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Telmisartan loaded Solid Lipid Nanoparticles augmented cytotoxicity in cervical cancer cells: Optimization and *in vitro* characterization

Rahul Sharma[a,b,*], Satish Sardana[c] Jitender Madan[d]

[a]Research Scholar, IKG-Punjab Technical University, Jalandhar, Punjab, India

[b]Hindu College of Pharmacy, Sonepat (Haryana) India

[c]Amity Institute of Pharmacy, Amity University, Guagaon, India

[d]Chandigarh College of Pharmacy, Mohali (Punjab)

ABSTRACT

Cervical cancer, a malignant cancer is leading second most cancer found in women. Telmisartan has 3000 times more affinity toward angiotensin II receptor type 1 (AT1) receptor than AT2 and inhibited neovascularization by down-regulating VEGF acting on endothelial cells with antagonized activity of Angiotensin II. Despite well-known therapeutic potential of telmisartan in malignant cancer, poor physicochemical properties and pharmacokinetic propertiesincluding meageraqueous solubility (0.078 mg/mL), low oral bioavailability (45-58%), and erratic biodistribution not only limit the therapeutic potential of telmisartan in treatment of malignant cancer but also appeal for development of dosage form with enhanced oral bioavailability.Telmisartan encapsulated stearic acid nanostructured solid lipid particles were developed by solvent diffusion method. On applying of box behnken design with three factors and three levels, 17 different formulations were yieldedand prepared with Response of particle size (Y1) and percentage drug entrapment (Y2) for 17 formulations were evaluated. The IC50 value of optimized telmisartan loaded lipid nanoparticle and market preparation, indicated telmisartan loaded solid lipid nanoparticleexpressed lower IC50 value of 30.28 µM with significant anticancer activity against HeLa cancer cell line in comparison to higher IC50 value 58.69 µM of market preparation. In conclusion, telmisartan loaded solid lipid nanoparticles may be a promising drug delivery systems for cervical cancer.

Keywords: Telmisarn, cervical cancer; solid lipid nanoparticles; cytotxicity

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***Address for Correspondence:**

Rahul Sharma, Hindu College of Pharmacy, Sonepat (Haryana) India

INTRODUCTION

Cervical cancer, a malignant cancer is leading second most cancer found in women, affects approximately 50000 million women and accountable for 200000 million deaths per year in developing countries including India. It is assumed that by 2030 rate of death via cervical cancer increases by 30% in low and middle developing with 73000 new cases in India.

Among all ARBs, telmisartan has been preclinically tested in the treatment of several cancers. Telmisartan has 3000 times more affinity toward angiotensin II receptor type 1 (AT1) receptor than AT2[1]and inhibited neovascularization by down-regulating VEGF acting on endothelial cellswith antagonized activity of Angiotensin II.

Despite well-known therapeutic potential of telmisartan in malignant cancer, poor physicochemical properties and pharmacokinetic propertiesincluding meageraqueous solubility (0.078 mg/mL), low oral bioavailability (45-58%), and erratic biodistribution not only limit the therapeutic potential of telmisartan in treatment of malignant cancer but also appeal for development of dosage form with enhanced oral bioavailability.[2-4]

Cytotoxic chemotherapeutic has been administered through various routes in the treatment of cervical cancer however oral route is the most prevalent route of administration of the drug in term of cost effective and patient compliance. However, the physiological barrier of GIT tract, structural geometry and characteristic propertiesof cervical tumors presentsvarious significant challenges including poor absorption, low oral bioavailability, multidrug resistance, less specificity, systemic toxicity, and low therapeutic efficacytowards the oral administration of

chemotherapeutic[5].Therefore, need the development of carrier system or drug delivery technologies which can reduce toxicity, multiple drug resistance andaugment absorption, drug concentration at a particular siteand bioavailability of drug therapeutic molecule.[6]

Recent finding indicated that among all carrier system solid lipid nanoparticles, physically engineering particles with a size range of 1-100 nm, are considered a good alternative to conventional chemotherapy because it canimprove drug bioavailability and decrease the toxicityof drug therapeutic molecule[7].Moreover, its characteristic properties including high volume to surface area ratio, augment absorption, enhanced dissolution, matrix encapsulation and deliver highly lipophilic drug pose them a good candidate for delivery of cancer chemotherapeutics via the oral route of administration in cervical cancer treatment.

In this context, solid lipid nanoparticles have been extensively investigated for delivery of cancer chemotherapeutics through oral route of administration. As reported in the literature, Solid lipid nanoparticle can easily transport a thorough gut wall to the systemic circulation. Small size and lipid matrix of solid lipid nanoparticle facilitateabsorption by intestine either as a particulate matter via M cells of payerpatches with a paracellular pathway[8] or as a lipase mediated mixed micelles through the gut wall and the encapsulated drug was further transferred to the systemic circulation through intestinal lymphatics via thoracic lymph duct. In addition, to augment the absorption of the drug, surfactant of solid lipid nanoparticle leads inhibition of Pgp-mediated efflux of the drug.

Therefore, the aim of present investigation was to develop and optimize telmisartan loaded stearic acid containing solid lipid nanoparticle using solvent diffusion method by employing box behnken design. The tailored formulation was subjected to several in vitro characterization technique including particle size, percentage drug entrapment, percentage drug loading, in vitro drug release and kinetics, Cell cytotoxicity studies, etc…

MATERIALS AND METHODS

Materials

Telmisartan (TEL) was a kind gift sample from Swiss Garnier Life Sciences, Indi, Stearicacid was obtained from Thomas Baker, India. All other chemicals used were of the highest analyticalgrade.

Development of telmisartan loaded nanostructure solid lipid particles

Telmisartan encapsulated stearic acid nanostructured solid lipid particles were developed by solvent diffusion method[1] in an aqueous surfactant solution. In brief,an appropriate weighed amount of stearic acid and 40mg of telmisartan was completely solubilized into an equivolume mixture of ethyl alcohol (6ml) and acetone (6ml). Furthermore, the resulting organic phase immediately dispersed into 100ml surfactant-containing aqueous phase at 70°C under continuous stirring by employing a hot plate magnetic stirrer for 30min. to complete evaporation of the organic solvent, Finally, the obtained nanodispersion was cooled at room temperature and subjected to freeze-drying to obtain a free-flowing powder of telmisartan loaded nanostructured solid lipid particles (TEL-SLN).

Experimental design for optimization

Currently, an approach comprising mathematical models based on statistics has been widely used for optimization and validation of process and formulation parameters for preparation of TEL-SLN. In this work, a three-factor three-level Box–Behnken experimental design was employed to optimized, validate and to elucidate the influence of independent parameters including drug lipid ration, the concentration of surfactant and stirring rpm and their interaction effect over the properties including particle size, Percentage drug entrapment and PI of telmisartan loaded solid lipid nanoparticle.Each independent parameter was studied at three different levels including low level (-1), medium level (0) and high level (+1), respectively as illustrated in Table.1.On applying of box behnken design[9] of experiment software a total 17 run were generated including 12 axial points and one central point with five replicates as displayed in Table 2 for which quadratic polynomial equation was generated is as given:

$$Response = \beta_0 + \beta_1.X1 - \beta_2.X2 - \beta_3.X3 + \beta_4.X1.X2 - \beta_5 X1.X3 + \beta_6.X2.X3 + \beta_7.X1^2 + \beta_8.X2^2 + \beta_9.X3^2$$

Response was the measurement of predicated value of each dependent variable, $\beta_{0\ is}$ intercept, β_1, β_2, β_3, are linear coefficient, β_4, β_5, β_6 are interaction coefficient, β_7, β_8, β_9 are quadratic coefficient and X1, X2, X3 are independent variable was selected on the basis of initial screening of parameters. All 17 formulations were prepared as per the independent variable selected in table no. and data was analyzed by employing design expert 7.0. The selection of best fit model was done by comparing various statistical parameters including p value, adjusted regression coefficient (R^2), predicated regression coefficient (R^2), and lack of fit, etc.ANOVA was employed to assess the significant value of each response parameter. The relationship between independent factor and response was expressed using 3D surface plot and the final formulation was be optimized with the maximum value of percentage drug entrapment with a minimum value of particle size.

