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Research Article

Preparation and In-Vitro Evaluation of Nanosuspension of Anti-Hypertensive Drug Using Factorial Design

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ABSTRACT

Telmisartan is member of BCS class II with oral bioavailability of 40% and antagonizes the action of non-peptide amphiphilic AT1 receptor. In present study, the author aimed to modify its dissolution profile by formulating in nano suspension form by using solvent evaporative technique. Various combination of stabilizer and a surfactant are used and their concentrations were optimized for cumulative percentage release, saturation solubility and particle size (nm) by the means of 3^2 full factorial design. The particle size of the optimized batches were found to be of 335.5 ± 8.45 nm, 554.6 ± 15.11 nm, respectively. The results of in vitro drug release study of optimized batches exhibited significant improvement in the dissolution rate and saturation solubility when compared with marketed formulation.

INTRODUCTION

Hypertension (HT) / high blood pressure is most common non-communicable cardio vascular disease, which affects mainly heart, brain, kidneys and blood vessels. Long term HT can cause ischemia, cardiac arrest, atherosclerosis, congestive heart failure (CHF) [1]. According to Global Health Observatory (GHO) data 2015, 1.13 billion people are affected by HT. In India almost 25.8 % people are affected by it, which means 1 in every 3 people [2]. Developing countries have high dominance of hypertension as compared to developed countries such as UK, USA, Canada and Korea. With the development and advancement in drug discovery, a large number of drugs are available. Many single and fixed combination doses of different strength are available in market to tackle HT related issues [3-4]. Many existing anti HT treatments or drug formulations are suffering poor solubility and permeability related issues. To overcome these situation modifications in aqueous solubility, hence enhancement of dissolution rate as well as bioavailibity is one of promising method [5-6]. Several methodologies such as complexation, use of co-solvents, micellar/ polymorphic formulations are currently used for enhancing solubility, dissolution rate and absorbability of marketed drugs [7]. Nanosuspension (NS) formulation is the one of the most significant strategies to get better solubility of poor water/lipid soluble molecules. They can be defined as stabilized colloidal biphasic system comprising of pure drug molecules dispersed in an aqueous vehicle, having diameter less than 1µm [8,9]. Various approaches are used generally for the preparation of nanosuspension such as top-down and bottom-up techniques [10, 11]. It was observed that, NS may lead to enhance the rate of flooding of the active compound, which ultimately maximizes plasma level of drugs [12-14]. Our present work has focus on preparation of optimum nanosuspension of drug telmisartan (TEL) to improve its saturation solubility and dissolution rate using factorial design. It is a highly selective non-peptide amphiphilic AT1 receptor antagonist and primarily used against hypertension. It is member of BCS class II with log P 7.7 and poor bioavailability (around 40-42 %) [15].

MATERIALS AND METHODS

Materials: Telmisartan was procured from Yarrow Chem Product, Mumbai, India. Polymers such as PVP K-30, HPMC and solvents used are

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purchased from Loba Chem Pvt. Ltd., Mumbai, India and were of analytical grade.

Preparation of Telmisartan Nanosuspension

Solvent evaporative technique was used prepare telmisartan nanosuspension. Preliminary studies were done to evaluate its solubility using various solvents. Acetone was selected as solvent along with surfactant PVP K30 and HPMC (hydroxyl propyl methyl cellulose) as a stabilizer. The process initiates with dissolution of PVP K30 in water (which further act as antisolvent) using homogenization at speed of 2000 rpm and labelled as solution A. Next, drug telmisartan will added in portion wise into a beaker containing HPMC E5 and acetone and labelled as solution B. The drug containing mixture (solution B) was stirred for almost 15 minutes to obtain a clear solution. After that, prepared aqueous solution (solution A) was mixed slowly with non-aqueous phase (solution B) over a time period of 20 minutes. Later the resulting solution was stirred for 12 hrs at 3000 rpm. The prepared suspensions were allowed to kept undisturbed to dissipate foam and subjected to particle size analysis.

