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Molecular modelling and multisimplex optimization of tocotrienol-rich Self Emulsified Drug Delivery Systems

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ABSTRACT

The objective of this study was to optimize a novel tocotrienol (TRF)-rich Self Emulsified Drug Delivery System (SEDDS). In the first part, an unusual phenomenon was investigated. It was observed that by substituting Tween® 80 with Cremophor® EL in the SEDDS it was possible to emulsify > 55% TRF (by weight of the formulation) into submicron (<200 nm) emulsion. With Tween®, only 17.5% of the loaded TRF could be emulsified into crude emulsion. The superiority of Cremophor® was attributed to the special arrangement of the surfactant at the oil/water interface, which was confirmed by modelling and docking studies. In the second part of this study, the composition of the secondary ingredients in the TRF-rich SEDDS were optimized by the modified Multisimplex® approach. SEDDS were manufactured at pre-defined step-size and tested for their dissolution behavior. Testing was performed sequentially until the optimum composition that can emulsify 50% of the loaded TRF into a stable < 150 nm submicron emulsion was obtained. Optimization end-point was identified when the "membership value" approached 1, which was confirmed by a second Multisimplex® run. Overall, this study demonstrated the utility of docking studies and the Multisimplex® approach in product development when little is known about the experimental "design space".

1. Introduction

Self-emulsifying drug delivery systems (SEDDS) are excellent candidates for the oral delivery of hydrophobic drugs (Aungst, 1993; Humberstone and Charman, 1997). They have been shown to overcome the obstacles that face the formulation of many weakly water-soluble drugs (Gursoy and Benita, 2004; Humberstone and Charman, 1997; Pouton, 1997). Furthermore, their physical stability and easiness to manufacture makes them a popular and commercially viable formulation approach. Strategies for the formulation of SEDDS and the efforts made to understand their mechanisms of action have been extensively reviewed (Constantinides, 1995; Gershanik and Benita, 2000). One of the most important factors in developing SEDDS formulations is the type of surfactant used. The most widely used surfactants in SEDDS are the non-ionic with a relatively high hydrophilic–lipophilic balance (HLB) values; typically within a concentration range between 30% and 60% (w/w) (Gursoy and Benita, 2004; Neslihan Gursoy and Benita, 2004). While amphoteric (e.g. lecithin or gelatin) and ionic surfactants (e.g. sodium palmitate) have also been used, the nonionic surfactants generally offer the most advantages. They are less toxic, less hemolytic, and tend to maintain near physiological pH values when in solution (Jonkman-De Vries et al., 1996). Based on these advantages, the widely used nonionic surfactant polysorbate 80 (Tween® 80) was selected as the backbone surfactant in many SEDDS formulations (Gursoy and Benita, 2004; Neslihan Gursoy and Benita, 2004). Previously, our laboratory has also reported on the optimization and lipolysis of α-tocopherol SEDDS formulation using Tween® 80 as the primary surfactant (Ali et al., 2008). It was shown that a maximum of 12.5% α-tocopherol could be incorporated into a Tween® 80 SEDDS formulation without causing phase separation upon dispersion in dissolution media.

For decades α-tocopherol, which is a member of the Vitamin E subfamily that is preferentially absorbed and accumulated in humans (Watson and Preedy, 2009), received much attention for its biological activity as a potent antioxidant. Recently, however, there was a shift in interest from tocopherols to palm oil rich in tocotrienols, commonly referred to as tocotrienol-rich-fraction or TRF, which constitute the other members of the Vitamin E subfamily (Fig. 1). A considerable body of studies have demonstrated that tocotrienols possess potent anticholesterolemic, antiatherosclerotic, antihypertensive, immunomodulatory, neuroprotective, and anticancer activities (Samant and Sylvester, 2006; Watson and Preedy, 2009). Aside from their own therapeutic activity and high solvent capacity, tocotrienols were also shown to potentiate the activity of many co-administered drugs. For example, in recent

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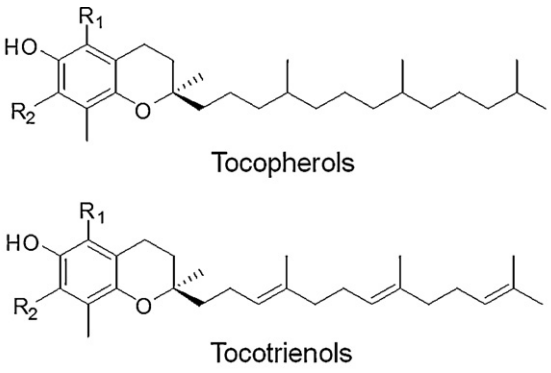

Compound	R1	R2
Alpha (α)	CH_3	CH_3
Beta (β)	CH_3	H
Gamma (γ)	H	CH_3
Delta (δ)	H	H

Fig. 1. The general chemical structure of Vitamin E showing its two isoforms (tocopherols and tocotrienols). All the isoforms have similar basic chemistry characterized by a phytyl side chain attached to a chromane ring. The main differences between these isoforms are the degree of methylation of their chromane ring and the level of saturation of their phytyl side chain.

studies, tocotrienols were shown to potentiate the antitumor activity of simvastatin (Wali and Sylvester, 2007), celecoxib (Shirode and Sylvester, 2010) and gefitinib (Bachawal et al., 2010). Tocotrienols, however, suffer from poor oral bioavailability (Sylvester et al., 2005). For example, it was found that the absolute oral bioavailability of α-tocotrienol in rats was approximately 28% whereas that of δ-tocotrienol and γ-tocotrienol was only 9% (Yap et al., 2003). Formulating TRF into a SEDDS may overcome their poor oral bioavailability, as SEDDS were shown to improve the oral bioavailability of many drugs with poor aqueous solubility such as halofantrine (Khoo et al., 1998), ontazolest (Hauss et al., 1998), cyclosporine (Klauser et al., 1997), and progesterone (MacGregor et al., 1997). Therefore, the primary objective of this study was to substitute α-tocopherol with TRF in the SEDDS formulation that was previously developed in our lab (Ali et al., 2008). The challenge, however, was to maximize the amount of TRF that could be incorporated in SEDDS beyond the 12.5% limit, which was observed with α-tocopherol (Ali et al., 2008).

