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Research Article

Development and Optimization of Nanoparticulate Drug Delivery System of Telmisartan by DoE Approach

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ABSTRACT

The present study was aimed to enhance the solubility and dissolution of BCS class II drug, Telmisartan (TEM), by nanoformulation approach. Several attempts were made to develop a nanosuspension by bottom-up and top-down techniques. Bottom-up techniques such as anti-solvent precipitation and emulsification solvent evaporation methods failed to reduce the size of the drug to nanoform by Poloxamer 108 and PVP K-30 at 1500–2000 rpm but resulted in micron-sized particles. However, the high pressure homogenization method has produced nanosuspension with a particle size of 112.6 nm and 0.119 PDI. Formulation and analytical development were carried out by statistical factorial design using the Design Expert software (version 11.0). The prepared nanosuspension was evaluated for particle size, entrapment efficiency, zeta potential and *in-vitro* dissolution. Zeta potential of optimized formulation was found to be −18.6 m V. Drug content and its release was estimated by the developed and validated *in-vitro* dissolution method. *In vitro* drug release studies on the optimized formulation have shown a drug release of 50.63% by the end of 6 h, whereas plain drug suspension has shown only 24.39% release, indicating a 2-fold increase of drug release with nanosuspension. It can be concluded that TEM, when formulated by high pressure homogenization technique as a nanosuspension, leads to enhanced solubility, dissolution, and stability. Thus, nanosuspension is promising approach to improve dissolution and bioavailability (BA) of Telmisartan

INTRODUCTION

Solubility is a very important characteristic of drugs; it mainly influences the efficacy of drugs in biological fluids. One of the major challenges in dosage form development of the drug molecule is its weak aqueous solubility [1]. The solubility of such poorly soluble drugs can be improved by solid dispersion technique, complexation with cyclodextrin, co-crystal approach, micronization, nanosization, etc. [2, 3]. Nanosization has been the recent and most widely used approach for solubility enhancement of drug molecules, in which the effective surface area of drug particles increases due to the reduction of drug particle size. There have been various techniques for nanoparticle formulation such as anti-solvent precipitation, high-pressure homogenization, ionic gelation, double emulsification, etc. [4, 5]. Most of the technologies used agitation, heat, sonication and organic solvents for the formulation of nanoparticles. However, this study mainly focused on ionic gelation technique because it is a simple and less time-consuming technique without using vigorous agitation, heat and organic solvent, and has industrial applicability [6]. Nanoparticulate drug delivery system with crystalline or amorphous nature has been a novel approach

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where active pharmaceutical ingredients can be stabilized by different stabilizers like polymers and surfactants [7, 8]. Higher energy state of amorphous nanoparticles has been mainly responsible for more aqueous solubility of drugs, but amorphous form has less stability as compared to crystalline form of drug. Freeze drying process has the ability to produce a stable amorphous product. This process yields porous powder with more wettability in aqueous medium. This process also has industrial applicability where larger equipment is capable to handle two steps of process (mainly freezing and drying) [9, 10]. Telmisartan has become popular for the treatment of hypertension acts, mainly on angiotensin II receptor. It appears as a white crystalline powder with poor aqueous solubility. A drug with inadequate water solubility generally shows less biological fluid availability, and ultimately leads to low physiological effect. So, attaining the desired physiological effect requires higher doses of drug ultimately leading to more toxicity. Telmisartan is class II drug of biological classification system which is rapidly absorbed through gastro-intestinal tract after dissolution. Thus, in spite of its high permeability through the biological membrane, dissolution is the rate limiting step for the absorption of telmisartan in biological system [11, 12]. The present study was aimed to enhance the solubility and dissolution of BCS class II drug, Telmisartan (TEM), by nanoformulation approach.

MATERIAL AND METHODS

Material

Telmisartan obtained as a gift sample from SVK Laboratories Pvt. Ltd, Hyderabad Maharashtra. Koliphor P-407 and Koliphor P-188 were procured from BASF The chemical Company. All the reagents used for nanoparticle development in the study were of analytical grade and were obtained from Fine Chemical, Mumbai.

