Design of Telmisartan Loaded Nanoparticles by Three Square Factorial Design Approach

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ABSTRACT

Telmisartan as an antihypertensive drug has poor solubility and high permeability. In the current study, efforts were taken to improve the solubility of telmisartan by the formulation of nanoparticulate drug delivery system. Nanosuspension was prepared by combining ionic gelation-ultrasonication technique. Later, nanosuspension was converted to powder by freeze drying technology. Three square factorial design approach was used for the investigation of the effect of concentration of trimethyl chitosan polymer and the rate of stirring on particle size and solubility of nanoparticles. The optimum condition was found to be 3.5 mg/ml of trimethyl chitosan and 9000 rpm stirring rate. All data was best fitted in quadratic model with high determination coefficient and F value. The average particle size of 281.5 nm was confirmed by dynamic light scattering. Differential scanning calorimetry and powder X-ray diffraction revealed reduced crystallinity of telmisartan. Freeze-dried nanoparticles were spherical-shaped under field emission scanning electron microscopy. The value of zeta potential was + 35.2 mV. In vitro dissolution study was performed by dialysis bag to investigate the improvement of the dissolution rate. The stability of developed nanoparticle was confirmed by the accelerated stability study of developed nanoparticles. Thus, the saturation solubility and dissolution rate were increased due to the particle size reduction and amorphous nature of the drug.

Key Words: *Nanoparticles, Ionic Gelation, Ultrasonication, Freeze Drying.*

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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]. According to the literature, two types of drugs that have low solubility and dissolution rate are grease ball and brick dust [2]. Grease ball molecules are highly lipophilic with log P value more than 4 and have weak intermolecular forces. Lipid-based formulation can be prepared for grease ball molecules to achieve maximum therapeutic effect. Whereas, brick-dust molecules are less lipophilic with low log P value, and have high intermolecular forces. Therefore, permeability is the rate-limiting step to get the therapeutic effect of brick dust molecules [3]. The solubility of such poorly soluble drugs can be improved by solid dispersion technique, complexation with cyclodextrin, co-crystal approach, micronization, nanosization, etc. [4-7].

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 [8, 9]. 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 [10]. A wide variety of natural and synthetic polymers have been used for the

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formulation of nanoparticles, among that chitosan has many applications in pharmaceutical and medical industries [11, 12]. Water-soluble chitosan derivatives have been attracting more attention in the development of nanoparticles as these are biodegradable, biocompatible, non-toxic and hydrophilic, which can effectively enhance the aqueous solubility of Class II and Class IV drugs of biological classification system [13].

Nanoparticulate drug delivery system with crystalline or amorphous nature has been a novel approach where active pharmaceutical ingredients can be stabilized by different stabilizers like polymers and surfactants [14, 15]. 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) [16, 17].

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 [18, 19]. Telmisartan has a log P value of 7.7 which is similar to grease ball molecules, but it is very difficult to prepare a lipid-based system of telmisartan because of its high melting point [20]. The solubility of such drug can be improved by using amorphous polymers, where melting point of drug can be reduced by adding plasticizer. Various approaches have been tried to improve the solubility of this drug such as biological porous starch foam, solid dispersion, and nanoparticles by the supercritical antisolvent process [6, 21].

The primary orientation of this study was to improve the solubility and dissolution of weak water-soluble telmisartan by ionic gelation technique. Full factorial design (3^2) was used for the optimization of freeze-dried nanoparticles, where the interaction between dependent and independent variables was analyzed to get an effective and optimized nanoparticulate drug delivery system.

MATERIALS AND METHOD

Materials

Telmisartan (Glenmark Pharmaceutical, Nashik, India), Meglumine (Ipca Laboratories, Mumbai, India), Chitosan (90% deacetylated) and Sodium Tripolyphosphate (Sodium TPP) was procured from Aditya Chemicals (Pune, India). Deionized water and analytical grade ingredients were used for the development of nanoparticles.

Formulation of nanoparticles of telmisartan by ionic gelation-ultrasonication-freeze drying technology

Prior to the development of nanoparticles' water soluble derivative of chitosan - Trimethyl Chitosan (TMC) - was synthesized by two step technique reported by Ushasree et al. [22]. The nanoparticles' formation involves ionic interaction between polymer- TMC and ion generating agent-Sodium TPP. The main functional groups involved in this interaction, are ionic charges of functionally active amine moiety of TMC with TPP as described by Calvo et al. [23]. The process involved the preparation of aqueous solution of TMC (2-5 mg/ml) at 25-27 °C with ultrasonication. The filtered aqueous sodium TPP solution (3mg/ml of pH 8) was used. A telmisartan solution in methanolic meglumine was incorporated to sodium TPP aqueous system. Afterwards, the drug containing solution was injected at controlled speed of 0.2 ml/min in TMC solution. The mixture was stirred by means of a mechanical stirrer (Emtek Instruments, Mumbai, India) at 3000-9000 rpm for 30 minutes. Then, it was sonicated with probe sonicator (Leelasonic 125-upp, Mumbai, India) at 5 Mm spindle for 15 minutes. Finally, all samples were centrifuged (Remi Instruments, Mumbai, India) for 30 minutes. The obtained suspension was frozen (α LD plus 1-2, Germany) at -40 °C (time required 4 Hrs). This study was continued using the controlled temperature, time and pressure (−48-0 ºC, 24 hrs with 10 mbar.). The freeze-dried nanoparticles were stored in tightly closed container at room temperature for further use.

Statistical analysis

The statistical performance of formulation was evaluated with Design-Expert software. Significant interactions of various factors of formulation were optimized with the statistical design analysis of variance (ANOVA).

