ORIGINAL ARTICLE

Development and Characterization of Self-nanoemulsifying Drug Delivery System Loaded with Fixed Oil of *Semecarpus anacardium* Linn.

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Abstract

Objective: Fruits and fixed oil of *Semecarpus anacardium* Linn. are used to treat various ailments in different Indian System of Medicine including Ayurveda. Despite having a wide array of therapeutic activities, it is also known for toxicity. Hence, there is a need to prepare a pharmaceutically acceptable dosage form to reduce its toxicity, enhance bio-availability, and explore the use of *S. anacardium* Linn. as a potent drug. In this study, an attempt has been made to prepare self-nanoemulsifying drug delivery systems (SNEDDS) for oral delivery of fixed oil. **Materials and Methods:** Various oils, surfactants and co-surfactants were investigated to prepare a stable SNEDDS of *S. anacardium* Linn. Emulsification time, droplet size and zeta potential, and dissolution studies were carried out to choose the best SNEDDS formulation. Stability studies of developed SNEDDS were carried out at 5°C, 25°C, and 40°C, respectively. **Results and Discussions:** Based on the emulsification time, droplet size and zeta potential after dispersion into aqueous phase, an optimized formulation (F_{19}) consisting of fixed oil, Labrafil M1944CS, Tween 80, Transcutol P (10:60:30% w/w) were prepared. The dissolution profile of fixed oil of *S. anacardium* Linn. loaded SNEDDS in various medium showed that 100% of fixed oil released within 10 min irrespective of the pH of the dissolution medium. The prepared SNEDDS was found stable for 3 months. **Conclusion:** It can be concluded that the poorly soluble fixed oil of *S. anacardium* Linn. can be successfully loaded in SNEDDS formulation.

Key words: Dissolution profile, formulation, pseudo-ternary phase diagram, self-nanoemulsifying, *Semecarpus anacardium* oil

INTRODUCTION

Active chemical constituents of fixed oil of *Semecarpus anacardium* Linn. responsible for different pharmacological activities include bioflavonoid, phenolic compound, bhilawanols, minerals, vitamins, and amino acids. Pharmacological and clinical studies indicated that fixed oil of *S. anacardium* Linn. exhibits a wide array of therapeutic activities such as anti-inflammatory activity, anti-arthritic effect, anti-tumor, anti-neoplastic, cytotoxic, cytostatic activity, hypolipidemic activity, hypocholesterolemic activity, antimicrobial activity, and antistress activity.[1] The major pharmacological actions of extract of *S. anacardium* Linn. and ayurvedic formulations are shown in Table 1.[2] Despite having many pharmacological uses fixed oil of *S. anacardium* Linn. is well-known for its toxicity.[3] It is also known to have a narrow therapeutic range.[2] The common effects of toxicity are generalized itching, vesication, erythematous patches, mucocutaneous popular eruptions, stomatitis, gastritis, proctitis, urethritis, etc.[2]

Apart from its toxicity, fixed oil of *S. anacardium* Linn. is insoluble in aqueous medium due to its highly lipophilic nature. Therefore, bioavailability of fixed oil is limited by

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Table 1: Reported pharmacological activities of *Semecarpus anacardium*

Extract/formulation	Pharmacological activity reported	*In vivo* models (humans/animals)	*In vitro* models (cell lines/chemical or microbial assay)	Dose	References
Nut extract	Breast cancer	-	T47D cell line	400 mg/mL	[13]
Seeds (Bioflavanoids)	COX inhibitors	Male Sprague-Dawley rats (Rat paw edema assay)	-	100 µg/kg	[14]
Nut milk extract	Antidiabetic	Male albino Wistar rats	-	300 mg/kg b.wt. (For 21 days)	[15]
Nut extract	Antiarthritic	Male albino Wistar rats	-	150 mg/kg	[16]
Nut milk extract	Antiarthritic	Male albino Wistar rats	-	150 mg/kg	[17]
Nut extract	Immunomodulatory and antiarthritic	Humans	-	1g/mL	[18]
Nut milk extract	Antiarthritic	Male albino Wistar rats (Freund's adjuvant induced arthritis)	-	150 mg/kg	[19]
Nut milk extract	Adjuvant antiarthritic with special reference to bone metabolism	Male albino Wistar rats (Freud's adjuvant induced arthritis)	-	150 mg/kg	[20]
Nut milk extract	Antiarthritic	Male albino Wistar rats	-	50, 100, 150, 200, 250 mg/kg	[21]
Chloroform extract of SA	Aphrodisiac	Male albino Wistar rats	-	150 and 300 mg/kg	[22]
Methanolic extract of SA nut	Cytotoxic	-	African green monkey kidney normal cell line (vero) and human epidermal larynx carcinoma cell line (Hep-2)	10-0.0196 mg/mL	[23]
Hydroalcoholic extract	Cardioprotective	Male Sprague-Dawley rats	-	100-500 mg/kg	[24]
Ethanol, acetone and aqueous extract	antioxidant	-	DPPH and ABTS assay	100 µg/mL (DPPH assay) and 0.01 to 0.5 mg/mL (ABTS assay)	[25]
Nut milk extract	Antidiabetic (type II)	Male Sprague-Dawley rats (Streptozotocin induced diabetes mellitus)	-	200 mg/kg	[26]
Nut milk extract	Cytoprotective (antidiabetic)	Male albino Wistar rats (Streptozotocin induced diabetes mellitus)	-	300 mg/kg	[27]