Methods

Formulation Development of TEM Nanosuspensions

Bottom-up Anti-Solvent Precipitation Method

Accurately weighed pure TEM was dissolved in solvent at room temperature. The above solution was poured into a beaker having a fixed volume of anti-solvent containing polymer at room temperature with a stirring speed of 1500 rpm using mechanical Remi stirrer. The above solution was stirred continuously for 2 h to evaporate the solvent. The formed nanosuspension was transferred into a container and stored at 25°C until further use (Table 1) [13].

Table 1: Formulation of Telmisartan loaded nanoparticulate drug delivery system

Sr. No.	Formulation code	Formulation Composition							
		TLM (mg)	PVP K30 (mg)	P- 407 (mg)	P- 108 (mg)	P- 188 (mg)	SOLUPLUS (mg)	PLG A (mg)	Water (Up to 100mL)
1	NCH1	100	100	100	-	-	-	-	100
2	NCH2	100	100	200	-	-	-	-	100
3	NCH3	100	100	300	-	-	-	-	100
4	NCH4	100	100	-	100	-	-	-	100
5	NCH5	100	100	-	200	-	-	-	100
6	NCH6	100	100	-	300	-	-	-	100
7	NCH7	100	100	-	-	100	-	-	100
8	NCH8	100	100	-	-	200	-	-	100
9	NCH9	100	100	-	-	300	-	-	100
10	NCH10	100	100	-	-	-	100	-	100
11	NCH11	100	100	-	-	-	200	-	100
12	NCH12	100	100	-	-	-	300	-	100
13	NCH13	100	100	-	-	-	-	100	100
14	NCH14	100	100	-	-	-	-	200	100
15	NCH15	100	100	-	-	-	-	300	100

Experimental Design

The formulation of nanosuspension was optimized by using Box-Behnken design. The three factors were concentration of polymer (Poloxamer-108), No. of HPH cycle and HPH pressure.

The effect of these factors was analyzed on the dependent variables particle size (Y1) and entrapment efficiency (Y2). A total of 13 runs were generated using software.

The data were treated using trial software Design-Expert (Version 12.0, Stat- Ease Inc., USA), and analyzed statistically using analysis of variance (ANOVA). The data were also subjected to 3-D response surface methodology and study the interaction of concentration of Poloxamer-108(polymer), No. of HPH cycle and HPH pressure on dependent variables. For optimization, the effects of independent variables upon the responses were modeled using the following mathematical model generated by Box- Behnken (Table 2).

Table 2: Factors (Independent variables), factor levels and responses (Dependent variables) used in Box-Behnken design

Factors	Type of Factors	Levels		Response	Type of Response
		-1	+1		
X1	Polymer conc.(%w/w)	100	300	Y1	Particle size
X2	HPH Cycle	10	40	Y2	Entrapment Efficiency
X3	HPH Pressure (bar)	500	1000	-	-

Characterization of Telmisartan-polymeric Nanosuspension:

Entrapment Efficiency

TLM loaded nanosuspension is centrifuged at 3000 rpm for 15min. After 15min, remove the free drug and collect the supernatant. Withdrawn 1mL supernatent with the help of micropipette and volume of 1mL supernatent is adjusted by organic solvent in such a way that gives a theoretical concentration. Determine the Conc. Of drug in organic solvent and back calculate actual loading.

Particle Size and Size Distribution

From the results obtained by above characterization tests, *in vitro* drug release study, drug content Batch, NCH6, NCH7, NCH12 selected for particle size and size distribution. The particle size and Particle size distribution of optimized batch NCH6, NCH7, NCH12 were analyzed using Malvern Particle size and zeta analyzer (Serial Number: MAL1098084, Zeta sizer Ver. 7.12). The sample was placed in cuvette with necessary dilutions and it was then kept in analyzer to determine the Particle size and Particle size distribution.

Zeta Potential

The Zeta Potential of Nanoparticles is a typical method for determining the surface charge property of Nanoparticles. It reflects a particle's electrical potential and is influenced by the particle's composition as well as the medium in which it is scattered. Then nanosuspension stability is determined by the zeta potential. The Zeta Potential of nanosuspension is governed by both the stabilizer and the medication. The Zeta Potential of batch was analyzed using Malvern Zeta analyzer (Serial Number: MAL 1098084, Zeta sizer Ver. 7.12).The sample was placed in cuvette with necessary dilutions and it was then kept in analyzer to determine the Zeta potential.

