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Effect of Surfactants and Co-surfactants on Phase Behaviour and Physicochemical Properties of Self-nanoemulsifying Drug Delivery System Loaded with Plumbagin

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ABSTRACT: Purpose: Plumbagin has wide pharmacological actions but limited solubility and low oral bioavailability. Self-nanoemulsifying drug delivery system (SNEDDS) has potential to improve solubility and dissolution rate. Selection of surfactant and co-surfactant is critical for a successful SNEDDS. Aim of present work was to investigate effect of different surfactants and co-surfactants on phase behaviour and physicochemical properties of SNEDDS loaded with plumbagin. Methods: Solubility of plumbagin in various oils, surfactants and co-surfactants was estimated. Out of those, Capmul®MCM (oil), Tween®20 and Tween® 80 (surfactants), polyethylene glycol 400 and propylene glycol (co-surfactants) in varying concentrations and combinations of surfactant: co-surfactant (Smix) were employed to construct pseudo-ternary phase diagrams. Thermodynamic stability, dispersibility, robustness to dilution, self-emulsification time, % transmittance and globule size tested. Results: Capmul®MCM, Tween®20, Tween® 80, polyethylene glycol (PEG) 400 and propylene glycol (PG) demonstrated the highest solubilisation and emulsification ability and studied further using ternary phase diagrams. Tween®20 showed larger self-emulsification region compared to Tween® 80 in combination with all co-surfactants studied. Tween® 80 could not form any nano-emulsion with PEG 400. Propylene glycol gave clear isotropic regions with 30% of oil. With Tween 20, it could emulsify up to 40% of oil. SNEDDSs prepared using Tween®20 with each of the co-surfactants passed dispersibility test. SNEDDSs containing Tween®20 and propylene glycol displayed smaller globule size, less self-emulsification time, high transmittance and than those prepared with Tween®20 and PEG 400. Conclusion: Tween®20 and propylene glycol are suitable surfactant and co-surfactant for plumbagin loaded SNEDDS.

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INTRODUCTION

Most of the new drug candidates and many existing drug molecules show poor aqueous solubility, which leads to poor oral bioavailability, high intra-and inter-subject variability and lack of dose proportionality [1]. The greatest challenge is to present the poorly water-soluble drugs into orally administered medications with sufficient bioavailability. To increase the oral bioavailability of poorly water soluble drugs, various formulation strategies have been adopted including the use of cyclodextrins, nanoparticles, solid dispersions, permeation enhancers and lipid-based formulations [2]. In recent years, considerable attention has been focused on lipid based formulations to improve the oral bioavailability of poorly water-soluble and lipophilic drugs. In fact, the most popular strategy is the incorporation of the drug molecule into inert lipid vehicles such as oils and surfactant dispersions, self-emulsifying formulations, emulsions, and liposomes with particular emphasis on self-nanoemulsifying drug delivery systems (SNEDDS) [3]. Self nanoemulsifying drug delivery systems (SNEDDS) are isotropic and thermodynamically stable mixtures of oil, surfactant, co-surfactant and drug that

have a novel property of forming fine oil-in-water (o/w) nanoemulsion in the nanometric range (10-100 nm) when introduced into the aqueous phase under gentle agitation [4]. Upon administration, the isotropic mixture will come in contact with the aqueous phase of gastrointestinal tracts and form an oil-in-water nanoemulsion with the aid of gastrointestinal motility. This spontaneous formation of nanoemulsion in the gastrointestinal tract presents the drug in a solubilized form, in small droplets of oil, all over its transit through the GIT [5]. The nano-sized droplets provide a large interfacial surface area for drug release and absorption. Apart from solubilization, the presence of oily phase in the formulation helps improve bioavailability by affecting the drug absorption [6]. Selection of a suitable self-nanoemulsifying formulation depends mainly upon the assessment of drug solubility in various components, the area of the self-nanoemulsifying region obtained in the phase diagram, and the droplet size of the resultant emulsion following self-emulsification [7]. Finally, SNEDDS offer the opportunity to deliver poorly water soluble drugs to the gastrointestinal tract in a dissolved state which leads to avoiding the dissolution step (which can limit the absorption rate of lipophilic drugs), reduction in inter-and intra-subject variability, reduction of food effect and ease of manufacturing and scale-up [8]. *Plumbago zeylanica* Linn (family Plumbaginaceae), is an erect semi-climbing shrub and grows throughout Asia, Africa and Australia. Traditionally, the aerial part of plant has long been used as medicine to treat rheumatic pain, scabies, skin diseases, dysmenorrhea, injury by bumping, wounds and even cancer. The roots of the plant and its constituents have been reported to possess potential antiatherogenic, cardiotonic, hepatoprotective, neuroprotective, antimicrobial and anti-ulcer activity. Plumbagin (5-hydroxy-2-methylnaphthalene 1, 4-dione) is a yellow crystalline naphthoquinone abundantly present in the roots of *P. zeylanica*. Plumbagin (PLB) has been explored for its anti-cancer, anti-inflammatory, anti-bacterial, anti-fungal, CNS stimulant and anti-ulcer activity [9]. Despite the great therapeutic interest, PLB showed low oral bioavailability (39%) due to its high lipophilicity (log P = 3.04) and poor aqueous solubility (79.3 ± 1.7 µg/ml); factors considered as major challenges in developing formulations for clinical efficacy [10]. Besides, the higher and frequent dose often causes severe side effects including diarrhea, skin rashes, increase in white blood cells and neutrophil count [9]. To overcome this problem a number of attempts have been made to enhance the solubility and, therefore, bioavailability of PLB. Singh and Udupa [11] prepared inclusion complex of PLB with beta-cyclodextrin employing neutralization method. Sheik et al. [12] developed nanoplumbagin encapsulated with cetyl trimethylammonium bromide or beta-cyclodextrin using ultrasonication. Chellampillai et al. [10] prepared surface modified PLB crystals by cold recrystallization technique. Rajalakshmi et al. [13] prepared different PLB crystals using anti-solvent precipitation, melt solidification, melt quenching, sonocrystallization and melt sonocrystallization processes. Sunil Kumar et al. [14] prepared PLB loaded long circulating PEGylated liposomes. Mandala et al. [15] prepared chitosan based PLB microsphere.