Characterization of freeze-dried nanoparticles

Analysis of particle size and Zeta potential

Particle size and particle size distribution of freeze-dried nanoparticles were determined by Horiba nanoparticles analyzer (Horiba SZ-100, ver. 1.90) which worked on the principle of Dynamic Light Scattering (DLS). The diluted samples were analyzed in triplicate at 25 ± 5 °C.

Laser Doppler Anemometry (Horiba SZ-100, ver. 1.90) technology was employed for predicting the potential differences between nanosized particles. Zeta potential

measurement was carried out in electrophoretic cell with applied potential of 3.3 V. Each sample was measured in triplicate.

Morphological Characterization

A Field Emission Scanning Electron Microscopy (FESEM) technology (Nova NanoSEM 450, India) was used for further particle size and shape analysis of telmisartan and freeze-dried nanoparticles. Before the analysis, the samples were coated with gold, and the photographs were taken by a different magnification power. All samples were observed with operating voltage of 5.00 kV, and the images were analyzed by the software FESEM xT microscope control.

Infrared spectroscopy (FTIR)

FTIR spectrum of telmisartan and freeze-dried nanoparticles were recorded by FTIR spectrophotometer (Jasco M- 4100 type A filter, Japan) to study the interaction of telmisartan with the other formulation ingredients. The samples were prepared for the analysis by KBr press pellet technique, where 3 mg of samples were dispersed in 9 mg of KBr, and pressed by applying hydrostatic force 8 torr per square centimeter for 1 minute.

Differential Scanning Calorimetry (DSC)

The thermal behavior of telmisartan, Physical mixture (PM-powder blend of drug and excipients) and freeze-dried nanoparticles were characterized by DSC (Shimadzu Co., Japan DSC-60). Three milligrams of the samples were placed in aluminium pan and heated within temperature range of 20 to 250 °C, with heating rate of 10 °C/min maintained in nitrogen atmosphere.

PXRD Study

PXRD diffractogram of telmisartan, PM and freeze-dried nanoparticles were recorded by X-ray diffractometer (Ultima-IV). The powder samples were placed in a glass sample holder and irradiated with the copper light source. The potential difference of 40 mV, and 40 mA current was applied along with scan velocity of 5°/min over a 2θ range from 5° to 80°.

Determination of saturation solubility

The saturation solubility of telmisartan and freeze-dried nanoparticles was performed by flask shaking technique. The solubility variation in different media like deionized water, and pH 7.5 phosphate buffer was evaluated. The excess samples were separately dispersed in 25 ml deionized water and pH 7.5 phosphate buffer in sealed vials. These vials were stirred on an orbital shaking thermo-stable incubator (Remi, Mumbai, India) overnight at 36°C - 38°C at 150 rotations per minute. Further, the samples were centrifuged and separated by the filtration. The analysis of filtered sample was performed in triplicate on spectrophotometer at wavelength of 296 nm. All data were recorded as average with standard deviation (SD).

Drug release study

In vitro dissolution study of freeze-dried nanoparticles and telmisartan was performed by the dialysis bag technique. Nanoparticles equivalent to 20 mg of telmisartan were transferred to the dialysis bag and both ends of bag were closed. Then, the bags were tied to paddle of the dissolution testing apparatus (Electrolab Dissolution Tester TDT-06P, India) containing deionized water and pH 7.5 phosphate buffer (900 ml) as the dissolution medium. The study was performed at temperature of 37 ± 0.5°C with the speed of 75 rpm. The sampling was performed at particular time intervals, and the amount of drug released from the nanoparticles was estimated by UV visible spectrophotometer (Analytical Technologies 20108+, Germany). The collected data were shown as the mean ± SD from three different independent release experiments.

Stability study

The accelerated stability study of freeze-dried samples were carried out in the humidity control chamber (LabHosp, India) at 40 ±2 °C/75 ± 5% relative humidity wherein the particle size, saturation solubility, dissolution rate, and retention of amorphous nature were analyzed over the period of six months.

RESULT AND DISCUSSION

Freeze-dried nanoparticles were fabricated by using the combination of TMC-Sodium TPP and meglumine as *in situ* pH adjuster. The pure telmisartan and optimized freeze-dried nanoparticles were analyzed by various techniques such as particle size, zeta potential (stability), FESEM (morphology study), FTIR, DSC (compatibility study), and XPRD (crystallinity study).

Statistical analysis

The interaction between different factors in the formulation of nano drug delivery system was more elaborated with the design expert software. A response surface model (RSM) was an effective technique for estimating the interaction between the factors by changing the values of all factors in parallel, and studying their interaction using the minimum number of experiments [24, 25]. In the present invention, 3^2 factorial design approach was used for the optimization of nanoparticulate drug delivery system. In total, nine batches were prepared, namely CN1-CN9. The concentration of TMC and the rate of stirring were chosen as independent variables, whereas the particle size (X) and the percentage solubility enhancement (Y) were selected as dependent variables. The graphical outcome of design in terms of Contour plots and 3-D surface plots have been shown in fig 1a-1d. Based on the preliminary study, three different levels of formulation variables were selected. All outcomes of the study were placed into linear, second order, and quadratic models. The statistical significance of the coefficients was

determined by ANOVA. From the obtained data, it was concluded that all the responses were best fitted in quadratic model instead of linear and second-order model. The polynomial equations used by Design expert software were:

$$X\ (nm) = +168.72 +76.83A -4.33B-5.25AB-58.53\ A2 +27.97\ B2$$

$$Y\ (\%) = +4.73 +0.63A+ 0.070B+0.040AB-3.26A2 -0.77\ B2$$

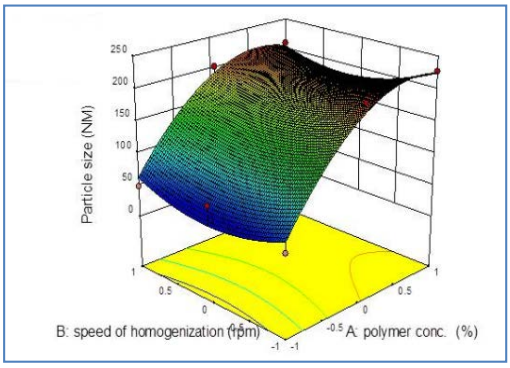
The concentration of TMC (A) and the rate of stirring (B) as an independent impacting factors could change the dependent collective output response of X and Y, as it was explained in the above polynomial equation.

