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Original Research Article

Optimization of Self Micro Emulsifying Drug Delivery System Containing Curcumin and Artemisinin Using D-Optimal Mixture Design

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Abstract: Curcumin and Artemisinin are a poorly water-soluble drugs and its poor oral bioavailability is very low. A new self-micro emulsifying drug delivery system (SMEDDS) has been magnificently developed to improve the solubility of curcumin and Artemisinin. Appropriate compositions of SMEDDS formulation were selected *via* solubility studies of curcumin and Artemisinin. The formulation of SMEDDS was optimized by D-Optimal Mixture design. The optimal formulation of SMEDDS was comprised of 3ml surfactant (Tween 80), 1 ml of co-surfactant (PEG 400) and 1 ml oil (Oleic acid). The solubility of curcumin (4.4 mg/3ml) and Artemisinin (73.4 mg/3ml) was increased considerably in SMEDDS. The average particle size of SMEDDS-containing curcumin and Artemisinin was 150.7 nm. The diffusion study (*in vitro)* showed that more than 63.81% of curcumin and 54.91% of Artemisinin in SMEDDS could be dissolved in medium with pH 1.2 in 1 hr 30 min. Our study illustrated that the developed SMEDDS formulation held great potential as a possible alternative to traditional oral formulations of curcumin and artemisinin.
Keywords: Curcumin, Artemisinin, SMEDDS, D-Optimal Mixture Design

INTRODUCTION

Curcumin is a naturally active constituent isolated from the plants of the *Curcuma longa*, it has a range of biological activities and pharmacological actions, such as anti-tumor, anti-inflammatory, anti-virus, anti-oxidant, anti-malarial and anti-HIV, and low toxicity with promising clinical application [1,2]. However, curcumin is slightly absorbed in the gastrointestinal tract due to its poor solubility in water the maximum solubility was reported to be 11 ng/ml in plain aqueous buffer pH 5.0) [3]. The oral bioavailability of curcumin is very low which is only 1% in rats. Artemisinin is a sesquiterpene-lactone endoperoxide isolated from *Artemisia annua* L., a plant with a long history of medical use against malaria in China. However it is a BCS class II drug having Low solubility and High Permeability this strikes to the mind of formulation scientist for developing a delivery system for increasing the solubility of artemisinin [4].

Several investigations have proved that curcumin, a lipophilic polyphenol extracted from the rhizomes of Curcuma longa, displays antimalarial activity both in vitro and in vivo [5]. Therefore, curcumin has been suggested as an eye-catching partner drug for Artemisinin due to its short-life (1–2 h), which closely matches the half-life of artemisinin derivatives. Curcumin acts as an adjunctive therapeutic approach in the artemisinin combination therapies to protect against parasite recrudescence and relapse. Certainly, Curcumin synergizes with artemisinin as an antimalarial to kill the parasites [6]. Due to its safety, relative abundance and cost effectiveness, Curcumin is a phytochemical of great interest in the treatment of several diseases, including malaria [7]. However, the oral therapy of Curcumin and Artemisinin is associated with low bioavailability of drugs due to poor water solubility, chemical instability at neutral and alkaline pH, poor absorption associated with high rate of metabolism and rapid elimination of these drugs [8-10].

To improve the bioavailability of curcumin and artemisinin, numerous approaches like formulation of liposomes and nanoparticles have been investigated [11]. These formulations require complex method of formation and high cost of ingredients involved. A simple technique is the Self Micro Emulsifying Drug Delivery System (SMEDDS) consisting of simple formulation method, less time of formulation and easily available cheap excipients [12].

Self-micro emulsifying drug delivery system (SMEDDS) has in recent times emerged as one of the most fascinating approaches to improve the solubility, dissolution and oral absorption for poorly water-soluble drugs. SMEDDS is an isotropic mixture of oil, surfactant, co-surfactant and drug substance, which can form a microemulsion under conditions of

gastrointestinal fluid and gastrointestinal motility after oral administration. The resultant microemulsion with a particle size less than 100 nm and the increasing solubility of hydrophobic drug can enhance the absorption in gastrointestinal tract [13].

The objectives of present study were to design, prepare, and characterize a SMEDDS formulation of curcumin and artemisinin, and evaluate its *in vitro* property. The solubility of curcumin and Artemisinin was determined in various vehicles. Pseudo Ternary phase diagram was constructed to determine efficient emulsified region and the resultant formulations loaded with curcumin and artemisinin were optimized by a D-Optimal Mixture Design. The optimal formulation of curcumin and artemisinin was further investigated for physicochemical characteristics [14].

MATERIAL AND METHODS

Materials

Curcumin was obtained as kind gift from Pharmanza Herbals PVT Limited, Khambhat.
Artemisinin was obtained as kind gift from Mangalam Organics PVT Limited, Vapi.
Oleic acid and PEG 400 was purchased from Astron Chemicals, Ahemdabad.
Tween 80 was purchased from S.D fine Chemicals, Ahemdabad.

Screening of SMEDDS Formulation

Solubility Studies

The solubility of Curcumin and Artemisinin in various oils such as Oleic acid, Olive oil, Corn oil, Coconut oil, Peanut oil, Sunflower oil. Surfactant such as Tween 20, 80 and Span 20, 80 and Co-surfactant such as Ethanol, PEG 200, PEG 400 and Propylene Glycol was measured, respectively. An excess amount of both drugs was added into each 3ml of vial containing oil and the mixtures were continuously stirred for 48 h at 37 °C under light shielding. After equilibrium was achieved, the mixtures were centrifuged at 12,000 rpm for 20 min. The supernatant was diluted appropriately and the concentration of drugs in the solution was assayed by UV spectroscopy (UV-3150, Shimadzu, Japan) at respective wavelength maxima [15].