(Contd...)

Table 1: (Continued)

Extract/formulation	Pharmacological activity reported	*In vivo* models (humans/animals)	*In vitro* models (cell lines/chemical or microbial assay)	Dose	References
Extract of stem bark	Cognitive enhancing	Mice (Morris water maze)	-	25, 50 and 75 mg/kg	[28]
Fruit extract	Hepatoprotective	Male albino Wistar rats	-	250 and 500 mg/kg	[29]
Vardhaman Bhallataki Rasayana processed with milk	Antirheumatic activity	Humans	-	3-6 nuts of bhallataka	[30]
Methanolic extract	Antifungal	-	Fungal strains (*Fusarium oxysporum, Rhizoctonia solani, Alternaria* spp., and *Sclerotium rolfsii*)	6.25, 12.5, 25, 37.5, 50 and 62.5 µg/mL)	[31]
Petroleum ether nut extract	Antibacterial	-	Microorganism (*Escherichia coli, Bacillus subtilis, Micrococcus luteus, Klebsiella pneumonia, Streptococcus aureus, Proteus vulgaris, Salmonella typhi*)	150 µl	[32]
Aqueous and organic extract	Antimicrobial	-	Microorganism (*Staphylococcus aureus, Shigella flexneri, Bacillus licheniformis, Vibro cholerae, Pseudomonas aeruginosa, Streptococcus aureus, Bacillus brevis*)	10, 50, 100 mg/ml 10 mg/ml	[33]
Ayurvedic formulations					
Amrut Bhallatak Avaleha (Electuary)	General tonic and vitalizer	Humans	-	1 to 2 teaspoonful for 2 times	[2]
Bhallatakasava (Wine)	Neuralgia and asthma	Humans	-	2 to 4 teaspoonful for 2 times	[2]
Suranvatak (Pills)	Piles and anorectal diseases	Humans	-	2 pills (500 mg pill) for 2 times	[2]
Sanjeevani Vati (Pills)	Dysentry and diarrhea	Humans	-	2 pills (250 mg pill) for 3 times	[2]
Bhallataka Parpati (Powder)	Rheumatic diseases	Humans	-	250 mg for 3 times	[2]
Narsimha Choorna (Powder)	General restorative	Humans	-	1 to 2 g for 2 times	[2]

oral route. To have a better therapeutic efficacy more dose of the drug has to be administered orally that could further lead to undesirable side effects. Hence, the development of pharmaceutically acceptable dosage forms for fixed oil of *S. anacardium* Linn. is required to achieve enhanced aqueous solubility as well as similar pharmacological activity at lower doses with minimal side effects.

The self-nanoemulsifying drug delivery system (SNEDDS), which is well-known for its potential to improve the aqueous solubility and oral absorption of lipophilic drugs. SNEDDS is an isotropic mixture composed of oil, surfactant, co-surfactant, and drug. It readily disperses in the aqueous environment of the gastrointestinal tract and forms a fine o/w emulsion with a droplet size in nanometer range, under gentle agitation and thereby enhances oral bioavailability of poorly soluble drugs.[4-6] As compared to metastable emulsions, SNEDDS is a thermodynamically stable formulation with the high solubilization capacity for lipophilic drugs and also can be filled directly into soft or hard gelatin capsules for convenient oral administration.[6,7] This study aims toward the development of SNEDDS of SA oil in a lower dose for oral administration and that can be further explored for various *in vivo* pharmacological efficacy evaluations.