The linear and adverse effect on response was concluded from the values of variables. It was observed that the independent variables of A and B pronounced effects on the response –Y, while the response-X was reduced with increasing rate of stirring B. The higher stirring speed could lower the particle size, and it was confirmed that the model was significant with F-values 33.54 and 60.79 for the particle size and the solubility enhancement; respectively, and 0.01% noise level.

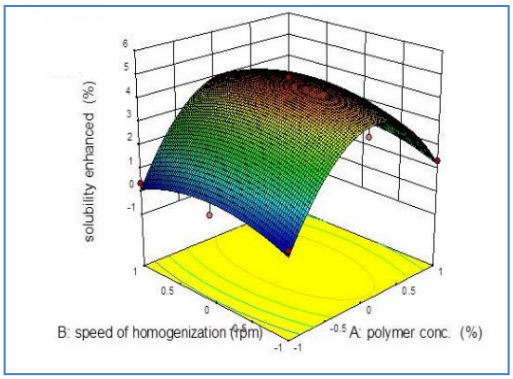
In equation, model terms A, A2, B2 controlled S/N. This ratio in given equation was higher than 4, indicating an adequate signal. The ratios of an adequate signal were 17.42 and 17.965, which confirmed that the model was significant. This was also proved by the observed P-value (0.0018). CN6 batch was found to be satisfactory, so further study was continued by using this batch.

Table 1: Independent and dependent variables' levels in Factorial design approach

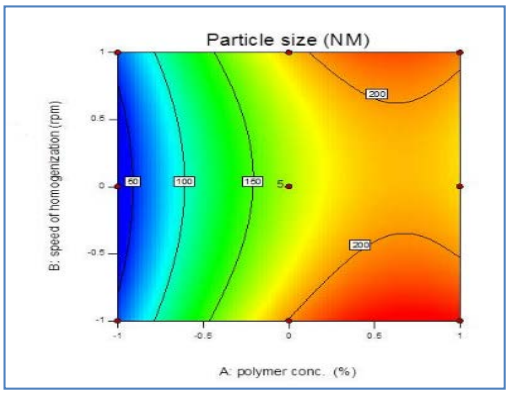
Independent variables	Levels		
	-1	0	1
A = Polymer concentration (w/v)	2	3.5	5
B = Rate of stirring (rpm)	3000	6000	9000
Dependent Variables	Constraints		
Particle Size (nm)	Minimize		
Solubility enhancement (%)	Maximize		



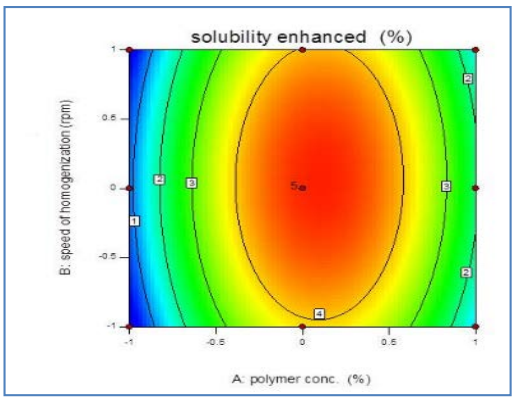
3D surface plot for effect on Particle Size



3D surface plot for effect on Solubility



Counter plot for effect on Particle Size



Counter plot for effect on Solubility

Fig. 1 Surface Response Graphs

Effect of Ultrasonication

At an initial stage, nanoparticles were formulated without the aid of ultrasonic energy, which in that case they were less homogeneous, and the average particle size was bigger (750-900 nm). The effect of ultrasonication on particle size was investigated. Nanosuspension was ultra-homogenized at different ultrasonic power input (100, 150, 200 & 250 W) for different time length (5-60 minutes). The probe diameter of 5 Mm was dipped in nanosuspension, where ultrasonic energy was travelling downwards and reflecting upwards. The power of ultrasonication did not show a pronounced effect on particle size. The particle size of nanosuspension was reduced to 281.5 nm at increasing time length of ultrasonication for 20 minutes. However, with ultrasonication for more than 20 minutes, the size of

particles again increased. Monir et. al. reported that, the application of ultrasonic energy could generate high energy ultrasonic waves. Intense shock waves could reduce the contact time between the particles and thereby, prevent the agglomeration of the particles. The ultrasonic waves also increased the adsorption of polymers on the particle surface, and caused the disruption of agglomerates [26]. But, longer time of ultrasonication did not help to reduce the particle size, because that could cause the disruption of the adsorbed polymeric film, which led to the agglomeration of particles. Hence, nanosize and homogenous particles were obtained by the probe sonication for 20 minutes. Later on, it was continuously used for the preparations of nanoparticles of telmisartan.

Analysis of Particle size and Zeta potential

Particle size and zeta potential have been the main factors for stability and *in vivo* faith of nanoparticles. Mean particle size and polydispersibility index of nanoparticles (CN6) were found to be 281.5 nm and 0.294; respectively (Fig. 2).