Poor water solubility and consequent restricted absorption is a major limitation with many drugs despite their good efficacy. SNEDDS provides an opportunity for the improvement of the *in vitro* and *in vivo* performance of poorly water soluble drugs and thus serves as an ideal carrier for the delivery of drugs belonging to Biopharmaceutics Classification System (BCS) classes II and IV [32]. Thus, SNEDDS is an important viable option for PLB oral delivery. The performance of a SNEDDS depend sound selection of its component system, most importantly, the surfactant and co-surfactant system.

The present study reports the effect of different surfactants and co-surfactants on the phase behaviour and properties of SNEDDS by use of ternary phase diagrams. This study helped in selection of product variables for optimization of PLB SNEDDS.

MATERIALS AND METHODS

Materials

Plumbagin (PLB) was purchased from PEE ESS Aromatics Chennai, India. Capmul® MCM EP was a kind gift from IMCD Ltd, Mumbai. Oleic acid was obtained from Research Lab Fine Chem. Sesame oil, Tween® 20, Tween® 80, Span® 80, PEG 200, PEG 400 were purchased from S.D. Fine Chem. Ltd., Mumbai, India. Cremophor® RH 40 were obtained from BASF, Germany. Labrafac® was a kind gift sample from Gattefosse Canada Inc. Castor oil was purchased from Himedia Pvt, Ltd. Propylene glycol (PG) (99.5%) was bought from BDH Laboratory, UK. Glycerol and Span®80 were obtained from Loba Chemie.

Methods

Solubility study

The solubility of PLB in various oils (Capmul® MCM , oleic acid, arachis oil, cinnamon oil, olive oil, sesame oil, castor oil, Labrafac®PG), surfactants (Tween® 20, Tween® 80, Cremophor® RH 40, Span® 80) and co-surfactants (PG, PEG 200, glycerol, PEG 400 was determined. An excess amount of PLB was added to 2 ml of each component in screw-capped glass vials and mixed continuously for 2 min using magnetic stirrer (REMI 1 MLH). The mixtures were then shaken (100 rpm) for 72 h at 25 ± 0.5°C in an orbital shaker (REMI-BL) followed by equilibrium for 24 h [16]. The equilibrated

samples were removed and centrifuged (REMI C-24 BL) at 5000 rpm for 30 min. The supernatant solution was taken and filtered through a Millipore membrane filter (0.45 μm) and then suitably diluted with methanol water system (50:50). The concentration of PLB was determined spectrophotometrically using UV-Visible spectrophotometer (JASCO V-630) at 268 nm using methanol water system (50:50) as a blank. The experiment was repeated in triplicates.

Preliminary screening of surfactants

Different surfactants for the per-oral use were screened for emulsification ability according to the method described by Date and Nagarsenker [17]. Briefly, 300 mg of each selected surfactant was added to 300 mg of the chosen oily phase (Capmul® MCM). The mixtures were gently heated at (45°C - 60°C) for homogenization of the components. 50 mg of each mixture was then diluted with double distilled water to 50 ml in a stoppered volumetric flask to yield fine emulsion. Ease of emulsification was judged by the number of flask inversions required to yield homogenous emulsion. The resulting emulsions were allowed to stand for 2 h and their % transmittance was evaluated spectrophotometrically at 638 nm by UV spectrophotometer (JASCO V-630) using double distilled water as a blank. Emulsions were furthermore observed visually for any turbidity or phase separation [18, 19].