***In Vitro* Drug Release Study**

Telmisartan release from NPs was investigated using the dialysis method at 37.2°C room temperature and compared to the pure drug solution. The drug solution and 20 mg of Telmisartan NPs were inserted in MWCO 12000 dialysis tubes and firmly sealed. The tubes were then submerged in 50 mL of PBS release medium (pH 6.8). The samples (5 mL) were withdrawn from the release medium at predetermined time intervals (1, 2, 3, 4, 5, 6, 24 hrs.), while stirring it with the magnetic stirrer at 300 rpm / min. was replaced with fresh medium. The 5 mL of material was extracted and evaluated using UV spectroscopic technique at 421 nm. NCH6, NCH7, NCH12 were chosen for Particle size and size distribution, Zeta Potential analysis based on the results of the above characterization tests drug content and *in vitro* drug release batch.

Optimization of Telmisartan Nanosuspension:
Optimization of Independent Variables:
Based on the study result i.e. effect of concentration on polymer. HPH pressure and No. of HPH cycles on the responses particle size and entrapment efficiency, the concentration of stabilizer and No. of HPH cycle were optimized.

Optimization of Dependent Variables
The effect of polymer concentrations, no. of HPH cycles and HPH pressure on particle size was evaluated using contour plot and 3D surface response plot. On the other hand the effect of polymer concentrations, no. of HPH cycles and HPH pressure on entrapment efficiency was evaluated using contour plot and 3D surface response plot.

Table 3: Statistically analysis summary of all design of experiments involving each dependent variable or a response

Variables	Analysis of Variance						
	f- value	p- value	R-Squared (R^2)	Adjusted R^2	Predicted R^2	Coefficient of Variance	Adequate Precision
Particle size	1. 50	0.3414	0.7298	0.2434	1.2057	4.63	3.983
Entrapment Efficiency	1.13	0.471	0.6705	0.0773	3.9403	5.06	3.954

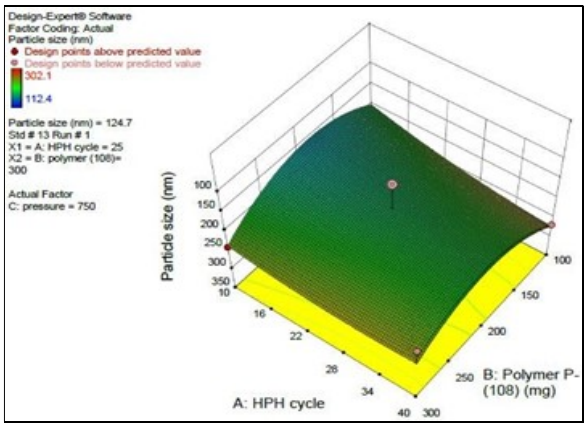

Figure 1: Surface response surface plot for dependent variables of nanosuspension with particle size as a response

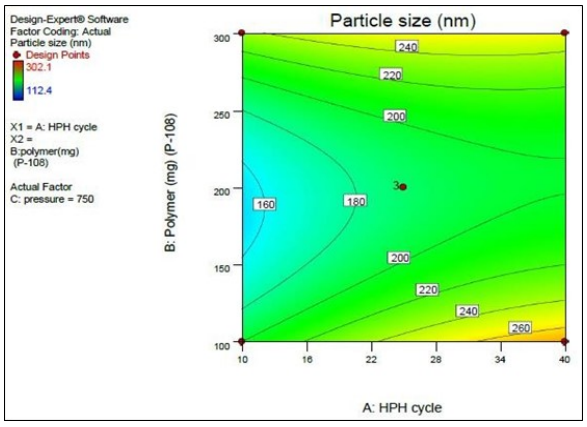

Figure 2: Contour plot for dependent variables of nanosuspension with particle size as a response

