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Effect of combined use of nonionic surfactant on formation of oil-in-water microemulsions

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Abstract

Purpose: This study evaluated the effects of combined use of two nonionic surfactants on the characteristics (i.e., appearance, emulsification time, and particle size) of oil-in-water microemulsions generated from flurbiprofen-loaded preconcentrates.
Methods: Three phase diagrams were constructed using Capmul PG8 (propylene glycol monocaprylate) as the oil, Tween 20 (polysorbate 20) and/or Cremophor EL (polyoxyl 35 castor oil) as surfactants. A number of preconcentrates were selected based on phase diagrams: $O_{20}T_{80}$ (20% Capmul PG8, 80% Tween 20), $O_{20}C_{80}$ (20% Capmul PG8, 80% Cremophor EL), $O_{20}T_{40}C_{40}$ (20% Capmul PG8, 40% Tween 20, 40% Cremophor EL). Flurbiprofen loading in preconcentrates was tested at 0%, 1%, 2.5%, and 5% (w/w). The resulting mixtures of these preconcentrates upon dilution 100-fold with aqueous medium were characterized by visual and microscopic observation, HPLC and photon correlation spectroscopy.
Results: (a) For preconcentrates using single surfactant, either $O_{20}T_{80}$ or $O_{20}C_{80}$, the dilution generated emulsions with visible cloudiness. The particle size increased as the drug loading increased; (b) for preconcentrates using two surfactants $O_{20}T_{40}C_{40}$, the dilution generated clear microemulsions with small particle sizes (10–11 nm), and the increased drug loading seemed to have little effect on the particle size. The microemulsions from preconcentrate $O_{20}T_{40}C_{40}$ was also found to be stable at ambient temperature over 20 days without significant change in particle size at different drug loadings. Dilution with different aqueous medium, either water, or simulated gastric fluid or simulated intestinal fluid, also did not change the particle sizes of the microemulsions.
Conclusions: The combined use of surfactants in preconcentrate showed the promise in generating desired self-emulsifying microemulsions with small particle size, increased drug loading, and improved physical stability. This will have significant implications in future dosage development for poorly water-soluble drugs in using self-emulsifying microemulsions drug delivery system (SMEDDS).

Keywords: Surfactant; Microemulsions; Preconcentrate; Flurbiprofen; SMEDDS; SEDDS

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1. Introduction

Microemulsion preconcentrate, also known as self-microemulsifying drug delivery systems (SMEDDS), is a mixture consisting of drugs, oils, and surfactants. Upon dilution with aqueous media and accompanied by gentle agitation, the preconcentrate spontaneously forms clear isotropic solutions, or microemulsions. Compared to ready-to-use microemulsions, it has improved physical stability profile upon long-term storage, and can be filled directly into soft or hard gelatin capsules for convenient oral delivery. Literatures indicate that the preconcentrate promotes drug solubilization, drug release at absorption sites, and ultimately drug oral bioavailability (Attwood and Florence, 1983; Humberstone and Charman, 1997; Pouton, 1997; Lawrence and Rees, 2000). As a drug delivery system, however, the preconcentrate has its limitations as a viable pharmaceutical dosage form (Pouton, 1997). The most significant one is that the dosage form uses a large amount of surfactants for the purpose of forming microemulsions. This has posed clinical liabilities as surfactants often have potential toxic effects (Humberstone and Charman, 1997; Pouton, 1997; Lawrence and Rees, 2000; Swenson et al., 1994; Cavanak and Sucker, 1986; Tibell et al., 1993) when used at high levels. Research efforts in this regard included the use of cosolvent (Pouton, 1997; Lawrence and Rees, 2000). The cosolvent is believed to act as a good solubilizer for both water and oil, and reduce surface tension by stabilizing film formation between the two phases (Humberstone and Charman, 1997; Pouton, 1997; Lawrence and Rees, 2000). However, cosolvents such as low-molecular weight alcohols may have undesirable effects upon dilution with aqueous medium, as they may partition out of the interfacial region and into the continuous phase. This leads to altered phase behavior, phase separation or drug precipitation (Humberstone and Charman, 1997; Pouton, 1997; Lawrence and Rees, 2000). Another approach that gained attention lately is the combined use of surfactants (Huibers and Shah, 1997; Engels et al., 1995; Weingarten et al., 1991; Moreno et al., 2003).