Preliminary screening of co-surfactants

The selected oily phase and surfactant were used for further screening of the different co-surfactants (PEG 400, PEG 200, PG, Glycerol) for their emulsification ability. Mixtures of 100 mg of co-surfactant, 200 mg surfactant, and 300 mg oil mixture were prepared and this mixture was homogenized with the aid of the gentle heat (45°C-60 ºC). The isotropic mixture, 50 mg, was accurately weighed and diluted to 50 ml with double distilled water to yield fine emulsion. The ease of formation of emulsions was noted by noting the number of flask inversions required to give uniform emulsion. The resulting emulsions were observed visually for the relative turbidity. The emulsions were allowed to stand for 2 h and their transmittance was measured at 638 nm by UV spectrophotometer (JASCO V-630) using double distilled water as blank. As the ratio of co-surfactants to surfactant/s is the same, the turbidity of resulting nanoemulsions will help in assessing the relative efficacy of the co-surfactants to improve the nanoemulsification ability of surfactants [20].

Construction of pseudo-ternary phase diagrams

The optimum concentrations or concentration ranges of oil, surfactant and co-surfactant necessary to promote self-emulsification are determined by construction of a pseudo ternary phase diagram [21]. Self-nanoemulsifying systems form fine o/w emulsions when introduced into aqueous media with gentle agitation. Surfactant and co-surfactant get preferentially adsorbed at the interface, reduce the tension, and provide mechanical barrier to prevent the globules from coalescence. The decrease in free energy required for emulsion formation consequently improves the thermodynamic stability [22]. On the basis of the solubility studies and preliminary screening of surfactants, Capmul® MCM was selected as the oil phase, Tween® 20 and Tween® 80 as the surfactants and PEG 400 and PG as the co-surfactants. Double distilled water was used as the aqueous phase for construction of phase diagrams. Pseudo-ternary phase diagrams of mixed surfactant and co-surfactant (Smix), oil and water but without drug incorporation were plotted, and each of them represents a side of the triangle. For any mixture, the total of surfactant, co-surfactant and oil concentrations always added to 100% [23]. Surfactant and co-surfactant were mixed in three ratios, namely; 1:1, 2:1 and 3:1 (Smix). For each phase diagram, oil and specific Smix ratio was mixed thoroughly in nine different ratios from 1:9 to 9:1 in different glass vials. The nine different combinations of oil and Smix; 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 were made so that maximum ratios were covered for the study to delineate the boundaries of phase precisely formed in the phase diagrams. Pseudo-ternary phase diagrams were developed using the aqueous titration method. Slow titration with aqueous phase was done to each weight ratio of oil and Smix. The total water consumed was noted during titration of oil–Smix ratio and observations were made for phase clarity [22]. The formation of the nanoemulsion was visually observed as clear/transparent and easily flowable/dispersible with low viscosity o/w nanoemulsion and marked on the pseudo-ternary phase diagram [24]. The point from clear to turbid and turbid to clear was considered as the end of titration. The amount of each component (oil, Smix and water) at this point was recorded and presented in a pseudo-ternary phase diagram [25].

Formulation of PLB loaded SNEDDS

Once the self-emulsifying region was identified, the desired component ratio of SNEDDS were selected (Table 1). PLB loaded SNEDDS were prepared by the method reported by Gupta et al [26]. The calculated amount of surfactant (Tween® 20) and co-surfactant (PG) were mixed in a vial on magnetic stirrer (Mix 1). The weighed amount of drug (PLB) was dissolved in the calculated amount of oil (Capmul® MCM) in a separate beaker (Mix 2). The oil phase (Mix 2) was added drop wise to the surfactant-cosurfactant mix (Mix 1) and stirring was continued for 1 h to obtain PLB loaded liquid SNEDDS.

Table 1: Composition of prepared PLB-SNEDDS formulations by using Capmul® MCM as an oil Tween® 20 and Tween® 80 as surfactants and PG and PEG 400 as co-surfactants.