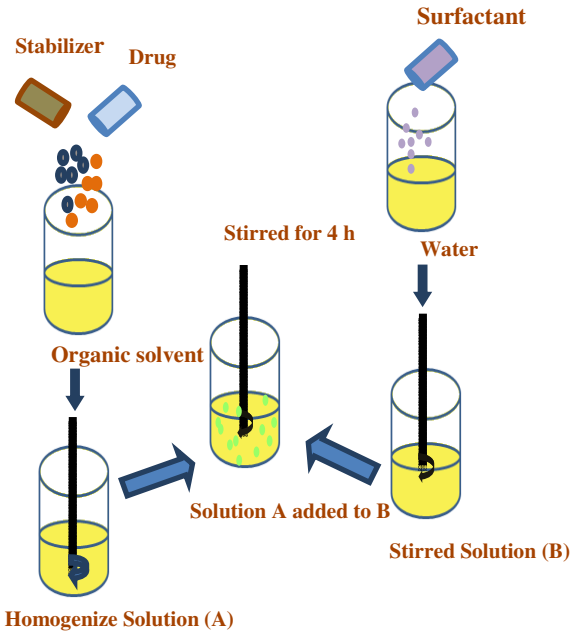

Figure 1: Preparation of Nanosuspensions

Experimental Design

The procedure involves application of full factorial design (3^2) to understand the outcomes of variables (stabilize and surfactant) at 3 levels on particle size of formulation. The formulation with nano metric size range were selected as optimized formulations which were characterized further like saturation solubility, viscosity, FTIR analysis, scanning electron microscopy, *in-vitro* drug release study and drug release kinetics [16]. Based on the experimental design it is evaluated by the imperial factorial by implementing 3^2 based factorial design on the outcome of critical factors like ratio of stabilizers, stirring speed properties of TEL NS. Experimental design consists of total of 9 runs (coded as F1-F9) and each of them is formulated as shown in the Table 1. The experimental factorial design involves total of 9 trials with 3 factors and 2 levels. The independent variables (X1, Y2 levels) selected for the study were PVP K30 and HPMC of different concentration and the dependent variable (Y) was the particle size of the NS.

Table 1: Layout of 3^2 factorial design batches of telmisartan nanosuspension

Batch code	variable levels (in code)		Actual value of variables	
	X1	X2	X1(PVPK30 in mg)	X2 (HPMC in mg)
F1	-1	-1	3	6
F2	0	-1	6	6
F3	1	-1	9	6
F4	-1	0	3	18
F5	0	0	6	18
F6	1	0	9	18
F7	-1	1	3	30
F8	0	1	6	30
F9	1	1	9	30

* X1-PVP-K30, X2-HPMC, Low (-1), Medium (0), High (1)

Table 2: Formulation of telmisartan nanosuspension

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug (mg)	30	30	30	30	30	30	30	30	30
PVP K30 (mg)	3	6	9	3	6	9	3	6	9
HPMC (mg)	6	6	6	18	18	18	30	30	30
Acetone (mL)	5	5	5	5	5	5	5	5	5
Water (mL)	20	20	20	20	20	20	20	20	20

Evaluation of Nanosuspension

Particle Size Analysis

For particle size analysis the prepared formulations (1 mL) of solution was diluted with (4 mL) of deionized water and vortexed for about 30 sec. Then the sample is kept in the cuvette of Malvern Nano-ZS (Malvern Instruments. UK) and the sample was run for the

analysis of particle size. On the basis of analysis, the best formulations which were having particle size in nano range were selected as optimized formulations and were further characterized using drug content, saturation solubility, viscosity, in-vitro release profile and the mechanisms of drug release (release kinetics) by the application of different mathematical models, FTIR and SEM.

FT-IR Spectroscopy Studies

Drug and the other excipients were analysed on FTIR (Shimadzu FTIR 8200-S) by using potassium bromide (KBr) pellet method. The finely grounded powder of Telmisartan drug was mixed with the powdered potassium bromide and was pressed with a specific hydraulic compression. The prepared KBr pellets were then put against the pathway on FTIR to record the spectrum of drug scanning in a range from 4000-400 cm^{-1}.

Drug-Excipients Compatibility Studies

Drug excipients compatibility was performed to ensure that the chosen excipients do not show any effect in the stability and safety of the drug during the formulation by effecting by the physical and chemical of the dosage form. It is the important phase during the preformulation stage for drug development which later effects on the bioavailability of the formulation. The drug excipients and physical mixture compatibility studies were carried out (Telmisartan, HPMC and PVP K30) in 1:1 ratio at room temperature. After 1 month samples were analysed based on the colour and physical changes and the data was recorded in Table 4 [17].

Evaluation of Drug Content

For the evaluation of drug content in the sample, the optimized batches (10mL each) were taken and centrifuged at 1000 rpm for 15 minutes. The aliquots was collected from supernatant and analysed at detected wavelength using UV-spectrophotometer after the proper dilutions with buffer. The content of drug was calculated using straight line equation obtained from the calibration curve [18].

Saturation Solubility Study

Saturation solubility study was performed by putting optimized formulations (30 mL each) in orbital shaker bath maintained at a temperature 37±1°C for a period of 48 hours. Simultaneously a solution of drug in pH 6.8 phosphate buffer was also prepared and exposed to same set of conditions. Finally samples were taken from each and analysed spectrophotometrically to find out and compare the solubility of drug solution and optimized formulations [19].

Viscosity Study of the Formulation

Viscosity of the optimized Telmisartan nanosuspensions was determined by using Brookfield Viscometer. A spindle (63) was used for the determination of viscosity of formulations. The prepared nanosuspension formulations were dipped into the spindle of viscometer up to the mark and the sample were run and the data value is recorded [20].