In this study, we aimed to investigate the effect of different surfactants, when used either alone or in combination, on microemulsion formation from preconcentrates. Based on preliminary results, this study employed Cremophor EL (polyoxyl 35 castor oil) and Tween 20 (polysorbate 20) as surfactants, and Capmul PG8 (propylene glycol monocaprylate) as oil. Both Tween 20 and Cremophor EL are nonionic and GRAS (generally-recognized-as-safe) excipients and are widely used in pharmaceutical preparations. With Tween 20 being more hydrophilic than the Cremophor EL, this surfactant combination (1/1 ratio, w/w) was found to be effective in drug emulsification in a number of early drug candidates at Novartis and some commercially available model compounds (data not shown here). A structured lipid, Capmul PG8 is immiscible with water. This excipient has been used as oil in a number of microemulsion patents over the years (Mulye, 2000; Hamied et al., 2001). The drug flurbiprofen was chosen as the model compound in this study. It is a phenylpropionic acid derivative, and is developed for the treatment of rheumatic disorders. In recent years, there were reports showing that flurbiprofen can be formulated into oil-in-water microemulsion (Park and Kim, 1999; Park et al., 1999). For example, Park and Kim (1999) prepared the flurbiprofen-loaded microemulsions by using ethyl oleate as oil, and Tween 20 as surfactant. It is of note that the commercial flurbiprofen product for parenteral administration is a prodrug, or flurbiprofen axetil, also formulated in oil-in-water emulsion (Lipfen, 50 mg/5 ml as flurbiprofen axetil, Green Cross, Japan). Experimentally, the following were conducted:

a. construct the phase diagram for oil-surfactant-water system. The surfactant will be either single one or in combinations (i.e., both Tween 20 and Cremophor EL);
b. analyze the above phase diagram, and select a number of appropriate preconcentrate compositions (oil and surfactant only);
c. load flurbiprofen onto these preconcentrates and dilute to 100-folds with aqueous medium, and characterize the resulting mixture with respect to appearance, emulsification time, and particle size.

2. Materials

Capmul PG8 (propylene glycol monocaprylate) and Cremophor EL (polyoxyl 35 castor oil) were gifts from Abitec Corporation (Columbus, Ohio) and BASF corporation (Mount Olive, NJ), respectively. Tween 20,

flurbiprofen and other chemicals were purchased from Sigma (St. Louis, MO). All chemicals were used as received.

3. Methods

3.1. Phase diagram

A titration method was employed to construct phase diagrams (von Corswant and Thorén, 1999). Briefly, mixtures of the oil (Capmul PG8) with surfactants (Tween 20, Cremophor EL, or a combination of the two surfactants at 1/1 (w/w) ratio) were prepared at ratios of 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10 into different vials. A small amount of water in 5% (w/w) increment was added into the vials. Following each water addition, the mixtures in vials were vortexed 2–3 min and were incubated at 25 °C for 48 h with gentle shaking (precision reciprocal shaking bath). The resulting mixtures were evaluated by visual and microscopic (equipped with crossed polarizer) observation. For the phase diagram, microemulsions (ME) was the region of clear and isotropic solutions that might also contain micelle solutions; LC (liquid crystal) was the region of gel-like phases with typical oil streaks or fan-shape textures, which showed birefringence under crossed polarizer microscope; EM (coarse emulsion) was the region of visibly cloudy dispersions even by visual observation. Except for LC, none of the other regions showed birefringence under the crossed polarizer. As to particle size, it is generally held that microemulsions (clear) are below 150 nm, while emulsions (visibly cloudy) are in the range of 150–1000 nm (Lawrence and Rees, 2000). A 48 h incubation period was used at each titration step to ensure phase equilibrium, as preliminary studies showed that phase diagrams generated from 48 h and one week incubation were almost identical. The phase boundary was determined in triplicate with accuracy better than ±2% (w/w) oil for each system.

3.2. Preconcentrate preparation

The preparation was proceeded as follows: (a) for placebo, blend surfactant and oil mixture in certain ratio (e.g., 4/1, w/w), followed by equilibrating this mixture on a shaker at 25 °C for 48 h; (b) for flurbiprofen-containing preconcentrate, first dissolve the drug in the oil, then mix the surfactant and equilibrate the mixture as in (a).