Construction of Pseudo Ternary Phase Diagram.

The pseudo ternary phase diagrams were constructed using the water titration method. A series of Emulsions was prepared by varying mass ratio of oil to Smix from 9:1 to 1:9. The ratio of Tween 80 to PEG 400 was optimized by varying their mass ratio from 1:0, 1:1, 2:1, 3:1, to 4:1. Each pre-concentrate mixture was titrated drop-wise with distilled water at room temperature and agitated after each drop. The end point of the titration was taken as the point when the solution became cloudy and turbid, and the quantity of water required was recorded. The pseudo ternary phase diagram was established to delineate the area of microemulsion and boundary of phases. The pseudo ternary phase diagrams were plotted using Prosim software [16].

Preparation of SMEDDS Formulation.

Accurately weighed 38mg of Artemisinin and 192mg of Curcumin was dissolved in required amount of oil and then add required quantity of Tween 80 and PEG 400 as per decided ratio from pseudo ternary phase diagram. The contents were mixed gently with magnetic stirrer until mixture turned to clear and then formulation was equilibrated for 24 hours at 37˚C.

Optimization of Curcumin and Artemisinin Loaded SMEDDS.

A D-Optimal Mixture Design was used to optimize the composition of SMEDDS. The design layout was generated by the trial version of Design Expert software 10. The independent variables were selected as ml of oil [X_1], surfactant [X_2] and Cosurfactant [X_3]. The measured responses (i.e. dependent variables) were Percentage Transmittance [Y_1] and Mean Droplet size [Y_2] and Poly dispersity index [Y_3]. The trials were run in a randomized fashion to eliminate bias in the experiment. In order to understand the importance (criticality) of variable, analysis of variance (ANOVA) and multiple linear regression was performed [20].

The responses for seventeen formulations were used to fit an equation for D-optimal model which can predict the properties of all possible formulations. Graphs of these properties in the form of contour plots were constructed. Polynomial model equation was developed as the best representation of the relationship between the Percentage Transmittance, Mean Droplet size and Polydispersity Index.

Characterization of Curcumin and Artemisinin Loaded SMEDDS.

pH and Viscosity measurement.

The pH of SMEDDS was measured by pH meter and the viscosities was measured to determine rheological properties of formulations. The viscosity of the prepared SMEDDS Batches was determined by using Brookfield LVDV-11 +Pro viscometer using a spindle in triplicate [17].

Thermodynamic Stability Studies.

For thermodynamic stability studies three stage process was carried out

1. Heating–cooling cycle: Six cycles between refrigerator temperature (4°C) and 45°C with storage at each temperature of not less than 48 h was conducted, and the formulations was examined for stability at these temperatures.

2. Centrifugation test: Formulations was centrifuged at 3,500 rpm for 30 min, and then looked for phase separation.
3. Freeze thaw cycle: Three freeze–thaw cycles between −21°C and +25°C, with formulation storage at each temperature for not less than 48 h, was performed. (16)

Dispersibility Test.

The efficiency of self-emulsification of oral Nano or micro emulsion was assess by using a standard USP XXII dissolution apparatus 2 for Dispersibility test. 1 ml of each formulation was added in 500 mL of water at 37 ± 1 0C. A standard stainless steel dissolution paddle was used with rotating speed of 50 rpm provided gentle agitation. The in vitro performance of the formulations was visually assess using the following grading system:
Grade A: Rapidly forming (within 1 min) Nano emulsion, having a clear or bluish appearance.
Grade B: Rapidly forming, slightly less clear emulsion, having a bluish white appearance.
Grade C: Fine milky emulsion that formed within 2min
Grade D: Dull, greyish white emulsion having slightly oily appearance that is slow to emulsify (longer than 2min).
Grade E: Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface.
Grade A and Grade B formulation are remain as Nano emulsion when dispersed in GIT. While formulation falling in Grade C could be recommend for SMEDDS formulation [17].

Droplet Size Analysis, Zeta Potential and Percentage Transmittance.

The droplet size of the SMEDDS was determined by photon correlation spectroscopy (which analyses the fluctuations in light scattering due to Brownian motion of the particles) using a The equipment is able to measure sizes between 10 and 5000 nm. Light scattering was monitor at 25°C at a 90° angle, after external standardization with spherical polystyrene beads. The Nano metric size range of the particle was retained even after 100 times dilution with water which proves the system's compatibility with excess water. Percent transmittance prove the transparency of formulation. The percent transmittance of the system was measured at particular wavelength using UV spectrophotometer by using distilled water as blank. The formulation was considered as transparent of the percentage transmittance was greater than 99% [17].

Electro conductivity study, Drug Content and Robustness to Dilution.

The SMEDDS system contains ionic or non-ionic surfactant, oil, and water. This test was perform for measurement of the electro conductive nature of system. The electro conductivity of resultant system was measure by electro conductometer. In conventional SMEDDSs, the charge on an oil droplet is negative due to presence of free fatty acids. Drug from pre-weighed SMEDDS was extract by dissolving in suitable solvent. Drug content in the solvent extract is analyse by suitable analytical method against the standard solvent solution of drug. For determination of Dilution potential prepared optimized SMEDDS were diluted to 50, 100 and 250 times dilution using 0.1 N HCl and the batches were observed for the phase separation. Ideal SMEDDS must remain clear [18].

In-vitro diffusion studies

SMEDDS containing Curcumin and Artemisinin were studied for drug release profile. One ml solution was put on the donor compartment. One ml solution was withdrawn from each in the time interval of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 min. The Samples were withdrawn at predetermined time intervals with fresh media replacement and analysed by HPTLC method[19].