***In-Vitro* Dissolution Study**

Dissolution studies were performed for the optimized formulations using 900 mL of pH 6.8 phosphate buffer solution maintained at a temperature 37±0.5 °C. USP Type II apparatus (paddle type) was employed for the study (Lab India DS-8000). Test was performed at 50 rpm and the samples of 5mL were withdrawn at time intervals of 5, 10, 15, 30, 60, 120, 240 and 480 minutes. They were filtered and diluted if necessary and the absorbance was taken on UV-Spectrophotometer at detected wavelength to find out the amount of drug released. The study was performed in triplicate.

Drug Release Kinetics Study

An appropriate drug release test is required to characterize the drug product and ensure batch-to-batch reproducibility and consistent pharmacological/biological activity. The dissolution data were analysed on the basis of zero order model, first order rate, Higuchi model, Hixson-Crowell model and Korsmeyer-Peppas model. The correlation coefficient (r^2=0.999) for each kinetic model was calculated. The appropriate equation was chosen on the basis of best fit line [21-23].

Scanning Electron Microscopy (SEM)

It is a type of electron microscope that creates the images of sample by scanning the surface with a focused beam of electrons in a raster pattern, with the interaction of atoms in the sample, by producing various signals that contain information about surface topography and composition of the sample.

Surface morphology of the optimized formulations was determined by using scanning electron microscopy. The samples were operated at the acceleration voltage of 20.0 kV of electron beam and scanned the surface of sample with an

acceptable depth of field. For SEM, samples were prepared by clinging the nanoparticles on the double adhesive tape stuck to the aluminium stub and were coated with gold and palladium under an argon atmosphere by using high vacuum and the samples were analysed [24].

RESULTS AND DISCUSSION

Particle Size Analysis Studies

Particle size and the size distribution are the crucial parameters in the case of long term stability. Particle size and size distribution analysis was performed for the prepared formulations of different batches. All the formulations were shown in the range of 335-1430 nm. Among all the formulations, F2 (335 nm) F7 (554.6 nm) were selected ass optimized formulations based on their nanometric size. Moreover, both of these formulations were having Polydispersity index (PDI) near to 0.4 which indicated their particle size uniformity and stability also. The nano metric size range with these formulations clearly indicated that a combination of medium level of surfactant and medium to high level of stabilizer were able to give formulations with desirable size range. Fig. 2 and 3 shows the optimized batch of F2 and F7 particle size.

Table 3: Response parameters for various runs of the formulation batches

Batch code	Variable levels (code form)		Actual variables		Dependent variables
	X1	X2	X1	X2	
F1	-1	-1	3	6	894
F2	0	-1	6	6	335
F3	1	-1	9	6	996.5
F4	-1	0	3	18	1430
F5	0	0	6	18	820.3
F6	1	0	9	18	1186
F7	-1	1	3	30	554.6
F8	0	1	6	30	1241
F9	1	1	9	30	780.8

