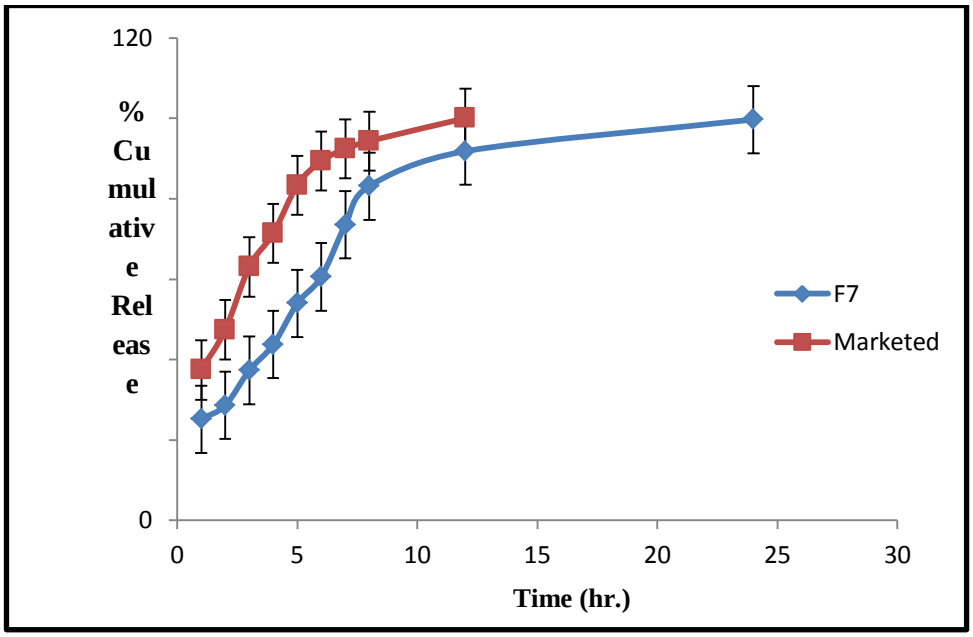

Figure- 12 % Cumulative Release in PBS pH 7.5

In PBS pH 1.2 TEL suspensions (marketed product) and polymeric micelles %CR at 24 hours was found 95.36 % and 90.89 % respectively. In PBS pH 6.8 TEL suspensions (marketed product) and polymeric micelles %CR at 24 hours was found 95.61 % and 88.50 % respectively. In PBS pH 7.4 TEL suspensions (marketed product) and polymeric micelles %CR at 24 hours was found 90.4 and 86.31 % respectively. In PBS pH 7.5 TEL suspension (marketed product) % CR at 12 hrs. was found 100 % so at 24 hr. there is no drug present in product for release and polymeric micelles % CR at 24 hr. was found 99.70%

Conclusion

The polymeric micelles were prepared by direct dissolution method followed by probe sonication of reproducible sizes in the range of 111.5 to 219.1 nm by addressing the effects of processing parameters. The application of 3^2 factorial design proved to be a useful tool for optimization of TEL-loaded polymeric micelles. Using the factorial design one can select a suitable composition of formulation to obtain TEL-loaded polymeric micelles in the size range of 111.5 to 219.1 nm depending on the application of the system. From saturation

solubility study data showed the increase in solubility of telmisartan by 100 folds. Also, the results of the *in-vitro* release study of TEL-loaded PMs has showed the drug remains for longer period of time may improve absorption of drug and hence increases bioavailability. So from all data concluded that there is increase in solubility as well as bioavailability of drug by formation of polymeric micelles.

Acknowledgement

The authors are thankful to the management of Sinhgad College of pharmacy Vadgaon Pune for providing the facilities and instruments to carry out this research work.

References

1. Kedar U.,Phutane P., Shidhaye S., KadamV., Advances in polymeric micelles for drug delivery and tumour targeting, Nanomedicine, 2010, 6, 714-729.
2. Huh K. M., Lee S. C., Cho Y. W., Lee J., Jeong J. H., Park K., Hydrotropic polymer micelle system for delivery of paclitaxel, J. Control. Release, 2005, 101, 59-68.
3. Strickley R. G., Solubilizing excipients in oral and injectable formulation, Pharm. Res., 2004,21, 201-230.
4. Kwon G. S. and Okano T., Polymeric micelles as new carriers, Adv. Drug Deliv. Rev.,1996,21, 107-116.
5. Trubetskoy V. S., Polymeric micelles as carrier of diagnostic agents, Adv. Drug Deliv. Rev., 1999, 37, 81-88.
6. GaucherG., Dufresne M. H., Sant V. P., Kang N., Maysinger D., Leroux J. C., Block copolymer micelles: preparation, characterization and application in drug delivery, J. Control. Release,2005, 109, 169-188.
7. Mohanty M. E., Vaidya J., Mishra A. K., A fluorescence study on interaction of telmisartan in triblock polymers pluronic P123 and F127, Spectrochi. Acta part A: mole. and Biomole. Spectroscopy, 2014, 121,330-338.
8. Pandya V. A. and Chaudhari S. P., Optimization and evaluation of a formulation containing low soluble antihypertensive agents, Int. j. current Pharm. Res. 2012, 4, 37-41.
9. Woraphatphadung T., Sajomsang W., Rojanarata T., Ngawhirunpat T., Opanasopit P., Polymeric micelles for enhanced solubility of meloxicam in oral drug delivery, Adv. Mate. Res., 2015, 1060, 7-11.
10. El-Dahmy R. M., Elsayed I., Elshafeefy A. H., Gawad N. A., El-Gazayerly O. N., Optimization of long circulating mixed polymeric micelles containing vinpocetine using simple lattice mixture design, in vitro and in vivo characterization, Int. J. Pharm., 2014, 477, 39-46.
11. Gambhire M. S., Bhalekar M. R., Gambhire V. M.,Statistical optimization of dithranol-loaded solid lipid nanoparticles using factorial design, Braz. J. Pharm. Sci., 2011, 47, 503-511.
12. Dhoranwala K. A.,PranavS., Shailesh S., Formulation Optimization of Rosuvastatin Calcium Loaded Solid Lipid Nanoparticles by 32 Full-Factorial Design, Nano. World Journal, 2015, 4, 112-121.

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