Formulation code	Oil (%)	Smix Ratio	Surfactant (%)		Cosurfactant (%)	
			Tween® 20	Tween®80	PG	PEG 400
1	10	01:01	45	-	45	-
2	15	01:01	42.5	-	42.5	-
3	20	01:01	40	-	40	-
4	25	01:01	37.5	-	37.5	-
5	30	01:01	35	-	35	-
6	10	02:01	60	-	30	-
7	15	02:01	56.66	-	28.33	-
8	20	02:01	53.33	-	26.66	-
9	25	02:01	50	-	25	-
10	30	02:01	46.66	-	23.33	-
11	10	03:01	67.5	-	22.5	-
12	15	03:01	63.75	-	21.25	-
13	20	03:01	60	-	20	-
14	25	03:01	56.25	-	18.75	-
15	30	03:01	52.5	-	17.5	-
16	10	02:01	60	-	-	30
17	15	02:01	56.66	-	-	28.33
18	10	03:01	67.5	-	-	22.5
19	15	03:01	63.75	-	-	21.25
20	10	01:01	-	45	45	-
21	15	01:01	-	42.5	42.5	-
22	20	01:01	-	40	40	-
23	10	02:01	-	60	30	-
24	15	02:01	-	56.66	28.33	-
25	20	02:01	-	53.33	26.66	-
26	10	03:01	-	67.5	22.5	-
27	15	03:01	-	63.75	21.25	-
28	20	03:01	-	60	20	-

Thermodynamic stability studies

The objective of these tests was to evaluate the stability and phase integrity of PLB loaded SNEDDS under different conditions of temperature variation and centrifugal force [25]. Three different tests were carried out in this study.

Centrifugation study [27]

Formulations were centrifuged (REMI C-24 BL) at 5000 rpm for 30 min and were then checked visually for instability such as phase separation, creaming, cracking or drug precipitation. The formulations that did not show any signs of instability were chosen for heating–cooling cycle.

Heating and cooling cycle [27]

Heating cooling cycle so performed involved three cycles between 4°C and 45°C with storage at each temperature for not less than 48 h. The formulations that passed at these temperatures, without undergoing any creaming, cracking, coalescence, phase separation or phase inversion, were chosen for freeze thaw stress test.

Freeze thaw cycle (accelerated aging) [27]

Freeze thaw cycle involved three freeze thaw cycles at temperatures between -20°C and +25 °C with storage at each temperature for not less than 48 h. The formulations were then visually observed for phase separation. Only formulations that were stable to phase separation were selected for dispersibility study.

Dispersibility study

The dispersibility studies were carried study the self-emulsification efficiency and self-emulsification time [22]. Self emulsification time is the time required by the preconcentrate to form a homogeneous mixture upon dilution, when disappearance of SNEDDS is observed visually [27]. Briefly, 0.5 ml of each formulation was added drop wise to 250 ml of double distilled water in a glass beaker with gentle agitation by placing it on a hotplate magnetic stirrer (REMI 1 MLH) at 100 rpm with temperature adjusted to 37 °C ± 0.5°C [22]. The time required for the disappearance of the SNEDDS was recorded [30]. The efficiency of self-emulsification of SNEDDS was visually assessed using the five grading system [28, 29].

Grade A: refers to rapidly forming (within 1 min) nanoemulsion, having a clear or bluish appearance.
Grade B: is rapidly forming (within 2 min), slightly less clear nanoemulsion, having a bluish white appearance.
Grade C: is fine milky emulsion that was formed within 2 min.
Grade D: is dull, grayish white emulsion having slightly oily appearance that is slow to emulsify (longer than 2 min).
Grade E: is a formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface (longer than 3 min). Formulations those of grade A or B were selected for further optimization.

Robustness to dilution

Robustness of selected plumbagin SNEDDS formulations to dilution was studied by diluting it 50, 100 and 1000 times with various diluents i.e. double distilled water, 0.1 N HCl and phosphate buffer pH 7.4. The diluted samples were stored for 24 h and observed for any signs of physical changes i.e. phase separation or drug precipitation [27, 18].

Globule size

The mean droplet size of the emulsion globules were determined using nanoparticle size analyzer (Horiba SZ-100, Kyoto, Japan). For droplet size analysis the dispersed formulation was measured after 100 dilutions with double distilled water. Light scattering was monitored at 25°C at 90° angle and Z average measured [30].

% Transmittance

The PLB-loaded SNEDDS were reconstituted with distilled water and the resulting nanoemulsion was observed visually for any turbidity. Thereafter, its % transmittance was measured at 638.2 nm using UV visible spectrophotometer (JASCO V-630) against distilled water as the blank. The studies were conducted after 50, 100, 250 and 1000 times dilution.

Self-emulsification time

A quantity of 1ml of each formulation was added to 900 ml of distilled water under continuous stirring (50 rpm) using USP 31 dissolution test apparatus II at 37 ± 0.5ºC. The time required to disperse the system completely and uniformly was determined, and the emulsification time was recorded in second [31].