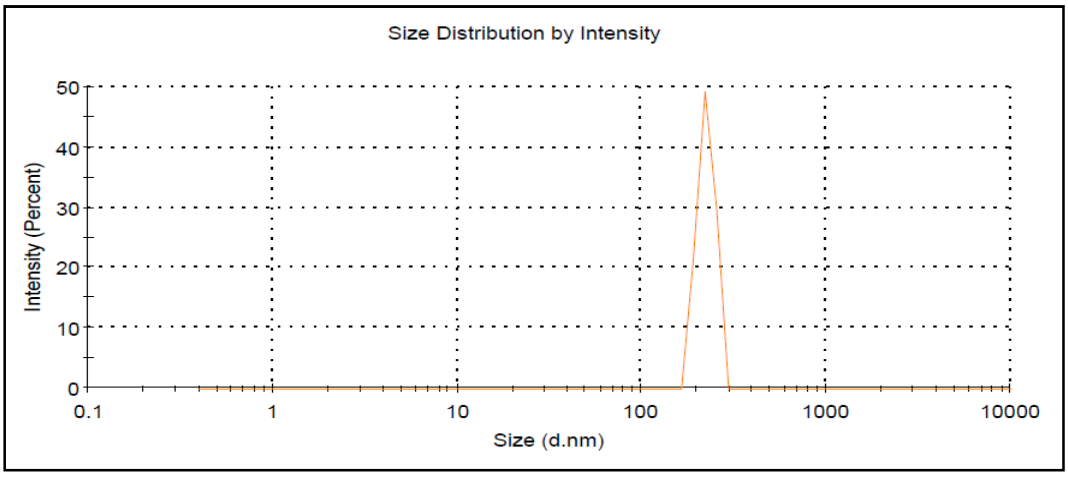

Figure 2: Particle size of batch F2

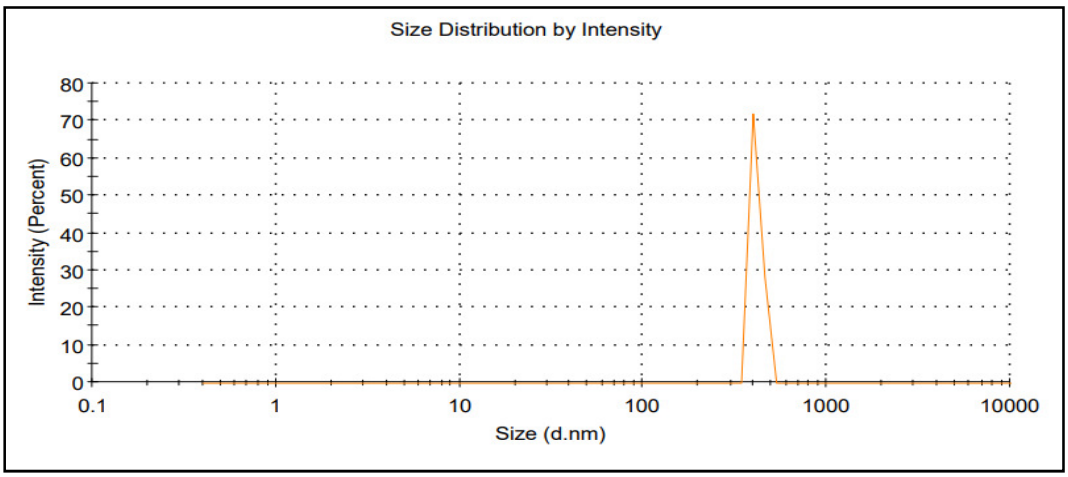

Figure 3: Particle size of batch F7

FTIR Analysis

The FTIR spectra of drug and excipients were recorded. FTIR spectrum showed different bands for specific functional groups, present in Telmisartan. the spectrum was recorded in the wavelength region of 4000–400 cm^{-1} as shown The drug telmisartan showed the stretch bands of O-H group at 3375 cm^{-1}, C-H stretching at 2970 cm^{-1}, C=O stretching at 1691 cm^{-1} and C-H bending at 1444 cm^{-1}, respectively. Thus the presence of mentioned groups with their corresponding band positions confirmed the identity of the drug. The Fig. 4 shows the spectrum of telmisartan and Fig. 5 and 6 shows the optimized formulations spectra.

Drug-Excipient Compatibility Studies

Compatibility studies of telmisartan with HPMC and PVP K-30 were analyzed for the physical changes and it was observed that no change in colour, appearance along with the other excipients under the stress conditions as shown in the Table 4.