To accomplish this objective, we discovered that substituting polysorbate 80 with polyoxyl 35 castor oil (Cremophor® EL) as the primary surfactant allowed for significantly higher TRF loading. Cremophor® EL and Tween® 80 are GRAS (Generally Recognized as Safe) ingredients that have been used in commercially available formulations. Due to the differences in their polarity and chemical structure, we speculated that polyoxyl 35 castor oil that contains three hydrophobic acyl side chains might be more efficient in emulsifying TRF than polysorbate 80 (Fig. 2). Furthermore, it has been reported that Vitamin E is more polar than triglycerides because of the hydroxyl group on the aromatic ring (Constantinides et al., 2006). Such polarity may therefore result in a higher solubility of polysorbate 80 in TRF, making it less available at the TRF/water interface. Consequently, adjusting the polarity of the primary surfactant in a SEDDS would more likely create a stabilized interface. To confirm this hypothesis, polysorbate 80 and polyoxyl 35 castor oil based SEDDS formulations were compared in this study for their physical behavior and their ability to produce stable submicron emulsions at high TRF loads. The second objective was to optimize the TRF-SEDDS by the sequential simplex optimization

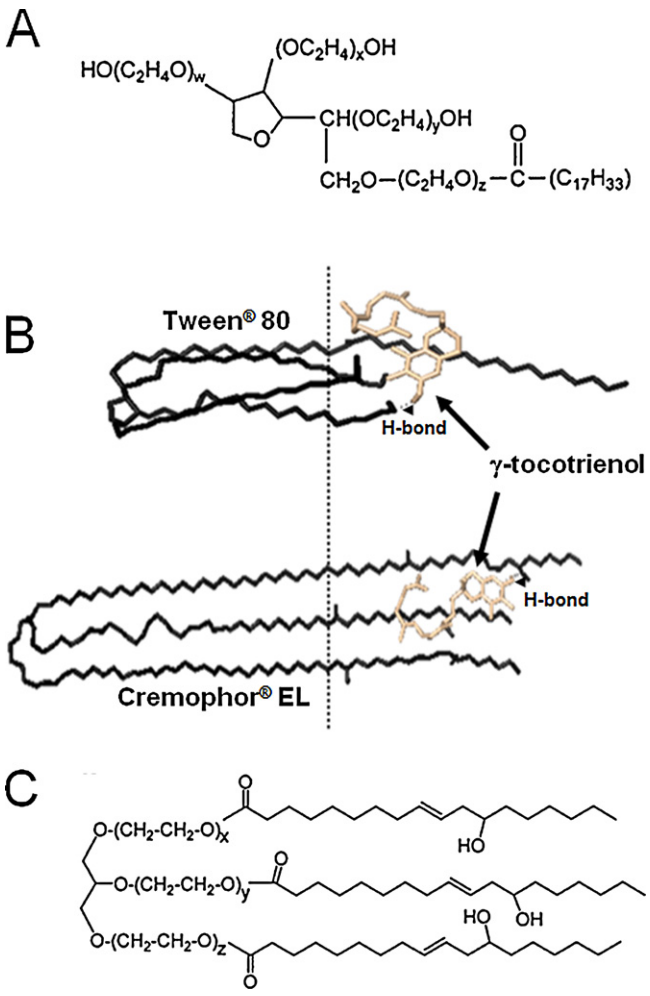

Fig. 2. (A) Chemical structure of polyoxyethylene sorbitan 20 monooleate (Tween® 80) where $w+x+y+z=20$, (B) docking structure of γ-tocotrienol to Tween® 80 and Cremophor® EL, (C) chemical structure of polyoxyethyleneglycerol 35 triricinoleate (Cremophor® EL) where $x+y+z=35$.

approach as an alternate optimization tool to the frequently used response surface methodology. In this study, we present our experimental results that demonstrated how sequential simplex optimization could be used for the development of highly loaded TRF-SEDDS formulations. While sequential simplex optimization has been successfully used in analytical method development (Ferreirós et al., 2006; Sanz et al., 2003; Shakerian et al., 2008), only few studies have been reported in which it was used in pharmaceutical and drug delivery applications (Dong et al., 2009) making this study a worthwhile introduction to this technique for future applications in drug product development.

2. Materials and methods

2.1. Materials

Tocotrienol-rich-fraction of palm oil (TRF), which contains approximately 30% α-tocopherol and 70% α, γ, and δ-tocotrienols was a gift from Beta Pharmaceutical Ltd (West Perth, Australia). Polyoxyethylated castor oil (Cremophor® EL), polysorbate 80 (Tween® 80), triglycerides of caprylic/capric acid (Captex® 355), and C8/C10 polyglycolyzed glycerides from coconut oil (Labrasol®) were provided by BASF (Mount Olive, NJ, USA), Uniqema (New Castle, DE, USA), Abitec Corporation (Janesville, WI, USA), and Gattefossé (Saint-Priest, Cedex, France), respectively. Ethyl alcohol USP

Table 1
Components of the two sets of TRF-SEDDS formulations in which Tween® 80 or Cremophor® EL was used as the primary emulsifier.

Formula No.	TRF (%, w/w)	Cremophor® or Tween® (%, w/w)	Labrasol® (%, w/w)	Captex® 355 (%, w/w)	Ethanol (%, w/w)
1	12.5	35.6	35.6	6.3	10
2	15	34.6	34.6	6.1	9.7
3	17.5	33.6	33.6	5.9	9.4
4	20	32.6	32.6	5.8	9.1
5	25	30.5	30.5	5.4	8.6
6	30	28.5	28.5	5.0	8.0
7	35	26.5	26.5	4.7	7.4
8	40	24.4	24.4	4.3	6.8
9	45	22.4	22.4	4.0	6.3
10	50	20.4	20.4	3.6	5.7
11	55	18.3	18.3	3.2	5.1
12	60	16.3	16.3	2.9	4.6
13	70	12.2	12.2	2.2	3.4

was purchased from AAPER Alcohol and Chemical Co. (Shelbyville, KY, USA). Empty hard gelatin capsules (size 0) were provided by Capsugel (Greenwood, SC, USA). Deionized water was obtained using the NanoPure purification system. All chemicals were used as supplied without further modification.