MATERIALS AND METHODS

Materials

Fixed oil was extracted from the fruits of *S. anacardium*. Labrafil M 1944 CS, Transcutol P, Labrafac CC, and Lauroglycol FCC were gifted by Gattefosse, Mumbai, India. Tween 20, 60 and 80, Span 20 and 80, olive oil, oleic acid, polyethylene glycol 400, polyethylene glycol 200, and propylene glycol were purchased from Loba Chemie (P) Ltd., Mumbai, Maharashtra, India. Hexane, octanol, and ethanol were purchased from Loba Chemie (P) Ltd, Mumbai, Maharashtra, India. Double distilled water of USP grade was used throughout the study.

Extraction of fixed oil

Fruits of *S. anacardium* were authenticated from pharmacognosy lab, institute for postgraduate teaching and research in Ayurveda (IPGT and RA), Jamnagar, Gujarat, India, with the authentication no. 6122/2014-15. About 50 g of SA nuts were taken, slightly crushed and added to the soxhlet condenser. In a round bottom flask, 250 ml of the hexane was taken and the temperature of solvent was maintained at 70°C (because hexane boiling point is 68°C). The extraction was allowed to run for 48 h for proper extraction of fixed oil. The viscous liquid obtained after extraction in round bottom flask was taken out and evaporated at 70°C on a water bath until the complete evaporation of hexane.[8]

UV analysis of fixed oil

To plot a calibration curve for the estimation of fixed oil, 0.1 ml (100 mg) of oil was dissolved in 100 ml of hexane in a standard volumetric flask (100 ml) to get a concentration of 1 mg/ml. From this 10 ml solution was withdrawn to another 100 ml standard volumetric flask and the solution was further diluted with 90 ml of hexane to get concentration of 100 µg/ml. From this 1, 2, 3, 4, and 5 ml of solutions were withdrawn and transferred to each 10 ml standard volumetric flask. The volume of each standard flask was adjusted to 10 ml using hexane to get concentration of 10, 20, 30, 40 and 50 µg/ml. The spectra of different concentration of fixed oil solutions were recorded in 1 cm quartz cells at a fast scan speed using hexane as blank solution in the range of 200-400 nm.

Solubility studies

To prepare SNEDDS prototypes, the solubility of fixed oil was evaluated in various oils, surfactant and co-surfactant [Table 2]. Fixed oil (150 mg) was added to 1 ml of listed vehicles, vortexed for 15 min and then kept shaken for 3 days on a water bath at 25°C. The resulting mixture of liquids was centrifuged at 3500 rpm for 15 min and supernatant was collected. Suitable dilutions were prepared and analyzed using UV-Visible spectrophotometer at 272.23. The study was repeated in triplicate and mean data was recorded.

Preparation of SNEDDS prototype and construction pseudoternary phase diagrams

The selected oil (Labrafil M 1944 CS), surfactant (Tween 80), and co-surfactant (Transcutol P) for the solubility studies were mixed as per their composition shown in Table 3. A series of self-nanoemulsifying system were prepared for each of 27 formulae [Table 3] with varying weight percentages of oil from 10% to 90%, surfactant from 3% to 60%, co-surfactant from 3% to 60%. To each of the SNEDDS prototypes 150 mg of fixed oil was loaded and the mixture was vortexed for 15 min. To evaluate the emulsification ability of selected oil, surfactant and co-surfactant, the prepared isotropic mixture were diluted to 250 ml with double distilled water, which was gently stirred using magnetic stirrer at 100 rpm at 37°C. The resulting emulsions were observed visually for the relative turbidity. The ease of formation of the nanoemulsion was also noted. The tendency to form nanoemulsion is judged as "good" when the droplets easily spread out in water and formed a fine transparent milky emulsion, and it was judged as "bad" when there was poor or no emulsion formation with the immediate coalescence of droplets, especially when stirring was stopped. The tendency to emulsify spontaneously and the progress of emulsion droplets spread were visually assessed using the grading criteria.[9] If a clear and slightly bluish or, a bluish white microemulsion is rapidly formed within 1 min, the corresponding region in the phase diagram

Table 2: Solubility of fixed oil of *Semecarpus anacardium* Linn. in various vehicles