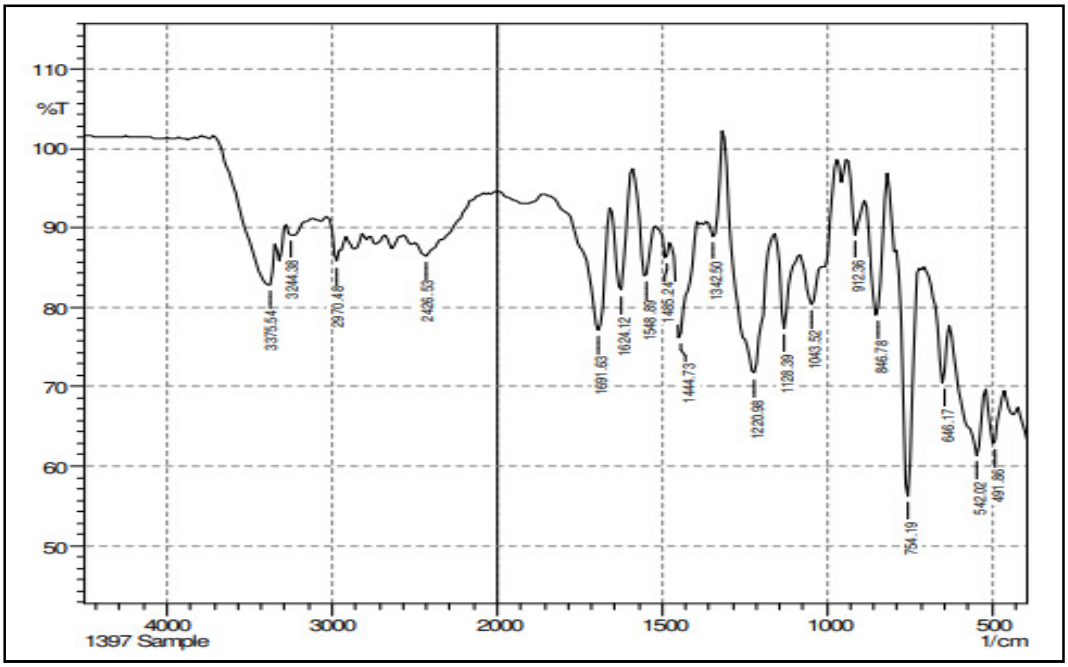

Figure 4: FT-IR spectrum of telmisartan

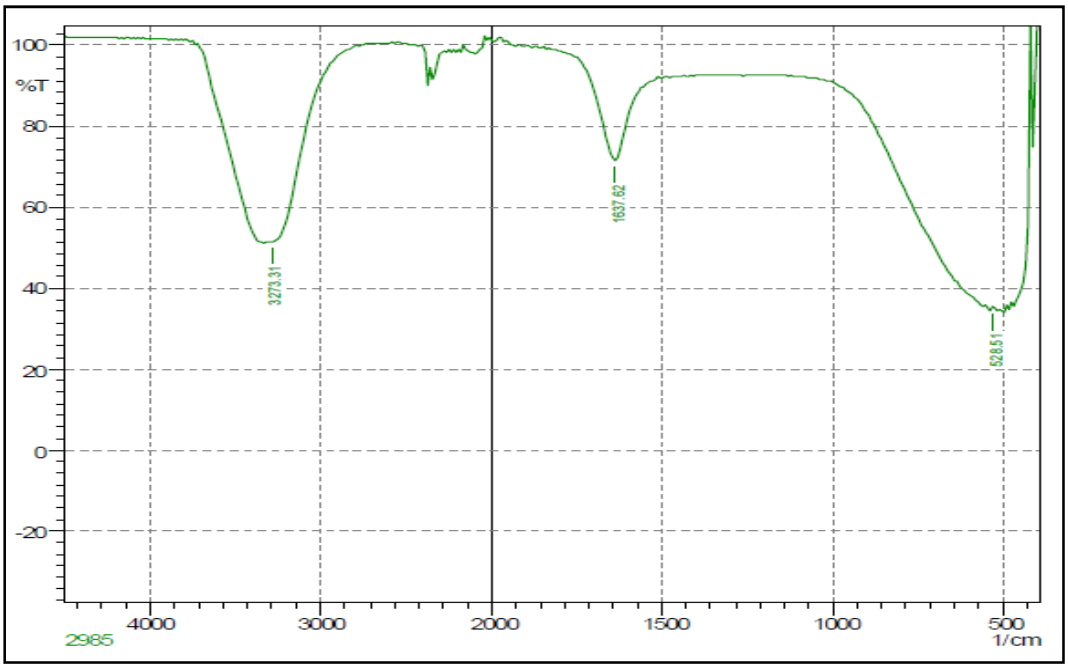

Figure 5: FT-IR spectrum of optimized batch F2

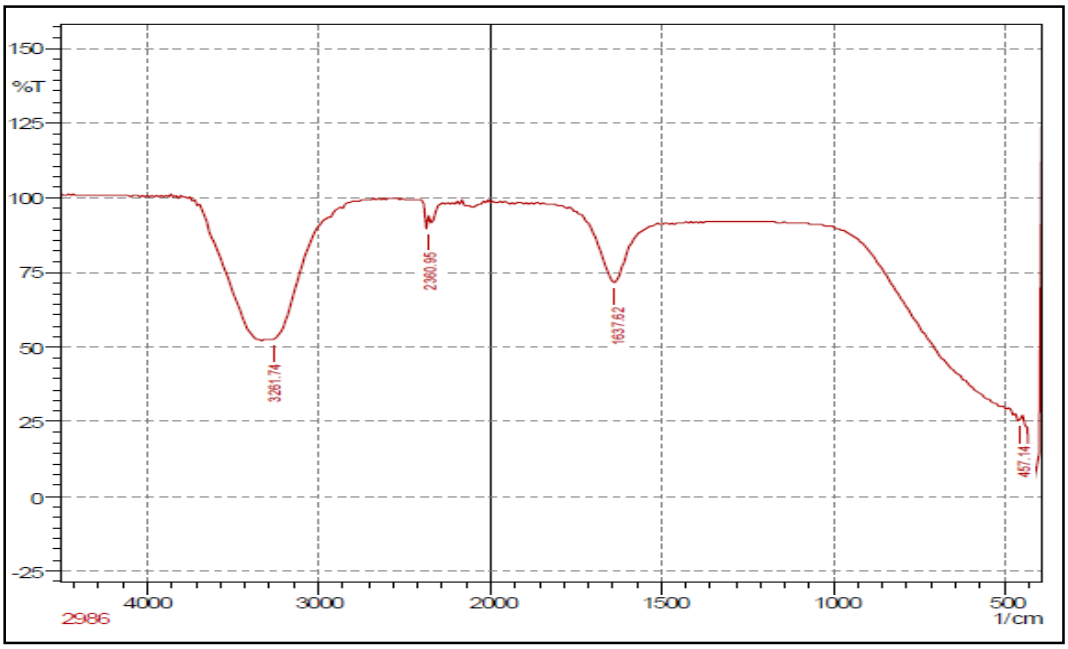

Figure 6: FT-IR spectrum of optimized batch F7

Table 4: Drug compatibility studies of drug, excipients and physical mixture.