3.3. Dilution

In an effort to mimic physiological dilution process after oral administration of preconcentrates, triplicate samples of selected preconcentrates containing various surfactants and oil compositions were diluted 100-fold with aqueous media including water, simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) (U.S. Pharmacopeia and National Formulary, 2003). The dilution was followed by gentle vortexing for 2 min at ambient temperature.

3.4. Emulsification

The emulsification time, i.e., the time needed for a preconcentrate to form homogeneous mixture upon dilution, was monitored by both visual observation and HPLC analysis for each preconcentrate in triplicate. Visual observation is to monitor the disappearance of preconcentrate “particles”, while the HPLC analysis is to measure the drug concentration in the dissolution medium over the time. A dissolution apparatus (DISTEK dissolution system 2100A) was employed with 500 ml simulated gastric fluid, and with a paddle speed of 50 rpm at 37 °C. The preconcentrates (1 ml) were delivered via a syringe pump at a rate of 10 ml/min at 1 cm below the surface of the dissolution medium. Samples of dissolution medium (2 ml each) were withdrawn at 1, 3, 5, 7, 10, 30, and 60 min and filtered through 0.45 μm TEFE syringe filters (Millipore) before HPLC analysis.

3.5. HPLC assay

An HPLC assay was developed to quantitate flurbiprofen concentrations. The HPLC system used a Waters 2690 (Waters Corporation) separation module and Waters 2487 dual wavelength absorbance detector (Waters Corporation). The HPLC chromatographic conditions were as follows: column-Synergi Max-RP, 150 cm × 4.6 mm (Phenomenex Corporation); flow rate 1 ml/min; detection wavelength 260 nm; injection volume 20 μl; mobile phase 40% acetonitrile and 60% of 0.1% triethylamine in 0.04 M ammonium acetate;

and flurbiprofen retention time 4.8 min. Standard solutions were found to be linear at concentrations of 0.5–100 μg/ml. The concentration was averaged values from triplicate samples with a relative standard deviation less than 2%. To simplify, only the average values of data were used in figures.

3.6. Particle size measurement

Photon correlation spectrometer using laser light scattering (Beckman Coulter N4 Plus) was employed to measure particle sizes of preconcentrate-generated emulsions/microemulsions (approximately 100-fold dilution of preconcentrate with aqueous). The samples were loaded onto 1 cm^2 cuvettes in a thermostated chamber. The sample viscosity and the water refractive index were factored in particle size measurement using the instrument software. Light scattering was monitored at a 90° angle and at a temperature of 25 °C. For microemulsions (particle size ≤150 nm), mean particle size values of triplicate samples with a relative standard deviation less than 5% were reported in Tables and Figure. For coarse emulsion (particle size >150 nm), mean particle size values of triplicate samples with a relative standard deviation less than 10% were reported in Table 1.

4. Results and discussions

Fig. 1(a–c) present the phase diagrams for three different oil-surfactant-water systems: System A (Capmul PG8, Tween 20, and water), System B (Capmul PG8, Cremophor EL, and water), and System C (Capmul PG8, Tween 20/Cremophor EL 1/1 ratio, and water). It can be seen that these phase diagrams contained different areas of clear, isotropic microemulsions (ME), liquid crystals, and coarse emulsions (EM). It can also be seen that System C with combined use of two surfactants appeared to have the largest region of microemulsion among the three systems. When the preconcentrate (surfactant plus oil) diluted with water, the System C also gave the largest range in oil composition for preparing the preconcentrate. This is evident

Table 1
Evaluation of mixtures formed upon dilution of preconcentrates with water

Composition	Drug loading (%)	Appearance	Emulsification time (min)	Particle fraction (%)	Mean particle size[a] (nm)
$O_{20}T_{80}$	0	Clear	3	100	12
	1	Cloudy	2	82	192
				10	10
				8	26
	2.5	Cloudy	3	75	209
				14	11
				11	47
	5	Cloudy	3	80	179
				12	14
				8	851
$O_{20}C_{80}$	0	Clear	50	100	13
	1	Slightly cloudy	52	56	164
				44	15
	2.5	Cloudy	58	55	242
				32	1000
				13	14
	5	Cloudy	62	95	236
				5	13
$O_{20}T_{40}C_{40}$	0	Clear	3	100	11
	1	Clear	4	100	11
	2.5	Clear	3	100	11
	5	Clear	4	100	10

[a] O for oil Capmul PG8 (propylene glycol monocaprylate), T for surfactant Tween 20 (polysorbate 20), C for Cremphor EL (polyoxyl 35 castor oil). For example, the $O_{20}T_{40}C_{40}$ indicates that the preconcentrate contained 20% Capmul PG8, 40% Tween 20, 40% Cremphor EL.

in Fig. 1(c), where as much as 40% oil can be incorporated in the preconcentrate and be diluted into microemulsions. This is significant in the preconcentrate formulation development, as more oil in the compositions helps to solubilize poorly water-soluble drugs in the preconcentrate.