2.2. Preparation of the TRF-SEDDS formulations

Two sets of TRF-SEDDS formulations (Table 1) using Tween® 80 or Cremophor® EL as the primary surfactant were prepared with increasing concentration of TRF from 12.5 to 70% (w/w). Labrasol®, Captex® 355, and ethanol were added to the SEDDS as co-surfactant, secondary oil, and co-solvent, respectively. One gram of each formulation was prepared by thoroughly mixing the pre-weighed SEDDS ingredients in a borosilicate vial at 1000 rpm for 5 min using IKA® UltraTurrax T8 mixer (IKA® Works Inc., NC, USA).

2.3. In vitro dissolution and physical characterization of the TRF-SEDDS formulations

Dissolution experiments were performed in 100 mL deionized water as the dissolution medium using mini USP Type II dissolution apparatus at a paddle speed of 100 rpm and bath temperature of 37 ± 0.5 °C (VK 7000, Varian Inc., NC, USA). In select runs, dissolution vessels were fitted with an ultra-fast fiber–optic probe to monitor the dispersion process. At the beginning of each experiment, size 0 hard gelatin capsule filled with 500 mg of the formulation was released into the dissolution medium. Capsules were held at the bottom of the vessel using stainless-steel sinkers. Dissolution experiments were allowed to run for 15 min. At the end of each experiment, a sample was collected to measure the percentage of TRF emulsified and the size of the emulsion droplets in the dissolution medium. The percentage of TRF emulsified was determined spectrophotometrically by measuring the UV absorbance of samples at 295 nm (Cary 50 probe UV/Vis spectrophotometer, Varian Inc., NC, USA). Samples for UV analysis were prepared by first diluting 100 μL of the sample collected from the dissolution medium with 4 mL methanol. Then, 100 μL of this blend was diluted to a final volume of 2 mL with methanol to obtain a clear solution. Intensity-weighed mean droplet size and population distribution (polydispersity index, PI) of the emulsion were measured by photon correlation spectroscopy (PCS) at 23 °C and a fixed angle of 90° using Nicomp™ 380 ZLS submicron particle size analyzer (PSS Inc., Santa Barbara, CA, USA). When needed, samples for size analysis were diluted with 0.2 mL-filtered and deionized water in order to minimize multiple-particle scattering and to achieve an optimal scattering intensity of 300 kHz. Analyses were performed in triplicates unless otherwise specified.

2.4. Molecular modelling and docking studies

Molecules of Cremophor® EL and Tween® 80 (Fig. 2) were built using Insight II molecular modelling software (Accelrys Inc., San Diego, CA, USA) and were energy minimized to obtain the optimized structures. Chemically, Cremophor® EL is made of three hydrophobic chains that contain oxyethylene groups. The length of each oxyethylene chain can vary depending on the number of oxyethylene groups with $x+y+z=35$, where x, y and z represent the number of oxyethylene groups (Meyer et al., 2002). Molecules with different oxyethylene chain lengths were built. Similarly, for Tween® 80, chain lengths with $x+y+z=20$ was used. Crystal structure of α-tocopherol from the structure of human α-tocopherol transfer protein (Meier et al., 2003) was used to build the molecule of γ-tocotrienol as a representative component of TRF. Double bonds were introduced to the hydrophobic chain of α-tocopherol and a methyl group was replaced by a hydroxyl group in the head group to obtain the 3D structure of γ-tocotrienol. All structures were energy minimized before docking experiments. Docking of γ-tocotrienol to Cremophor® EL and Tween® 80 molecules was performed by autodock software (Cosconati et al., 2010; Huey et al., 2007). A grid box of 120 Å × 120 Å × 120 Å was created around Cremophor® EL/Tween® 80 molecule to cover the entire molecule with fatty acid side chain. One molecule of Cremophor® EL/Tween® 80 occupied the grid box created. γ-Tocotrienol could be placed anywhere inside the grid box to evaluate the interaction between γ-tocotrienol and Cremophor® EL/Tween® 80 molecules. Lamarkian genetic algorithm was then used to find the different docked conformations of γ-tocotrienol to Cremophor® EL or Tween® 80. Detailed analyses of the ligand–receptor interactions were carried out and the final coordinates of the ligand and receptor were saved as protein data bank file format. PyMol software (Schrodinger LLC, Portland, OR) was used for final display of molecules and to analyze the hydrogen bonding interactions. Preliminary docking calculations and analysis of the docking results were performed on a Linux computer. Ten million energy evaluations with 50 runs in docking were performed on a Linux Cluster computer (High performance computing center at Louisiana State University, Baton Rouge, LA) via the Louisiana Optical Network Infrastructure (LONI). Final low-energy docked structures were used as representative structures to show the interaction between γ-tocotrienol and Cremophor® EL or Tween® 80.

2.5. Sequential simplex optimization approach

2.5.1. Theory

Traditionally, and with a lack of a systematic approach, formulations are optimized by "trial-and-error" or by changing one control variable at a time while holding the rest constant. Such methods are not efficient in finding the true optimum, since they

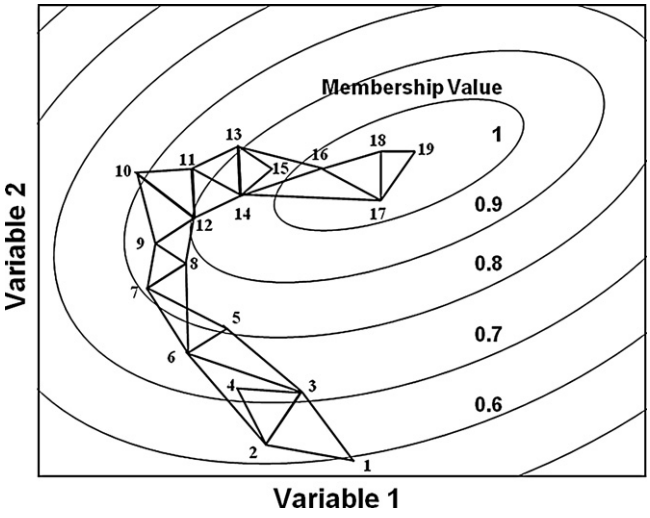

Fig. 3. Hypothetical graph demonstrating the principles of sequential simplex optimization of two variables. The starting simplex consists of vertexes 1, 2 and 3, which gave the worst response (lowest membership value). Variables are then modified to yield the second simplex, which consists of vertexes 2, 3, and 4. Each subsequent change in the two primary variables, results in the movement of the simplex towards a higher membership value until the optimum level of variables is achieved. In this example, the optimum is given by vertex 19, which had the highest membership value.

require a large number of experiments and completely ignore the role of factor interactions in formulation development. Alternatively, design of experiments (DOE) and the application of response surface methodologies became the norm in optimization studies. DOE was extensively used in our laboratory in process and formulation optimization (Ali et al., 2010; El-Malah and Nazzal, 2006; El-Malah et al., 2006). While DOE is commonly used, it requires a well-defined design space with specific upper and lower limits for each factor.