Excipients	Drug compatibility studies in different time intervals (in days)			
	1	7	21	28
Telmisartan Drug				
Colour	✓	✓	✓	✓
Appearance	✓	✓	✓	✓
State	✓	✓	✓	✓
HPMC E5				
Colour	✓	✓	✓	✓
Appearance	✓	✓	✓	✓
State	✓	✓	✓	✓
PVP K30				
Colour	✓	✓	✓	✓
Appearance	✓	✓	✓	✓
State	✓	✓	✓	✓
Telmisartan: HPMC E5				
Colour	✓	✓	✓	✓
Appearance	✓	✓	✓	✓
State	✓	✓	✓	✓
Telmisartan: PVP K30				
Colour	✓	✓	✓	✓
Appearance	✓	✓	✓	✓
State	✓	✓	✓	✓
Telmisartan:HPMC E5:PVP K30				
Colour	✓	✓	✓	✓
Appearance	✓	✓	✓	✓
State	✓	✓	✓	✓

*✓ indicates that no changes observed in the drug and excipient and physical mixture

Evaluation of Drug Content

The optimized formulations F2 and F7 were estimated for the drug content by taking the sample in to the ependroff tubes and centrifuging at 10000 rpm for 15 min. The supernatant was analyzed at λmax of 296 nm by UV spectrophotometer and drug content was evaluated from the standard curve. Formulations F2 and F7 showed drug content of 0.218 mg/mL and 0.185 mg/mL respectively in comparison to 0.194 mg/mL of pure drug in pH 6.8 phosphate buffer.

Estimation of Saturation Solubility

Saturation solubility of optimized formulations were studied by keeping the samples in the incubator shaker for 48 hours at a speed of 50 rpm and the samples are analyzed using UV spectrophotometer at 296 nm. The saturation solubility of F2 and F7 batch was found to be 0.415 mg/mL and 0.281 mg/mL when compared with the pure drug which was 0.086 mg/mL.

Evaluation of Viscosity of the Formulations

For the evaluation of viscosity the instrument was maintained at room temperature (27±2°C) and the spindle S63 was connected to the instrument. The samples were rotated at a speed of 50 rpm and the values obtained were recorded. It was shown that the F2 and F7 cP value around 87-144.

In-Vitro Evaluation

In-vitro studies were performed by USP apparatus II (paddle type). The study was

performed in phosphate buffer pH 6.8 by setting the temperature at 37±0.5°C at a speed of 50 rpm. Sample of about 5mL were withdrawn at time intervals of 5, 10, 15, 30, 60, 120, 240 and 480 minutes and equal amount of fresh volume is added to maintain sink condition. The samples were analyzed using UV-spectrophotometer at 296 nm and the study was conducted in triplicate. The percentage drug release was found to be 83.6% and 81.6% respectively. Fig. 7 shows the comparison of the both optimized formulations (F2& F7).