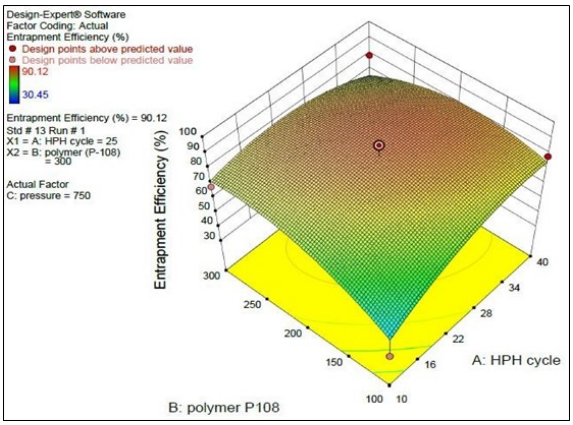

Figure 3: Surface response surface plot for dependent variables of nanosuspension with entrapment efficiency as a response

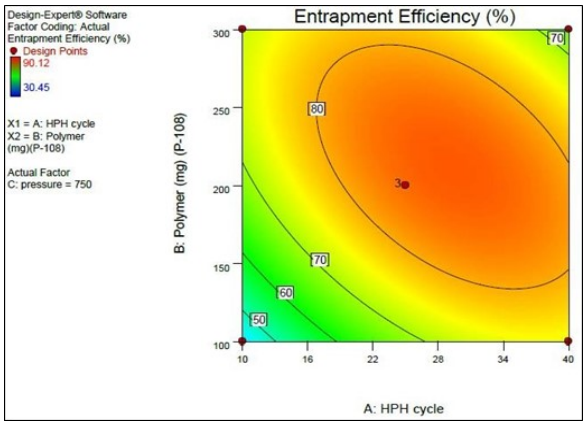

Figure 4: Contour plot for dependent variables of nanosuspension with entrapment efficiency as a response

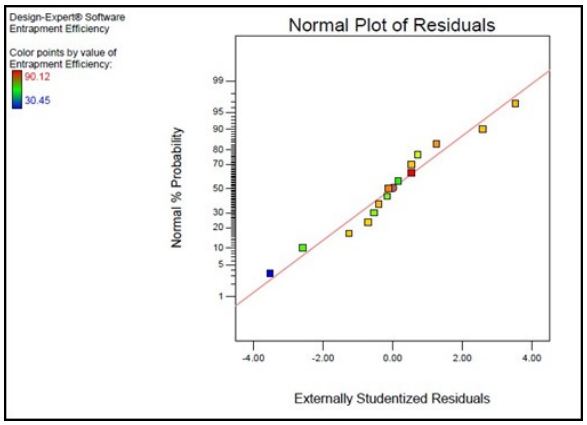

Figure 5: Normal residual plot for entrapment efficiency as a response

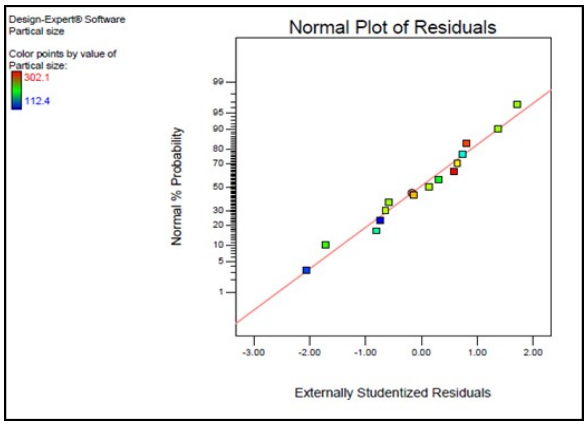

Figure 6: Normal residual plot for particle size as a response

These plots further help to understand the correlation between independent and dependent variable (response).

Response 1 (Particle Size Y1)

ANOVA was applied to estimate the significance model and individual response parameters. The Model F-value of 1.50 implies the model is not significant relative to the noise. There is 34.14 % chance that a F-value this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case there are no significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Lack of Fit F-value" of 0.50 implies the Lack of Fit is not significant relative to the pure error. There is a 71.80% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit. A negative "Pred R-Squared" implies that the overall mean may be a better predictor of your response than the current model. "Adeq Precision" measures the signal to noise ratio. A ratio of 3.98 indicates an inadequate signal and we should not use this model to navigate the design space.