Furthermore, it is a structured experimentation design that requires a large number of experiments at a minimum number of factor levels (Prater et al., 1999). For example, a simple two-level factorial design requires 2^k experiments, where k is the number of factors for which the system is being optimized (Prater et al., 1999). In contrast, sequential simplex optimization (Walters et al., 1991) eliminates the need to specify the design space by moving a geometric figure (the "simplex", Fig. 3) in a step-wise pattern through a factor space via a relatively simple geometric algorithm until an optimal response is achieved (Dong et al., 2009). The starting simplex is composed of $K+1$ experiments, where K is the number of variables. The experiments are then performed one by one with each experiment representing a different formulation composition. Formulations that cannot be manufactured or those that yield failing results are defined as "impossible" trials for which the algorithm would suggest an alternate composition. Once the experiments are carried out, the results of each experiment are recorded and one new experiment is suggested. The procedure is then repeated until the optimum formulation is reached. For each step, the simplex method requires only one additional experiment regardless of the number of factors being evaluated, which drastically lowers the number of experiments required to reach the optimum (Walters et al., 1991). In order to measure the closeness of the results to the desired optimum, sequential simplex optimization makes use of a "membership value". This value ranges from 0 to 1 and takes into account the results of all responses generated during the optimization process. Optimized conditions are achieved when the membership value is close to 1 (Ferreirós et al., 2006). It is worth noting, however, that while the multisimplex approach can solve many optimization problems, it has certain limitations. For example, multisimplex assumes that the control and response variables are continuous variables. Therefore, true categorical variables are difficult to include in an optimization study without user interaction in every step. Another limitation is excessive noise, which can be overcome with an increase in step size and/or repeated trials. For additional and in-depth discussion on the simplex method, the reader is referred to specialized literature (Walters et al., 1991).

2.5.2. Experimental design and optimization

Optimization of the TRF-SEDDS formulation was carried out using the modified simplex method to identify a formulation that can emulsify ≥ 50% (w/w) TRF into a stable submicron emulsion. Control and response variables including the optimization criteria (reference values, step size, and the upper and lower limits of the desired responses) are given in Table 2. Step size is the range within which control variables are allowed to vary in each step or experiment. Since information is not available on the approximate location of the optimum formulation, a wide step size (200% of the reference values) was used to allow for the evaluation of a larger design space with lower number of experiments or trials. Reference values are the initial formulation composition around which the software adjusts the composition with each subsequent experiment. The composition of the formulations that were evaluated in each trial and the observed responses are given in Table 3, with formulations 1–6 representing the starting "simplex" of the sequential simplex optimization process. The exact composition of each SEDDS formulation was specified by the Multisimplex® software (Grabitech Solutions AB, Sweden), which was used to guide the sequential simplex optimization process. Trials that suggested negative control values or when the theoretical percentage of TRF in the formulation was less than 50% were discarded as impossible trials for which no "membership value" was generated. From each

Table 2
Control and response variables that were used for the construction of the first Multisimplex® run.

Control variables					
	TRF (mg)	Cremophor® (mg)	Labrasol® (mg)	Captex® (mg)	Ethanol (mg)
Reference value[a]	300	122.1	122.1	21.6	34.2
Step size[b]	600	244.2	244.2	43.2	68.4

Response variables			
	Cumulative % TRF emulsified	Particle size (nm)	% TRF loaded in the SEDDS
Objective	Target value: 100%	Minimization	Maximization
Influence (0–1)	High (1)	High (1)	Very low (0.01)
Lower limit	0	1	50
Upper limit	101	100,000	100

[a] Reference value based on a 600 mg SEDDS formulation.
[b] Step size = 200% of the reference value.

Table 3
The composition of the SEDDS and the observed responses for the trials that were performed sequentially as part of the first Multisimplex® run.

Trial No.	TRF (%, w/w)	Crem (%, w/w)	Lab (%, w/w)	Cap (%, w/w)	Eth (%, w/w)	Diss (%)	Load (%)	Size (nm)	Mem. value
1	0.0	0.0	0.0	0.0	100.0	–	–	–	–
2	0.0	40.7	40.7	7.2	11.4	–	–	–	–
3	84.3	0.0	0.0	6.1	9.6	–	–	–	–
4	71.1	0.0	28.9	0.0	0.0	–	–	–	–
5	65.7	26.8	0.0	0.0	7.5	25.5	65.7	5756	0.48914
6	0.0	85.0	0.0	15.0	0.0	–	–	–	–
7	74.4	−5.0	20.2	−0.9	11.3	–	–	–	–
8	39.4	37.4	10.7	6.6	6.0	–	–	–	–
9	59.0	19.7	16.0	3.5	1.8	80.6	59	195.5	0.88976
10	60.0	20.0	16.3	3.5	0.1	66.6	60	203	0.80958
11	65.6	9.9	17.8	1.8	4.9	51.6	65.6	16,783	0.65287
12	95.4	0.8	2.2	0.1	1.5	–	–	–	–
13	42.5	22.9	23.6	4.1	7.0	–	–	–	–
14	49.3	24	25.7	−0.2	1.3	–	–	–	–
15	70.6	9.4	10.1	3.6	6.3	5.9	70.6	36,800	0.19383
16	80.1	7.9	9.3	0.6	2.2	–	–	–	–
17	55.6	17.7	18.6	2.8	5.3	100	55.6	166	0.98835
18	57.9	27.1	3.0	3.9	8.1	84.5	57.9	209	0.91027
19	54.8	26.4	12.2	1.9	4.8	89.9	54.8	163.2	0.93665
5 REV	65.7	26.8	0.0	0.0	7.5	25.5	65.7	5756	0.48914
20	54.7	16.9	20.7	4.3	3.5	100	54.7	162.5	0.98751
21	47.4	32.9	10.6	4.9	4.3	–	–	–	–
9 REV	59.0	19.7	16.0	3.5	1.8	79	59	201.3	0.88090
22	60.9	15.8	15.9	2.6	4.8	31	60.9	1922	0.54886
23	54.3	22.1	12.1	2.8	8.6	100	54.3	167	0.98705
24	50.0	28.2	10.8	3.8	7.2	100	50.1	129	0.96893
25	58.1	19.0	14.7	2.9	5.4	79	58.1	200	0.88045
26	53.1	14.1	27.9	2.1	2.9	100	53.1	156.4	0.98549