RESULT AND DISCUSSION.

Screening of SMEDDS Formulation

Solubility studies

The screening of oil was based on the solubility of curcumin and artemisinin in oil as shown in Figure 1 and Figure 2. Higher the drug solubility in oil greater is the drug loading capacity.

Various oils such as Corn oil, Oleic acid, Olive oil, Castor oil, Coconut oil, Sunflower oil, Peanut oil were taken for the solubility study of both drugs. Out of which the solubility of curcumin and artemisinin was greater in Corn oil but due to formation of turbid emulsion upon dilution which results in precipitation of drugs it was not chosen secondary Olive oil was then taken as an oil but due to phase separation it was not taken hence oleic acid was chosen as oil. (4.4 mg/3ml for curcumin & 73.4mg/3ml for artemisinin) Oleic acid is a monounsaturated omega-9 fatty acid, abbreviated with a lipid number of 18:1 cis-9. It has the formula $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$. Moreover, formulation of SMEDDS with oil of low drug solubility would require incorporation of more oil to incorporate the target drug dose, which in turn would require higher surfactant concentration to achieve oil solubilization, which might increase the toxicity of the system. After selection of Oleic acid as the oil phase, the goal was to identify the surfactant that has the highest solubilization capacity for the oil.

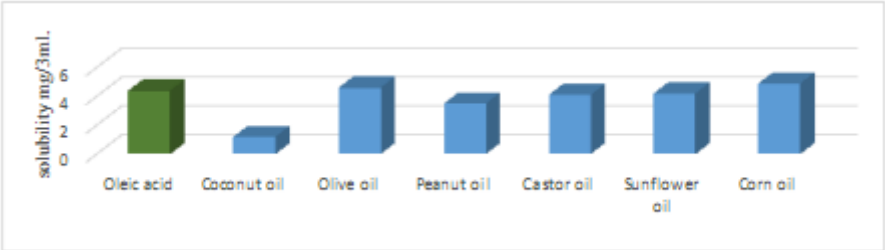

Fig-1: Solubility of Curcumin in various oils

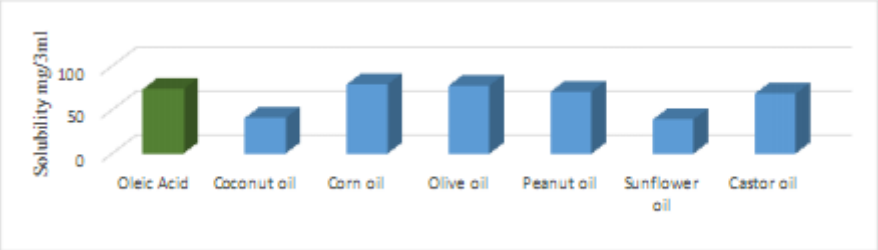

Fig-2: Solubility of Artemisinin in various oils

The screening of surfactant was based on the solubility of curcumin and artemisinin in surfactant. Higher the drug solubility in surfactant greater is the drug loading capacity.

Various surfactants such as Tween 20, 80 and Span 20, 80 were taken for the solubility study of both drugs. Out of which the solubility of curcumin and artemisinin was greater in Tween 80 (69.1mg/3ml for curcumin & 84.5 mg/3ml for artemisinin)

The screening of co-surfactant was based on the solubility of curcumin and artemisinin in surfactant. Various Co-surfactant such as Ethanol, PEG 200, PEG 400 and Propylene glycol were taken for the solubility study of both drugs.(Figure 3 and Figure 4) Out of which the solubility of curcumin and artemisinin was greater in PEG 400 as it was chosen as co-surfactant.

