ORIGINAL ARTICLE

Development of Self-emulsified Nanoemulsion of Telmisartan by Low-energy Method Using D Optimal Mixture Matrix Design Approach as a Tool for Optimization Methodology

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Abstract

Introduction: Telmisartan is one of the promising, long-lasting antihypertensive agents that act as an angiotensin II receptor blocker. It belongs to biopharmaceutics classification system (BCS) class II having low water solubility and very low oral bioavailability. **Materials and Methods:** The objective of the study is to design and develop self-emulsified nanoemulsion (SNEDDS) of telmisartan by low-energy method using a factorial design approach as a tool for optimization methodology. The solubility of the drug in different oils, surfactants, and cosurfactants was analyzed. Pseudo-ternary phase diagram was used to identify the self-nanoemulsifying region along with the optimized concentration of oil, surfactant, and cosurfactant. D-optimal mixture matrix design approach was applied to selected elements to find out the optimized batch. A total of 16 batches were analyzed for particle size, drug content zeta potential along with other physiochemical characteristics. **Results:** Telmisartan SNEDDS was developed using oleic acid as oil (15%), tween 20 (surfactant): Transcutol P (cosurfactant) (3:1 ratio) (70%) as nanoemulsion system components. D-optimal mixture matrix design was used to find out different possible batches having an optimal blend of mixture components. From the various combinations optimized batch was further evaluated for different physicochemical properties and found within acceptable range. The droplet size of the selected optimized nanoemulsion was within 200–250 nm, polydispersity index 0.240, zeta potential -40.6 mV, determined using a particle size analyzer. **Conclusion:** The solubility of poorly water-soluble telmisartan had improved with a selfanoemulsifed nanoemulsion drug delivery system. Oleic acid as oil (15%), tween 20 (surfactant): Transcutol P (cosurfactant) (3:1 ratio) (70%) found to be compatible and suitable for formation of stable nanoemulsion system. Using D-optimal mixture matrix design approach and applying the low energy method telmisartan-containing nanoemulsions were prepared. The optimized formulation showed the improved drug content and drug released as compared to the plain drug. The developed optimized formulation with design experts gives promising strategies to improve the efficacy and effectiveness of poorly soluble drugs such as telmisartan.

Key words: Mixture matrix design expert, SNEDDS, telmisartan

INTRODUCTION

Hypertension is one of chronic disease which is increasing with very fast rate in India as well in global.[1] World Health Organization estimated 1.28 billion adults having the age between 30 and 79 years worldwide have hypertension.[2] Hypertension is one of the causes of cardiovascular diseases include its morbidity and mortality. The use of antihypertensive agents is the solution to control and treat hypertension with better results. Telmisartan is one of the promising, long-lasting antihypertensive agents act as an angiotensin II receptor blocker belonging to BCS class II. Telmisartan is used in a single dosage form as well as in combined dosage form with other drugs like hydrochlorothiazide, amlodipine, cilnidipine, metformin, ramipril, and azelnidipine. Telmisartan has high tolerability with high lipophilicity which enhances

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tissue penetration, absorption, bioavailability, and vascular protection. It has the great potential to reduce risky early morning high blood pressure which is associated with increased cardiovascular risk.[3,4]

The molecular mechanism of telmisartan involves the selective reversible binding and inhibition of the angiotensin II type 1 (AT1) receptor. Angiotensin II plays an important role in the renin–angiotensin system in high blood pressure. The potent effects include vasoconstriction of vascular smooth muscles causing them to narrow leading to increased blood pressure. Telmisartan has a high affinity toward AT1 receptor and inhibits the activity of the angiotensin II receptor. This inhibition affects in vasodilation and reduces vascular resistance. Angiotensin II stimulates the adrenal cortex to release aldosterone, which is responsible for sodium and water retention by the kidneys. This phenomenon contributes to an increase in blood pressure. The blockage of AT1 receptor reverses the effect and decreases sodium and water retention which results in lower blood pressure. In addition, telmisartan also shows agonist activity for peroxisome proliferator-activated receptor gamma provides protective effects for cardiovascular diseases.[5,6]

Despite its advantages, there are some challenges associated with telmisartan which include low solubility in biological fluid which leads to poor bioavailability after oral administration (about 42%), late onset of action. One of the reasons to have low bioavailability is its high first-pass metabolism.[7]

Many research highlight different effective strategies for improving the solubility of telmisartan including solid dispersion techniques, co-crystallization with suitable conformers, complexation method, microemulsion, and nanoemulsion drug delivery systems.[8-13] Self-nanoemulsified drug delivery system is one of the promising drug delivery systems help in the improvement in solubility of poorly soluble drugs.[14-16]

D-optimal mixture design is the exceptionally effective approach used in food and pharmaceutical industry. It has many advantages including capability to analyze different interactions between various factors and variables and identify the possible limitations. This helps in the reduction in experimental runs and improves the performance of the method. The overall use of design expert makes the analysis easy, less time-consuming, and cost-effective. The present study aims to develop self-emulsified nanoemulsion with D-optimal mixture design using selected oil, surfactant and cosurfactant to get desired nanoemulsifed globules. Meanwhile, the interactive effect between various dependent and independent variables and their effect on particle size was examined.[17-19]

The goal of the present study to improve the water solubility and dissolution rate of BCS class II telmisartan by reducing particle size with novel drug delivery approach like nanoemulsions. Oil in water type of emulsion is best suitable emulsion for efficient delivery of telmisartan, in which poorly soluble telmisartan is dissolved in the oil phase and dispersed in water phase. Low-energy methods of nanoemulsions preparation include the phase inversion method, sonication method, and high-pressure homogenizer used effectively to achieve the formulation of nanoemulsions. The evaluation and characterization of the prepared self nanoemulsion prove the suitability of nanoemulsion.[20]

MATERIALS AND METHODS

Materials

Telmisartan standard drug sample was kindly received from J.B. Chemicals and Pharmaceuticals Limited, Mumbai. Labrafil M2125CSand capryol 90 oil samples were received as gift samples from Gattifose, India Pvt. Ltd. Others such as oleic acid, tween 20, and transcutol P were procured from LobaChemie Pvt. Ltd. All chemicals and reagents were used of analytical grade.