Crem, Cremophor® EL; Lab, Labrasol®; Cap, Captex® 355; Eth, ethanol; Diss, %TRF emulsified in the dissolution medium; Size, Mem. value, membership value. Trails No. 1, 2, 3, 4, 6, 7, 8, 12, 13, 14, 16 and 21 were considered "impossible" runs and therefore data from these trials were not generated and/or reported (–). These trials were considered "impossible" due to one or more of the following reasons: (a) the percentage of TRF in the formulation was <50%, which was outside the effective boundaries of the control variables as defined in Table 2, (b) the concentration of any of the ingredients was given in a negative value by the software, and (c) the formulation completely phase separated in dissolution medium and consequently no data could be generated. REV, reevaluation run. These runs were reevaluated to prevent the simplex from being trapped around a false favorable response.

trial, droplet size of the emulsion, percentage of TRF emulsified, and the theoretical percentage of TRF in the formulation were used as the response variables. At the conclusion of each experiment, the results from each response were used to calculate the "membership value". Experiments were performed in a stepwise manner and were continued until the optimum conditions (Table 2) were reached, i.e. until the membership values were consistently close to 1.

3. Results and discussion

3.1. Tween® 80 and Cremophor® EL SEDDS formulations

Previously, a SEDDS formulation of α-tocopherol was developed in our lab. It was stabilized with Tween® 80 and Labrasol® as the primary surfactant and co-surfactant, respectively. While it formed a nanoemulsion upon dispersion, it had a limited capacity to hold α-tocopherol or TRF. To increase TRF loading into SEDDS, Tween® 80 was replaced with Cremophor® EL. It has been established that for the development of a SEDDS formulation, the correct choice of surfactant is essential (Craig et al., 1995). To demonstrate the differences between Tween® 80 and Cremophor® EL with respect to their emulsifying potential, two sets of formulations were prepared (Table 1). The formulations in each group were loaded with increasing concentration of TRF from 12.5 to 70% (w/w), while maintaining the ratio between Labrasol®, Captex® 350, ethanol, and the primary surfactant constant. The formulations were then subjected to dissolution studies. From the dissolution test the percentage of TRF emulsified into the dissolution medium was estimated, which was used as a measure of surfactant affinity to the TRF/water interface. To amplify the differences between the formulations and to allow for more accurate optimization process, a mini USP Type II dissolution vessel with 100 mL of dissolution medium was used. Droplet size and polydispersity of the dispersed formulations was also measured to determine whether the dispersions lay within the accepted known range of nanoemulsions. In general, no differences in lag phase and/or emulsification rate were observed between the formulations when the dissolution process was analyzed by real-time spectroscopy. The formulations were completely released from the ruptured capsules and dispersed into the dissolution medium within 10 min (data not shown).

The percentage of TRF emulsified into the dissolution medium as a function of TRF loading in the formulations is shown in Fig. 4.

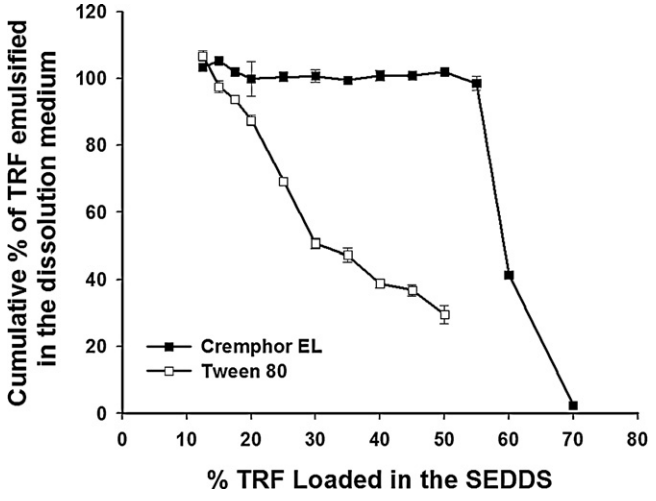

Fig. 4. Cumulative percentage of TRF emulsified in dissolution medium as a function of the percentage of TRF loaded in either Tween® or Cremophor® SEEDS.

For Tween® 80 based formulations; a significant decrease in % TRF emulsified was observed when TRF was loaded at concentrations above 17.5%. In formulations where the % TRF emulsified was < 100%, the emulsion broke and a visible oil layer was seen on the surface of the dissolution medium. In contrast, when Cremophor® EL was used as the primary surfactant, it was possible to load the SEDDS formulations with up to 55% TRF with 100% of the drug emulsified in the dissolution medium (Fig. 4), which indicated that an increase in TRF loading did not adversely impact the quality of the SEDDS or its capacity to readily emulsify into the dissolution medium, albeit it may have had an impact on droplet size and PI as discussed later. When SEDDS were loaded with >55% TRF, however, phase separation was observed and an oily layer was seen on the surface of the dissolution medium. This could be attributed to the coalescence of unstable globules of the emulsion due to a decrease in the amount of surfactant and co-surfactant available at the oil/water interface.