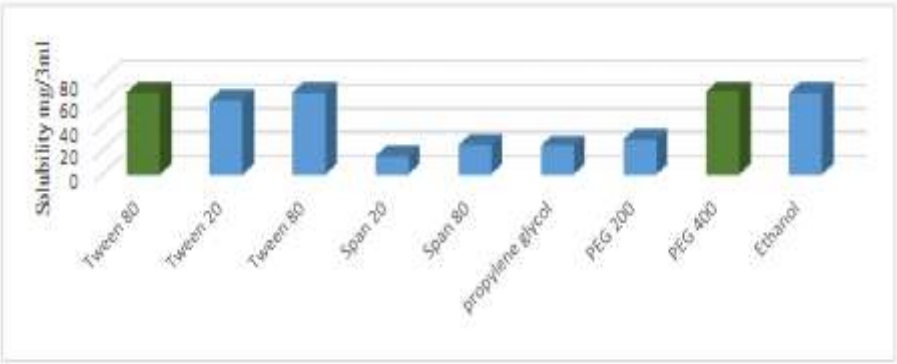

Fig-3: Solubility of Curcumin in various Surfactants and Co-surfactant

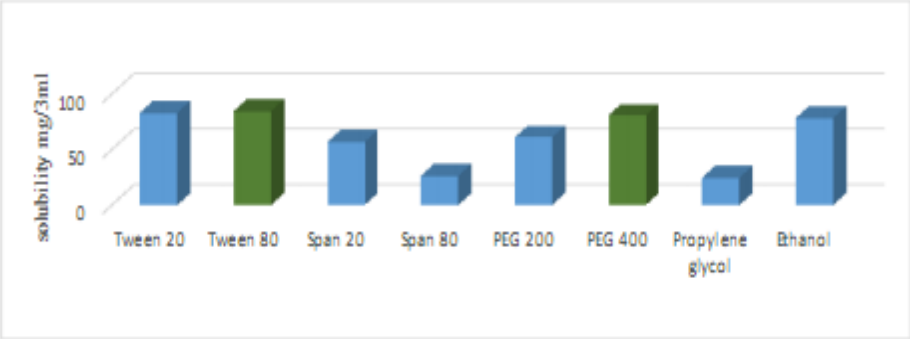

Fig-4: Solubility of Artemisinin in various Surfactants and Co-surfactants

Pseudo Ternary Phase Diagram

The construction of pseudo ternary phase diagrams was used to obtain appropriate concentration ranges of components which includes oil, surfactant and co surfactant in the areas of forming micro emulsions. The pseudo ternary phase diagrams of O/W micro emulsions consisted of the mixture of Oleic acid, Tween 80, PEG 400 and distilled water with various Smix (Tween 80 to PEG 400) ratio such as 1:0, 1:1, 1:2, 1:3, 2:1, 3:1. All micro emulsion formulations tested at various Smix could be diluted with water without limit. In first four Smix ratios, [1:0,1:1,1:2, 1:3] increasing concentration of co surfactant and puts surfactant concentration constant, in other two ratio [2:1,3:1] increasing concentration of surfactant and puts co surfactant concentration constant. When the co surfactant concentration with respect to surfactant was increased to the [Smix 1:3], it was observed that the micro emulsion area increased as compared to [Smix 1:1]. It was also observed that decreasing the oil level led to an increase in the area of micro emulsion formation. As the level of oil increased from 1 ml to 9 ml decreased in micro emulsion area. This fact suggested that the oil constitutes the inner phase of the micro emulsion droplets, which is consistent with a direct o/w-type structure On further increasing the surfactant concentration, i.e., at [Smix 3:1] the micro emulsion region increased in size as compared to the region in Smix 1:0, 1:1, 1:2, 1:3. In case of [Smix 1:1, 1:2, 1:3] decreased in the micro emulsion area compared to [Smix 3:1] so that these Smix ratio was exclude from the study. Micro emulsion region was dependent on surfactant and co surfactant ratio and best result was found to be obtained in the batch contained higher amount of surfactant which stretches close to the water apex with good dilution potential with no risk of drug precipitation in GIT and good in vivo performance as seen in the [Smix 3:1](Figure 5).

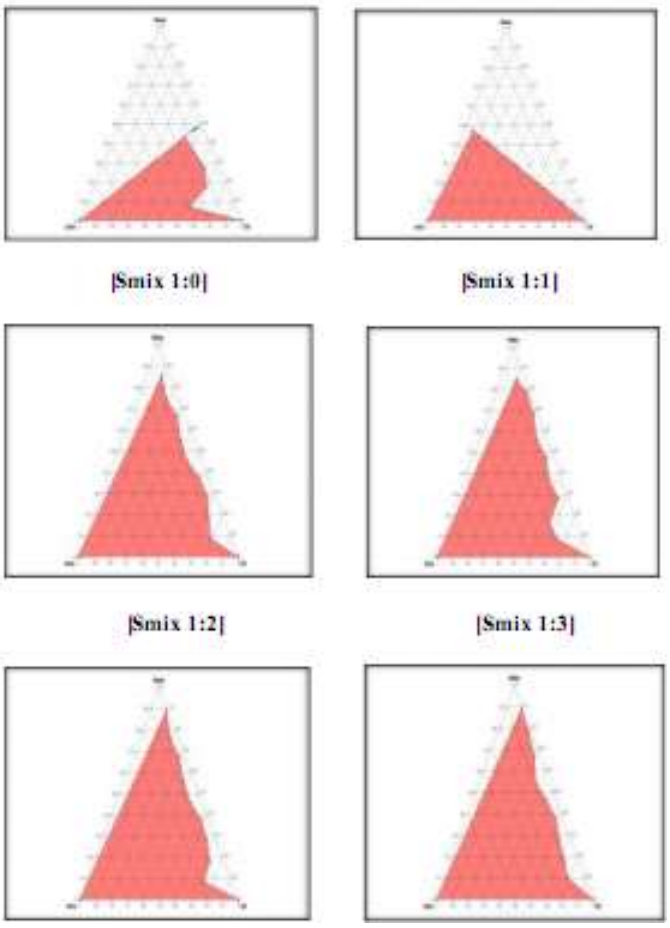

Fig-5: Pseudo Ternary Phase Diagrams containing various Smix Ratios

Optimization of Curcumin and Artemisinin Loaded SMEDDS.

Total Seventeen experimental trials involving three independent variables were generated using Design Expert Software and the % Transmittance, Mean Droplet size and Poly Dispersity Index were set as response variables. The measured responses were shown in Table 1. A factor that gives higher P value i.e. above 0.05 was taken as non-significant.

Table 1: Variables in D-Optimal Mixture Design

Independent variables	Level			
	Coded value		Transformed value	
	Low	High	Low	High
A= Conc. of oil (ml)	-1	1	0.5	1.5
B= Conc. of surfactant (ml)	-1	1	2.5	3.5
C= Conc. of co-surfactant (ml)	-1	1	0.5	1.5
Dependent Variables	% Transmittance (More than 80%) Mean Droplet size (150-600 nm) Polydispersity index (Less than 0.5)			

A statistical model incorporating interactive and polynomial terms was used to evaluate the responses. The % Transmittance, Mean Droplet size and P.D.I for 17 batches (B1 to B17) have been taken. The fitted equations (full and reduced) relating the responses R1, R2, R3 have been investigated. The polynomial equations can be used to draw conclusion. The % Transmittance was found to be more than 89.84. Hence all the batches can be considered as clear and transparent. Where batch 15 was superior to other batches having 98.27 % transmittance, mean droplet size was within the range of 150 to 601 nm, whereas batch 15 was showing the lowest droplet size. Poly dispersity Index was found to be in the range of 0.188 to 1.000. Where batch 15 was having lowest P.D.I.