RESULTS & DISCUSSION

Solubility study

Solubility of drug in excipients plays an important role in determining stability of formulation, as many formulations undergo precipitation before undergoing *in situ* solubilization [16]. Formulations of self-emulsifying property essentially contain combination of oils, surfactants, and co-surfactants. This mixture should be clear, isotropic, monophasic liquid at room temperature [33] and should have good solubilizing capacity to incorporate dose of drug in minimum volume of the mixture [34]. The solubility of plumbagin in various oils, surfactants and co-surfactants is presented in Table 2. Among various vehicles screened, Capmul® MCM was selected as the oil phase as it demonstrated the highest solubilization capacity (128.10 ± 2.64 mg/ml). Similarly, Tween® 20 (34.10±1.50 mg/ml) and propylene glycol (15.79 ±1.41mg/ml) showed highest solubilization capacity. Previous reports demonstrated that medium chain monoglyceride like Capmul® MCM shows good solvent capacity for hydrophobic drugs and also promote water penetration and self-dispersibility of lipid formulations upon hydration. Additionally, Capmul® MCM is likely to increase the interfacial fluidity of surfactant boundaries in the micelles because the entrapment of Capmul® MCM in high HLB surfactant enhances the emulsification process upon dilution with an aqueous medium [34, 35].

Screening of surfactant and co-surfactant for their emulsification ability

Emulsification process and efficiency are controlled by multiple variables including lipid-surfactant affinity, hydrophilic-lipophilic balance (HLB) value of surfactant and visco elasticiy of the emulsion base [36]. HLB value of surfactant affects the spontaneity of emulsification and emulsion droplet size. Surfactants with HLB >10 are suitable

for formation of o/w nanoemulsions [20]. Non-ionic surfactants are often considered for pharmaceutical applications and nanoemulsion formulations since these are less toxic [18], less affected by pH and ionic strength changes [37] and typically have lower critical micelle concentration compared to their ionic counterparts [36,38]. They are usually accepted for oral ingestion [39]. The selected surfactants were compared for their emulsification efficiencies using Capmul® MCM as the oily phases. It has been reported that well formulated SNEDDS is dispersed within seconds under gentle stirring conditions [39]. Transmittance values as well as number of flask inversions of different mixtures are given in Table 3. The results inferred that highest % transmittance, i.e. highest emulsification efficiency, is demonstrated by Tween® 20 (HLB 16.7) Tween® 80 and (HLB 15.0). The observed difference in their emulsifying ability could be attributed to the HLB values of the examined surfactants, where Tween® 20 possessed more HLB value than Tween® 80 in addition to the difference in their structure and chain length [17, 19]. Higher HLB surfactants lead to the formation of more stable nanoemulsion upon exposure to water [20]. Tween® 20 and Tween® 80 showed highest solubility as well as emulsification efficiency (yielded clear nanoemulsions requiring shorter time for nanoemulsification). Among various vehicles screened, Capmul® MCM was selected as the oil phase showing the highest solubilization capacity. Tween® 20 and Tween® 80 used as surfactant and PG and PEG 400 co-surfactant possessing the high solubilities and emulsification for further study.

Table 2. Solubility of PLB in various oils, surfactants and co-surfactants.

Vehicle	Solubility (mg/ml)	Vehicle	Solubility (mg/ml)
Oleic acid	112.00±1.23	Cremophor® RH 40,	24.89±1.74
Arachis Oil	109.44±2.24	Tween® 20	34.10±1.50
Sesame oil	87.46±2.35	Tween® 80	29.12±1.96
Cinnamon oil	73.86±1.57	Span® 80	2.30±0.64
Labrafac® PG	55.51±2.22	PEG 400	8.33±1.46
Olive oil	34.45±0.816	PEG 200	4.72±2.25
Castor oil	21.47±2.52	Propylene glycol	15.79±1.41
Capmul® MCM	128.10±2.64	Glycerol	1.27±0.56

Data expressed as mean ± SD (n = 3).

Table 3. Emulsification ability of selected surfactants using Capmul® MCM as an oily phase and emulsification ability of selected co-surfactants using Capmul® MCM and Tween® 20 as an oily phase and surfactant respectively.

Sr. No.	Surfactant	No of inversions	%Transmittance
1	Cremophor® RH 40,	7.33±0.57	81.55±1.32
2	Labrasol®	10.00±1.00	57.16±0.44
3	Tween® 20	3.33±1.52	97.44±0.64
4	Tween® 80	5.33±0.577	92.88±2.60
5	Span® 80	17.00±1.00	46.33±02.10
Sr. No.	**Co-surfactant**	**No of inversions**	**Transmittance**
1	PEG 400	6.00±1.00	95.7±2.19
2	PEG 200	11.66±1.04	87.6±1.70
3	Propylene glycol	3.00 ±1.00	99.16±1.04
4	Glycerol	19.33±1.52	55.15±1.63

Data expressed as mean ± SD (n = 3).