Solubility of drug in different solvents

Solubility of telmisartan in different solvents including distilled water, acidic buffer (pH 1.2), phosphate buffer (pH 6.8) andvarious oil, surfactant, and cosurfactant were investigated. The linearity regression equations were established using methanol and further utilized in the analysis.

Screening of oil, surfactant, and cosurfactant for nanoemulsion formulation

The selection of oil, surfactant, and cosurfactant was determined using shaking incubator. The solubility of telmisartan was determined by adding an excess of drug in 5 mL of selected excipients such as oil (oleic acid, caproyl, labrafil, natural oils almond oil, peppermint oil, castor oil), surfactant (tween 80, tween 20, span 80, span 20), cosurfactant (propylene glycol, transcutol P) in 15 mL of capacity stoppered vials, initially stirred with vortex mixer and kept on incubator shaker with 1000 rpm speed at ambient temperature for 72 h. Sample suspensions were centrifuged at 3000–3500 rpm for 20 min to separate undissolved drug. The supernatant solution was filtered through 0.45 µm filter. 1 mL of filtered solution was further diluted with methanol, the absorbance was recorded using ultraviolet (UV) – visible spectrophotometer (Shimadzu UV-1800) at 296 nm. The concentration of soluble drug was determined by regression equation obtained from the standard curve method by plotting concentration of telmisartan in methanol versus absorbance.

Selection of surfactant and co surfactant ratio

The effect of surfactant and cosurfactant ratio was further evaluated for further optimization of the Smix ratio. The

pseudoternary phase diagram helps to find out the most suitable nanoemulsified region with selected oil. The phase diagram was constructed for the various ratios 1:1, 2:1, 3:1, 4:1 of selected surfactant and cosurfactant, 3:1 Smix ratio was further selected depending on stability.

Preparation and preliminary screening of self nanoemulsion

This work was performed for preliminary screening on the series of (o/w) nanoemulsion prepared by combined ultrasonication and temperature inversion methods. The effect of oil (oleic acid 15%), Smix (tween 20 and transcutol (3:1) 70%), and water were investigated by keeping oil and Smix at low, intermediate, and high concentration. The minimum and maximum values of the respective component were finalized for additional evaluation using design expert. The oil phase and mixture of Smix were prepared separately. Drug was dissolved in oil with 50–55°C heat as required and transfer it drop wise and mixed thoroughly every time with Smix with continues stirring on magnetic stirrer having speed 2000–2500 rpm at 50–55°C. 5 mg/mL of the telmisartan was dissolved in the nanoemulsion system. The prepared emulsion concentrate (1 mL) was further checked for the emulsion formation ability by adding in 100 mL of distilled water and visual inspection was done to categorize grade of nanoemulsion formed.

The initial preliminary study was performed to find out the minimum and maximum concentration that could be useful to get desired nanoemulsions. According to the Schiff's D optimal mixture design sum of the percentage of components mathematically must be equal to 100%. (Means A [Oil] + B [S Mix] + C [Water] = 100%). The total 16 runs were suggested by software and all sixteen formulations were prepared and evaluated for droplet size, drug content, and zeta potential. Best suitable batch was taken further for evaluation and characterization.

Optimization of experimental design for preparation of telmisartan loaded oil in water self nanoemulsions using D optimal mixture matrix design approach

Experimental design

The study employed a three-factor D optimal mixture design to assess the effect of three independent variables oil (A), surfactant, and cosurfactant ratio (Smix) (B) and water (C). Particle size, drug content, and zeta potential were considered as dependent variables. Different 16 batches were suggested by the design expert 13 software. The maximum and minimum values of oil, surfactant, and cosurfactant in percentage were added in software. D optimal mixture matrix suggested the quadratic Scheffe model with significant Analysis of Variance (ANOVA) values. Each design was assessed independently to observe the influence of the composition of each variable toward three responses. D-optimal design replicated one design points to estimate the design error. The replicates were observed in runs 1 and 4, 11 and 13.

Statistical analysis using ANOVA

ANOVA was done with the help of D-optimal mixture design to assess the significant difference between each of the independent variable. The experimental data analysis shows the quadratic ANOVA analysis was significant for particle size, drug release, and zeta potential having $P < 0.05$.

The best formulation for SNEDDS was one who had given lowest particle size with optimum zeta potential and drug content. The samples were prepared and analyzed in triplicate. The % prediction error (PPE %) was calculated using the equation to assess the adequacy of the selected model.

$$PPE\,(\%) = \frac{Difference\ between\ experimental\ value\ and\ predicted\ value}{Experimental\ value} \times 100$$

Evaluation of developed SNEDDS

Thermodynamic stability study

Stability studies were performed for evaluate stability of the nanoemulsions under the influence of various environmental conditions including light, temperature, and humidity. Thermodynamic stability study was done in three steps. First was heating and cooling cycle which stated the stability of nanoemulsions against the variation in temperature. Nanoemulsion was exposed to six cycles between +40°C and −40° for 48 h for each cycle. Formulation was found stable and hence further centrifuged at high speed (5000 rpm) for 30 min and observed for any objectionable changes like phase separation, creaming, or cracking. In the third step of the stability study, freeze and thaw cycle was performed by varying temperature between −21°C and +25°C. The collected sample was analyzed for physical changes and efficiency of self-emulsification.