Response 2 (Entrapment Efficiency Y2)

ANOVA was applied to estimate the significance model and individual response parameters. The Model F-value of 1.13 implies the model is not significant relative to the noise. There is a 47.11 % chance that a F-value this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case there are no significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Lack of Fit F-value" of 8.43 implies the Lack of Fit is not significant relative to the pure error. There is a 10.79% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit. A negative "Pred R-Squared" implies that the overall mean may be a better predictor of your response than the current model. "Adeq Precision" measures the signal to noise ratio. A ratio of 3.95 indicates an inadequate signal and we should not use this model to navigate the design space.

RESULT AND DISCUSSION

% Entrapment Efficiency:

EE was determined using UV spectroscopy by liquid formulation and results of that were shows in the Table 4. Entrapment efficiency of batch NCH6 shows maximum entrapment efficiency i.e. 90.18% as compared to other 14 batches.

Table 4: % Entrapment efficiency batches

Formulation Code	Entrapment Efficiency %	Formulation Code	Entrapment Efficiency %
NCH 1	30	NCH 9	39.50
NCH 2	83.74	NCH 10	80
NCH 3	23.2	NCH 11	43.43
NCH 4	87.81	NCH 12	76.75
NCH 5	56.76	NCH 13	52.3
NCH 6	90.18	NCH 14	59.3
NCH 7	81.60	NCH 15	69.9
NCH 8	73.18		

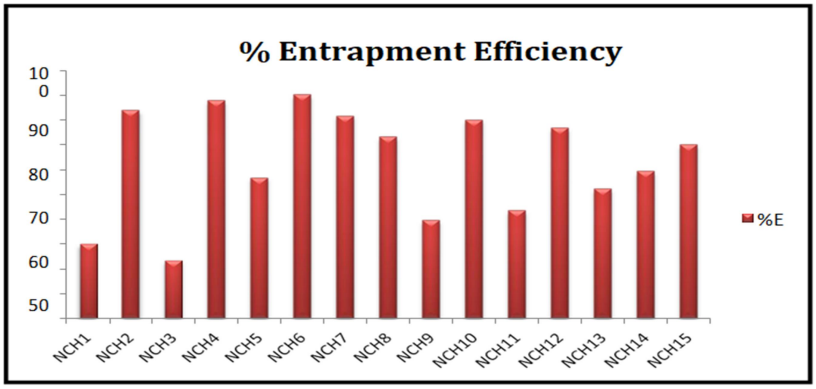

Figure 7: %EE of Nanosuspension

Particle Size and Size Distribution:

NCH 4, NCH 6, NCH 7 and NCH 10 show the higher entrapment efficiency as compare to other formulation, hence this four formulation selected for particle size and size distribution analysis (Fig. 8 to 11). The sizes of Polymeric nanoparticles were found in nm; therefore we could expect better drug release as compared to pure drug. Particle size above batches reduces due to high pressure homogenization method & its impact. High pressure homogenization method helps to reduce the Particle size from micro to nano & hence improves solubility and rate of dissolution. From the result of particle size analysis batch NCH6 shows minimum particle size 112.6nm as compared to other 14 batches. So batch NCH6 is considered as optimized batch from result of both drug content and particle size analysis (Table 5).