Zeta potential measures the electrostatic potential, and depends on the surface charge of particle. The value of zeta potential may be positive or negative depending on the nature of polymers used. Zeta potential of freeze-dried nanoparticles (CN6) was 35.2 mV. The positive value of Zeta potential depended on the cationic nature of TMC. A positive value of zeta potential was observed indicating the surface of nanoparticles to be positively charged, and helped to maintain a good redispersibility of nanoparticles. The morphology and nanosize of nanoparticles were also analyzed, and confirmed by FESEM.

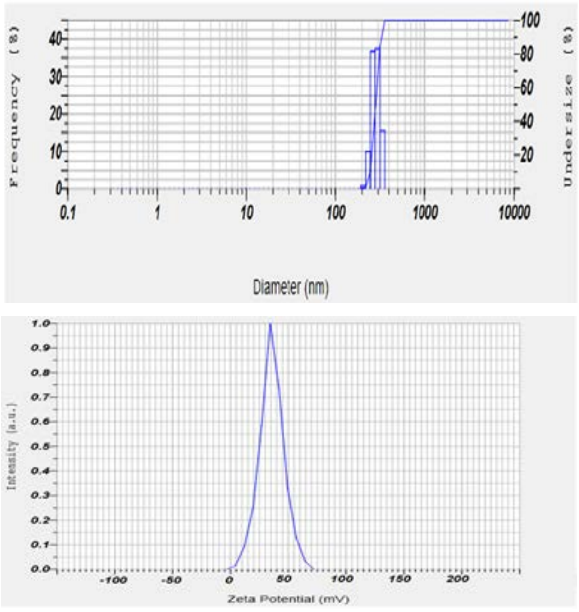


Fig. 2: A) Particle size of CN6 batch, B) Zeta potential of CN6 batch

Morphology Characterization

The particle size, shape and surface morphology of pure drug and freeze-dried nanoparticles (CN6) were analyzed by using Field Emission Scanning Microscopy (FESEM). As shown in FESEM photographs (Fig.3), pure drug was appeared as broad and needle-shaped microcrystals. An agglomerated nanoparticle was observed at lower magnification power (10000 times). A careful observation of FESEM image of nanoparticles at higher magnification power (100000 times) revealed that the agglomerated particles were composed of spherical-shaped distinct nanoparticles.

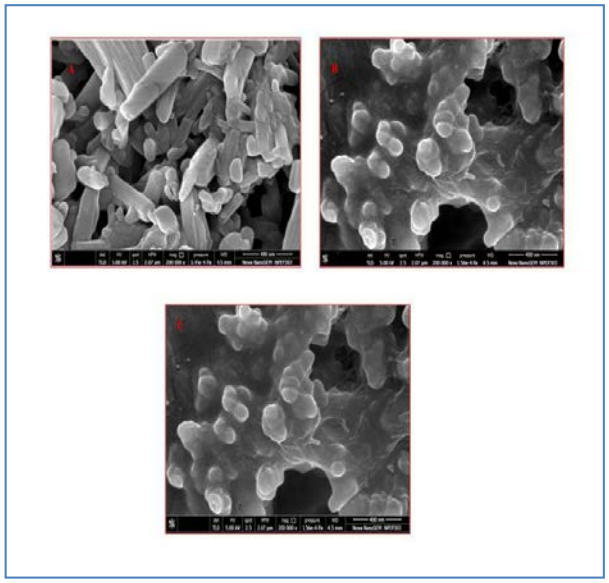


Fig. 3: A) FESEM of telmisartan, B) FESEM of CN6 BATCH, C) FESEM of CN6 batch after 6 months

FTIR analysis

FTIR analysis was performed to investigate the chemical compatibility between drug and polymers. FTIR spectrum of drug, PM and freeze-dried nanoparticles have been portrayed in Fig.4. IR spectral pattern of telmisartan was characterized by C-H (2867 cm^{-1}), O-C=O group (1698.98 cm^{-1}), C-H bend (1458.89 cm^{-1}), and COOH (1267 cm^{-1}), and PM by NH_4 (3466 cm^{-1}), C-H (2854 cm^{-1}), O-C=O group (1664.62 cm^{-1}), C-H bend (1456.3 cm^{-1}), and COOH (1271.13 cm^{-1}). The IR spectral pattern of freeze-dried nanoparticles (CN6) revealed peaks at NH_4 (3462 cm^{-1}), C-H (2865 cm^{-1}), O-C=O group (1668.48 cm^{-1}), C-H bend (1453.57 cm^{-1}), and COOH (1267 cm^{-1}), whereas after 6 months, IR pattern of freeze-dried nanoparticles (CN6) was as NH_4 (3458 cm^{-1}), C-H (2867.13 cm^{-1}), O-C=O group (1652.7 cm^{-1}), C-H bend (1458.39 cm^{-1}), and COOH (1248 cm^{-1}). All the prominent peaks were obtained in optimized formulation to ensure that drug and other excipients were compatible with each other in the developed formulation.