Vehicle (Oil)	Solubility of fixed oil (mg/ml)	Vehicle (surfactant)	Solubility of fixed oil (mg/mL)	Vehicle (Co-surfactant)	Solubility of fixed oil (mg/mL)
Water	0	Labrafac CC	0.335±0.95	Transcutol P	8.809±0.87
Olive oil	0.572±1.44	Tween 20	0.896±0.56	PEG 400	7.096±0.35
Castor oil	0.150±0.88	Tween 60	0.780±0.34		
Sesame oil	0.100±0.76	Tween 80	9.000±0.98		
Mustard oil	0.500±0.13	Propylene glycol	3.950±0.64		
Oleic acid	0.200±0.45	Span 20	0.500±0.56		
Sunflower oil	0.200±0.98	Span 60	0.409±1.56		
Mineral oil	0.112±0.56	Span 80	0.120±1.65		
Corn oil	0.150±1.23	Labrasol	0.390±0.23		
Cotton seed oil	0.340±0.63	Lauroglycol FCC	0.986±1.90		
Peanut oil	0.187±0.87	Cremophor EL	0.709±1.65		
Labrafil M1944CS	15.578±0.97	Caproyl 90	1.324±0.35		
Coconut oil	0.324±1.65				

Each value represents the mean±SD, number of replicates of the study. *n*=3. SD: Standard deviation

will be labeled as SNEDDS to describe the best efficient self-emulsification region. If a bright white emulsion (translucent emulsion) is formed within 2 min, the region is labeled as self-micro emulsifying drug delivery system (SMEDDS) and will still be considered to have met the criterion for self-emulsification. However, if a dull greyish opaque white emulsion with slightly oil appearance (i.e., a formulation exhibiting either poor or minimal emulsification with the large oil droplets floating on the surface) is formed slowly (i.e. longer than two min.), the composition for this formulation will be labeled as emulsion in the phase diagram.[6] All studies were repeated in triplicate, with similar observations being made between repeats.

Turbidity measurements

The turbidity of the resultant emulsions given in nephelo turbidity unit was measured using Orbeco-Hellige model 966, Orbico Analytical system Inc., Framingdale, New York, USA.

Droplet size and zeta potential analysis

The mean droplet size and polydispersity index (PDI) of the optimized batch of SNEDDS were determined by using Malvern nano zeta seizer instrument (DTS Ver. 5. 10). The PDI reflects uniformity of droplet diameter and can be used to depict the size distribution of micro-emulsion population. The sensitivity ranges from 10 nm to 5 µm and the data shown by computer calculation using the Mie equations of light scattering. The measurements performed at 25°C at a fixed angle of 90°. The measurement time was 2 min and each run underwent 12 sub runs. The selected batch of formulation (0.1 ml) was dispersed into a value of 100 ml of water, pH 1.2, pH 3.0, and pH 6.8 buffers under gentle stirring in a glass beaker. Then, 1 ml aliquot was withdrawn and added into a sample cell for droplet size measurement. Each size value reported was the average of at least 3 independent measurements. Zeta potential measurement was carried out on the same diluted sample using the same equation and operating conditions, and the zeta potential values were calculated according to the Smoluchowski equation.[6]

In vitro dissolution study

The optimized batch (F_{19}) as it has shown better droplet size, zeta potential, and self-emulsifying ability of SNEDDS was filled in hard gelatin capsules. To evaluate the effect of pH on the *in vitro* dissolution of fixed oil loaded SNEDDS, *in vitro* release studies of SNEDDS was studied using USPXXIII apparatus I at 37°C ± 0.50°C with a rotating speed of 50 rpm in dissolution media (900 ml) namely, water, pH 1.2, 3.0 and 6.8 buffers. During the study, 1 ml of aliquots were withdrawn at predetermined time intervals (10, 20, 30 and 45 min) from the dissolution medium and replaced with fresh buffer. The withdrawn aliquots were filtered using 0.25 µm membrane filter and determined by UV spectrophotometer at 272.22 nm. In a similar way, pure fixed oil was filled in capsule and the dissolution was carried in the same way in water pH 1.2, 3.0 and 6.8 buffers. The dissolution profiles of fixed oil loaded SNEDDS and pure fixed oil were compared.

Stability studies

The optimized batch of SNEDDS (F_{19}) was subjected for stability studies at 25°C ± 2°C/65% ± 5% R.H., 40°C ± 2°C/75% ± 5% R.H. for 3 months instability chamber (Remi Electro Technique, India) and 5°C ± 0.5°C in refrigerator for 3 months, respectively. The aged samples

were analyzed for drug precipitation and change in droplet size.