Construction of pseudo-ternary phase diagrams

Ternary phase diagrams were constructed in the absence of PLB to identify the self-nanoemulsifying region and to select a suitable concentration of oil, surfactant and co-surfactant for the development of liquid SNEDDS formulations. These phase diagrams play important role in studying phase behaviour of the formed nanoemulsions [16, 28]. A simple ternary phase diagram comprises oil, water, and Smix, where each corner in the phase diagram represents 100% of that particular component. Based on the data obtained from the solubility study and preliminary screening of surfactants, Capmul® MCM was used as the oil phase, Tween® 20 and Tween® 80 as surfactants and PG and PEG 400 as co-surfactant for constructing different phase diagrams. The pseudo ternary phase diagrams of oil (Capmul® MCM), surfactant (Tween® 20, Tween® 80) and co-surfactant (PG, PEG 400) were constructed with different surfactant/co-surfactant ratio of 1:1, 2:1 and 3:1. Fig. 1 shows the constructed phase diagrams, where the translucent and low viscosity nanoemulsion area is presented as shaded area in the phase diagrams. It was clearly shown that the use of PEG 400 as co-surfactant at Smix ratio (1:1) with Tween® 20 could not form clear isotropic nanoemulsion region while at Smix ratio (2:1) and (3:1) could form nanoemulsion but with not more than 20% oil. Employing Tween® 80 could not form any nanoemulsion region with PEG 400 as co-surfactant. The use of PG as co-surfactant gave clear isotropic regions employing not more than 30% of oil for all constructed phase diagrams except for Tween® 20/PG (3:1) system which could emulsify

up to 40% of oil. Tween® 20 showed larger self emulsification region compared to Tween® 80, due its higher HLB and hence more hydrophilic nature. Higher HLB value is required for forming a good o/w emulsion [20] as higher hydrophilicity property favors faster emulsification of the oil–surfactant mixture in contact with water [22]. Self-nanoemulsification is spontaneous and the resulting dispersion is thermodynamically stable [24]. Free energy of nanoemulsion formation depends on the extent to which the surfactant lowers the surface tension of the oil–water interface and the change in dispersion entropy [38], and the present results demonstrated that increasing surfactant proportion (Smix 1:1, 2:1 and 3:1) led to a more favorable formation of nanoemulsion. The surfactant forms a layer around oil globule in such a way that polar head lies toward aqueous and non-polar tail pull out oil and thereby reduces surface tension between oil phase and aqueous phase [40]. Further, increasing the concentration of surfactant increased the spontaneity of the self emulsification process [22]. The increase in co-surfactant decreases the region of emulsion formation, as co-surfactants have very little effect on reducing the interfacial tension directly rather they help the surfactants to reduce the interfacial tension [20].

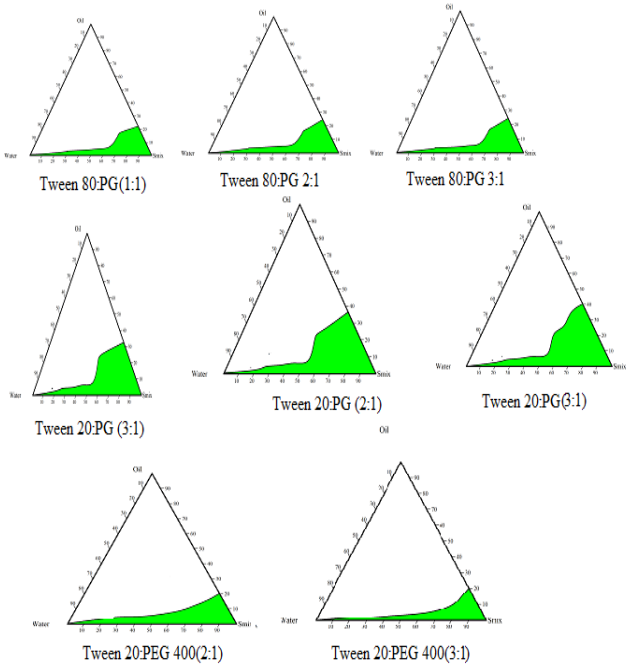
Thermodynamic stability studies

The main difference between emulsions and nano emulsions is kinetic stability, reflecting the thermodynamic stability of the two systems. SNEDDS undergoes in situ solubilisation to form nano emulsion system, and it should have stability such that it does not undergo precipitation, creaming or cracking. However, in many cases, prolonged storage might cause the drug to precipitate from the nanoemulsion; seed crystals start to appear and might grow to large crystalline materials that will precipitate out at the bottom of the vessel [16]. Therefore, to check the stability, formulation was exposed to centrifugation study, heating and cooling cycle and freeze thawing cycle in order to eliminate the metastable ones. Twenty two formulations passed the test (Table 4) i.e. there was no sign of phase separation, turbidity or drug precipitation observed. Formulation 5, 10 and 15, containing Capmul MCM in a concentration more than 25% with Tween® 20 and and formulation 22, 25 and 28 containing concentration more than 15% with Tween® 80 were unstable and showed phase separation as well as turbidity, which may be due to the coagulation of the internal phase which led to phase separation [41]. The stable formulations were further tested for dispersibility.