In vitro characterization of telmisartan loaded nanostructured solid lipid particles

Percentage of drug encapsulation efficiency and drug loading

The percentage drug encapsulation efficiency and drug loading capacity of TEL-SLN18was determined using the ultracentrifuge filtration method. This method comprising the determination of free drug available over the surface of solid lipid nanoparticle by dispersing separately 10mg weighed quantity of freeze-dried telmisartan loaded solid lipid nanoparticle of each formulation in 10ml of 0.1N NaoH. TEL-SLN18 dispersion was ultra-centrifuged at 50000 rpm for 4 hr. at 4°C and consequently, the obtained supernatant was filtered off by employing 0.22µm membrane filter. The concentration of telmisartan in the supernatant was determined through UV spectrophotometer at λmax 298 nm. All analysis was carried out in triplicate (n=3).

Percentage drug encapsulation efficiency and drug loaded capacity was determined according to the equation are as given below.

$$\%\ Drug\ encapsulation\ efficiency = \frac{\text{Total amount of drug added} - \text{Amount of drug extracted in the supernatent}}{\text{Total amount of drug added}} \times 100$$

$$\%\ Drug\ loading\ efficiency = \frac{\text{Amount of drug encapsulated}}{\text{Total amount of solid lipid nanoparticle}} \times 100$$

Particle size and Zeta Potential

Average particle size, size distribution, and zeta potential of optimized tailoredTEL-SLN18 formulations were determined by employing Malvern Nano ZS, respectively. To get desired particle size separately solubilized 10mg of weighed quantity of freeze-dried nanoparticle of each formulation in 10ml of phosphate buffer solution pH 6.8 and subjected to particle size analyzer at 25±1°C, an electric field of 150mv with a 90° angel of scattering. All measurements were taken in triplicate (n=3)

Transmission electron microscopy (TEM)

Geometry, particle shape and surface topography of optimizedTEL-SLN18 formulation were investigated by transmission electron microscopy (TEM, FTI Tecnai F20). Briefly, a dispersion of telmisartan loaded nanoparticle formulation was preparedin phosphate buffer solution pH 6.8 and drop of the sample was soaked onto a carbon-coated copper grid, and the grid was subjected to air-dried at room temperature. The air-dried grid was loaded into a microscope which was already maintained at 80 kV and observed. The images were captured and analyzed by software.

Fourier transforms-infrared (FT-IR) spectroscopy

FT-IR analysis was carried out to allocution the existence of any interaction between the functional group of drug and excipients. FTIR spectrum of pure drug telmisartan, physical mixture of drug and excipients, blank lipid nanoparticle and tailored formulation TEL-SLN18 were recorded by scanning in a range of 400-4000 cm^{-1} at a resolution of 4 cm^{-1}. Each sample was prepared into KBR disc by employing hydrostatic press at a force of 40 psi.

Powder X-ray diffraction (PXRD) pattern

To investigate the polymorphic state of the drug into nano-size range lipid matrix powder XRD analysis was carried out using powder x-ray diffractometer(Ultima-4, Rigaku Company, Japan)with Ni-filtered, Cu K-radiation, a voltage of 60 kV and a current of 50 mA. The PXRD diffract gram of pure drug telmisartan, physical mixture of drug and excipients, blank lipid nanoparticle and TEL-SLN18 were recorded by scanning over a rangeof5 to 50° diffraction angel at a rate of 1°/min.

In vitro drug release study

In vitro drug release study was performed to study the release pattern of telmisartan from our tailored formulation and compared with the release pattern of the market preparation. It was carried out by using dialysis diffusion tubing technique[1] employing a dialysis membrane with molecular weight 12-14 KDA in fresh phosphate buffer solution pH 6.8 An accurately weighed quantity of tailored formulation equivalent to 40mg of telmisartan and market preparation was resuspended separately into 5ml of phosphate buffer 6.8 pH and filled in dialysis tubing. The dialysis tube was immersed into 900 ml of phosphate buffer 6.8 pH (without serum), maintained at 37° and stirred at constant speed 100rpm. At predetermined specified time interval 5ml of dissolution medium was withdrawal up to 26hr from receiver compartment and simultaneously replenished with the same amount of media 6.8pH (without serum) to simulate sink condition. The aliquots were appropriately dilute with media and concentration of drug in each withdrawal aliquots were determined using UV spectrophotometer at 298nm.

In vitro drug release of tailored formulation was fitted into various mathematical model including zero order, first order, Higuchi and korsmeyer-pepaas model to determined mechanism to characterize of drug release pattern from tailored formulation. Regression value of regression coefficient, intercept and n (exponent) was calculated for each model to determine the best fit model.

Cell cytotoxicity study

A standard colorimetric MTT assay, as an in vitro cytotoxicity assay, was employed to investigatetheeffect of market preparation of telmisartan and TEL-SLN18over the cell viability using 96 well microtiter plates. The principal of the assay is based on the metabolic conversion of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5diphenyltetrazolium bromide) into a formazan dye by the mitochondrial enzyme succinate dehydrogenase of the viable cells and that formazan dye further absorbs light at 550nm. The cervical cancer cell line was seeded at a density of 5×10^3/well in 200µL of serum DMEM medium in (3-[4,5-dimethylthiazol-2-yl]-2,5diphenyltetrazolium bromide). After an incubation period of 24hr medium is replaced with serum-free DMEM and subsequently seeded cells were exposed to a gradient concentration of telmisartan, telmisartan loaded nanostructure solid lipid particle equivalent to 20-100µM for 72hr. At the end of the predetermined incubation period 20µL MTT dye (mg/ml) was added to each well and each plate was incubated for a period of 4 hr at 37°C. Following cell lysis, the supernatant was decanted and consequently obtained formazan crystal was dissolved in 100µL of DMSO. The absorbance of the mixture was measured at 5670nm and 630nm as a reference wavelength by employing ELISA reader. All steps of the Study were carried out in triplicates

RESULTS AND DISCUSSION

Preparation of telmisartan loaded nanostructure lipid solid lipid particles

Solvent diffusion method was successfully employed for the preparation of TEL-SLN. An organic lipid phase containing telmisartan was dispersed into an aqueous surfactant solution and subsequently, telmisartan loaded nano-size range lipid emulsion droplets was formed. Heating step triggered thediffusion of organic solvents consequently increases the concentration of lipid inside the droplets. Upon cooling, solidification of lipid emulsion droplets and absorption of poloxamer around the surface of spherical lipid particles were accomplished simultaneously.

Optimization and validation using box behnken design

To obtained stable telmisartan loaded nanoformulation with low particle size and high drug encapsulation efficiency, optimization and validation of various process and formulation parameters was required Before optimization process, preliminary screening was accomplished by conducting various trails to determine most anticipated formulation and process independent variables by evaluating their effect over the physicochemical properties of drug-loaded nano-size range solid lipid particulates. Based on a result of preliminary screening, three most critical independent variables including percentage lipid to drug ratio (X1), amount of surfactant (X2)and stirring rpm (X3) with three levels (low, middle and high) were selected as illustrated in table no 1and evaluated their effect on the physicochemical property of drug loaded solid lipid nanoparticle. For three-factor and three levels, box behnkendesign offer advantage in terms of no. of run among all types of design, because it was yielding only 17 runs in comparison of central composite design and factorial design, were yielding high numbers of the run. Based on experiment efficiency and no. of run box behnken design was selected for

optimization and validation of telmisartan loaded solid lipid nanoparticle.