Three preconcentrates having 20% oil and 80% surfactants (either alone or in combination) were

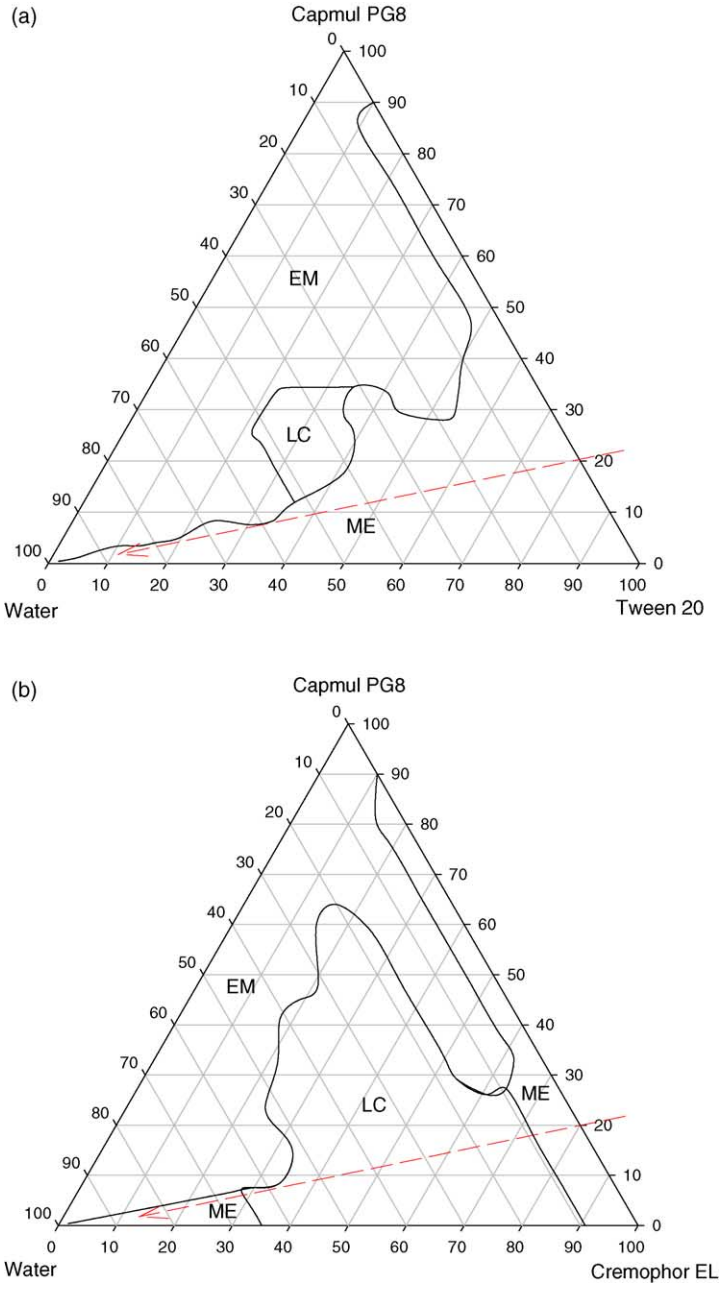

Fig. 1. Phase diagrams for (a) Capmul PG8, Tween 20, and water, (b) Capmul PG8, Cremophor EL, and water, and (c) Capmul PG8, Tween 20, Cremophor EL, and water.