3.2. Droplet size and polydispersity index (PI)

Another measure to differentiate between the formulations is the droplet size of the emulsions after SEDDS dispersion into the dissolution medium. In the case of Tween® 80 formulations, the droplet size increased significantly with an increase in TRF loading (Fig. 5A). The size of the nanoemulsion increased from 100 nm at 12.5% TRF loading to approximately 550 nm at 17.5% TRF loading. The significant increase in droplet size could be attributed to the positioning of the TRF molecules at the water/emulsion interface as discussed in the subsequent section. When TRF was loaded at concentrations above 17.5% the droplets coalesced resulting in phase separation. Similarly, a gradual increase in droplet size was observed with Cremophor® EL based formulations as the % TRF loaded increased from 12.5 to 50% (Fig. 5A). In contrast to the Tween® 80 formulations, however, the size of the dispersions did not exceed 200 nm at the highest TRF concentration.

Another parameter, polydispersity index (PI), was used as a measure of the homogeneity and width of the distribution of the emulsion droplets within the medium. Perfectly monodisperse population will yield a PI of 0 (Müller et al., 1998). While there was a decrease in PI with an increase in TRF loading in the Tween® 80 based formulations, the dispersions maintained high PI values (>0.5), which indicated a heterogeneous system with a very broad size distribution (Fig. 5B). In contrast, PI values for the Cremophor® EL based preparations varied with TRF loading. At low TRF loads, the PI did not exceed 0.2, which indicated high degree of homogeneity and narrow droplet size distribution. PI then gradually increased to a maxima of 0.47 at 30% TRF reflecting a heterogeneous system of small and large emulsion droplets. With further increase in TRF loading, the capacity of Cremophor® EL to emulsify TRF reached its limit. Consequently and as observed with Tween® 80 formulations, the PI decreased as the smaller droplets coalesced to form a homogenous dispersion of larger droplets.

3.3. Molecular modelling and docking studies

It could be conceded from the results that Cremophor® EL is more efficient in emulsifying TRF than Tween® 80. Cremophor® EL is less hydrophilic (HLB value between 12 and 14) than Tween® 80 (HLB = 15). This gives Cremophor® EL an advantage over Tween® 80 in emulsifying TRF as TRF is more hydrophilic than oils, mostly triglycerides that are commonly used as the oil phase in SEDDS formulations. The significant difference in emulsification potential between Cremophor® EL and Tween® 80, however, could not be explained only by the difference in their HLB values. We speculated that the structural differences between the two surfactants

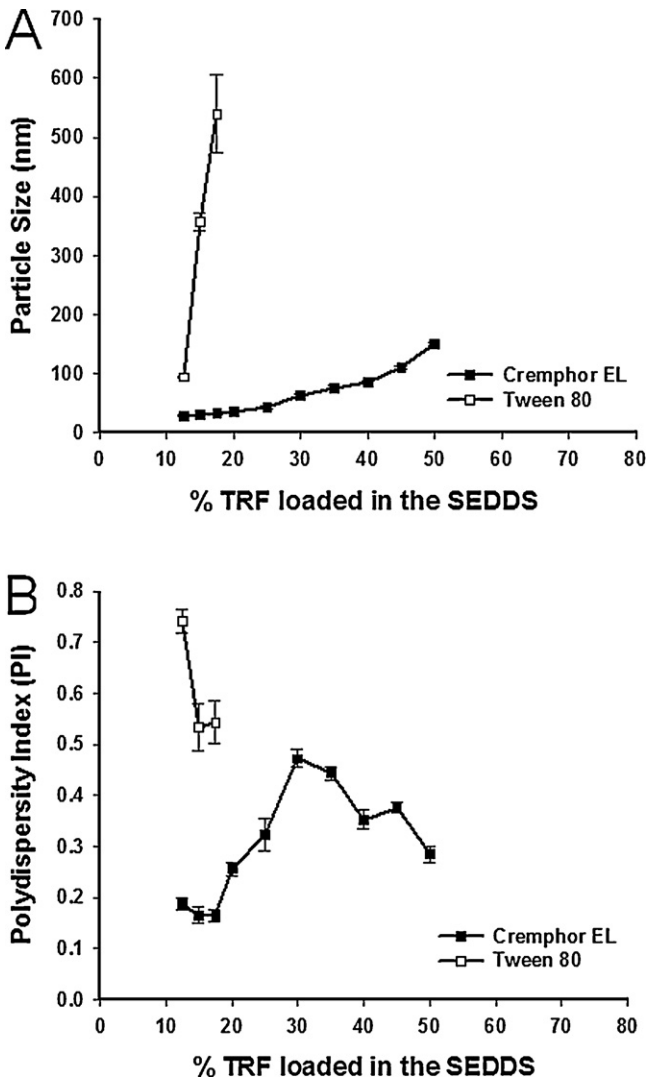

Fig. 5. Physical properties of the emulsified SEDDS when formulated using Tween® or Cremophor® as the primary surfactant. The figure shows the change in (A) droplet size of the emulsion and (B) polydispersity index (PI) of the resultant dispersions as a function of TRF loading in the SEDDS.

and their spatial arrangement and positioning in the aqueous media in relation to TRF plays a major role.

Several studies have shown that TRF efficiently partitions inside the cell membrane, which consists mainly of phospholipids (Atkinson et al., 2008). While there is a structural resemblance between Cremophor® EL (Fig. 2A) and phospholipids, the presence of a hydroxyl group on the acyl chains may allow the partitioning of TRF within Cremophor® EL micelles or emulsion droplets and away from the water/emulsion interface. This was demonstrated by molecular modelling and docking studies that were performed to better understand the differences in emulsifying capacity between Tween® 80 (Fig. 2A) and Cremophor® EL. In docking studies, the ligand (γ-tocotrienol) was allowed to perform random walks around the receptor (Cremophor or Tween). At each step, the ligand was moved by small increment and orientation, which resulted in different configurations or structure for which interaction energy was calculated with a free-energy expression based on previously defined grid surface (Huey et al., 2007). For Cremophor® EL, it was observed that most of the low energy structures (−6.25 kcal/mol of docking energy) were formed when the isoprenyl group of γ-tocotrienol was docked near the hydrophobic acyl chains forming a hydrogen bond with the hydroxyl group of Cremophor® EL

(Fig. 2B). In the case of Tween® 80, low energy structures were obtained when γ-tocotrienol (−3.5 kcal/mol) docked near the interface of oxyethylene moiety and the long hydrophobic chain. At this configuration, the hydroxyl group of γ-tocotrienol would form a hydrogen bond with the hydroxyl group on the polar head of Tween® 80, whereas the isoprenyl group of γ-tocotrienol would form hydrophobic interactions with the hydrophobic acyl chain of Tween® 80 (Fig. 2B). Such positioning of γ-tocotrienol at or near the water/emulsion interface may explain the instability of the emulsions and the increase in droplet size with increase in TRF loading. On the other hand, with Cremophor® EL, the surfactant molecules would encapsulate TRF within the emulsion droplet and away from the interface, thereby increasing the stability of the emulsion and decreasing its susceptibility to an increase in droplet size with an increase in TRF loading.