On applying of box behnken design with three factors and three levels, 17differentformulations were yieldedand prepared with Response of particle size (Y1) and percentage drug entrapment (Y2) for 17 formulations were evaluated as summarized in Table 2.Data of all 17 formulations were simultaneously fitted into box behnkendesign[10] to obtain predicted value of both response. For each response Y1 and Y2, statistical parameters are including mathematical model analysis, adjusted R^2 and predicted R^2 value was determined and summarized in Table 3 respectively. It was evident that all three independent variables have individual and interactive influence on the responses (Baig et al. 2016). Significance of each statistical parameter on responsewas depending on the p value asdetermined by employing ANOVA. Table 4-5 summarize the effect of each as well as a p value of all terms for all the response. Variables associated with p value <0.05 were considered to be significant only.Reckoning of the statistical parameter for each response attributedquadratic mode is the best fit model for each response as having high R^2 value for both Y1 and Y2 response, among all other mathematical models. Higher non-significant value of Lack of fit measures the suitability of the model for an experimental result.Positive and negative sign before each coefficient in software generated quadratic polynomial equation bearing individual and combined factor revealedboosting or castrating impact of variableson the response respectively. Interaction and quadratic terms described the non-linearity between factors and responses. The intercept represented the mean of the response.

Perturbation graph were presenting to explain effect of each factor on response in better way. If design having more than two factor then perturbation plot has been a best diagrammatic tool to identify the most influencing factor end their effect on response. These plot demonstrated sensitivity of each response toward particle factor, while all other factor remain constant at response point by changing in curvature of graph.

Three dimensional plotswere identified as a useful diagrammatic tool to study the interaction and quadratic effect of two predetermined variables while retaining the third factor as a constant at one time on the observed response of particle size, polydispersity index, and drug encapsulation efficiency.

Effect on particle size

Particle size is the most vital parameter that governs the therapeutic efficacy and stability of drug loaded solid lipid nanoparticles in situ biological milieu. Hence the aim of this study experiment was to optimized formulation with minimum particle size. In order to determine the established relationship between all selected independent variable and particle size of telmisartan loaded solid lipid nanoparticles several different mathematical models including linear, 2FI, Quadratic and cubic were fitted to obtain the best fit model.[10] Selection of model is depending on the value of regression coefficient as summarized in Table 3 was found maximum 0.998313 forthe quadratic model. According to ANOVA report (Table 4) F value and the p value of model was found to be 460.22 and <0.0001 implies that the quadratic model was the best fit model while the F value of lack of fit value of 0.13 also confirm the significance of quadratic model relative to the pure error. Furthermore,drug to lipid ratio (X1), concentration of surfactant (X2), stirring rpm (X3), X1X2&X2X3 (interactive term), $X1^2$&$X3^2$ are significant model terms because they all were having p value <0.0001 and model had good agreement between "Pred R-Squared" of 0.9951 and "Adj R-Squared" of 0.9961. The particle size of all prepared formulation was found in a range of 68.14±1.15 to 132.1±2.19 nm with average particle size 91.910nm, respectively.Influence of all selected independent variable on the particle size was determined by employing quadratic equation in term of coded factor as described in equation no 1.

Particle size (Y1) = 80.76 + 22.78 X1 -5.98X2 -6.75 X3 -6.67 X1X2 + 0.81 X1 X3 –2.21X2 X3 +10.09 $X1^2$+ 1.09 $X2^2$+ 12.52 $X3^2$(1)

Equation no 1 clearly showedparticle size was significantly increased synergistically by increasing drug to lipid ratio (X1), $X1^2$&$X3^2$ and decrease in antagonist way by increasing concentration of surfactant (X2), Stirring rpm (X3), X1X2&X2X3 (interactive term). The large value of coefficient (22.91) depicted that particle size largely influenced by the lipid to drug ratio in comparison to a low coeeficent value of surfactant (-5.98) and stirring rpm (-6.75). The reasons could probably be that higher drug to lipid ratio would enhance the viscosity of dispersion which makes difficult to disrupt emulsion droplets, and reduced the emulsification capacity of surfactant leading to enhance coalescence of particles, yielding particle with large size. Similarly, the amount of surfactant had a contrast effect on particle size. Positive gradient concentration of surfactant produces uniform and stable nanoparticles by reducing the interfacial tension between lipid and external phase.

Despite the amount of surfactant, stirring speed also had negative effect on particle size. Positive increment of stirring rpm would increase kinetic energyallowingparticles to collide with the greater force,resulting in particles with small size. **Figure1A**as showed that independent variable X1(lipid to drug ratio) displayed more curvature in graph among all three factors, illustrated more pronounced effect on the particle size than the variable X3 (stirring rpm) and X2 (concentration of surfactant).

Interaction between the variables X1X2 and X2X3 had negative effects on the particle size and X1X3 interactive term had a positive effect on particle size. This interaction can be utilized to tailor particle size according to the values of factors. 3D surface response graph as shown in figure no.1B describing that drug to lipid ration had a positive effect while the concentration of surfactant and stirring rpm had a negative effect on the particle size and in the same way interactive and quadratic terms X1X2, X1X3, X2X3, $X1^2$, $X2^2$, and $X3^2$alsohad an influence on the particle size of drug loaded solid lipid nanoparticle.