Dispersibility studies

To evaluate the efficiency of self-emulsification of nanoemulsions dispersibility studies were performed by using a standard USP XXII dissolution apparatus. 2 ml of each of the formulation was incorporated into 500 ml of distilled water at 37 ± 0.5°C. A standard stainless steel dissolution paddle was rotated at 50 rpm for providing proper agitation. Different batches of nanoemulsions were evaluated visually.

Viscosity

Viscosity of emulsion is an important physicochemical property that was evaluated with the help of a Brookfield viscometer.

Refractive index (RI)

RI of nanoemulsions was determined by Abbes refractometer at 25 ± 0.5°C by placing the drop of nanoemulsions on the slide. RI of nanoemulsions was compared with the RI of water (1.333).

Robustness to dilution test

Robustness to dilution test is considered very important in self nanoemulsions which focus on the stability of nanoemulsions on large dilution with continuous phase including distilled water, acidic buffer (pH 1.2), and phosphate buffer (pH 6.8).

Percent transmittance

Using UV spectrophotometer at a particular wavelength using distilled water as a blank percentage transmittance was determined at 296 nm. Transparent nanoemulsions show more than 99% of transmittance.

p^H measurement

p^H meter is a very regularly used instrument for the determination of p^H. p^H shows irritability of the solution in a given biological system. Dilute the nanoemulsion with distilled water, place the p^H electrode into the solution, and allow it to equilibrate. Calibrated p^H meter will provide the accurate p^H value of sample.

Characterization of developed SNEDDS

Measurement of droplet size and polydispersity index (PDI)

Droplet size and PDI of different formulations were determined in terms of Z average diameter using zetasizernano ZSP instrument (Malvern instrument, UK) based on photon correlation spectroscopy. Zetasizer monitors the variation in the light scattering due to the Brownian motion of particles against the time. As smaller is the particle size higher is the velocity. Laser beam get distracted because the particles present in the solution. Rapid variation in the intensity of the scattering laser intensity respect to the mean value at a fixed angle caused by particle size. Zetasizer allow to warm up for about 30 min and then allows the software to launch in system. One ml of the nanoemulsions was diluted with 10 mL double distilled water to have homogeneous dispersion. Sonicate the solution for 2–3 min as required. Fill the measurement cell cautiously to avoid air bubbles. After checking all the measurement settings insert the filled cell into zetasizer and measure the particle size. It provides accurate particle size along with PDI.

Measurement of zeta potential

Zeta potential measures the surface charge of the particle in a liquid state. It is one of important parameter used to determine the stability of developed dispersion. The measured value of zeta potential depends on the physicochemical properties of drug, surfactant, cosurfactant, vehicle like water and their adsorption. One ml of the nanoemulsions was diluted with 10 mL double distilled water to have homogeneous dispersion and measured by Malvern zetasizer to detect its electrophoretic mobility of oil droplets. Zeta potential ±30 mV is considered considerable for the stability of nanoemulsions.

Fourier transform infrared spectroscopy (FTIR) spectral analysis

FTIR analysis is carried out to assess the interaction between the drug and excipient, polymerization, cross-linking, and drug loading. FTIR of pure drug provides the figure print of the molecule which was compare with formulated nanoemulsions to assess the correctness of formulation. FTIR analysis was completed using Shimadzu FTIR instrument suitable for liquid as well as solid samples with direct measurement.

RESULTS AND DISCUSSION

Solubility of drug in different solvents

Solubility of telmisartan in different solvents including distilled water, acidic buffer (pH 1.2), phosphate buffer (pH 6.8) and various oil, surfactant and cosurfactant were investigated. The telmisartan is very less soluble in distilled water and phosphate buffer (pH 6.8) and more soluble in acidic buffer (pH 1.2) [Table1]. The linearity curve and regression equation of drug in methanol were determined. This analysis involved plotting the relationship between the concentration of the solutions and their corresponding absorbance, allowing for the establishment of a linear model. The resulting regression equation ($R^2 = 0.9972$) provides a mathematical representation of this relationship ($y = 0.0516x + 0.0084$), enabling predictions and further insights into the behavior of these solutions under varying conditions [Figure1].

Screening of oil, surfactant, and cosurfactant for nanoemulsion formulation

Depending upon the solubilization capacity of the oleic acid as oil (15%), tween 20 (surfactant): transcutol P (cosurfactant) (3:1 ratio) (70%) respectively [Table 1]. Along with solubilization hydrophilic lipophilic balance (HLB) values of the selected component also considered which helps to identify the emulsion with stability to be formulated. pKa value of telmisartan 3.5, 4.1, 6.0 shows weak acidic characteristic, Oleic acid (HLB 1) shows high lipophilicity, tween 20 (HLB 16.7) shows hydrophilicity and transcutol P (HLB 4.2) shows lipophilicity combination of these components helped to formulate more stable emulsion. Oleic acid having better solubility of the telmisartan also provides the antioxidant property to the formulation. Non-ionic surfactant was

selected because they are known to be less affected by pH and ruggedness to words ionic strength. Transcutol P is a powerful solubilizer which improves the telmisartan solubility and possesses a lower irritation potential.