Dispersibility study

As SNEDDSs are released in the lumen of the gastrointestinal tract, it disperses to form a fine nanoemulsion with the aid of GI fluid. Thus, it is important that formed nanoemulsion does not undergo precipitation following phase separation with infinite dilution in the GI fluids. It is observed more prominently with drugs having poor aqueous solubility or nanoemulsion, which undergoes phase transition [16]. The SNEDDS should disperse completely and quickly when subjected to dilution under mild agitation of GIT due to peristaltic activity [16, 27].

Figure 1. Pseudo-ternary phase diagrams of Capmul® MCM, Tween® 20, Tween® 80, PEG 400 and PG at Smix ratios 1:1, 2:1 and 3:1 indicating the clear o/w nanoemulsion region as shaded regions.



It has been reported that self-emulsification mechanism involves the erosion of a fine cloud of small droplets from the monolayer around emulsion droplets, rather than progressive reduction in droplet size [16]. The ease of emulsification was suggested to be related to the ease of water penetration into the colloidal or gel phases formed on the surface of the droplet [42]. Accordingly, dispersibility study in double distilled water was conducted. In the present study, double distilled water was used as a diluent for self-nano emulsification test because it is well known that there is no significant difference in the SNEDDS prepared using pharmaceutically acceptable surfactants, dispersed in either water or GI fluids [38]. All SNEDDS that passed this test either in grade A or B were selected for further investigation, because grade A or B formulations will remain as SNEDDS when dispersed in GI fluids [46**].** It was clearly revealed that only SNEDDSs prepared using Tween® 20 as the surfactant i.e. Formulation 1, 2, 3, 6, 7, 8, 11, 12, 13, 18 and 19 could pass the test (Table

4). This might be explained by the higher HLB value (16.7) possessed by Tween® 20 and consequently higher hydrophilicity compared to Tween® 80 (15.0). Higher HLB value is required for forming a good o/w emulsion [20] since higher hydrophilicity property favors faster emulsification of the oil–surfactant mixture in contact with water [22], in addition to reduced free energy of the system [43]. It was also observed that the increase in oil concentration in formulation 4, 5, 9, 10, 14 and 15, and decrease in Tween® 20 concentration in formulation 16 and 17 required more time for emulsification. The reason that can be put forward is the increase in interfacial tension between larger volume of oil and aqueous phase with a net decrease in surfactant system, which makes the emulsification time longer [44]. Pouton found that the formulations containing more hydrophilic components was superior in self-nanoemulsifying ability and provided smaller droplets than those containing more lipophilic components [5].

Table 4. Evaluation of thermodynamic stability and dispersion study, globule size, self-emulsification time and % transmittance of PLB loaded SNEDDS formulations.

Formulation code	Centrifugation	Heating and cooling	Freeze thaw	Dispersion		Globule size (nm)	Self emulsification time (sec)	% transmittance (%)
				Grade	Inference			
1	Passed	Passed	Passed	A	Passed	52.16 ± 1.10	20.66 ± 2.51	99.28 ± 0.5
2	Passed	Passed	Passed	A	Passed	58.46 ± 1.85	35.00 ± 2.0	97.32 ± 1.31
3	Passed	Passed	Passed	A	Passed	200.26±1.85	40.33 ± 1.52	87.08 ± 1.21
4	Passed	Passed	Passed	E	Fail	-	-	-
5	Fail	Fail	Fail	-	-	-	-	-
6	Passed	Passed	Passed	A	Passed	50.53 ± 1.75	17.00 ± 1.00	99.21 ± 0.69
7	Passed	Passed	Passed	A	Passed	54.3 ± 1.05	27.00 ± 1.73	99.16 ± 0.28
8	Passed	Passed	Passed	A	Passed	106.16±1.94	37.66 ± 1.52	95.15 ± 1.01
9	Passed	Passed	Passed	D	Fail	-	-	-
10	Fail	Fail	Fail	-	-	-	-	-
11	Passed	Passed	Passed	A	Passed	50.4 ± 1.01	17.66 ± 2.08	99.28 ± 0.50
12	Passed	Passed	Passed	A	Passed	55.46 ± 1.70	25.00 ± 1.00	99.19 ± 0.74
13	Passed	Passed	Passed	A	Passed	82.83 ± 1.40	36.33 ± 2.08	96.36 ± 1.36
14	Passed	Passed	Passed	C	Fail	-	-	-
15	Fail	Fail	Fail	-	-	-	-	-
16	Passed	Passed	Passed	D	Fail	-	-	-
17	Passed	Passed	Passed	D	Fail	-	-	-
18	Passed	Passed	Passed	A	Passed	245.34±2.87	95.15 ± 2.45	84.15 ± 1.65
19	Passed	Passed	Passed	B	Passed	315.56±2.45	115.34 ± 1.65	78.23 ± 2.04
20	Passed	Passed	Passed	C	Fail	-	-	-
21	Passed	Passed	Passed	E	Fail	-	-	-
22	Fail	Fail	Fail	E	Fail	-	-	-
23	Passed	Passed	Passed	E	Fail	-	-	-
24	Passed	Passed	Passed	E	Fail	-	-	-
25	Fail	Fail	Fail	-	-	-	-	-
26	Passed	Passed	Passed	E	Fail	-	-	-
27	Passed	Passed	Passed	E	Fail	-	-	-
28	Fail	Fail	Fail	-	-	-	-	-