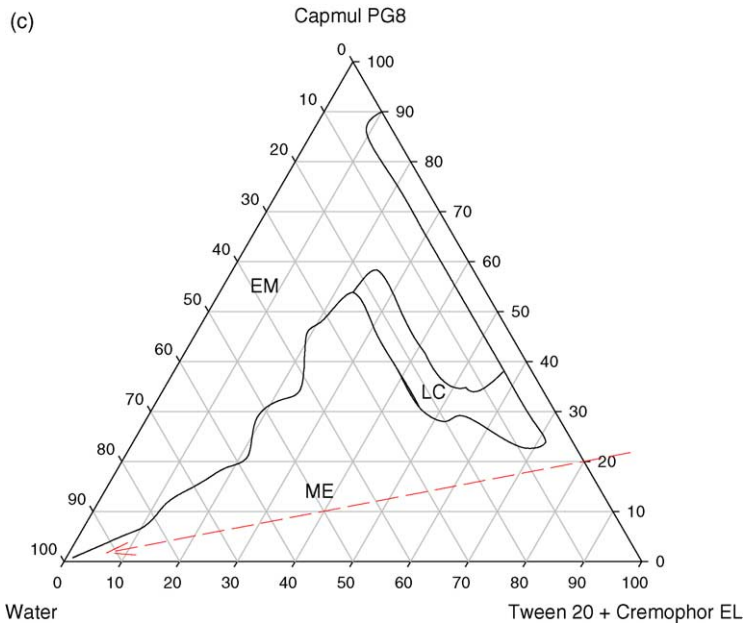

Fig. 1. (*Continued*).

selected: $O_{20}T_{80}$ (20% Capmul PG8, 80% Tween 20), $O_{20}C_{80}$ (20% Capmul PG8, 80% Cremophor EL), and $O_{20}T_{40}C_{40}$ (20% Capmul PG8, 40% Tween 20, and 40% Cremophor EL), and the drug flurbiprofen was loaded at different levels: 0%, 1%, 2.5%, 5% (w/w). These preconcentrates were diluted 100-fold with water and the resulting mixtures were evaluated with regard to their appearance, emulsification time, as well as the mean particle size. These results are given in Table 1. It is evident form Table 1 that when flurbiprofen was absent, the generated mixtures were clear microemulsions, with negligible difference in particle sizes (11–13 nm). Unlike drug-free preconcentrates, when flurbiprofen was loaded, the mixtures generated by single surfactant-containing preconcentrates, either $O_{20}T_{80}$ or $O_{20}C_{80}$ showed emulsions with observed cloudiness, and the particle size increased greatly as compared to that of drug-free microemulsions noted earlier, and the distribution was split into a number of peak areas. This became more apparent as the drug loading was increased. However, mixtures generated from combined surfactant-containing preconcentrate $O_{20}T_{40}C_{40}$ upon dilution were still clear microemulsions for all drug loadings (up to 5%), and the particle sizes of these microemulsions remained practically unchanged (<11 nm). This is an important observation, suggesting that the combined use of surfactants (Tween 20 and Cremophor EL) is significantly more effective in generating microemulsions, and especially for flurbiprofen-containing preconcentrates.

The emulsification time, or the time needed to reach the emulsified and homogeneous mixture, remained low for both $O_{20}T_{80}$ and $O_{20}T_{40}C_{40}$ (<4 min) for all drug loadings, but high for $O_{20}C_{80}$ (approximately 1 h) by visual examination. The difference is surprisingly large. This suggests that combined surfactants can be used to modify the emulsification time for the flurbiprofen-containing preconcentrates.

The above three preconcentrates at 5% flurbiprofen loading were further evaluated for their emulsification by monitoring the drug concentration in simulated gastric fluid using a dissolution apparatus. The drug percentage was used to indicate the drug emulsified into SGF. Fig. 2 shows that at the first minute (the first sampling), more than 95% flurbiprofen were found in respective dissolution media from both $O_{20}T_{80}$ and $O_{20}T_{40}C_{40}$, while only 60% from $O_{20}C_{80}$. In fact it took approximately 10 min for

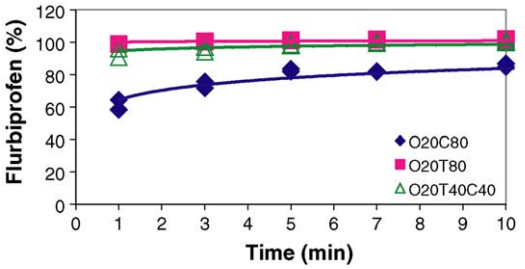

Fig. 2. Emulsification of preconcentrates $O_{20}T_{80}$, $O_{20}C_{80}$ and $O_{20}T_{40}C_{40}$ loaded with 5% flurbiprofen.