Table 1: Solubility of telmisartan in different solvent

Component	Solubility (mg/mL)
Distilled water	0.5±0.0590
Acidic buffer (pH 1.2)	4±0.05
Phosphate buffer (pH 6.8)	2±0.0570
Oleic acid	8±0.0590
LabrafilM2125CS	9±0.0425
Caproyl oil	4±0.0233
Castor oil	6±0.0451
Almond oil	8±0.0521
Peppermint oil	7±0.0348
Pomegranate oil	3±0.0254
Lemon grass oil	2.5±0.0471
Tween 80	2±0.0321
Tween 20	3±0.0450
Transcutol P	3.5±0.0781
Propylene Glycol	4.5±0.0249

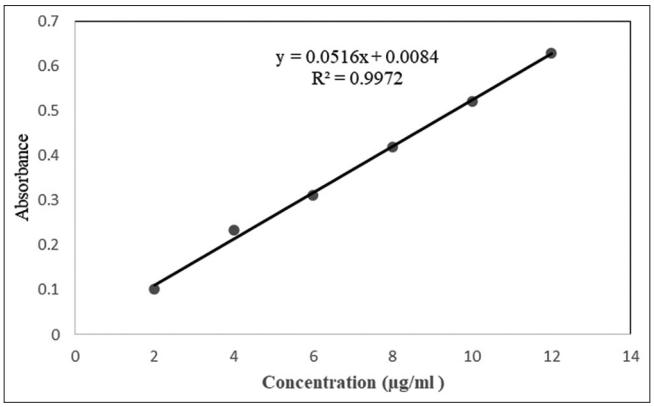

Figure 1: Linearity curve of telmisartan in methanol

Selection of the oil, surfactant and cosurfactant is the major challenge in the development of SNEDDS for telmisartan as it is very less soluble in the number of solvents as shown in Table 1. The oil which has good solubility like almond oil showed the unstability of the emulsion after 48 h in the form of separation of the emulsion into two separate layers. Increased concentration of Smix helps to maintain stability but at the same time, it increases the chances to have irritation and inflammation within the body after administration.

The initial preliminary study was performed to find out the minimum and maximum concentration of oleic acid as oil (10–15%), tween 20: Transcutol P as Smix in proportion 3:1 in the concentration range 65–70% was given required nanoemulsions with acceptable nanoglobules depicted in Table 2.

Selection of surfactant and cosurfactant ratio

Based on the pseudoternary phase diagram surfactant: cosurfactant (Smix) ratio 3:1 was finalized, as 2:1 and 1:1 showed the narrow zone for emulsion as compared to 3:1 [Figure 2].

Along with the solubility and HLB value the optimized oil: Smix ratio satisfies the formulation parameters including emulsion stability. When the concentration of oil increases above 15% the phase separation is observed after 48 h. Similarly, when the 1:1 and 2:1 Smix ratios showed the turbidity and unstability of the emulsion. Optimal Smix ratio ensured efficient emulsification with minimum energy method. Optimized oleic acid as oil (15%), tween 20

Table 2: Different variable along with level considered for design expert

Variable	Level of variable	
	Low	High
Oleic Acid	10%	15%
S mix (Tween 20 and Transcutol P 3:1)	65%	70%
Water	15%	20%

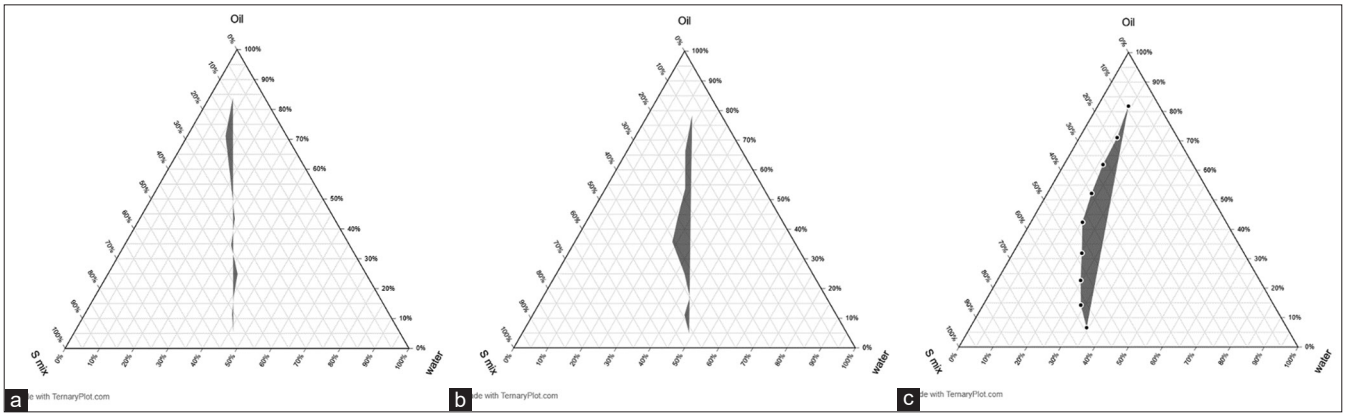

Figure 2: Pseudotenary phase diagram of oil, surfactant cosurfactant mixture (smix) and water (a) Smix 1:1 (b) S mix 2:1 (c) Smix 3:1

(surfactant): transcutol P (cosurfactant) (3:1 ratio) (70%) showed efficiency in terms of the uniformity of the dispersion, droplet size control, drug loading capacity, viscosity, and phase behavior management.

Optimization of experimental design for preparation of telmisartan loaded oil in water self nanoemulsifiednanoemulsion using D optimal mixture matrix design approach

Model fitting and statistical analysis

Composition factors on particle size, drug content, zetapotential of nanoemulsions which were experimentally based on optimal design. The predicted values and experimental values matching with each other with very small difference. Prediction data was made on the experimental data [Table 3].

The final equation which depicts the effect of independent variable Oil (A), Smix (B), water (C) on dependent variable like particle size, drug content, and zeta potential as mentioned in Equation 1, 2, 3.