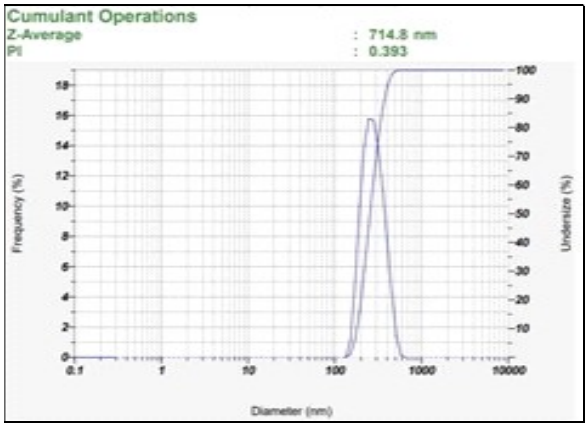

Figure 8: Particle size and size distribution NCH 4

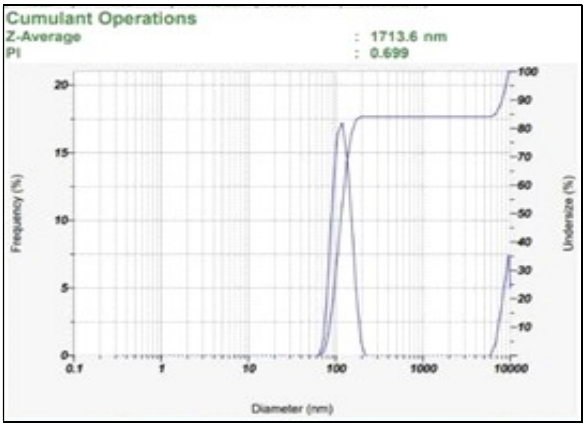

Figure 9: Particle size and size distribution NCH 6

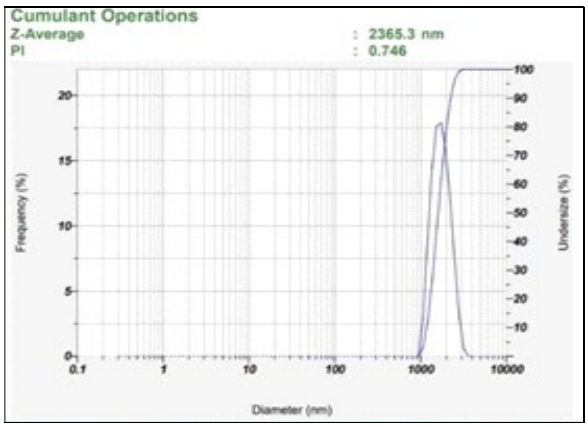

Figure10: Particle size and size distribution NCH 7

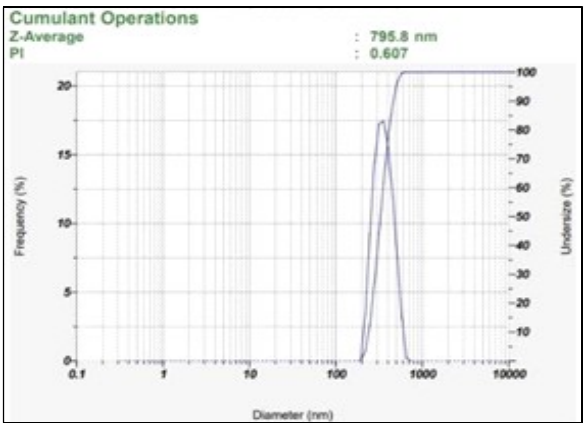

Figure 11: Particle size and size distribution NCH 10

Table 5: Particle size of different batches

Formulation Batch	Particle size	PDI
NCH4	1665.0 nm	0.746
NCH6	112.6 nm	0.370
NCH7	165.1 nm	0.457
NCH8	235.7 nm	0.482

Zeta Potential:

Batches (NCH4, NCH6, and NCH7) had Zeta potentials of 26.2mV, 18.6mV, and 18.7mV, respectively (Fig. 12 to 14). Colloidal stability is achieved when the voltage is greater than +30 mV or lower than -30 mV; however, a value approaching zero indicates instability and fast coagulation. Even though the measured Zeta Potential was low, batch NCH6 stabilized Telmisartan nanoparticles were relatively stable in our investigation (-26.2) Using this standard range of zeta potential, it was determined that all of the manufactured batches were colloidally stable (Table 6).