It is worth noting, however, that the docking studies presented here only serve as a model to represent possible interactions between γ-tocotrienol and the individual emulsifier based on their physicochemical properties. The limitations of the method is that Cremophor® EL and Tween® 80 were used as individual molecules and the effect of other SEDDS ingredients was ignored. Assembly of these amphipathic molecules in micellar environment is a more appropriate model to represent the interaction of γ-tocotrienol with the emulsifiers. Nonetheless, docking studies presented here provided possible binding sites for γ-tocotrienol on these amphipathic molecules and provided a model to explain the macroscopic behavior of the blends. The low energy docked structures clearly suggested that γ-tocotrienol binds to Cremophor® EL deep inside the hydrophobic pocket whereas in Tween® 80, it binds at the interface of the hydrophobic and polar head groups (Fig. 2B).

3.4. Sequential simplex optimization

The higher TRF loading capacity, smaller droplet size, and greater homogeneity obtained with Cremophor® EL based SEDDS suggested that Cremophor® EL is significantly more effective in generating nanoemulsions than Tween® 80. These SEDDS formulations, however, were not simple binary blends of surfactant and TRF. Rather, they were blends with co-surfactant, co-solvent, and secondary oil at a fixed ratio, which were previously shown to play a critical role in emulsifying α-tocopherol when Tween® 80 was used as the primary surfactant (Ali et al., 2008). The significance of these ingredients in Cremophor® EL based SEDDS is, however, unknown. What was known from preliminary experiments is that a simple binary blend with Cremophor® EL is not sufficient to emulsify TRF. This raised a question whether a nanoemulsion with lower droplet size at high TRF loads (≥50%) can be obtained by adjusting the concentration of each non-TRF ingredient. Since the concentration limits (upper and lower) of each ingredient that can be used without compromising the % TRF emulsified is unknown, a traditional response surface methodology cannot be used. Instead, the sequential simplex method is better suited for the optimization process since it does not require prior knowledge of factor limits. It systematically and sequentially adjusts the composition of the SEDDS formulation until it identifies a composition that yields the desired responses, such as a nanoemulsion with a lower droplet size. Another advantage of sequential simplex optimization is that it can handle several optimization criteria simultaneously to find the optimum SEDDS composition with a minimum number of practical trials.

The optimization procedure was carried out as described in Section 2.5.2 using the parameter outlined in Table 2. The reference formulation composition (starting simplex) was the Cremophor EL® SEDDS at 50% TRF loading, which was identified as formula number 10 in Table 1. Although it was shown from the dissolution

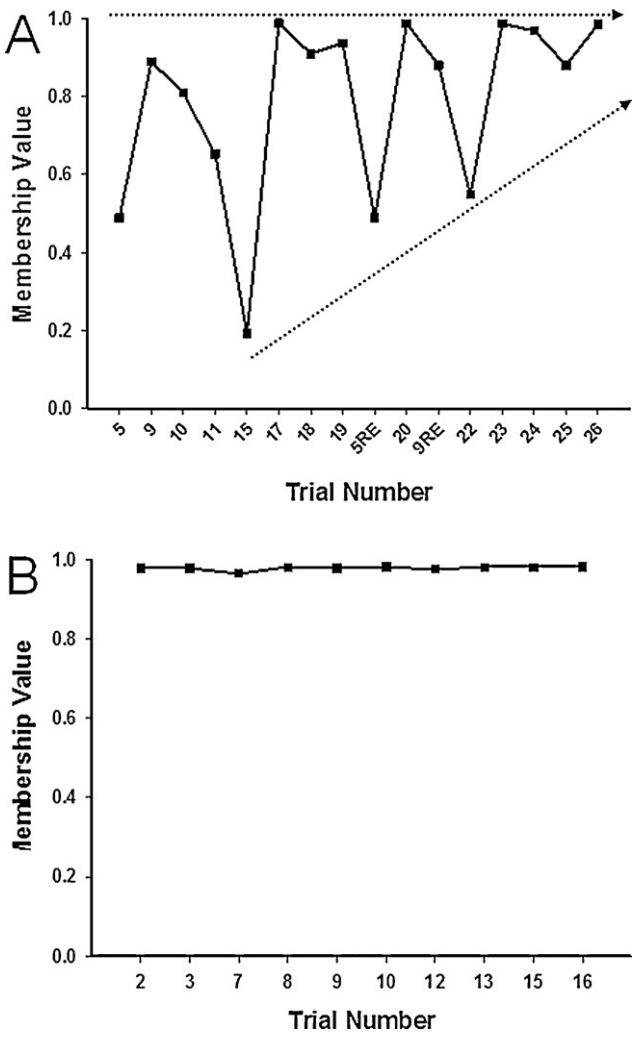

Fig. 6. Evolution of the membership value with each successful trial in the (A) first multisimplex run and (B) second multisimplex run.

study that a maximum 55% TRF could be loaded into a Cremophor EL based formulation, 50% TRF loading was selected as the starting simplex to avoid optimizing the formulation around the borderlines. The composition of each subsequent formulation suggested by Multisimplex® and the results obtained are given in Table 3. Each formulation was prepared as previously described and sequentially analyzed one at a time. Based on the dissolution, size, and PI data generated from each experiment a new composition was suggested and the procedure was repeated until an acceptable "membership value" was obtained. The target of the "membership value" and the overall optimization process was to identify a SEDDS composition (if any) with TRF loading ≥50% that could emulsify 100% of the loaded TRF into a <150 nm nanoemulsion. The % TRF loaded into the formulation was specified as both a control and response variable. This was essential in order to force the program to execute the optimization process for only formulations with TRF loading ≥50%.