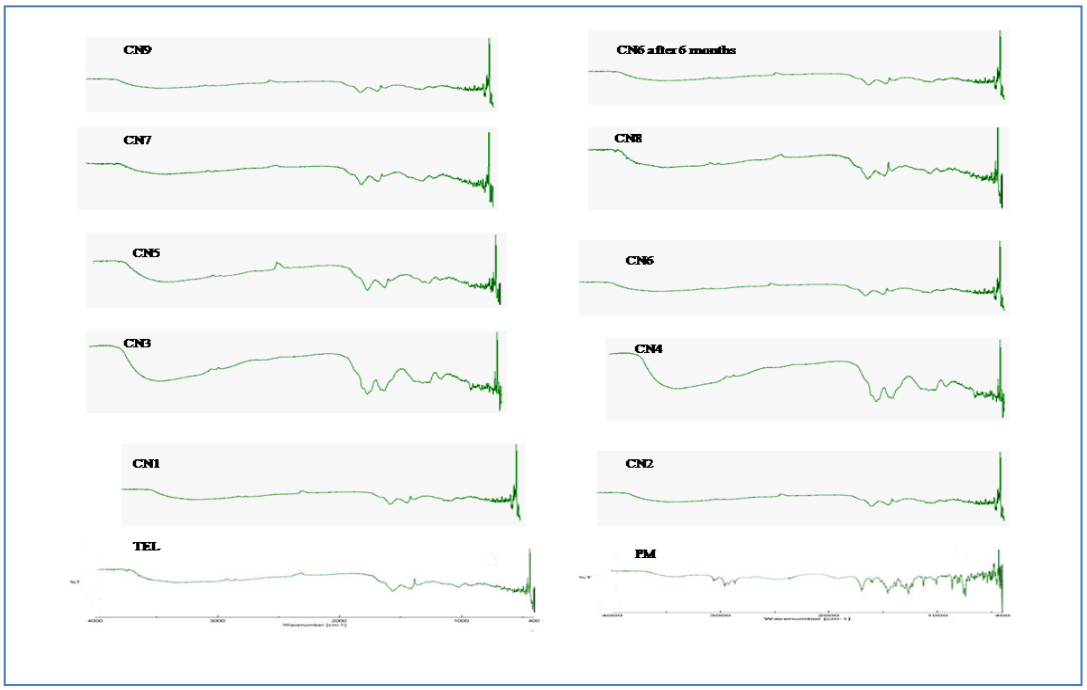


Fig. 4: FTIR Study

DSC

Many researchers reported that the ultrasonication and freeze drying processes could reduce the crystallinity of drugs. Therefore, the physical state of nanoparticles was investigated by DSC and PXRD technique. DSC thermogram of drug, PM and nanoparticles have been shown in Fig. 5. The endotherm of telmisartan showed sharp and intense peak at 269.67 °C (enthalpy of 396.70 mJm/g), whereas PM showed a peak at 269.17°C (enthalpy of 230 mJm/g), that indicated the drug and polymer were compatible with each other. A broader endothermic peak at -89 °C with low enthalpy was observed in DSC thermogram of freeze-dried nanoparticles (CN6). This clearly revealed that nanosized drug was converted to amorphous form, and was totally entrapped in polymeric chain. A similar finding was reported by Manik P. et al, where drug peak was not seen in DSC thermograms [27].

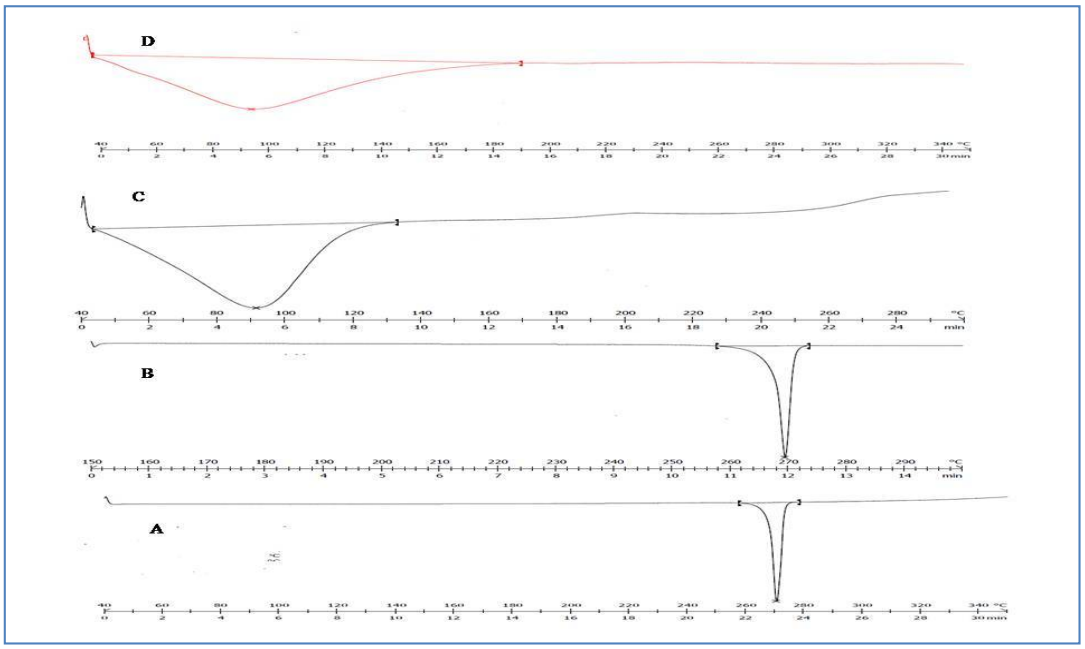


Fig. 5: A) DSC thermogram of telmisartan, B) DSC thermogram of PM, C) DSC thermogram of CN6 batch, D) DSC thermogram of CN6 batch after 6 months

PXRD

The diffraction spectra of telmisartan, PM and freeze-dried nanoparticles have been portrayed in Fig. 6. The characteristic intense and sharp peaks of telmisartan at 2θ values 6.6°, 14.04°, 22.18 and 24.28° were observed, which suggested that the drug was in its crystalline form. Also, the crystalline peaks of telmisartan did not change in physical mixture. The diffraction peaks were absent in nanoparticles (CN6), instead of that the broad and diffused peaks with less peak intensity were observed. Hence it was concluded that the crystallinity of the drug was reduced due to the entrapment of drug in polymer matrix, leading to a higher surface disorder, and so in a higher saturation solubility than crystalline materials and enhanced dissolution rates. The reduction of peak intensity also revealed that the particle size was reduced. The PXRD results agreed with those results measured with DSC, and these combined techniques clearly demonstrated that the reduction of particle size could reduce the crystallinity of the drug [28].