Equation 1

$$Y1 \text{ (Particle Size)} = -93.22\,A + 393.51\,B + 559.87\,C + 337.32\,AB + 115.24\,AC + 198.46\,BC$$

Equation 2

$$Y2 \text{ (Drug Content)} = +141.37\,A + 73.86\,B + 93.20\,C - 33.68\,AB - 71.03\,AC + 6.08\,BC$$

Equation 3

$$Y3 \text{ (Zeta potential)} = +17.48\,A - 147.57\,B - 90.00\,C + 100.21\,AB + 2.75\,AC + 371.35\,BC - 1429.21\,A^2BC + 530.57\,AB^2C - 411.40\,ABC^2$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the mixture components are coded as +1 and the low levels are coded as 0. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

The residual analysis was performed using plots of predicted versus actual value all three dependent components which gives good agreement between actual and predicted response [Figure 3].

ANOVA was use to perform statistical data analysis of experimental data. It helped to find out best fitted model for three independent variables; particle size, drug content, and zeta potential [Table 4].

Statistical data given by the design expert were analyzed by considering different parameters (*P*-value, lack of fit value, coefficient of determination (R^2), adjusted R^2, predicted R^2, adequate precision). Data used to obtain the best fitting mathematical equation is shown as Equation 1, 2, and 3, respectively.

According to statistical data analysis quadratic model was found to best significant model for particle size and drug content and special quadratic model for zeta potential.

Table 3: Actual and predicted values of different dependable variables

Run/Batch no.	Oil	SmixA (3:1)	Water	Actual particle size	Predicted particle size	Drug content	Predicted drug content	Zeta potential	Predicted zeta potential
1	10	70	20	525.23	526.31	85.12	85.05	−24.56	−25.95
2	15	66.66	18.33	256.00	263.94	99.31	99.75	−39.25	−37.62
3	11.66	69.16	19.16	448.00	447.59	87.23	86.95	−29.23	−29.91
4	10	70	20	532.15	526.31	85.12	85.05	−27.45	−25.95
5	12.5	67.5	20	412.00	415.30	90.16	90.18	−38.12	−37.95
6	12.5	70	17.5	358.50	387.59	90.23	87.68	−38.26	−38.89
7	15	65	20	257.00	262.14	99.12	99.53	−35.58	−35.58
8	15	68.33	16.66	245.58	254.72	99.52	99.64	−35.00	−34.73
9	12.5	70	17.5	413.00	387.59	85.12	87.68	−39.52	−38.89
10	15	67.5	17.5	256.42	260.71	99.41	99.74	−34.52	−35.98
11	15	70	15	237.50	234.47	99.35	99.20	−40.00	−40.00
12	14.16	66.66	19.16	365.00	317.24	98.53	95.80	−39.86	−41.22
13	15	70	15	240.50	234.47	99.25	99.20	−40.00	−40.00
14	12.5	67.5	20	399.00	415.30	89.28	90.18	−38.23	−37.95
15	13.33	68.33	18.33	355.00	359.06	90.93	91.85	−37.86	−36.84
16	15	65	20	257.00	262.14	99.34	99.53	−35.58	−35.58

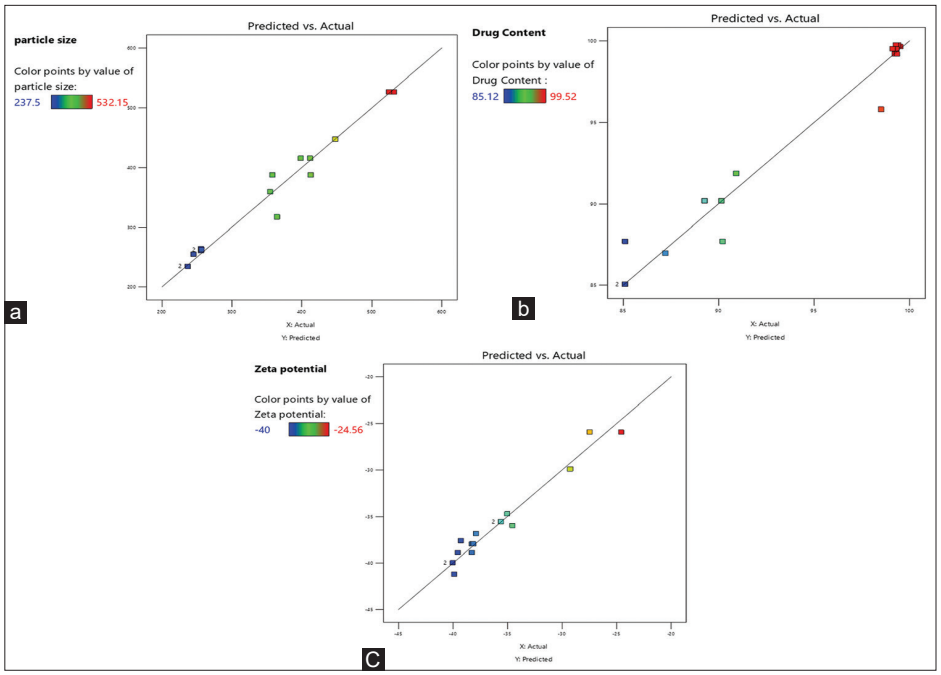

Figure 3: Scatter plot of predicted versus actual (a) Particle Size (b) Drug content (c) Zeta potential