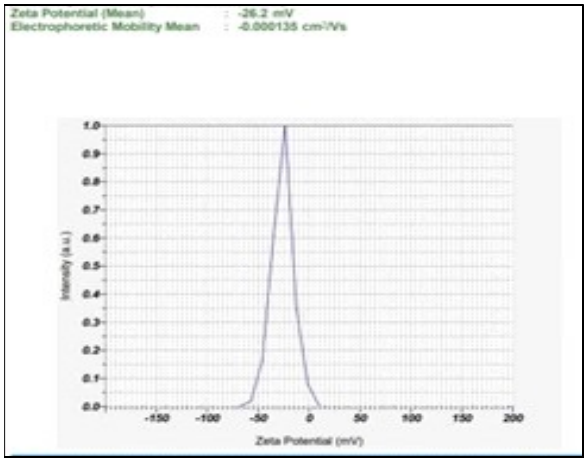

Figure 12: Zeta Potential NCH4

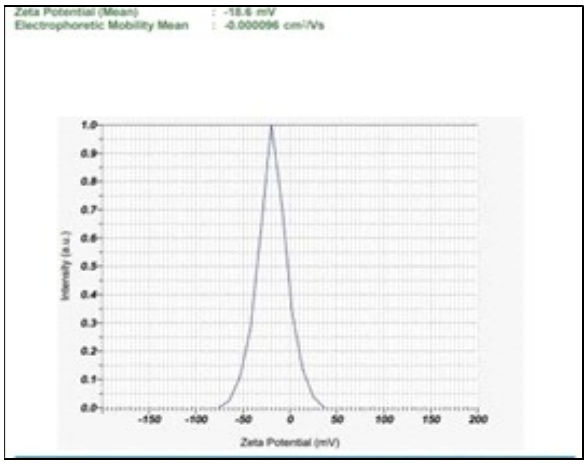

Figure 13: Zeta Potential NCH6

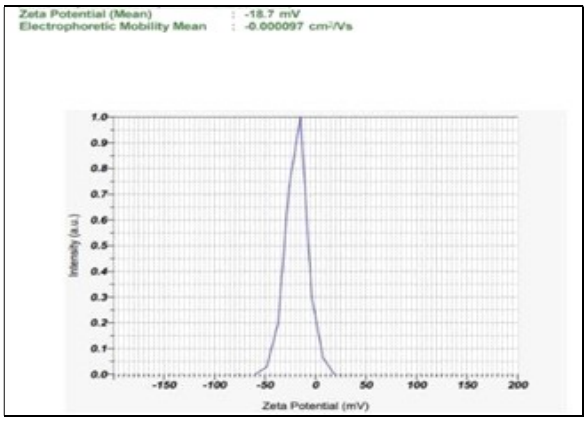

Figure 14: Zeta Potential NCH 7

Table 6: Data of analysis of Zeta Potential for bathes

Formulation Batch	Zeta Potential
NCH7	-26.2 mV
NCH6	-18.6 mV
NCH12	-18.7 MV

***In Vitro* Dissolution Using Dialysis Bag:**

From the results obtained by above characterization tests % entrapment efficiency batch NCH6, was selected for *in-vitro* drug release study (Table 7 and Fig. 15).

Table 7: Drug release study of plain drug Telmisartan and optimized batch

Sr. No	Time in hrs	Plain Telmisartan	Optimized batch (NCH6)
1	1	2.05±0.0081	6.23±0.0124
2	2	4.44±0.0124	12.29±0.0169
3	3	8.32±0.0125	13.48±0.0205
4	4	11.40±0.0128	24.12±0.0124
5	5	14.58±0.0167	40.92±0.0124
6	6	20.14±0.0169	47.38±0.0205
7	24	34.26±0.0163	56.63±0.0124

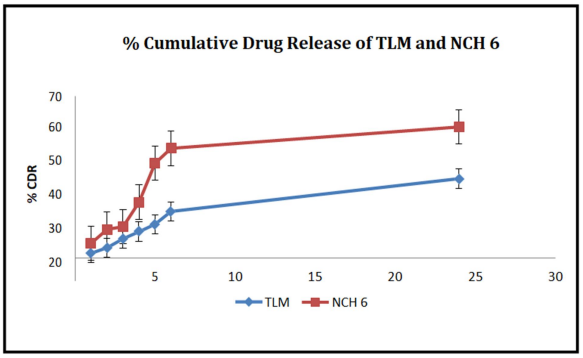

Figure 15: % cumulative drug release of Telmisartan and optimized Batch Using Dialysis

CONCLUSION

Nanoparticulate Drug Delivery System of Telmisartan by DoE Approach can be successfully prepared by Bottom-up anti-solvent precipitation method with different polymers like PVP K30, P-407, P-108, P-188, Soluplus and PLGA. Based on drug release and Nanoparticle properties, NCH 6could be considered as promising formulations.

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