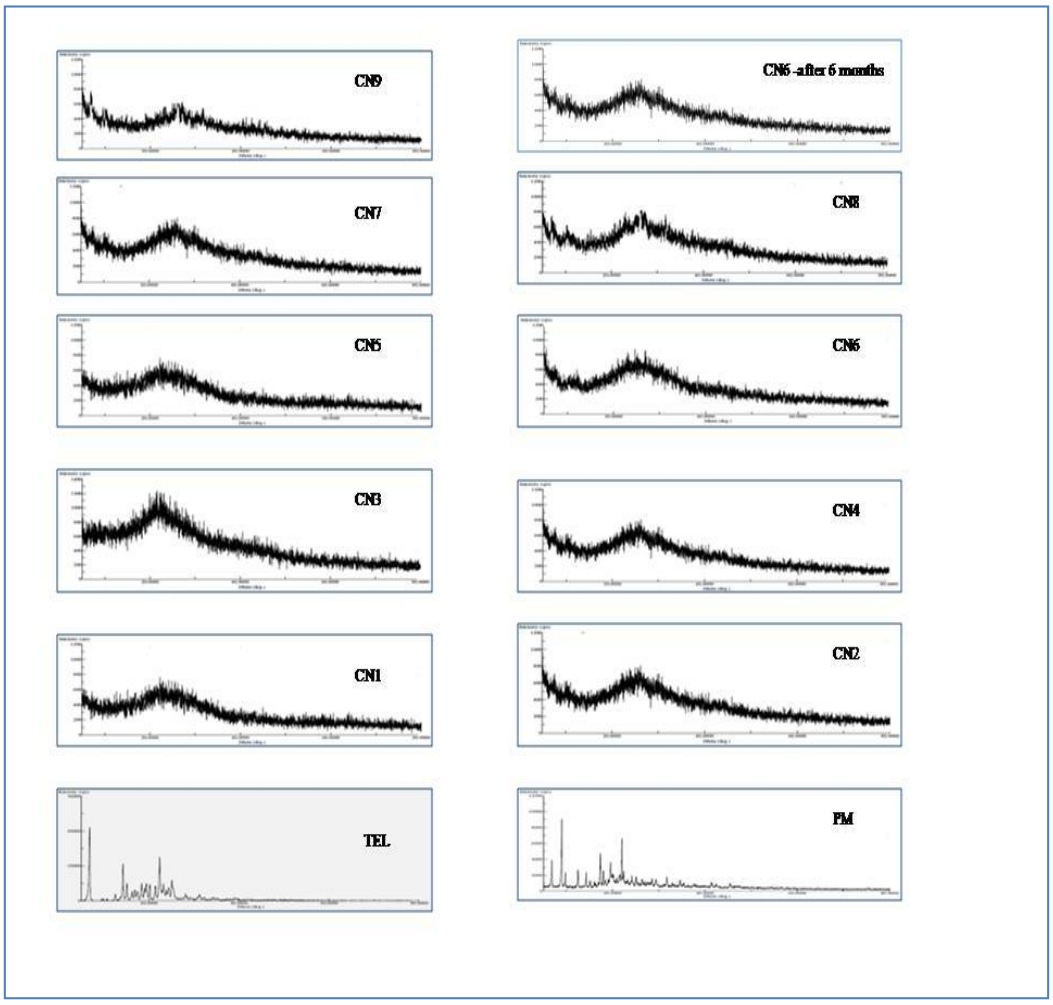


Fig. 6: PXRD study

Saturated Solubility

Telmisartan is practically insoluble in water. The significant solubility enhancement of telmisartan in the form of freeze-dried nanoparticles (CN6) was observed (table 1). The mechanisms for the solubility enhancement were nanosization of drug molecule with the increased surface area, the formation of porous and amorphous structure with a high vapor pressure of nanoparticles as revealed in PXRD and DSC data, the electrostatic interaction between drug and polymer [29].

Table 2: Saturated Solubility Study of drug and freeze-Dried Nanoparticles.

Sr. No	Medium	Solubility of Pure drug (Mean ± SD*)	Solubility of freeze-dried nanoparticles (Mean± SD*)	Solubility of freeze-dried nanoparticles (Mean± SD*) (After 6 months)
1	Water	0.0632 ±0.0003	0.2443 ±0.004	0.2463 ±0.0003
2	pH 7.5 Phosphate buffer	0.0698 ± 0.0006	0.2553 ±0.003	0.2554 ±0.0003

*SD= Standard Deviation

Drug release study

In vitro dissolution study has been very essential for the investigation of the dissolution rate of drug which ultimately influences the pharmacological effect of drug in the biological system. The results of *in vitro* drug released were obtained by plotting the graph as the cumulative percentage drug released versus time. The dissolution rate of nanoparticles was compared with the dissolution rate of freeze-dried nanoparticles (CN6). Figure 7 reveals the dissolution profile of telmisartan and the freeze-dried nanoparticles. The highest cumulative dissolution rate was achieved within 45 minutes. This phenomenon might be occurred due to the nanosized drug molecule with the high surface area, the formation of *in situ* alkaline medium, and the formation of soluble complex between drug and stabilizers. The results of X-RD and DSC also revealed that the decreased crystallinity of nanoparticles would lead to the higher saturation solubility and enhanced dissolution rate [30, 31].