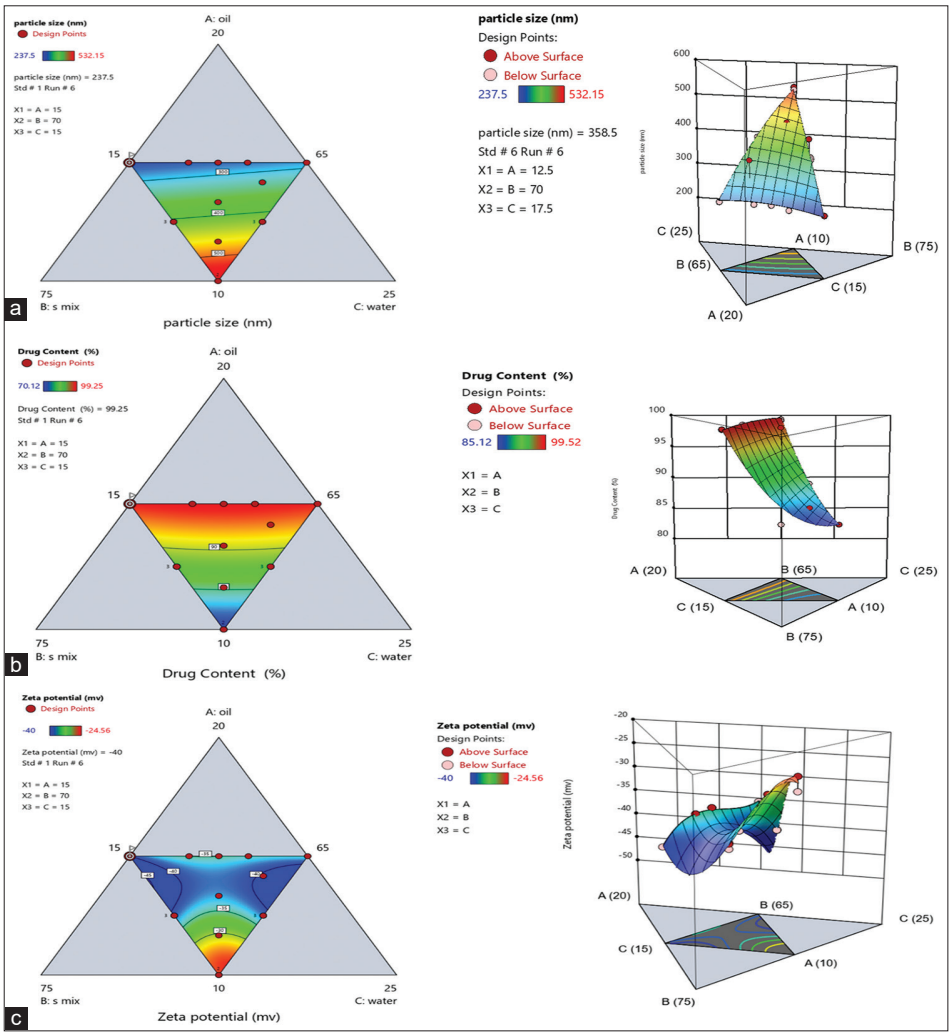

Figure 4: Contour plot and three-dimensional plot showing interaction between three variables A (Oil), B (Smix), C (Water) with respect to (a) particle size (b) drug content (c) zeta potential

Table 4: ANOVA for Quadratic model*

Source	Sum of squares	df	Mean square	F-value	P-value	Significance
Particle size						
Model	1.507E+05	5	30133.30	69.46	<0.0001	Significant
Linear mixture	1.498E+05	2	74884.60	172.63	<0.0001	
AB	684.85	1	684.85	1.58	0.2375	
AC	78.10	1	78.10	0.1800	0.6803	
BC	248.85	1	248.85	0.5737	0.4663	
Residual	4337.93	10	433.79			
Lack of fit	2744.36	5	548.87	1.72	0.2827	Not significant
Pure error	1593.57	5	318.71			
Cor total	1.550E+05	15				
R^2=0.9720 Adjusted R^2=0.9580 Predicted R^2=0.9396 Adequate Precision=22.8811						
Drug content						
Model	536.57	5	107.31	47.03	<0.0001	Significant
Linear mixture	500.09	2	250.05	109.59	<0.0001	
AB	6.83	1	6.83	2.99	0.1144	
AC	29.67	1	29.67	13.00	0.0048	
BC	0.2335	1	0.2335	0.1023	0.7556	
Residual	22.82	10	2.28			
Lack of fit	9.34	5	1.87	0.6935	0.6511	Not significant
Pure error	13.47	5	2.69			
Cor total	559.38	15				
R^2=0.9592 Adjusted R^2=0.9388 Predicted R^2=0.8994 Adequate Precision=15.8964						
Zeta potential						
Model	327.39	8	40.92	21.55	0.0003	Significant
Linear mixture	163.24	2	81.62	42.98	0.0001	
AB	0.7161	1	0.7161	0.3771	0.5586	
AC	0.0006	1	0.0006	0.0003	0.9862	
BC	11.47	1	11.47	6.04	0.0436	
A^2BC	24.92	1	24.92	13.12	0.0085	
AB^2C	4.04	1	4.04	2.13	0.1881	
ABC^2	1.49	1	1.49	0.7860	0.4047	
Residual	13.29	7	1.90			
Lack of fit	8.32	2	4.16	4.18	0.0857	Not significant
Pure error	4.98	5	0.9952			
Cor total	340.68	15				
R^2=0.9610 Adjusted R^2=0.9164 Predicted R^2=0.6365 Adequate Precision=14.7779						

*Values obtained from design expert 13 software. ANOVA: Analysis of Variance

It has significant *P*-value (<0.0001), a lack of fit value (nonsignificant relative to pure error), the Predicted R^2 were in reasonable agreement with the adjusted R^2 (the difference is <0.2). Adequate precision measures the signal to noise ratio (>4 is desirable). So, considering all parameters quadratic model was considered as best fitted model.

D-optimal analysis

Self-emulsifiednanoemulsion more effective in the range of 200–300 nm. Smaller the size of nanoemulsion gives enhanced permeability and hence improves the bioavailability and efficacy.