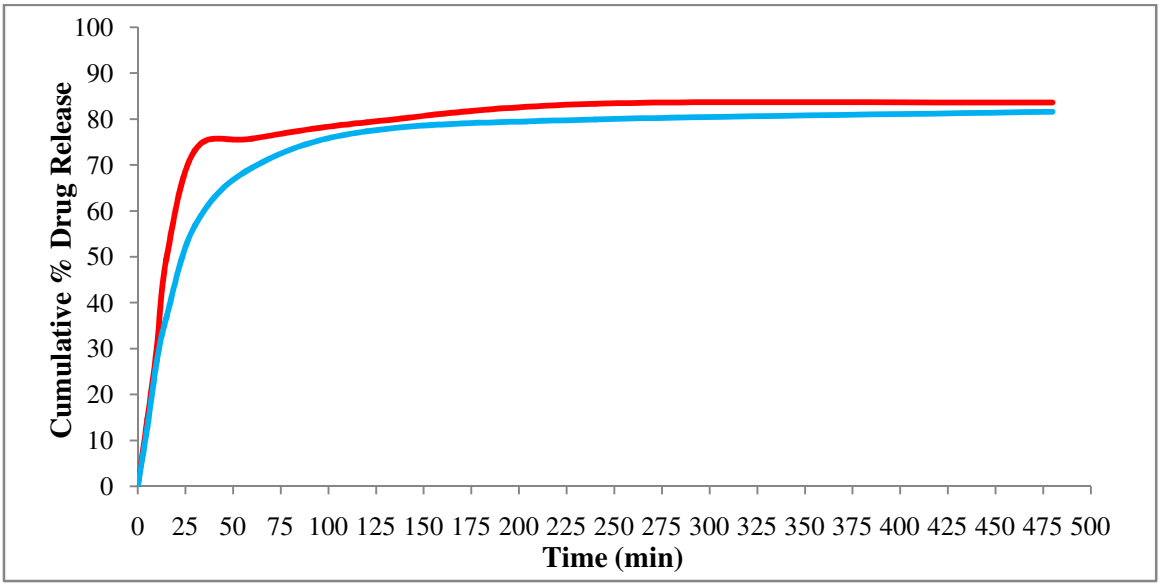

Figure 7: Dissolution profile of formulation F2 and F7

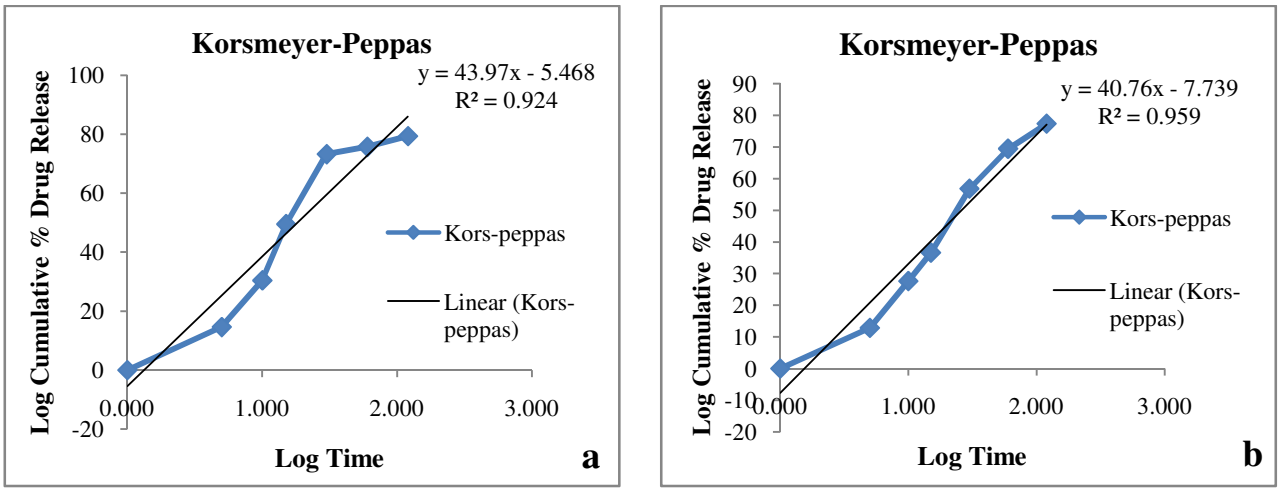

Figure 8: Drug release kinetics of a) optimized formulation F2, b) formulation F7

Evaluation of Drug Kinetic Profile Study

Drug kinetics models were applied to the mathematical models of telmisartan nanosuspension formulations and evaluation is done on graphical representation. The drug release kinetic data was recorded from the data obtained from in-vitro studies. From all mathematical models, the graphical representation of log cumulative percentage drug release against time follows the principle of Korsmeyer-Peppas model with correlation coefficient r^2=0.9242 of optimized telmisartan formulation F2 and it were observe that the r^2=0.9591for the optimized formulation F7.

Surface Morphological Evaluation

SEM is used to determine the surface morphology of the nanoparticles. The study revealed formulations of telmisartan from the particles in that optimized formulations of Telmisartan appears to be needle crystals with smooth surface. The particles were observed to be in clusters as shown in the Fig. 9 (a, b).

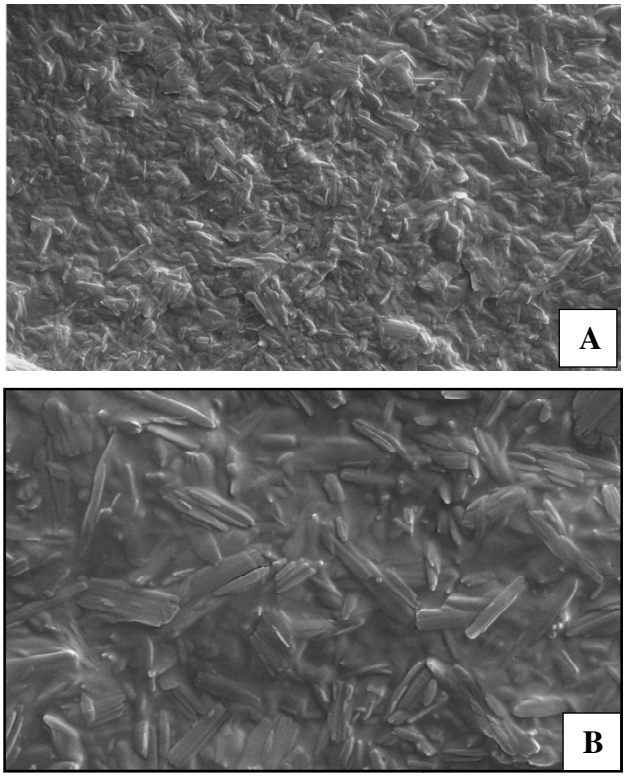

Figure 9: Surface morphological of optimized formulations a) F2 and b) F7

CONCLUSION

To tackle the problem of low solubility and bioavailability of anti-hypertensive drug telmisartan, nanosuspension was prepared using full factorial design (3^2). The dissolution data was subjected to drug release kinetics and it was found that the both formulations were following Korsmeyer-Peppas kinetics. So, finally it can be concluded that the developed formulations showed appreciable in-vitro performance, however, in-vivo potential is yet to be evaluated.