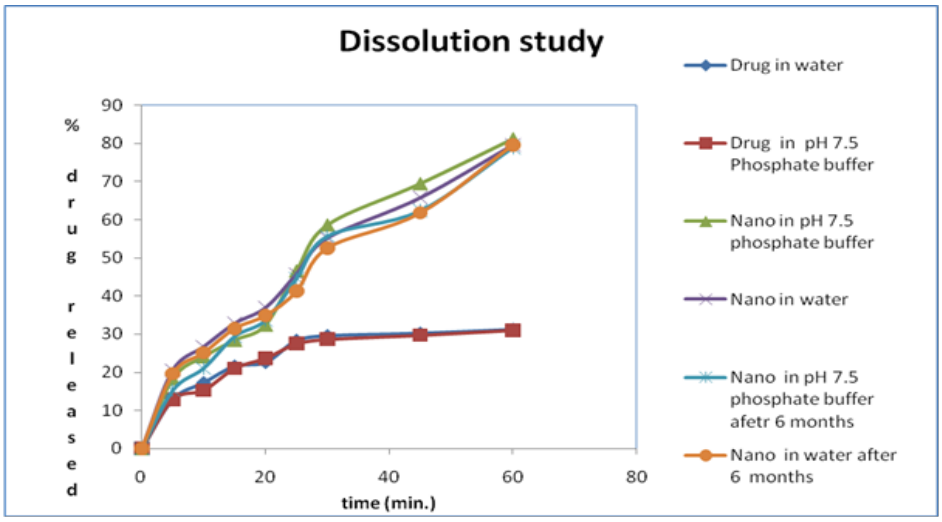


Fig. 7: Drug Released Study of nanoparticles.

Stability study

Freeze drying technology has mainly produced porous and amorphous dosage form after the sublimination of water molecules. Vast studies have also been done for the formulation of freeze-dried nanoparticles, but most of the times, the formed amorphous mass converted to crystalline form in the presence of residual moisture during the storage [32]. Since, the physicochemical transitions might lead to less solubilisation and dissolution of freeze-dried nanoparticles, a solid-state stability study of CN6 batch was conducted by monitoring the particles' size and the retention of amorphous nature by the SEM, DSC, PXRD and FTIR. The data obtained after 6 months' study revealed no significant changes in freeze-dried nanoparticle formulations when stored at 40±2 °C/75±5%, relative humidity suggesting no re-crystallization during the storage. The size of particles was maintained within the range after the storage for 6 months.

CONCLUSION

The development of nanoparticles for the enhancement of solubility and the dissolution rate of telmisartan was the goal of the study. TMC as water soluble derivative of chitosan was successfully prepared by two step technique, and further utilized for the development of nanoparticles. Three square factorial design approach was used for the optimization of nanoparticles. The effect of process variables on the solubility and particle size was identified. The optimum concentration of TMC was 3.5 mg/ml. The ultrasonication was proven to be a simple and efficient technology for reducing the particle size. The positive value of zeta potential was obtained by DLS study. DSC and PXRD study confirmed the amorphous nature of drug particles. The saturation solubility study revealed 3.9 times solubility enhancement compared to the pure telmisartan. *In vitro* studies revealed that nanoparticles were capable to increase the dissolution rate of the drug. Furthermore, the results of the accelerated stability study showed its solubility, and the particle size retained within the range, as well, the amorphous state of nanoparticles was not altered. This study concluded that the developed nanoparticles could enhance the solubility and dissolution rate of telmisartan. Future works shall consist of the oral bioavailability study of telmisartan nanoparticles.

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REFERENCES

[1] Savjani K., Gajjar A. and Savjani J., 2012. Drug Solubility: Importance and Enhancement Techniques. ISRN Pharmaceutics, 2012, pp 1-10.

[2] Roya Y., Krasimir V. and Spomenka S., 2014. Nanosuspension Technologies for Delivery of Poorly Soluble Drugs. Journal of Nanomaterials, 2015 (), 13 pages.

[3] Jorwakar P., Pathak A.A. and Choudhari P.D., 2012. Formulation development of Aceclofenac loaded nanosuspension by three square factorial designs. International Journal of Pharmaceutical sciences and nanotechnology, 4(4), 1575-1582.

[4] Kumari S., Santanu K., Samrat M., Jinu I. and Ghosh A., 2014. Solubility Enhancement of Ezetimibe by a Cocrystal Engineering Technique. Cryst. Growth Des., 14(9), 4475–4486.

[5] Varaporn B. J. and Boontida M., 2015. Nanocrystals for enhancement of oral bioavailability of poorly water-soluble drugs. Asian Journal of Pharmaceutical Sciences, 10 (1), 13-23.

[6] Eun-Sol Ha, Jeong-Soo K., In-hwan B. and Sung-Joo H., 2015. Enhancement of dissolution and bioavailability of ezetimibe by amorphous solid dispersion nanoparticles fabricated using supercritical antisolvent process. Journal of Pharmaceutical Investigation, 45 (7), 641–649.

[7] Sareen S., Mathew G. and Joseph L., 2012. Improvement in solubility of poor water-soluble drugs by solid dispersion. Int J Pharm Investig. 2(1), 12–17.

[8] Gabriel O., Yvonne T., and Kok-Khiang P., 2016. Enhancement of norfloxacin solubility via inclusion complexation with β-cyclodextrin and its derivative hydroxypropyl-β-cyclodextrin. Asian Journal of Pharmaceutical Sciences, 11(4), 536-546.

[9] Catarina P., Ronald J.N., Antonio J.R. and Francisco V., 2006. Methods for preparation of drug-loaded polymeric nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine, 2 (10), 8–21.

[10] Rao P. J. and Geckeler K. E., 2011. Polymer nanoparticles: Preparation techniques and size-control parameters. Progress in Polymer Science, 36 (7), 887–913.