Contour plots and response surface analysis

Two-dimensional contour plots and three-dimensional response surface plots are presented in Figure No.6 and 7, which is useful to study the interaction effects of the factors on the responses. The relationship between the dependent and independent variables was elucidated by constructing response surface plots.

% Transmittance

The % Transmittance of all seventeen batches are presented in Table 2. The % transmittance ranged from 89.84-98.27% which indicates that all batches quickly gets converted to fine emulsion upon dilution. The selected cubic model was used to generate following equation for % Transmittance.

$$Y=84.53X_1+115.25X_2+99.06X_3-33.55X_1X_2-0.071X_1X_3-51.79X_2X_3+155.70X_1X_2X_3+5.23X_1X_2(X_1X_2)-0.41\ X_1X_3(X_1X_3)-41.95\ X_2X_3(X_2X_3)$$

Figure 6 indicates the influence of microemulsion components on % Transmittance The high % transmission of micro-emulsion deceipt the efficient emulsification of oil phase into water phase. In contour plot it was observed that % of transmission is maximum at medium level of surfactant. Low level of oil, High level of surfactant cause efficient emulsification of oils into small globules.

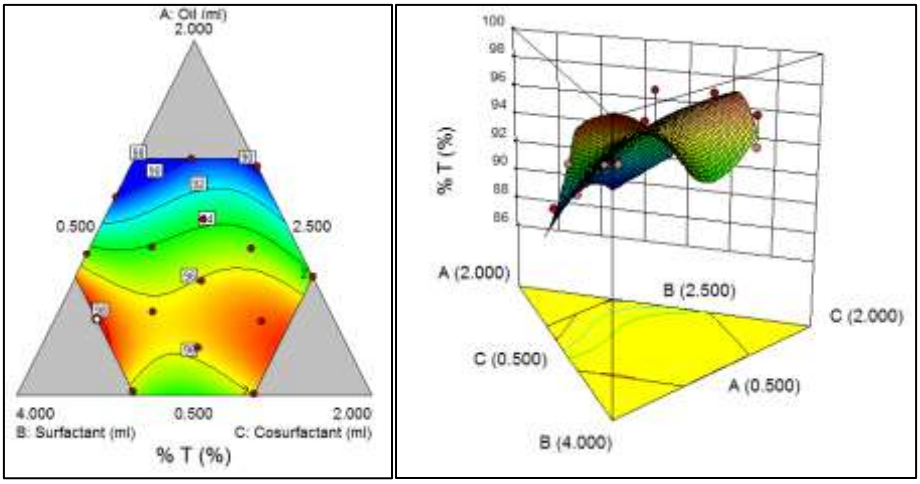

Fig-6: Contour plot (i) and response surface (ii) plot showing the relationship between various levels of independent variables (Concentration. of oil, Surfactant and Co-surfactant) on % Transmittance.

Mean Droplet size

The Mean Droplet Size of all seventeen batches are presented in Table 2. The Mean Droplet Size ranged from 150.7-601.8 nm which indicates that all batches quickly gets converted to fine emulsion upon dilution. The selected cubic model was used to generate following equation for Mean Droplet Size.

$Y=1834.5X_1+1313.8X_2+1647.1X_3-4788.8X_1X_2-6166.6X_1X_3-5900.4X_2X_3+12704.6X_1X_2X_3+921.4X_1X_2(X_1X_2)-516.7X_1X_3(X_1X_3)+949.5X_2X_3(X_2X_3)$

The influence of micro-emulsion components on mean droplet size is shown in contour plot. As indicated in figure 7 the mean droplet size increases with the increase in oil phase and decreases with the increase in the surfactant phase. Thus we can say that increased surfactant concentration leads to formation of small droplet size globules by efficient emulsification.

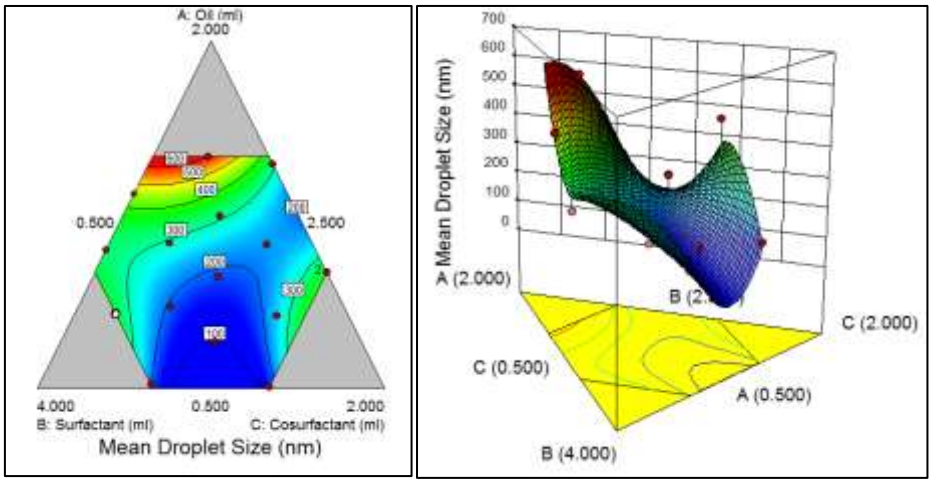

Fig-7: Contour plot (i) and response surface (ii) plot showing the relationship between various levels of independent variables (Concentration. of oil, Surfactant and Co-surfactant) on Mean Droplet Size

Poly Dispersity Index

The Polydispersity Index of all seventeen batches are presented in Table 2. The Polydispersity Index ranged from 0.188-1.00 which indicates that all batches quickly gets converted to fine emulsion upon dilution. The selected cubic model was used to generate following equation for Polydispersity Index.