REFERENCES

[1] Cohuet G, Struijker-Boudier H. Mechanisms of target organ damage caused by hypertension: therapeutic potential. Pharmacology & therapeutics. 2006; 111(1):81-98.

[2] Dorans KS, Mills KT, Liu Y, He J. Trends in Prevalence and Control of Hypertension According to the 2017 American College of Cardiology/American Heart Association (ACC/AHA) Guideline. J Am Heart Assoc. 2018; 7(11).

[3] Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. Journal of the American College of Cardiology. 2017; 70(1):1-25.

[4] Correction to: 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Hypertension. 2018; 72(3):e33.

[5] Assessment SP. Guidance for Industry. Center for Biologics Evaluation and Research (CBER). 2002.

[6] Lis Y, Roberts MH, Kamble S, J. Guo J, Raisch DW. Comparisons of Food and Drug Administration and European Medicines Agency Risk Management Implementation for Recent Pharmaceutical Approvals: Report of the International Society for Pharmacoeconomics and Outcomes Research Risk Benefit Management Working Group. Value in Health. 2012;15(8):1108-18.

[7] Desai PP, Date AA, Patravale VB. Overcoming poor oral bioavailability using nanoparticle formulations–opportunities and limitations. Drug Discovery Today: Technologies. 2012; 9(2):e87-e95.

[8] Shid RL, Dhole SN, Kulkarni N, Shid SL. Nanosuspension: a review. Technology. 2013; 30:34.

[9] Sutradhar KB, Khatun S, Luna IP. Increasing possibilities of nanosuspension. Journal of nanotechnology. 2013; 2013.

[10] Lakshmi P, Kumar GA. Nanosuspension technology: A review. Int J Pharm Sci. 2010; 2(4):35-40.

[11] Katteboinaa S, Chandrasekhar V, Balaji S. Drug nanocrystals: A novel formulation approach for poorly soluble drugs. International journal of pharmtech research. 2009; 1(3):682-94.

[12] Sunder S, Nair R. Methods of nanonization of drugs for enhancing their dissolution. Eur J Adv Eng Technol. 2016;3(8):101-10.

[13] Salazar J, Ghanem A, Müller RH, Möschwitzer JP. Nanocrystals: comparison of the size reduction effectiveness of a novel combinative method with conventional top-down approaches. European journal of pharmaceutics and biopharmaceutics. 2012; 81(1):82-90.

[14] Ghosh I, Schenck D, Bose S, Ruegger C. Optimization of formulation and process parameters for the production of nanosuspension by wet media milling

technique: effect of vitamin E TPGS and nanocrystal particle size on oral absorption. European journal of pharmaceutical sciences. 2012; 47(4):718-28.

[15] Deppe S, Boger RH, Weiss J, Benndorf RA. Telmisartan: a review of its pharmacodynamic and pharmacokinetic properties. Expert Opin Drug Metab Toxicol. 2010;6(7):863-71.

[16] Rao MR, Bajaj A. Study of effect of variables on particle size of telmisartan nanosuspensions using box-Behnken design. Drug Res (Stuttg). 2014; 64(12):663-7.

[17] Pokhariya P, Ganarajan G, Kothiyal P. Formulation and evaluation of mouth dissolving tablet of telmisartan using natural superdisintegrants. Indo American Journal of Pharmaceutical Sciences. 2016; 3(7):713-27.

[18] Yadav U, Ray Chowdhuri A, Sahu S, Husain N, Rehman Q. Formulation of nanoparticles of telmisartan incorporated in carboxymethyl chitosan for the better drug delivery and enhanced bioavailability. Asian Journal of Pharmaceutical and Clinical Research. 2017; 10:236.

[19] Dubey A, Kharia A. Enhancement of Aqueous Solubility and Dissolution of Telmisartan Using Solid Dispersion Technique. International Journal of Pharmaceutical Sciences and Research. 2014; 5(10): 4478-4485.

[20] Manishaanjane, Shikha A, Amreen K. Formulation and Evaluation of Nanosuspension of Valsartan. International Journal of Current Pharmaceutical Research. 2018; 10(2).

[21] Bruschi ML. Strategies to modify the drug release from pharmaceutical systems: Woodhead Publishing; 2015.

[22] Zhang Y, Huo M, Zhou J, Zou A, Li W, Yao C, et al. DDSolver: an add-in program for modeling and comparison of drug dissolution profiles. Aaps j. 2010; 12(3):263-71.

[23] Reddy KR, Mutalik S, Reddy S. Once-daily sustained-release matrix tablets of nicorandil: formulation and in vitro evaluation. AAPS pharmscitech. 2003; 4(4):480-8.

[24] Ahuja BK, Jena SK, Paidi SK, Bagri S, Suresh S. Formulation, optimization and in vitro–in vivo evaluation of febuxostat nanosuspension. International journal of pharmaceutics. 2015; 478(2):540-52.