Data expressed as mean ± SD (n = 3).

Robustness to dilution

Uniform emulsion formation from SNEDDS is very important at different dilutions because drugs may precipitate at higher dilution in vivo which affects the drug absorption significantly [27, 37]. SNEDDS formulations were exposed to different folds of dilution in different media to mimic the in vivo conditions where the formulation would encounter gradual dilution. Hence, each formulation was subjected to 50, 100 and 1000 times dilution in double distilled water, 0.1 N HCl, and phosphate buffer pH 7.4. Even after 24 h, all formulations, which passed dipersion study, showed no signs of precipitation, cloudiness or separation, which ensured the stability of the reconstituted emulsion. This reveals that the SNEDDS were robust to dilutions by fluids of different pH. These findings will ensure the prospect of uniform drug release profile in vivo.

Characterization of formulations

Globule size

Droplet size is one of the most important characteristics of nanoemulsion for stability evaluation and a critical step in the pathway of enhancing drug bioavailability [27]. Smaller droplet size leads to larger interfacial surface area for drug absorption and hence may lead to more rapid absorption and improved bioavailability [16]. Hence, the droplet size of the nanoemulsion may govern the effective drug release [22]. The droplet sizes of formulations are given in Table 4. The average droplet size of the formulations at 100 times dilution ranged from 50.4 ± 1.01 to 315.56 ± 2.45 nm, which indicated that emulsion droplets are in nanometric range. The formulation, which contain PG as co-surfactant showed small globule size (< 200 nm) compared to PEG 400 (< 200 nm), might be due to high emulsification ability of PG than PEG 400. The droplet size decreased as the concentration of oil decreased i.e. this could be attributed to an increased surfactant proportion relative to co-surfactant, which is probably explained by stabilization of the oil droplets as a result of the localization of the surfactant molecules at the oil–water interface [16]. The surfactant may cause the interfacial film to condense and stabilize, resulting in smaller droplet diameters, whereas the addition of the co-surfactant may cause the film to expand [41]; thus, the relative proportion of surfactant to co-surfactant has varied effects on the droplet size [32]. These observations are in consistent with earlier author reports [41].

% Transmittance

The primary means of assessment of self-emulsification is visual evaluation [45]. Hence, the PLB loaded SNEDDS were diluted with distilled water and the resultant nanoemulsions were observed visually. They were found to be non-turbid and were bluish-white appearance, indicating spontaneous nanoemulsification. The transmittance values (Table 4) ranges from 78.23 ± 2.04 % to 99.28 ± 0.50%. High transmittance value showed formulation, which contain PG than PEG 400.

Self emulsification time

Self emulsification time of PLB loaded SNEDDS found to be in ranges 17.00 ± 1.00 sec to 115.34 ± 1.65 sec (Table 4). It is observed that SNEDDS containing PG emulsify within 1 min while for SNEDDS containing PEG 400 more than 1 min it indicates spontaneous nanoemulsion formation on contact with physiological fluids observed in SNEDDS containing PG.

CONCLUSION

From the ternary phase diagrams, thermodynamic stability study and characterization it was observed that Capmul® MCM (10% to 20% concentration) Tween® 20 and PG were found to be the best suitable oil, surfactant and co-surfactant respectively. The study helped in arriving at a suitable working range for concentration of surfactant and co-surfactant. Further optimization can be done using a suitable DOE.

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