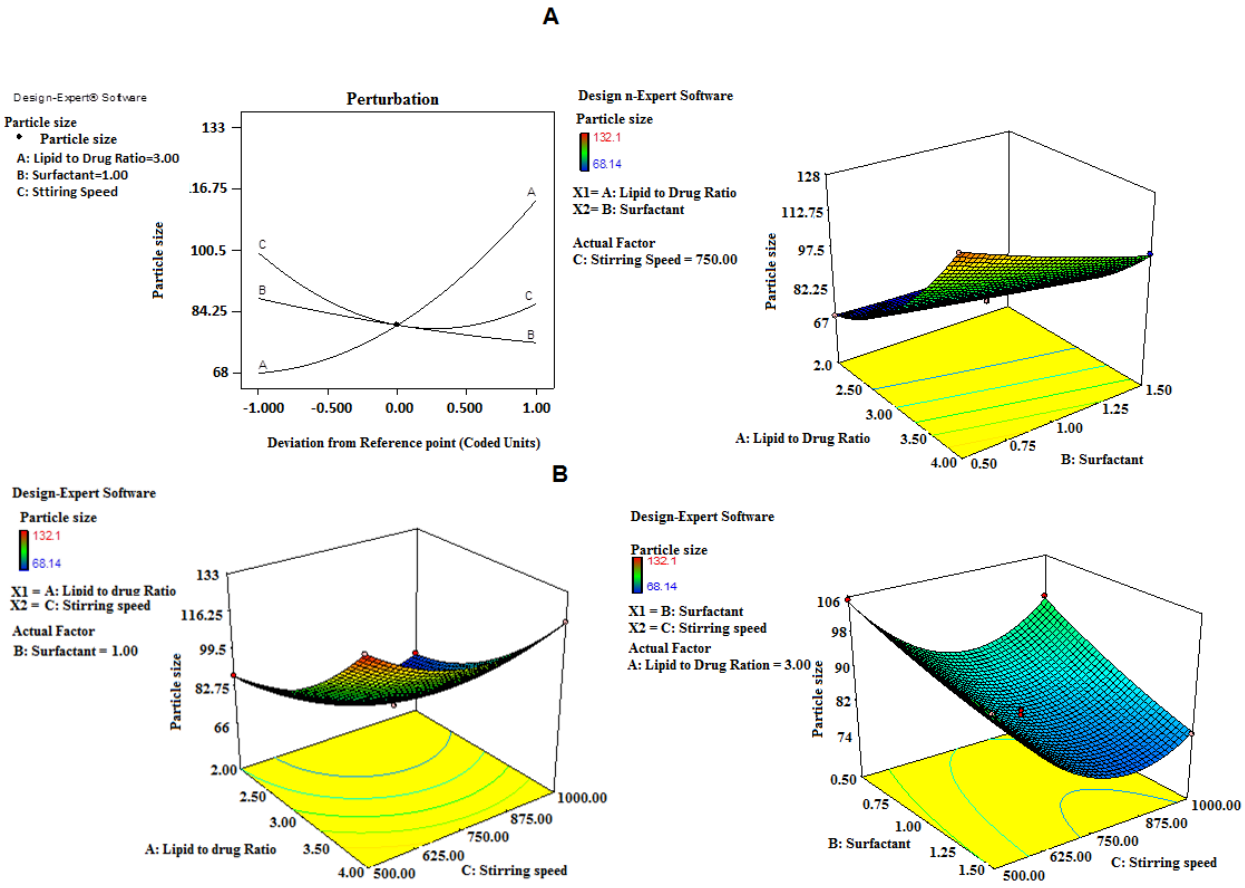

Figure 1: Effect of process variables during preparation of telmisartan loaded solid lipid nanoparticles

Effect on percentage drug entrapment

Parentage drug encapsulation for all prepared formulations was found to be in the range of 62.21±0.377 to 86.04±0.15 with a mean value of 77.399. All the data were fitted in a different mathematical model and evaluated for the response of percentage drug encapsulation efficiency. According to ANOVA report model, F value (307.50) implied that the quadratic model is significant with a p value <0.0001 and lack of fit value 0.19 as illustrated in Table 5.There was a significant correlation between the Pred R-Squared" of 0.9916 and Adj R-Squared" of 0.9942 Despite the quadratic model, independent variable like X1, X2, X3, interactive terms (X1X2, X1X3 &X2X3) and quadratic terms ($X1^2$&$X2^2$&$X3^2$) are also significant with a p value less than<0.005.

The quadratic polynomial equation for the response% drug encapsulation in term of coded factor is as follow:

Percentage drug encapsulation efficiency (Y2) = +83.69 + 9.23 X1 -0.85X2 -0.51 X3 -1.23X1X2 + 1.53X1 X3 –3.46X2 X3 -5.65 $X1^2$-3.33 $X2^2$-4.38$X3^2$……(2)

In quadratic equation 2 positive and negative sign before coefficient indicates the synergistic and antagonist effect of variables on response encapsulation efficiency of drug loaded solid lipid nanoparticle while the magnitude of each coefficient suggested the contribution of each factor to the response. Quantitativeestimations of the model illustrated that drug to lipid ration displayed significant influence on percentage drug encapsulation efficient with F value 1806.55 and p value <0.0001.By increasing the internal phase, more amount of lipid was available to solubilize the drug and decreased the partition of the drug into the external phase. Moreover, higher lipid enhanced the viscosity of the dispersion that would offer résistance to the diffusion of drug molecule into the aqueous phase resulting in higher drug entrapment efficiency.

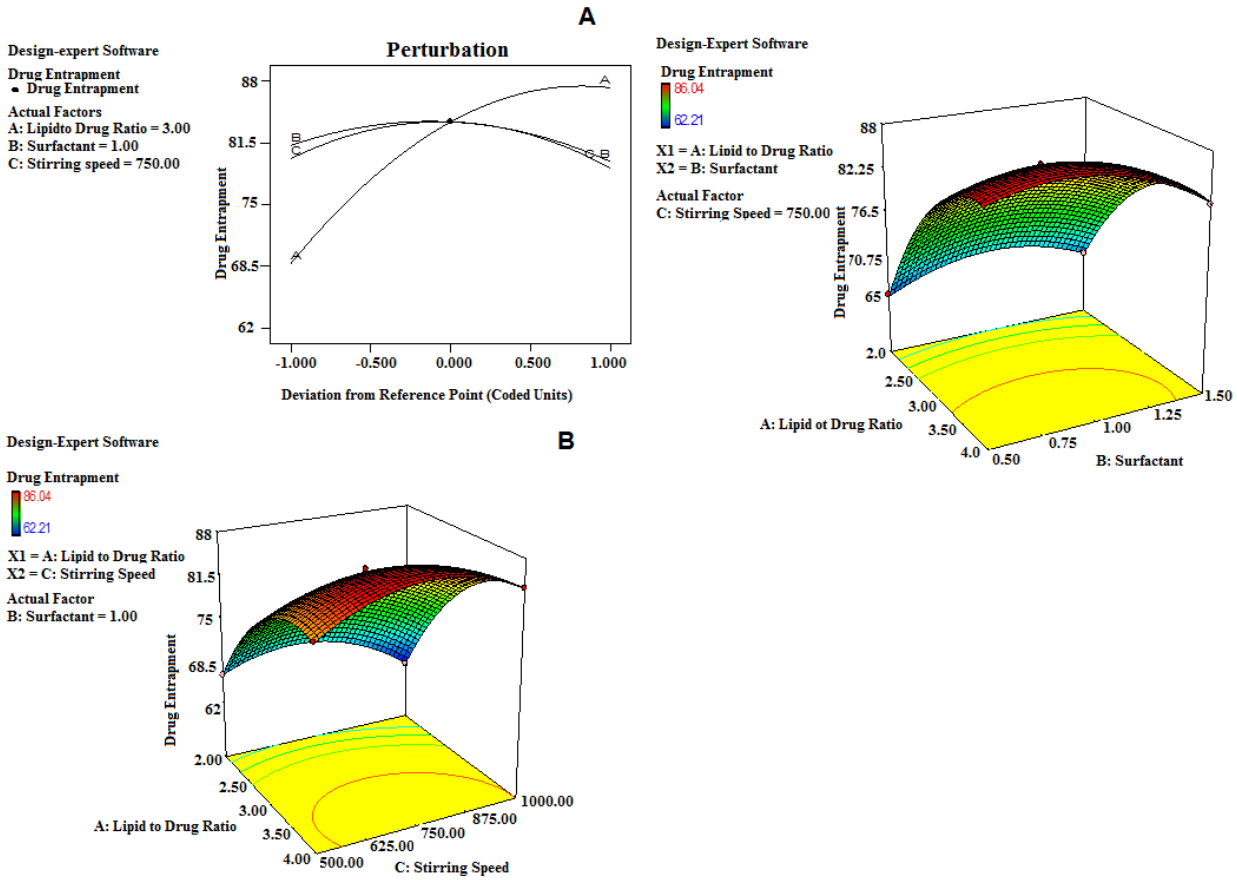

Figure 2: Study of independent variables

Perturbation graph 2A showed that independent variable X1(lipid to drug ratio) displayed more curvature in graph among all three factors, illustrated more pronounced effect on the percentage drug encapsulation than the variable X3 (stirring rpm) and X2 (concentration of surfactant). The physicochemical property of drug also governed the drug encapsulation efficiency of the drug into lipid nanoparticleslike telmisartan was hydrophobic in nature having more tendencies

to solubilize into lipid thereby yielding higher drug encapsulation efficiency.As lipid to drug ratioincreased encapsulation of drug into lipid matrix of solid lipid nanoparticles would simultaneously enhance up to a maximum value and then decreased in an umbrella shape pattern depend on the affinity of drug towards the lipid stearic acid as depicted in 3D surface response graph as shown in Figure 2B.In contrast to the lipid to drug ratio, the concentration of surfactant and stirring rpm were inversely proportional to percentage drug encapsulation. High surfactant concentration in the external phase might favor the partition of the drug from the internal phase to external aqueous dispersing medium,leadingsolubilization of drug into an aqueous solution and decreased encapsulation efficiency. Similarly increasing in stirring rpm resulted in more expulsion of the drug from lipid nanoparticle then reduced the drug entrapment efficiency. Effect of both independentvariables was not prominent because telmisartan is a hydrophobic and strongly associated with lipid molecule resulting in encapsulation of drug in lipid core.