Table 3: Composition of various SNEDDS formulations

Batch number	Oil (%)	Surfactant (%)	Co-surfactant (%)
F_1	0.1 (10)	0.45 (45)	0.45 (45)
F_2	0.2 (20)	0.40 (40)	0.40 (40)
F_3	0.3 (30)	0.35 (35)	0.35 (35)
F_4	0.4 (40)	0.30 (30)	0.30 (30)
F_5	0.5 (50)	0.25 (25)	0.25 (25)
F_6	0.6 (60)	0.2 (20)	0.2 (20)
F_7	0.7 (70)	0.15 (15)	0.15 (15)
F_8	0.8 (80)	0.10 (10)	0.10 (10)
F_9	0.9 (90)	0.05 (5)	0.05 (5)
F_{10}	0.1 (10)	0.3 (30)	0.6 (60)
F_{11}	0.2 (20)	0.27 (27)	0.53 (53)
F_{12}	0.3 (30)	0.23 (23)	0.47 (47)
F_{13}	0.4 (40)	0.2 (20)	0.40 (40)
F_{14}	0.5 (50)	0.17 (17)	0.33 (33)
F_{15}	0.6 (60)	0.13 (13)	0.27 (27)
F_{16}	0.7 (70)	0.1 (10)	0.2 (20)
F_{17}	0.8 (80)	0.07 (7)	0.13 (13)
F_{18}	0.9 (90)	0.03 (3)	0.07 (7)
F_{19}	0.1 (10)	0.6 (60)	0.3 (30)
F_{20}	0.2 (20)	0.53 (53)	0.27 (27)
F_{21}	0.3 (30)	0.47 (47)	0.23 (23)
F_{22}	0.4 (40)	0.40 (40)	0.2 (20)
F_{23}	0.5 (50)	0.33 (33)	0.17 (17)
F_{24}	0.6 (60)	0.27 (27)	0.13 (13)
F_{25}	0.7 (70)	0.2 (20)	0.1 (10)
F_{26}	0.8 (80)	0.13 (13)	0.07 (7)
F_{27}	0.9 (90)	0.07 (7)	0.03 (3)

SNEDDS: Self-nanoemulsifying drug delivery systems

RESULTS AND DISCUSSIONS

Development of calibration curve

The overlay of UV spectra of various concentrations (10-50 µg ml) of fixed oil is shown in [Figure 1a]. The λ_{max} of the fixed oil of *S. anacardium* Linn. in hexane was found to be 272.23 nm. The calibration plot was found linear in the range of 10-50 µg/ml with a correlation coefficient of 0.999 [Figure 1b].

Formulation of SNEDDS

Solubility studies of fixed oil in oil, surfactant, and co-surfactants

Among all the oils, Labrafil M 1944 CS has shown the maximum solubility of 15,578.90 ± 0.97 µg/ml, among all the surfactants selected tween 80 has shown the maximum solubility of 9000.56 ± 0.98 µg/ml and among co-surfactant, Transcutol P has shown solubility of 8809.23 ± 0.87 µg/ml. Therefore, Labrafil M 1944 CS (HLB value 4) was selected as oil, tween 80 (HLB value 15) as surfactant and Transcutol P (HLB value 3.5) was selected as co-surfactant for the formulation of SNEDDS prototypes. The results are shown in Table 2.

Screening of oils, surfactants and co-surfactants for emulsification ability and construction of ternary phase diagram

A series of SEDDS were prepared and their self-emulsifying properties were visually observed [Table 4]. The formed emulsions were judged as SNEDDS, SMEDDS and normal emulsion on the basis of their turbidity measurements and visual observations for transparency. Pseudoternary phase diagram was constructed in the presence of fixed oil to identify the self-emulsifying region and to optimize the concentration of oil, surfactant and co-surfactant in the SEDDS formulation. The phase diagram of the system containing Labrafil M1944 CS, Tween 80, and Transcutol P as the oil, surfactant and co-surfactant, respectively, is shown in Figure 2. It was observed that incorporation of the co-surfactant (Transcutol P) within the self-emulsifying region increased the spontaneity of the self-emulsification process. The efficiency of emulsification

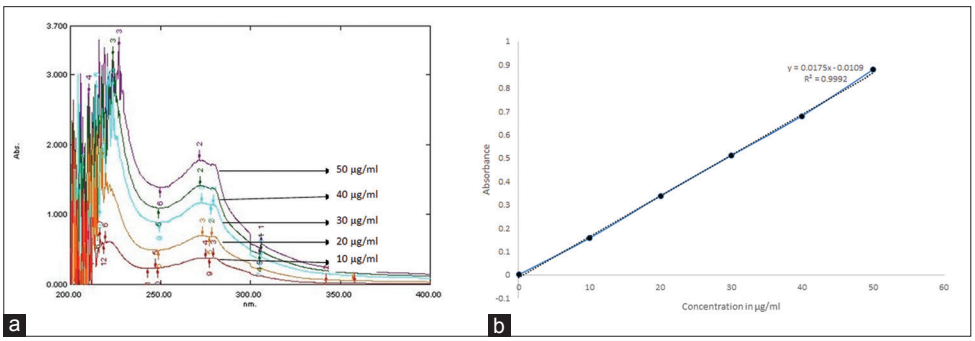

Figure 1: (a) Overlay of UV spectra of various concentrations of fixed oil of *Semecarpus anacardium* Linn. (b) Calibration curve of fixed oil of *S. anacardium* Linn. in hexane