$Y=1.64X_1+2.73X_2+1.67X_3-7.35X_1X_2-6.04X_1X_3-8.96X_2X_3+25.00X_1X_2X_3-2.68X_1X_2(X_1X_2)+0.53X_1X_3(X_1X_3)-2.79X_2X_3(X_2X_3)$

The influence of micro-emulsion components on Polydispersity index is shown in contour plot. As indicated in figure 7 the poly dispersity index increases with the increase in concentration of oil and surfactant has the main effect on P.D.I as a result increased surfactant concentration leads to efficient emulsification and less P.D.I.

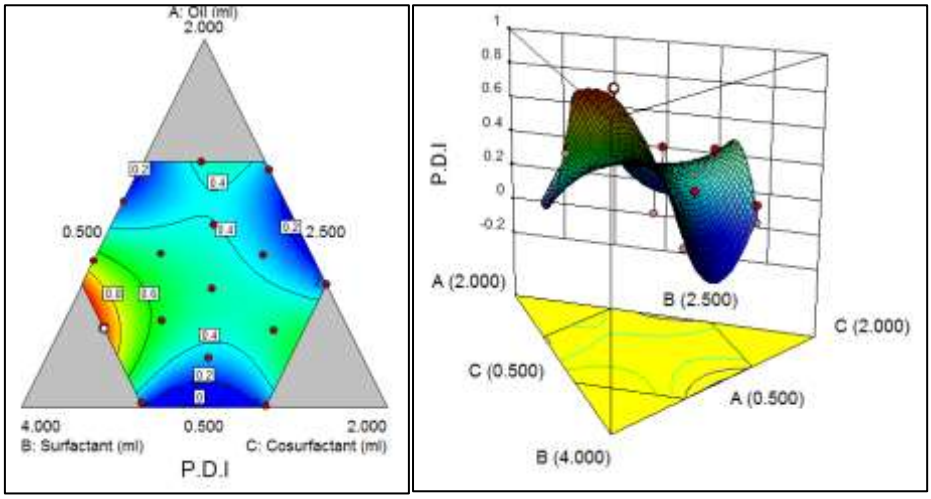

Fig-7: Contour plot (i) and response surface (ii) plot showing the relationship between various levels of independent variables (Concentration. of oil, Surfactant and Co-surfactant) on Polydispersity Index

It is clear from the developed equations that optimum response in terms of Higher % Transmittance (>99.99), smaller droplet size (<170 nm) and lesser Polydispersity Index (>0.5) was achieved at low concentrations of oil and higher concentrations of surfactants and low concentration of co surfactant. Hence optimized batches, B15 containing 1ml of oil, 3ml of surfactant and 1ml of co-surfactant showed the responses (Y1=98.27%, Y2=150.7, Y3=0.188) which were in close agreement with the predicted ones. Therefore the developed model was found reliable.

Characterization of Curcumin and Artemisinin Loaded SMEDDS

pH and Viscosity Measurement

pH is an important parameter influencing the stability of the formulation. pH of the formulation depends on the excipients used in the formulation which influence the zeta potential and finally the stability of formulation. All formulation batches B1-B17 having pH in the range of 6.8-7.6 so that no issue of the stability because drug was not diffused in the external phase and remained in oil phase. Since, water was the external phase entire system showed pH of water. Results are as shown in Table 2.

Table 2: Design matrix and Response of D-Optimal Mixture Design

Batch no	X_1	X_2	X_3	% T (%)	Droplet Size analysis (nm)	Poly Dispersity Index
B1	1.500	2.761	0.739	89.84	601.8	0.395
B2	1.124	3.115	0.761	93.56	281.4	0.514
B3	0.852	3.247	0.900	97.12	174.7	0.631
B4	0.514	3.500	0.986	95.82	164.1	0.218
B5	0.701	3.132	1.168	94.33	154.9	0.388
B6	1.242	2.840	0.918	92.56	300.8	0.441
B7	0.812	2.808	1.380	97.86	180.7	0.523
B8	1.095	3.405	0.500	94.54	284.2	0.612
B9	1.338	3.162	0.500	90.45	478	0.295
B10	1.000	2.500	1.500	95.25	304.7	0.252
B11	0.504	2.996	1.500	95.23	166.9	0.21
B12	0.504	2.996	1.500	97.30	166.54	0.304
B13	1.120	2.700	1.180	94.78	291.4	0.472
B14	1.468	2.500	1.032	90.00	304.8	0.296
B15	0.981	2.977	1.042	98.27	150.7	0.188
B16	0.819	3.500	0.681	98.12	407.5	1
B17	1.000	2.500	1.500	93.00	456.2	0.265

The viscosity of micro emulsion systems was measured by standard rheological techniques. It depends on the excipients used in the formulation. It was observed that the viscosity of all the formulation batches B1-B17 was in between 1.92 to 7.38. From the result it was revealed that as the concentration of Surfactant increases it leads to increase in viscosity of formulation. Due to low viscosity of the formulation SMEDDS forms o/w micro emulsion water remains as external phase and viscosity of SMEDDS is near to water. The results of viscosity are as shown in Table 2.

Thermodynamic Stability Studies.