[11] Nicoletta R., Elena F., Ilaria A., Rebeca H., Carmen M., Rossana P. and Jose M., 2015. Use of Alginate, Chitosan and Cellulose Nanocrystals as Emulsion Stabilizers in the Synthesis of Biodegradable Polymeric Nanoparticles. Journal of Colloid and Interface Science, 445 (1), 31-39.

[12] Smitha K.T., Anithaa A., Furuikeb T., Tamurab H., Naira S. V. and Jayakumara R., 2013. In vitro evaluation of paclitaxel loaded amorphous chitin nanoparticles for colon cancer drug delivery. Colloids and Surfaces B: Biointerfaces, 104, 245–253.

[13] Anderson M., Eliezer J., Alessandro J. and Petr S., 2013. Physicochemical aspects behind the size of biodegradable polymeric nanoparticles: A step forward. Colloids and Surfaces A: Physicochem. Eng. Aspects, 436, 1092– 1102.

[14] Fonte P., Araujo F., Silva C., Pereira C., Reis S., Santos H. and Sarmento B., 2015. Polymer-based nanoparticles for oral insulin delivery: Revisited approaches, Biotechnol Adv., 33(6), 1342-54.

[15] Jog R. and Diane J. B., 2017. Pharmaceutical Amorphous Nanoparticles. Journal of Pharmaceutical Sciences, 106 (1), 39-65.

[16] Wassim A., Ghania D., Serge S. and Hatem F., 2007. Freeze-Drying of Nanoparticles: Formulation, Process and Storage Considerations. Advanced Drug Delivery Reviews, 58(15), 1688-713.

[17] Aranaz I, Gutierrez MC, Ferrer ML and Del Monte F. 2014. Preparation of chitosan nanocomposites with a macroporous structure by unidirectional freezing and subsequent freeze-drying. Mar. Drugs, 12 (11), 5619–5642.

[18] M.P. Maillard, C. Perregaux, C. Centeno, J. Stangier, W. Wienen, H.R. Brunner and M. Burnier, 2002. In vitro and in vivo characterization of the activity of telmisartan: an insurmountable angiotensin II receptor antagonist. J Pharmacol Exp Ther. 302 1089-1095.

[19] Patel P. and Patravale V., 2010. Commercial Telmisartan Tablets: A Comparative Evaluation with Innovator Brand Micardis. Int. J Phar Sci and Research, 1, 282-292.

[20] Arona E. and Dasan P., 2016. Formulation, Characterization and Pharmacokinetic Evaluation of Telmisartan Solid Dispersions. J Mol Pharm Org Process Res, Vol 4(1): 131 DOI: 10.4172/2329-9053.1000131

[21] Patel B., Parikh R. and Swarnkar D., 2012. Enhancement of dissolution of Telmisartan through use of solid dispersion technique surface solid dispersion. J Pharm Bioallied Sci. 4(1), S64–S68.

[22] Ushasree M., Zenab A., Krishnamurthy B. and Shaila L., 2016. Acyclovir Entrapped N-Trimethyl Chitosan Nanoparticles for Oral Bioavailability

Enhancement. Current Nanoscience, 12 (999), 378-385.

[23] Calvo P., Remunan-Lopez C., Vila-Jato J. L. and Alonso M. J., 1997. Novel Hydrophilic Chitosan-Polyethylene Oxide Nanoparticles as Protein Carriers. Journal of Applied Polymer Science. 63,125-132.

[24] Singh B, Kumar R and Ahuja N., 2005. Optimizing drug delivery systems using systematic "design of experiments". Part I: Fundamental aspects. Crit Rev Ther Drug Carrier Syst. 22 (1), 27–105.

[25] Shah U., Joshi G. and Sawant K., 2014. Improvement in antihypertensive and antianginal effects of felodipine by enhanced absorption from PLGA nanoparticles optimized by factorial design. Materials Science and Engineering, 35, 153–163.

[26] Monir N., Shahidan R. and Azmi Z., 2014. Influence of sonication on the stability and thermal properties of Al2O3 nanofluids. Journal of nanomaterials, 2014, Article ID 612417, 10 pages.

[27] Manik P. M., Dhruthiman M., Indika P., Hareesha V., Alan R. and Kenneth A., 2013. Characterization of crystalline and amorphous content in pharmaceutical solids by dielectric thermal analysis. J Therm Anal Calorim, 111(3), 1987–1997.

[28] Shete G., Puri V., Lokesh K., and Bansal A., 2010, Solid State Characterization of Commercial Crystalline and Amorphous Atorvastatin Calcium Samples. AAPS PharmSciTech., 11(2), 598–609.

[29] Richard A. G., Grace A. L., Elena Y. G., Demaurian M.M., Levon A. B., and Tarun K.M., 2015. Formulation and Evaluation of Biodegradable Nanoparticles for the Oral Delivery of Fenretinide. Eur J Pharm Sci. 76, 1–9.

[30] Mosharraf M and Nystrom C., 1995. The effect of particle size and shape on the surface specific dissolution rate of microsized practically insoluble drugs. International Journal of Pharmaceutics, 122 (1-2), 35–47.

[31] Mazumder S., Dewangan A. and Pavurala N., 2017, Enhanced dissolution of poorly soluble antiviral drugs from nanoparticles of cellulose acetate based solid dispersion matrices. Asian Journal of Pharmaceutical Sciences, 12(6), 532-541.

[32] Antonio R., Massimiliano B., Paolo B. Barbara B. and Attilio C., 2013. Chitosan nanoparticles: Preparation, size evolution and stability. Int. J. Pharm., 455(1-2), 219–228.