Table 4: Observations recorded for various SNEDDS formulations

Batch number	Oil (%)	Surfactant (%)	Co-surfactant (%)	Turbidity (NTU)	Visual observation
F_1	0.1 (10)	0.45 (45)	0.45 (45)	2.567	Clear bluish white
F_2	0.2 (20)	0.40 (40)	0.40 (40)	>20	Opaque emulsion
F_3	0.3 (30)	0.35 (35)	0.35 (35)	17.18	Translucent emulsion
F_4	0.4 (40)	0.30 (30)	0.30 (30)	>20	Opaque emulsion
F_5	0.5 (50)	0.25 (25)	0.25 (25)	18.23	Translucent emulsion
F_6	0.6 (60)	0.2 (20)	0.2 (20)	19.16	Translucent emulsion
F_7	0.7 (70)	0.15 (15)	0.15 (15)	16.22	Translucent emulsion
F_8	0.8 (80)	0.10 (10)	0.10 (10)	17.18	Translucent emulsion
F_9	0.9 (90)	0.05 (5)	0.05 (5)	>20	Opaque emulsion
F_{10}	0.1 (10)	0.3 (30)	0.6 (60)	4.879	Clear bluish white
F_{11}	0.2 (20)	0.27 (27)	0.53 (53)	>20	Opaque emulsion
F_{12}	0.3 (30)	0.23 (23)	0.47 (47)	>20	Opaque emulsion
F_{13}	0.4 (40)	0.2 (20)	0.40 (40)	>20	Opaque emulsion
F_{14}	0.5 (50)	0.17 (17)	0.33 (33)	>20	Opaque emulsion
F_{15}	0.6 (60)	0.13 (13)	0.27 (27)	>20	Opaque emulsion
F_{16}	0.7 (70)	0.1 (10)	0.2 (20)	>20	Opaque emulsion
F_{17}	0.8 (80)	0.07 (7)	0.13 (13)	>20	Opaque emulsion
F_{18}	0.9 (90)	0.03 (3)	0.07 (7)	16.18	Translucent emulsion
F_{19}	0.1 (10)	0.6 (60)	0.3 (30)	5.876	Clear bluish white
F_{20}	0.2 (20)	0.53 (53)	0.27 (27)	>20	Opaque emulsion
F_{21}	0.3 (30)	0.47 (47)	0.23 (23)	>20	Opaque emulsion
F_{22}	0.4 (40)	0.40 (40)	0.2 (20)	>20	Opaque emulsion
F_{23}	0.5 (50)	0.33 (33)	0.17 (17)	>20	Opaque emulsion
F_{24}	0.6 (60)	0.27 (27)	0.13 (13)	>20	Opaque emulsion
F_{25}	0.7 (70)	0.2 (20)	0.1 (10)	>20	Opaque emulsion
F_{26}	0.8 (80)	0.13 (13)	0.07 (7)	13.18	Translucent emulsion
F_{27}	0.9 (90)	0.07 (7)	0.03 (3)	19.16	Translucent emulsion

SNEDDS: Self-nanoemulsifying drug delivery systems

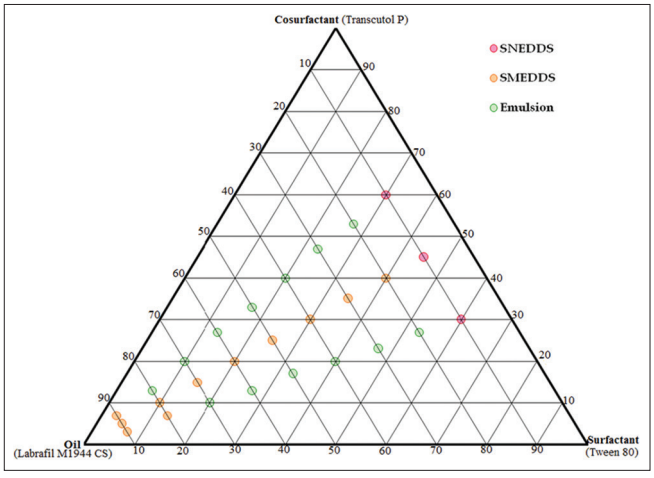

Figure 2: Ternary phase diagram showing self-nanoemulsifying drug delivery systems, emulsion and phase separation region