The Prepared batches were subjected to thermodynamic stress test which include Heating Cooling cycle, Centrifugation cycle and Freeze thaw testing study. Thermodynamic stability gives long shelf life to the micro emulsion as compared to ordinary emulsions. It differentiates them from emulsions that have kinetic stability. Formulation Batches B1-B17 were subjected for thermodynamic stress test and observed for phase separation, turbidity and cracking out of 17 batches the batches B1, B2, B6, B9, B13 and B14 failed the Heating-cooling, centrifugation cycle and freeze thaw testing it would be due to large concentration of oil in formulation. Higher the concentration of oil large amount of surfactant is required and lesser the concentration of oil less surfactant is required. An Ideal Emulsion is one in which there are no signs of Cracking, Phase separation and Turbidity. Batches no B3, B4, B5, B7, B8, B10, B11, B12, B15, B16, B17 passes the stress test.

Dispersibility Test

The ability of SMEDDS to maintain the drug in solubilised form is greatly influenced by the solubility of the drug in oil phase. If the surfactant or co-surfactant was contributing to the greater extent in drug solubilisation then there could be a risk of precipitation, as dilution of SMEDDS will lead to lowering of solvent capacity of the surfactant or cosurfactant, hence it is very important to determine stability of the system after dilution. This is usually done by diluting a 1 ml of SMEDDS in 250ml of

distilled water and solutions were kept for 10 min and the percentage transmittance was observed by single beam U.V. Visible spectrophotometer at 436 nm and the solution was observed for drug precipitation if any. Ideally SMEDDS should keep the drug solubilized for four to six hours assuming the gastric retention time of two hours. Formulations that passed dispersibility test in Grade A and B were taken for further study, as Grade A and B formulations may remain as micro emulsions when dispersed in GIT. From the results it has been cleared that formulations B10, B11, B12, B15, B16 and B17 fall in grade A, while rest of the formulations are in C and B grade. Results are as shown in Table 2.

Droplet Size and Percentage Transmittance.

The droplet size of the emulsion is an important factor in self-emulsification performance because it determines the rate and extent of drug release as well as drug absorption. It has been reported that smaller the particle size of the emulsion droplets higher the absorption and improved bioavailability. Decrease in particle size may be the result of non-ionic surfactants being available to stabilize the oil water interface. Furthermore, the decrease in the droplet size behaviour reflects the formation of a better closed packed film of the surfactant at the oil water interface, thereby stabilizing the oil droplets. The smaller the droplet size, the larger the interfacial surface area will be provided for drug absorption. It appeared that the particle size was inversely proportional to the concentration of oil and directly proportional to the concentration of Smix Smaller particle size of SMEDDS may impart faster release of drug. Transparency of formulation was determined in the termed of Transmittance (%T). Transmittance value greater than or equal to 98% indicates the high clarity of micro emulsion. Other formulation batches has %T values were between 95% to 97% suggesting less clarity of the formulation may be due to larger particle size of the formulation. %Transmission value of all batches were in between 89.8% to 98.27% which indicated that formulation batches of SMEDDS were more clear and transparent. As increase in the particle size, oil globules may reduce the transparency of micro emulsion and thereby higher values of %T. Results are as shown in Table 2.

Zeta Potential Measurement.

The zeta potential governs the stability of micro emulsion, it is important to measure its value for stability samples. The high value of zeta potential indicates electrostatic repulsion between two droplets. According to DLVO theory states that electric double layer repulsion will stabilize micro emulsion where electrolyte concentration in the continuous phase is less than a certain value. A negative force means a negative potential between the droplets. Many physiological studies have proved that the apical potential of absorptive cells, as well as that of all other cells in the body, is positively charged with respect to the mucosal solutions in the lumen. A novel SMEDDS, which results in the negatively charged dispersed oil droplets upon dilutions with an aqueous phase, leads to adhesion to the intestinal mucosa and thereafter drug uptake from the mucosa. Thus, these formulations enhanced the oral absorption and thus oral bioavailability. In this study it was observed that zeta potential of optimised batch was found to be -18 mv. This result attribute due to presence of non-ionic surfactant in SMEDDS.

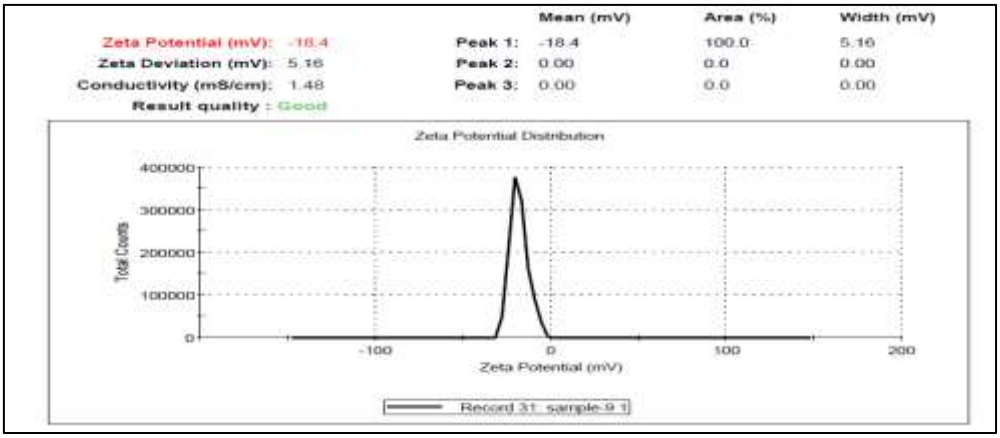

Fig-8: Zeta Potential of Optimized Batch

Electro conductivity Study, Drug Content and Robustness to dilution.