Optimization and validation

Effect of all independent variable on both response particle size and percentage drug encapsulation was demonstrated by employing quadratic polynomial equation was further optimized and validatedto obtain stable formulation with minimum particle size and maximum drug encapsulation efficiency.The optimal value predicted for independent variables including lipid to drug ration, concentration of surfactant and stirring rpm were 3.02:1, 1.09% w/v and 779.43 with desirability 0.856. 18 The value of desirability illustrated the closeness of value toward the target value and varied from 0 to 1depending on the desired and non-desired situation of responsepredicted value for response including particle size and percentage drug encapsulation efficiency was found to be 79.6001nm and 83.4651, respectively. To confirm predicated formula, validation was carried out by preparing a new batch of telmisartan loaded solid lipid nanoparticle and determine the magnitude of error between the observed experimental value and predicted value.The observed experiment value for particle size and percentage drug encapsulation efficiency was found to be 78.90± 1.8 nm and 82.88± 2.4 were in reasonable agreement with a predicted value suggesting the selected box Behnken optimization design were truthful and trustworthy for tailored telmisartan loaded solid lipid nanoformulation.

Particle size polydispersity index and Zeta Potential

Size of particle plays a vital role in the therapeutic efficacy of nanoformulation because of nanoparticle with particle size <100 can easily lymphatic system through peyer patches resulting in enhanced drug delivery via oral administration. The particle size of optimized formulation was found to be 78.90±1.8as given in table no.9 and figure no. 3Arespectively. Nanoparticle with particle size <200nm was reported to be easily escaped through reticuloendothelial system recognition; therefore, it can release the drug in a sustained manner over a long period of time in systemic

circulation.Membrane wrapping process was known to be responsible for uptake of nanoparticle depending on size (30-50nm). It is based on the principle that small particle with size range 30-50nm engender free energy to uphold the internalization while particle with size range >50nm did no generated sufficient energy to initiate this membrane wrapping process.Polydispersity index defined the uniformity of particle into dispersion and varied from 0 to 1. Low value of PDI indicated uniform homology between particles. Table 6 described PDI value for all formulations was found to be in the range of 0.09±0.21 to0.152±0.61. In addition, PDI value for the optimized formulation was observed to be 0.119±0.879respectively, indicated that particle was uniform in prepared solid lipid nanoparticles.

Zeta potential attributed the magnitude of charge present on the surface of the nanoparticle and plays a significant role in illustrating stability and in vivo fate aqueous dispersion of nanoparticle. The ideal range of zeta potential was reported to be +30mv to -30mv to obtain a stable aqueous dispersion. The reason could probably the existence of attractive and repulsive force between particles which is responsible for agglomeration or deagglomeration of particles in aqueous dispersion. Despite the stability of aqueous dispersion, zeta potential also determines the in vivo pharmacokinetic behavior, circulation, and immune response. Zeta potential of tailored optimized formulation was found to be 38.19±1.98,respectively as shown in Figure 3A. The negative value of zeta potential was due to functional group COO- of stearic acid.

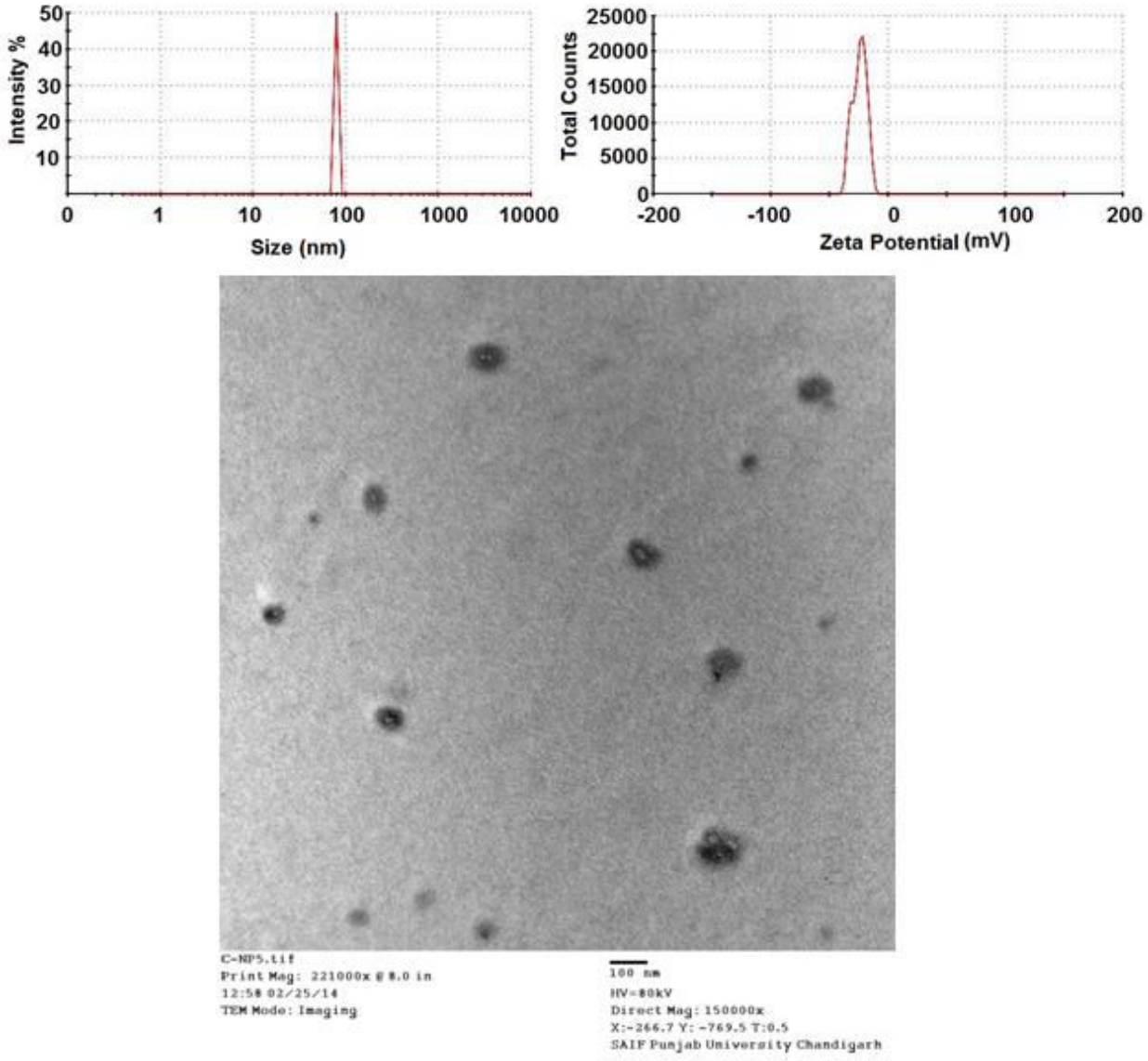

Figure 3: Particle size, zeta-potential and transmission electron microscopy of telmisartan loaded solid lipid nanoparticles

TEM analysis

The surface architecture of telmisartan loaded lipid nanoparticle depicted in Figure 3B expressing that prepared nanoparticle were spherical, discrete, uniform in shape and free form any surface deformability 33 with a size range 20-50nm.