was good when the surfactant/co-surfactant concentration was more than 30% v/v of the SEDDS formulation. It was observed that only 3 formulations F_1, $F_{10,}$ and F_{19} have shown very good emulsification region with transparency after dilution in water. These formulations were considered to form nanoemulsions and were subjected for mean droplet size, PDI and zeta potential analysis. It was also noted that addition of surfactant and co-surfactant up to 90% v/v in the SEDDS formulation, only 10% v/v of Labrafil M1944 CS containing SA oil got solubilized. About 1 ml of each, F_1, F_{10} and F_{19} SNEDDS formulation was sufficient to dissolve 150 mg of fixed oil.

Characterization of optimized batch of SNEDDS

From the ternary phase diagram, it was observed that formulations, F_1, F_{10} and F_{19} resulted in the formation of

SNEDDS after dilution in water. Therefore, these three batches were selected for % drug loading, mean droplet size, PDI, and zeta potential analysis. The % drug loading of F_1, F_{10} and F_{19} of SNEDDS was found to be 95 ± 0.02, 96.26 ± 1.1 and 98.12% ± 0.06%, respectively. The mean droplet size, zeta potential and PDI of F_1, F_{10} and F_{19} are shown in Table 5. It was observed that all three formulations showed their mean droplet size in nanometers range when diluted in 200 ml water. This was possible as surfactant strongly localized to the surface of the emulsion droplet reduced interfacial free energy and provided a mechanical barrier to coalescence resulting in a thermo-mechanically spontaneous dispersion.[10,11] Furthermore, co-surfactant increased interfacial fluidity by penetrating into the surfactant film creating void space among surfactant molecules.[5,11,12] To further evaluate the effect of change in pH on the droplet size, the nanoemulsions (F_1, F_{10} and F_{19}) were diluted with 200 ml of water, pH 1.2, 3.0 and 6.8 buffers, respectively [Table 5]. The fixed oil loaded SNEDDS showed fairly similar mean droplet size within range of 170-190, 130-160 and 95-110 nm, for formulations F_1, F_{10} and F_{19}, respectively, when diluted with various dilution medium differing in pH. The time required for formation of nanoemulsions after dilution with various dilution media was just 2 min for all selected formulations. The resulting nanoemulsions were bluish white transparent in appearance and did not show any signs of phase separation and drug precipitation even after 24 h.

The zeta potential of the resulting SNEDDS was found in the range of −16 to −32 mV, which is sufficient to stabilize the formed nanoemulsion [Table 5]. Since formulation F_{19} has shown least droplet size and highest zeta potential hence it was subjected further for *in vitro* dissolution and stability studies.

In vitro dissolution study

The *In vitro* dissolution profile of optimized batch (F_{19}) of fixed oil loaded SNEDDS in various dissolution media is shown in Table 6. The dissolution profile of fixed oil loaded SNEDDS in various medium showed that within 5 min about 95% of fixed oil was released and within 10 min 100% of fixed oil released irrespective of the pH of the dissolution medium. Whereas, only 10% of pure fixed oil released in

Table 5: Mean droplet size zeta potential and polydispersity index of fixed oil loaded SNEDDS in water and different buffer pH conditions

Formulation	Dissolution medium (200 ml)	Droplet size (nm)[a]	Polydispersity index[b]	Zeta potential[b] (mV)
F_1	Water	183.56±1.16	0.712	−20.22±1.08
	pH 1.2	178.18±0.88	0.796	−18.68±1.89
	pH 3.0	189.36±1.12	0.812	−22.15±1.13
	pH 6.8	172.11±1.08	0.702	−16.15±1.49
F_{10}	Water	136.45±1.56	0.809	−18.15±1.16
	pH 1.2	132.12±1.12	0.812	−16.22±1.45
	pH 3.0	156.14±0.88	0.766	−21.15±1.12
	pH 6.8	138.16±1.54	0.716	−18.56±1.38
F_{19}	Water	110.15±1.18	0.512	−31.34±1.16
	pH 1.2	112.16±1.86	0.512	−25.22±1.41
	pH 3.0	102.96±0.33	0.762	−23.22±1.05
	pH 6.8	95.16±1.36	0.816	−26.51±1.34