Figure 5: Self nanoemulsifed drug delivery system for batch 11

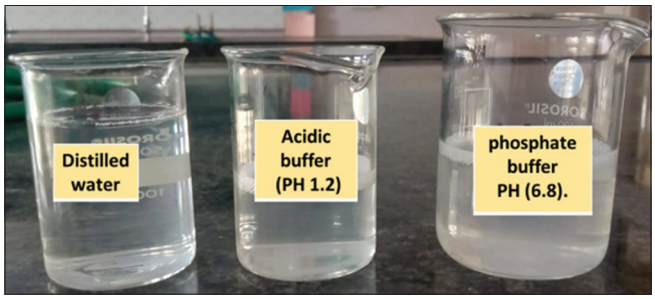

Figure 6: Robustness to dilution test in distilled water, acidic buffer (pH 1.2), and Phosphate buffer (pH 6.8) for batch 11

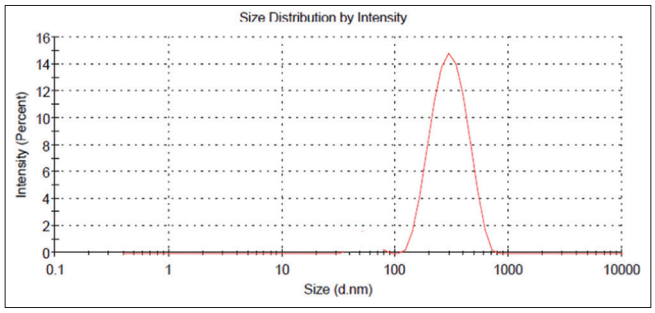

Figure 7: Particle size of optimized batch 11

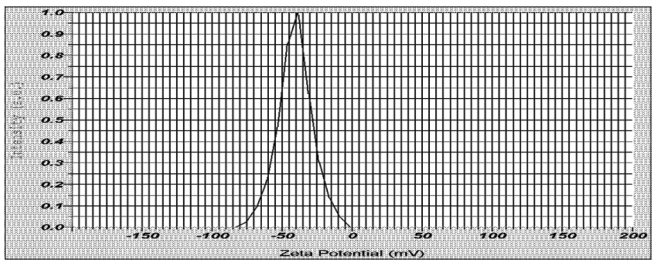

Figure 8: Zeta potential of optimized batch 11

For the optimization of telmisartan-loaded self-emulsified nanoemulsions, contour, and three-dimensional surface graphs were plotted using design expert software. Three dimensional graphs explained the effect of various concentration combination of oil, Smix, and water on the particle size, drug content, and zeta potential. Particle size was decreased with increased in oil concentration and increased with Smix concentration. Oil in combination with only Smix increases the particle size and oil with water increases the particle size. Drug content get improved with increased concentration of oil, Smix, and water. Zeta potential get increased with oil decreased with addition of Smix and water. Overall combining effect of oil, Smix, and water gives the required acceptable value of zeta potential [Figure 4].

Optimization of the telmisartan loaded nanoemulsions by D-optimal design mixture

Optimum formulation should have a minimum particle size, with acceptable drug content and zeta potential. The run no. 11 found to be optimized formulation which having experimental particle size 237.5 ± 5 nm, drug content 99.35 ± 0.05%, and zeta potential value of 40 ± 1 mV. All these values suggest the best suitable and stabilized nanoemulsions [Figure 5].

Evaluation studies

Thermodynamic stability study

Thermodynamic study shows physical properties viscosity and appearance remained consistent with no sign of phase separation. The drug content remains stable with various thermodynamic conditions.

Dispersibility study

All the batches showed an emulsification time less than one minute highlights the effectiveness of the formulation and processing techniques employed. It also shows that optimizing factors oil, Smix ratio, mixing techniques, temperature control gives stable and effective formulation.

Viscosity

Viscosity of emulsion was evaluated with the help of Brookfield viscometer and found 85 ± 1cP. Viscosity provides insights of physical properties and directly influences the stability, texture and flow properties. The 85 ± 1cP viscosity is considered as moderate viscosity which indicates favorable flow characteristics and good stability.

Refractive Index (RI)

RI of nanoemulsions was compared with the RI of water (1.333). It was found to be 1. 3160 ± 0.5. The observed RI is nearly equal to water suggesting the better dispersion of drug within the emulsion matrix. RI near to water explained that emulsion is clear. It also shows there are no significant changes in composition and phase separation during storage.

Robustness to dilution test

Dilutability test is considered as very important in self nanoemulsions which focused on stability of nanoemulsions on large dilution with continuous phase. There was no separation of phase had observed as shown in Figure 6 for optimized batch no.11.

Percent transmittance

Percent transmittance was obtained by using UV spectrophotometer at 296 nm wavelength using distilled water as a blank. Optimized nanoemulsion showed the more than 99% of transmittance indicating excellent clarity and stability.

p^H measurement

p^H of the nanoemulsions in distilled water was found in the range of 6.8–7.3. It shows the stability and non-irritability of nanoemulsions.

Characterization studies

Measurement of droplet size and PDI

Optimized formulation (batch 11) has resulted in particle size 237.3 ± 5 nm with PDI 0.240 which suggests the stability and possible effectiveness of the developed system for poorly soluble drug telmisartan. The lower value of PDI shows greater stability by homogenization index [Figure 8].