Percentage drug loading of telmisartan on lipid nanoparticle

Percentage drug encapsulation for all TEL-SLNswere found to be in the range of 6.24±0.032% to 8.60±0.015% as shown in table no.6. Furthermore, for final optimized formulation it was measured to be 8.34±0.025%.

FTIR spectrum analysis

FTIR spectrum of telmisartan, physical mixture of telmisartan, poloxamer 188 and stearic acid in equimolar ratio, blank lipid nanoparticle and optimized TEL-SLN18 was apprehended to probe the chemical incompatibility and existence of chemical bond between lipid and drug characteristics stretching and bending vibration in term of wavenumber of drug, lipid, physical mixture, blank lipid nanoparticle, and drug-loaded lipid nanoparticle was depicted in figure no. 4. FTIR spectrum of pure drug telmisartan showed the characteristic peaks at wavenumber of 3350.42 cm^{-1}, 2970.32 cm^{-1}, 1696.04 cm^{-1}, 1377.52 cm^{-1} and 746.32 cm^{-1} corresponding to the aromatic C-H stretch, aliphatic C-H stretch, carboxylic acid, OH bending and C-O stretching of carboxylic acid, OH bending and C-O stretching of carboxylic acid, respectively.

FTIR spectrum of TEL-SLN18 showed a slight shift in wavenumber at 2915.83 cm^{-1} (aliphatic C-H stretch) and 2849.42 cm^{-1} for CH_3/CH_2 groups and 1697.32 cm^{-1} for -OC=O concerning original peaks of drug telmisartan.However, in comparison to drug lipid nanoparticle, the physical mixture did not display a shift in wavenumber of theses peaks in both components (**Figure 4**). Therefore FTIR spectrum analysis confirmed the absence of any chemical incompatibility between components as well as evidence that drug was persisted with their chemical functional groups and their chemical stability was not affected by lipid matrix during encapsulation.

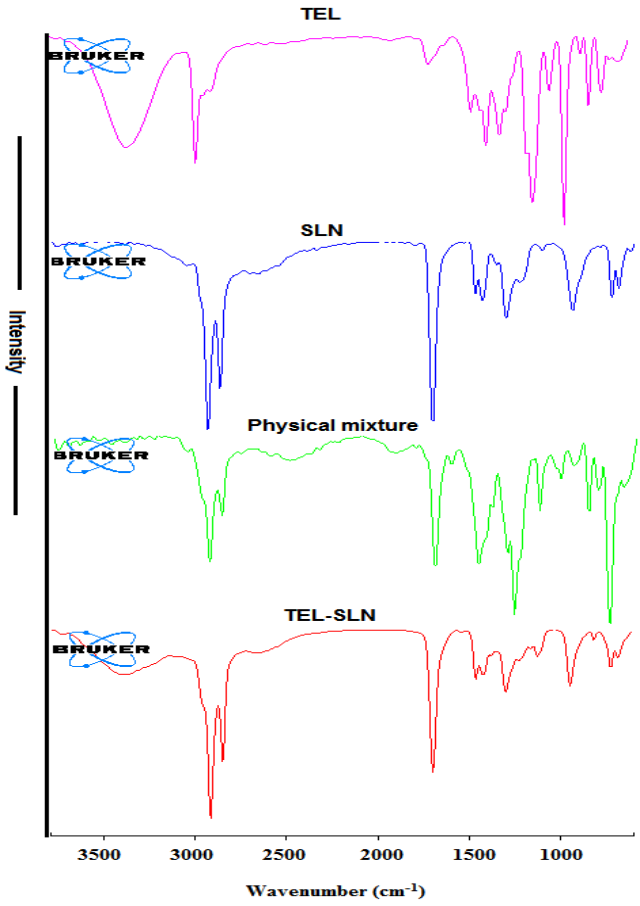

Figure 4: FT-IR spectrum of (A) telmisartan (B) blank solid lipid nanoparticles (C) physical mixture and (D) telmisartan loaded solid lipid nanoparticles

PXRD Analysis

Polymorphic state of drug and lipid in solid lipid nanoparticle was elected by comparing the diffractogram of pure drug telmisartan, physical mixture, blank lipid nanoparticle, and TEL-SLN18 as shown in Figure 5, respectively. The XRD pattern of pure drug telmisartan and physical mixture displayed sharp and intense characteristic peaks indicating the crystalline state of telmisartan in bulk form and physical mixture as well as implying that there was no incompatibility between drug and lipid.However, the characteristic peak of telmisartan was disappeared in PXRD pattern of telmisartan loaded solid lipid nanoparticle provides support to inference the telmisartan was well molecularly dispersed and encapsulated in lipid matrix of solid lipid nanoparticle and stabilized amorphous state (**Figure 5**). Moreover, in telmisartan loaded solid lipid nanoparticleprincipal peaks of stearic acid was not completely absent, rather appeared with reduced intensity. The reason may elucidate that incorporation of the drug between crystal lattice of lipid, leading to changes in the lattice structure of lipid in solid lipid nanoparticle.

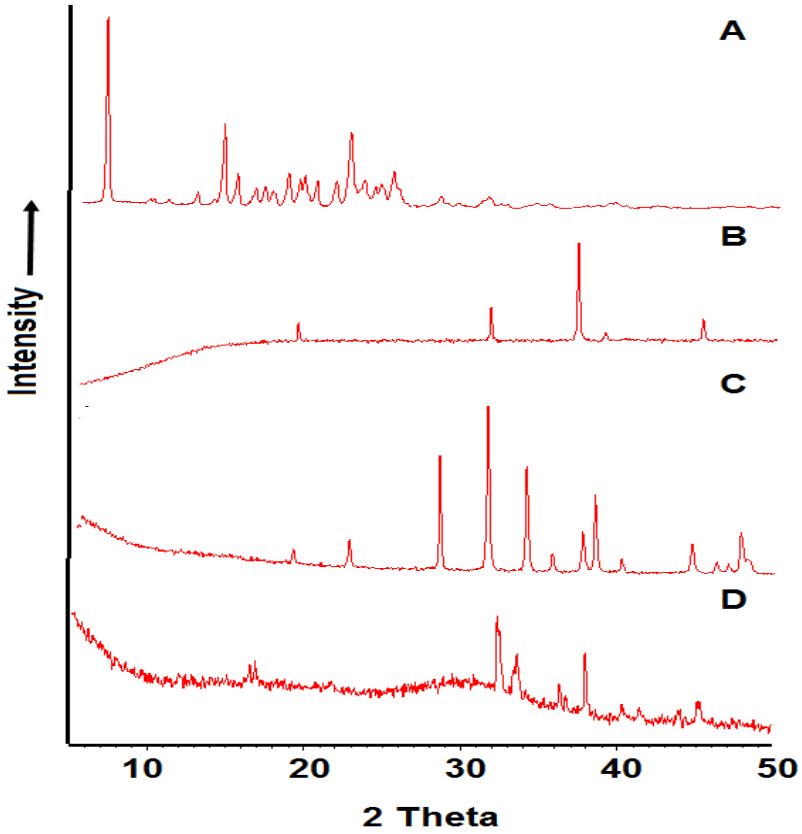

Figure 5: XRD pattern of (A) telmisartan (B) blank solid lipid nanoparticles (C) physical mixture and (D) telmisartan loaded solid lipid nanoparticles

In vitro drug release

In vitro drug release study of TEL-SLN18and market preparation Telvas 40 was carried out by employing dialysis tubing method with a semipermeable dialysis membrane. Percentage drug release for telmisartan loaded solid lipid nanoparticle and market preparation was found to be in the range of 6.81±1.73% to 20.39±0.85% and 9.11±2.95% to 78.40±3.17% as can be clearly visualized in Figure 6,TEL-SLN18 showed drug release in the biphasic pattern including burst release 11.65±3.03%of telmisartan at initial time interval within 1hr, following by releasing 77.84±2.83%of the drug in a sustained manner up to 24hr. 24.