From the attempted formulations (Table 3), it could be seen that the maximum concentration of TRF that can be successfully loaded into a SEDDS was approximately 55%, i.e. the simplex method could not identify a successful composition loaded with >55% TRF, which was in agreement with earlier observations. Also, it could be seen from the table that the ratio between the ingredients is not critical and that the composition could be slightly adjusted without compromising the quality of the formulations. The results

Table 4
Control and response variables that were used for the construction of the second Multisimplex® run.

Control variables				
	Cremophor® (mg)	Labrasol® (mg)	Captex® (mg)	Ethanol (mg)
Reference value[a]	122.1	122.1	21.6	34.2
Step size[b]	244.2	244.2	43.2	68.4

Response variables		
	Cumulative % TRF emulsified	Particle size (nm)
Objective	Target value: 100%	Minimization
Influence (0–1)	High (1)	High (1)
Lower limit	0	1
Upper limit	101	3000

[a] Reference value based on a 600 mg SEDDS formulation.
[b] Step size = 200% of the reference value.

Table 5
The composition of the SEDDS and the observed responses for the trials that were performed sequentially as part of the second Multisimplex® run.

Trial No.	TRF (%, w/w)	Crem (%, w/w)	Lab (%, w/w)	Cap (%, w/w)	Eth (%, w/w)	Diss (%)	Size (nm)	Mem. value
1	50	0.0	50.0	0.0	0.0	–	–	–
2	50	23.0	23.0	4.1	0.0	100	132.1	0.97790
3	50	21.9	21.9	0.0	6.1	100	131.45	0.97801
4	50	0.0	0.0	19.4	30.6	–	–	–
5	50	50.0	0.0	0.0	0.0	–	–	–
6	50	0.0	38.3	4.5	7.2	–	–	–
7	50	15.4	34.6	0.0	0.0	100	207	0.96504
8	50	19.6	24.5	2.3	3.7	100	121	0.97979
9	50	21.4	22.0	2.5	4.0	100	125.3	0.97906
10	50	29.5	20.5	0.0	0.0	100	112	0.98132
11	50	35.6	16.8	−0.9	−1.5	–	–	–
12	50	33.5	8.4	3.2	5.0	100	144	0.97587
13	50	28.4	15.7	2.3	3.6	100	122	0.97962
14	50	26.0	19.8	−0.3	4.5	–	–	–
15	50	24.1	21.7	2.4	1.8	100	121	0.97979
16	50	27.1	20.3	2.5	0.2	100	114	0.98098

Crem, Cremophor® EL; Lab, Labrasol®; Cap, Captex® 355; Eth, ethanol; Diss, %TRF emulsified in the dissolution medium; Size, Mem. value, membership value. Trail No. 1, 4, 5, 6, 11, and 14 were considered "impossible" runs and therefore data from these trials were not generated and/or reported (–).

also demonstrated the relative importance of each ingredient. For example, formulations in trials number 5 and 11 were loaded with approximately 65% TRF, with trial number 5 having significantly higher concentration of the primary surfactant Cremophor® EL. Nonetheless, absence of Labrasol® and Captex® 350 in trial number 5, in spite of the high concentration of Cremophor® EL, led to only 25% TRF emulsification as opposed to 51% in trial number 11. This suggested that the inclusion of these secondary components is essential to improve the physical properties of the formulations and the emulsification of TRF. Specific conclusions on the effect of each secondary component on TRF loading and emulsions stability, however, could not be made from the data generated from the multisimplex runs. Nonetheless, the overall role of each ingredient on the emulsification of α-tocopherol was previously discussed by Ali et al. (2008).

Only formulations/trials with 100% of the TRF emulsified had a "membership value" > 0.9. These included trials number 17, 20, 23, 24, 26, with trial number 24 having the lowest droplet size and consequently one of the highest "membership value". Formulation in trial 24 was loaded with 50% TRF and resulted in a high "membership value" (0.969) and a dispersion with a droplet size smaller than the size of the reference formulation, thereby satisfying the requirements of the optimization process. The evolution of the "membership value" with each trial is shown in Fig. 6A. The narrowing gap between the high (desired) and low (undesired) "membership value" with each new experiment reflects the self-learning algorithm of the sequential simplex optimization process that aims to reach the optimum conditions with least number of trials.

Since the "membership values" was very close to 1 as seen with trial number 24, it was concluded that further adjusting formulation composition would not significantly reduce droplet size and that the optimal formulation is not far from the composition given by trial number 24. To confirm this conclusion, a second sequential simplex optimization run was carried out. This time the % TRF loaded was fixed at 50% and only formulations that can emulsify 100% of the loaded TRF were considered in the optimization process (Table 4). Formulation compositions that resulted in <100% emulsification were deemed "impossible" and were excluded from the optimization process. For this experiment, a total of 16 trials were performed (Table 5), of which 6 were "impossible". As seen from the table, the droplet size of the dispersions was within the 112–207 range and all the formulations had a "membership value" > 0.965 (Fig. 6B). The similarity in droplet size and "membership value" of trials in the second multisimplex run to the results obtained with trial number 24 from the first multisimplex run demonstrated that once a "membership value" close to 1 is attained, no significant changes in the responses would be expected. This should help the formulator identify the optimal composition and decide when to stop the multisimplex run.

4. Conclusion

The choice of formulation ingredients has a tremendous impact on the quality of SEDDS formulations. For example, SEDDS made with Cremophor® EL were superior to those made with Tween® 80. A significantly higher concentration of TRF could be loaded into Cremophor® EL formulations without compromising the ability

of the SEDDS to emulsify the drug into emulsions in aqueous dissolution media. This was attributed to the spatial position of TRF within the emulsion droplets as supported by docking studies. γ-Tocotrienol was found to bind to the hydroxyl group deep in the hydrophobic region of Cremophor® EL whereas in Tween® 80 it binds closer to the head group at the water/emulsion interface. For the optimization process, the sequential simplex method proved effective in optimizing the concentration of TRF and non-TRF components of the SEDDS within minimal number of trials without prior knowledge of the design space. The multisimplex process was also found to be useful in identifying the range within which the concentration of the individual components in a SEDDS could be adjusted while maintaining the desired response. While this information could be used to construct a design space around the reference formulation, the multisimplex method is more suitable for identifying the optimal formulation composition or process parameters. The versatility of this method should make it an ideal Quality by Design tool in pharmaceutical product development.

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