SNEDDS: Self-nanoemulsifying drug delivery systems. [a]Droplet size expressed as mean (*n*=2) where relative standard deviation was <10%. [b]Data expressed as mean (*n*=3)

Table 6: *In vitro* dissolution study of fixed oil loaded SNEDDS (F_{19}) and pure fixed oil

Time (min)	% Cumulative release (*Mean±SD)							
	SAO loaded SNEDDS (F_{19})				Pure SAO			
	Water	Buffer pH 1.2	Buffer pH 3.0	Buffer pH 6.8	Buffer pH 1.2	Buffer pH 3.0	Buffer pH 6.8	Water
5	96.18±2.0	94.32±2.17	92.16±1.18	91.12±2.0	2.13±1.18	1.16±2.0	2.91±2.32	3.1±1.20
10	101.18±1.18	100.16±1.17	100.18±2.00	100.02±1.34	4.51±2.16	3.16±1.31	5.22±2.00	6.52±2.14
15	101.54±2.1	100.28±2.68	100.48±1.64	100.16±2.24	8.12±2.9	10.12±1.82	7.19±2.18	11.55±1.22
30	101.82±1.6	100.36±2.68	100.67±0.87	101.22±1.74	16±1.45	14.56±2.64	12.56±2.11	14.18±1.16
45	102.102±2.0	101.12±2.00	100.91±1.16	101.36±1.98	20.16±2.0	17.42±1.46	19.15±1.50	20.24±1.46

*Mean of six replicate studies. SNEDDS: Self-nanoemulsifying drug delivery systems, SD: Standard deviation

Table 7: Stability study results of fresh and aged fixed oil loaded SNEDDS kept at different stability conditions for 3 months

[a,b]Mean droplet size (nm)				Drug precipitation			
Fresh	Aged			Fresh	Aged		
	25°C±2°C/ 65%±5% R.H.	40°C±2°C/ 75%±5% R.H.	5°C±0.5°C		25°C±2°C/ 65%±5% R.H.	40°C±2°C/ 75%±5% R.H.	5°C±0.5°C
103±1.12	120.10±1.36	145.46±2.18	107.15±1.62	No precipitation	No precipitation	No precipitation	No precipitation

[a]Droplet size expressed as mean (n=2) where relative standard deviation was <10%. [b]When diluted in 100 ml water.
SNEDDS: Self-nanoemulsifying drug delivery system

45 min in all the dissolution medium. It was also noted that in the case of pure fixed oil, oil was floating in the form of small globule in the dissolution medium (immiscible phase). This showed that SNEDDS is having a very high potential to deliver the lipophilic/oily drugs orally.

Stability studies

The result of stability studies is shown in Table 7. There was no drug precipitation observed in any of the samples kept at different temperature and humidity conditions for 3 months. The minor change in mean droplet size was observed in samples kept at 25°C ± 2°C/65% ± 5% R.H. and 5°C ± 0.5°C, respectively. However, a large change in mean droplet size was observed in the case of samples kept at accelerated conditions (40°C ± 2°C/75% ± 5% R.H.). However, still the droplet size was in nanometer range and was observed less than 350 nm. Hence, it can be safely concluded that the developed fixed oil loaded SNEDDS were stable at different temperature and humidity conditions.

CONCLUSION

This study has clearly demonstrated the potential utility of SNEDDS for formulating ZTO with improved aqueous dispersibility, stability and oral bioavailability. In the formulated SNEDDS, the fixed oil itself could serve as a partial lipid phase with the dual advantages of increasing drug loading as well as minimizing the amount of the inert oils required. Based on the emulsification time, droplet size and zeta potential formulation (F_{19}) consisting of fixed oil, Labrafil M1944CS, Tween 80, Transcutol P (10:60:30% w/w) was selected as best SNEDDS formulation. Upon mixing with water, the fixed oil loaded SNEDDS was rapidly dispersed into fine droplets with a mean size of 103 ± 1.12 nm and zeta potential of −31.34 ±1.16 mV. The dissolution profile of fixed oil loaded SNEDDS in various medium showed that 100% of fixed oil released within 10 minutes irrespective of the pH of the dissolution medium. The active components remained stable in the optimized SNEDDS stored at 5°C, 25°C for 3 months with no drug precipitation and major change in mean droplet size. A change in droplet size was observed with samples kept at 40°C, however, the droplets were still in nanometer range. Hence, it can be concluded that the poorly soluble fixed oil can be successfully loaded in SNEDDS formulation and can be further explored for various *in vivo* pharmacological efficacy evaluations for the treatment of diseases exemplified in Table 1.

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