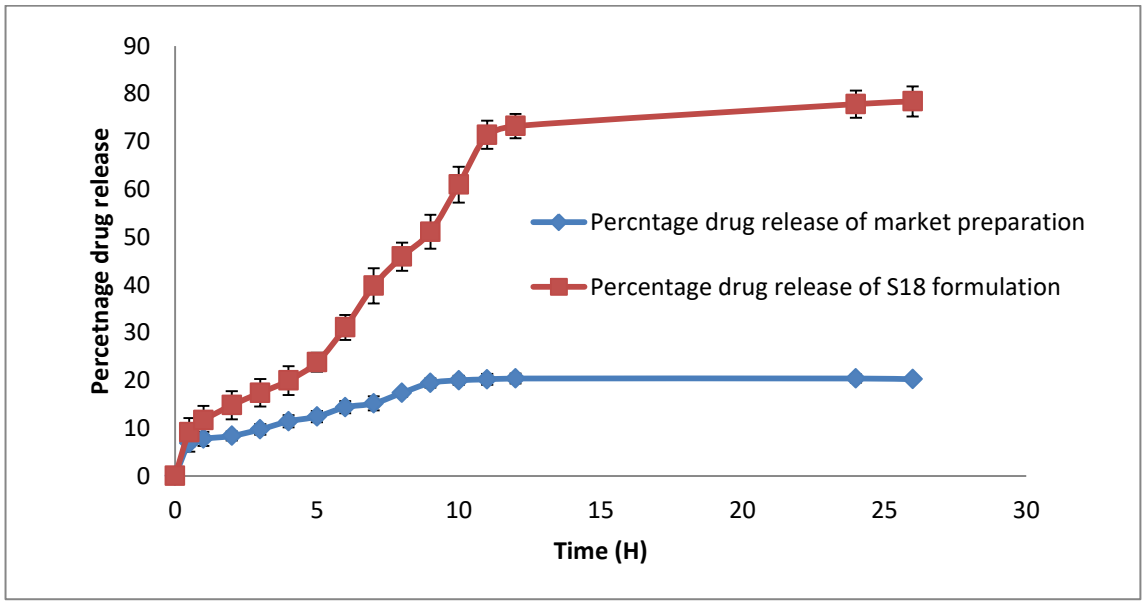

Figure 6: In vitro drug release from telmisartan and telmisartan loaded solid lipid nanoparticles

The initial burst can be attributed to the small size, large surface area and unentrapped drug adsorbed around the surface of tailored formulation.Whereas, in later stage slow release of telmisartan from solid lipid nanoparticle provides support to the assumption that drug was homogenously encapsulated into the inner core of solid material of formulation and diffuse out through both diffusion and dissolution mechanism. In contrast to solid lipid nanoparticle, marketed preparation displayed very less release of drug 20.39±0.85%within 24 hrin comparison to

sustained release of telmisartan loaded solid lipid nanoparticle.

In order to suggest a mechanism of drug release from tailored formulation experimental data was fitted into different mathematical modal and check the goodness of fit of the model by comparing the value of statistical parameters including regression coefficient, intercept and release exponent as depicted in Table 7. The regression coefficient value was found to be maximum for Korsmeyer-Peppas model 0.9181 followed by Higuchi 0.8799 and first-order 0.848, respectively.The release exponent value 0.6485. Indicated that the release of telmisartan from tailored formulation followed nonfickian diffusion, indicating the release of molecularly encapsulated drug was controlled by diffusion and erosionof the core of solid lipid of the tailored formulation. Similar release kinetic for drug-loaded solid lipid nanoparticle was reported in previous literature.

In vitro anti-cancer cell line study

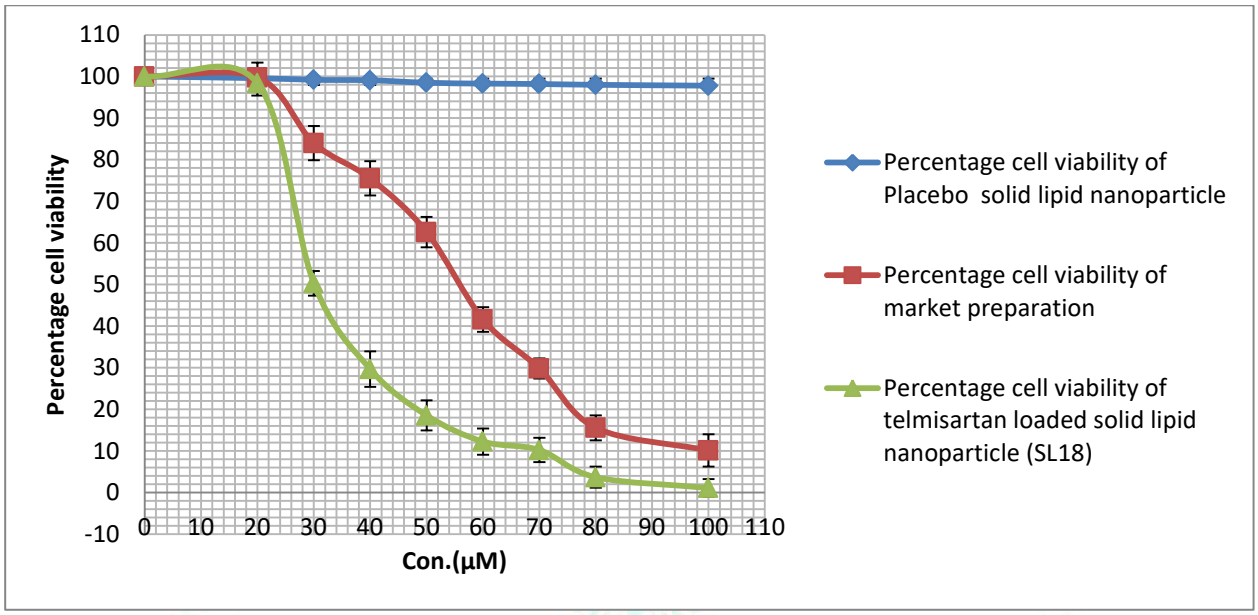

Figure 7: In vitro cytotoxicity assay of telmisartan, telmisartan loaded solid lipid nanoparticles and blank solid lipid nanoparticles against human cervical cancer cells, HeLa

To determine the toxic effect of tailored nanoformulation and market preparation over cancer cells, in vitro cancer cell line cytotoxicity was carried out using MTT assay against HeLa cells (Human cervical cancer cell line) and measured IC50 value of each formulation. In this case, placebo lipid nanoparticle was used as control. IC50 value defined as a minimum concentration of formulation required to kill 50% of cells. **Figure 7** described the IC50 value of telmisartan loaded lipid nanoparticle and market preparation, indicated telmisartan loaded solid lipid nanoparticle expressed lower IC50 value of 30.28µM with significant anticancer activity against HeLa cancer cell line in comparison to higher IC50 value 58.69µM of market preparation. Moreover, placebo solid lipid nanoparticles did not show anticancer activity against HeLa cancer cell line.The higher cell cytotoxicity and low IC50 value was evident to the higher uptake of tailored nanoformulation by cancer cell line.