This test is performed for measurement of the electro conductive nature of system. The electro conductivity of the resultant system was measured by using an electro conductometer. Based on electrical conductivity, the phase system of micro emulsion were determined.(Figure 9). From the result of conductivity it was noted that as the increased in addition of ml of water, increased in the conductivity of the formulation which determined that the micro emulsion system of o/w type. The Drug content of Optimized Formulation

was found to be 98.26% for Curcumin and 97.52% for Artemisinin. Emulsion must remain clear upon dilution into G.I tract so optimized batch was diluted to 50, 100 and 250 times and was clear and have good dilution potential.

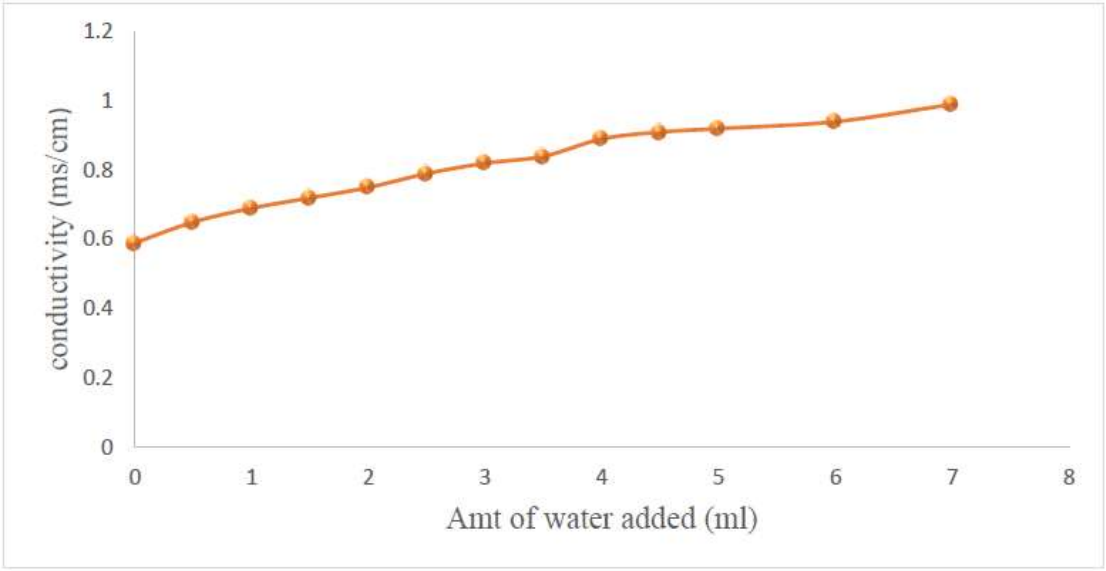

Fig-9: Electro Conductivity Chart of Optimized Formulation

In vitro Diffusion studies

After 1.30 h of diffusion 63.81% of the curcumin and 54.91% of Artemisinin was diffused from SMEDDS, while from drug solution the diffusion was found to be 35.16 % of curcumin and 31.50% of artemisinin. Thus, the amount of the drug diffused through the membrane has more permeation capacity when it is given in the form of SMEDDS. The enhancement in diffusion is due to formation of micro emulsion droplets in Nano-meter range and improved permeation of the SMEDDS because of the presence of surfactant, which reduces the interfacial tension of formulation (Figure 10).

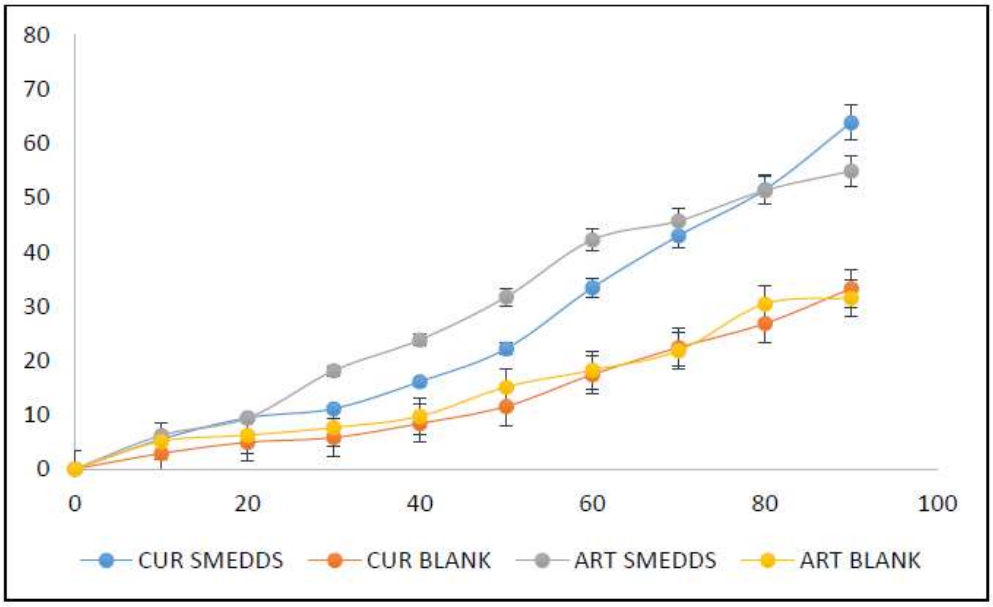

Fig-10: In-vitro Diffusion study of SMEDDS

CONCLUSION

A SMEDDS formulation for Curcumin and Artemisinin was developed and optimized with D-Optimal mixture design, and the optimum formulation was containing 50 % surfactant (Tween 80), 12.5% co-surfactant (PEG 400) and 12.5% oil (oleic acid). The formulation was found to be novel, effective, safe, stable and patient friendly. It also overcomes the

drawbacks associated with drugs solubility. If the afore mentioned formulation will scaled-up to manufacturing level, it will have increased solubility and attain higher bioavailability.

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