Measurement of Zeta potential

Zetapotetial of optimized formulation was 40± 1 mV which showed the stability of the nanoemulsions. The zeta potential observed as a combine effect of drug, oil, Smix and water. It also shows the system suitability for the nanoemulsions. A zeta potential value 40 ± 1 suggests the nanoemulsion particles possess a strong negative charge [Figure 7]. This charge creates electrostatic repulsion between the particles

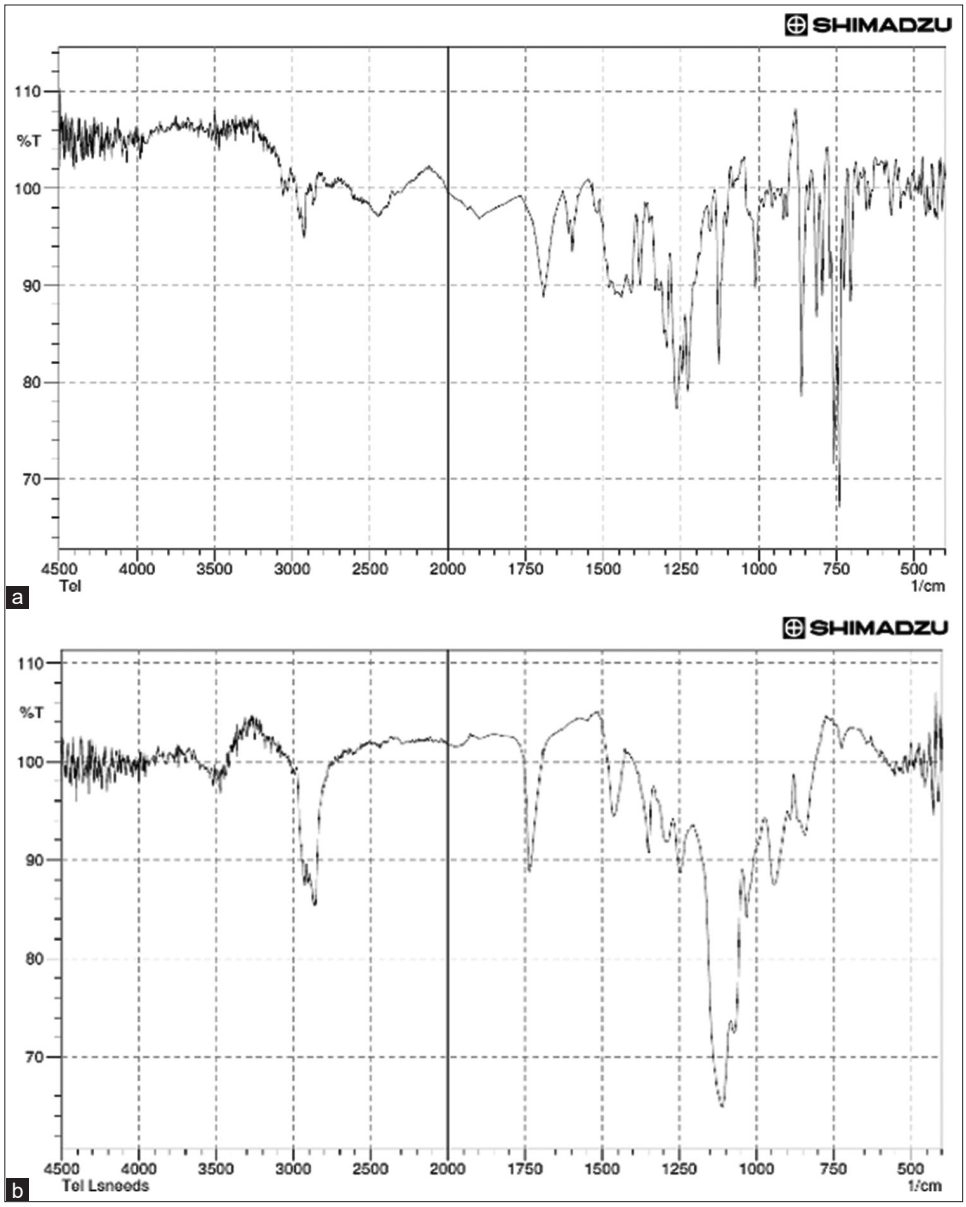

Figure 9: Fourier transform infrared spectrum of (a) Telmisartan (b) Telmisartan loaded self-emulsified nano emulsion (for batch 11)

having the same charges and reduce aggregation. The zeta potential greater ± 30 considered as sufficient to ensure the physical stability of the nanoemulsion.

FTIR spectral analysis

The FTIR analysis of the pure drug and the optimized nanoemulsion revealed distinct differences in their spectral profiles. These variations indicate that the drug is completely dissolved within the nanoemulsion system. The absence of characteristic peaks associated with the pure drug in the FTIR spectrum of the nanoemulsion suggests that the drug has undergone significant interactions with the emulsifying agents and the solvent matrix, confirming its effective incorporation into the nanoemulsion formulation [Figure 9].

CONCLUSION

The study was demonstrated that by using D optimal mixture matrix design approach telmisartan containing self-emulsified nanoemulsion prepared effectively using oleic acid as oil (15%), tween 20 (surfactant): Transcutol P (cosurfactant) (3:1 ratio) (70%). The ANOVA showed the model fitness with significant F value (for particle size 10.47, drug content 19.98, zeta potential 42.40) and low *P*-value of all (< 0.0001) and a nonsignificant lack of fit. The research provides not only the guidelines on effective use of D optimal mixture matrix design but also provides interaction of various independent variables with dependent variables. The formulated optimized self nanoemulsion of telmisartan evaluated for physicochemical characteristics included particles size (237.3 ± 5 nm), drug content (99.35 ± 0.05%), zeta potential (−40 ± 1 mV) and found the effective in improving the drug content with stability proved with FTIR analysis. In future scope, as liquid SNEDDS are difficult to handle need to develop as solid SNEDDS. Solid SNEDDS will be filled in capsule or developed as tablet dosage form.

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