Table 1: Independent and dependent variables with their levels in Box-Behnken design for the development of telmisartan loaded solid lipid nanoparticle

Independent variables					
Factor	Name	Units	Low Actual	High Actual	Mean
			Low (-1)	High (1)	Mean (0)
X1	Lipid to Drug Ratio		2:1	4:1	3:1
X2	Surfactant (Poloxamer 1)88	(%w/v)	0.5	1.5	1
X3	Stirring speed	RPM	500	1000	750
Dependent variables					
Particle size (nm)					
Percentage drug entrapment					

Table 2: Observed value and predicted value of all response in Box-Behnken design for the formulation of telmisartan loaded solid lipid nanoparticle (n=3)

Formulation code	**Independent Variables**			**Dependent Variables**	
	Factor 1(X1) Lipid to Drug	Factor 2 (X2) Surfactant	Factor 3 (X3) Stirring speed	Particle size (Y1)	Percentage of drug entrapment (Y2)

	Ratio	(%w/v)	RPM	Observed Value	Predicted value	Observed Value	Predicted value
S1	3	1	750	81.1	80.756	84.46	83.688
S2	3	1.5	500	97.01	97.3425	79.19	79.1025
S3	3	1	750	82.09	80.756	83.15	83.688
S4	4	0.5	750	127	127.36625	86.04	86.0075
S5	2	1	1000	73.02	73.03125	62.21	62.39
S6	2	1	500	88.11	88.14375	66.42	66.475
S7	2	0.5	750	68.14	68.46125	65.37	65.1025
S8	2	1.5	750	70.21	69.84375	65.82	65.8525
S9	3	1.5	1000	79.08	79.435	71.36	71.1475
S10	3	0.5	500	105.24	104.885	73.67	73.8825
S11	3	1	750	82.3	80.756	83.1	83.688
S12	3	0.5	1000	96.14	95.8075	79.69	79.7775
S13	3	1	750	79.07	80.756	84.58	83.688
S14	4	1	500	132.1	132.08875	82.05	81.87
S15	3	1	750	79.22	80.756	83.15	83.688
S16	4	1.5	750	102.39	102.06875	**81.58**	**81.8475**
S17	4	1	1000	120.25	120.21625	83.95	83.895

Table 3: Summary of the value of regression coefficient analysis of the models for responses Y1 and Y2 of telmisartan loaded solid lipid nanoparticle

Response 1 (Y1) Particle size						
Model	R-Squared	Adjusted R-Squared	Predicted R-Squared	Std. Dev.	CV %	Remark
Linear	0.776528	0.724957535	0.630693311	10.31075251	11.22	
2FI	0.808879	0.694206173	0.427439223	10.87188484	11.83	
Quadratic	0.998313	0.996143656	0.995145191	1.220895163	1.33	Suggested
Cubic	0.998467	0.993866085		1.539782452	1.68	
Response 2 (Y2) Percentage drug entrapment						
Model	R-Squared	Adjusted R-Squared	Predicted R-Squared	Std. Dev.	CV %	
Linear	0.658695	0.579932085	0.470061392	5.239448328	6.77	
2FI	0.719248	0.550797399	0.324541767	5.418099013	7	
Quadratic	0.997477	0.994233373	0.991616042	0.613884587	0.79	Suggested
Cubic	0.997785	0.991139067		0.760966491	0.98	

Table 4: Summary of the value of Analysis of the variance of the calculated model and independent variables for response particle size Y1 of telmisartan loaded solid lipid nanoparticles

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F	
Model	6174.014105	9	686.0015672	460.2230448	< 0.0001	significant
X1-Lipid to Drug Ratio	4152.33845	1	4152.33845	2785.71061	< 0.0001	
X2-Surfactant	285.9636125	1	285.9636125	191.8465653	< 0.0001	
X3-Stirring speed	364.0951125	1	364.0951125	244.2632339	< 0.0001	
X1X2	177.9556	1	177.9556	119.3864154	< 0.0001	
X1X3	2.6244	1	2.6244	1.76065102	0.2262	
X2X3	19.492225	1	19.492225	13.07689598	0.0086	
X12	428.7294129	1	428.7294129	287.6249344	< 0.0001	
X22	4.986476053	1	4.986476053	3.345314794	0.1101	
X32	660.3443813	1	660.3443813	443.010215	< 0.0001	
Residual	10.434095	7	1.490585			
Lack of Fit	0.950375	3	0.316791667	0.133614939	0.9350	not significant

Table 5: Summary of the value of Analysis of the variance of the calculated model for response and independent variables for response percentage drug encapsulation Y2 of telmisartan loaded solid lipid nanoparticles

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1042.977	9	115.8862793	307.5095169	< 0.0001	significant
X1-Lipid to Drug Ratio	680.805	1	680.805	1806.547055	< 0.0001	
X2-Surfactant	5.81405	1	5.81405	15.42784631	0.0057	
X3-Stirring speed	2.1218	1	2.1218	5.630292876	0.0494	
X1X2	6.027025	1	6.027025	15.99298516	0.0052	
X1X3	9.333025	1	9.333025	24.76560664	0.0016	
X2X3	47.95563	1	47.955625	127.2524337	< 0.0001	
X12	134.5414	1	134.5414003	357.0117294	< 0.0001	
X23	46.76725	1	46.76725289	124.0990342	< 0.0001	
X32	80.69345	1	80.69345289	214.1237501	< 0.0001	
Residual	2.63798	7	0.376854286			
Lack of Fit	0.3217	3	0.107233333	0.185181987	0.9013	not significant

Table 6: Percentage drug loading and PDI of all telmisartan loaded solid lipid nanoparticles (n=3)

Formulation code	Percentage of drug Loading	PDI
S1	8.47±0.009	0.118±0.23
S2	7.96±0.013	0.11±0.59
S3	8.31±0.009	0.115±0.64
S4	8.60±0.015	0.19±0.39
S5	6.24±0.032	0.09±0.21
S6	6.62±0.018	0.105±0.44
S7	6.53±0.013	0.135±0.57
S8	6.54±0.034	0.11±0.75
S9	7.15±0.023	0.113±0.53
S10	7.36±0.005	0.145±0.6
S11	8.31±0.151	0.111±0.36
S12	7.91±0.014	0.151±0.42
S13	8.45±0.016	0.116±0.08
S14	8.24±0.025	0.134±0.11
S15	8.31±0.009	0.113±0.96
S16	8.10±0.036	0.145±0.41
S17	8.33±0.016	0.152±0.61

Table 7: Value of regression coefficient for different Mathematical model used for evaluation of drug release kinetic of telmisartan loaded solid lipid nanoparticle

Formulation Name	Zero-order		First-order		Higuchi		Peppas	
	R^2	K_0	R^2	K_0	R^2	K_0	R^2	K_0
SL18 Formulation	0.783	2.99	0.848	-0.027	0.8799	19.13	0.9181	0.6458

CONCLUSION

In conclusion, we have successfully prepared and scaled up the solid lipid nanoparticles of telmisartan for drug delivery

to cervical cancer cells.Further, optimization techniques help to achieve a good yield of nanoparticles with minimum use of organic solvents. The optimized formulation exhibited low IC50 value as compared to telmisartan. Hence, we could predict that telmisartan loaded solid lipid nanoparticles would be a potential drug delivery system.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical Approval: No ethical approval is required as no animals or humans have been used in the study.

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