$O_{20}C_{80}$ to reach 85%, and almost an hour to reach 100%. The increasing order of emulsification time ($O_{20}T_{80} \approx O_{20}T_{40}C_{40} \ll O_{20}C_{80}$) was consistent with the visual observation. It is speculated that the slow emulsification found in $O_{20}C_{80}$ might be a result of forming lamellar liquid crystal (LC) on the dilution path (see arrow in phase diagram, Fig. 1(b)). There were reports indicating that highly ordered liquid crystal increased apparent viscosity of oil/surfactant/water mixtures (U.S. Pharmacopeia and National Formulary 2003; Constantinides and Scalart, 1997; Alany et al., 2001; Ninham et al., 1984), and hence slowed hydration rate for the preconcentrate.

In Table 1, particle sizes of microemulsion immediately after their formation were reported. To determine the physical stability of microemulsions with time, the preconcentrates with $O_{20}T_{40}C_{40}$ composition at various flurbiprofen loading (0%, 1%, 2.5%, 5%, w/w) were evaluated at ambient temperature over a 20-day period (Fig. 3). Both visual observation and particle size measurement indicated that the microemulsions

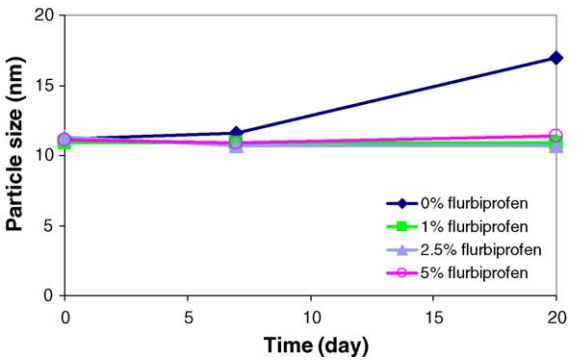

Fig. 3. Mean particle sizes as a function of time when $O_{20}T_{40}C_{40}$ having different drug loadings were diluted with water.

Table 2
Mean particle sizes of microemulsions formed at different flurbiprofen loadings in $O_{20}T_{40}C_{40}$ upon dilution with water, simulated gastric fluid (SGF), and simulated intestinal fluid (SIF)

	Mean particle size (nm)				
Diluent	0% drug	1% drug	2.5% drug	5% drug	10% drug
Water	11.2	10.9	11.3	11.1	40.0
SGF	12.4	13.7	14.3	15.1	15.3
SIF	12.2	10.9	11.3	11.1	13.6

formed at various drug loading were stable, and that the drug loading up to 5% did not produce meaningful particle size change (<18 nm) over the test period. Furthermore, even at 10% drug loading, $O_{20}T_{40}C_{40}$ preconcentrates formed clear microemulsions without any phase separation into cloudy emulsions. It was also observed that there were no significant differences in microemulsions generated when either water, or simulated gastric fluid, or simulated intestinal fluid was used as dilution medium. Table 2 shows the particle sizes of microemulsions formed when $O_{20}T_{40}C_{40}$ preconcentrate (to 10% drug) was diluted with water, SGF, and SIF. The result comparison suggests that, for flurbiprofen (acidic, with $pK_a = 4.2$) (Li and Zhao, 2003) loaded preconcentrate, drug ionization state may have little influence in microemulsion formations.

The combined use of surfactants showed apparent advantages over the single use of surfactant: the microemulsion region was greatly increased in the phase diagram. The oil compositions were also broadened so that high drug loading became possible. Evaluation on microemulsions generated from $O_{20}T_{40}C_{40}$ as well as $O_{20}T_{80}$ and $O_{20}C_{80}$ revealed that there certainly a benefit to be gained by screening mixed surfactant systems in developing SMEDDS for poorly water-soluble drugs. In this study, we have used Tween 20, which is the most hydrophilic in Tween series (HLB = 17, Ash and Ash, 1997), and Cremophor EL (HLB = 12–14, Ash and Ash, 1997), which is less hydrophilic but more capable of solubilizing hydrophobic drug components. The results of this study seem to indicate that, as opposed to the use of single surfactant in preconcentrate, combined use of surfactants might have provided a better surfactants' hydrophilic–lipophilic balance. As a result, it enhanced the flexibility of surfactant layer that was formed, it also enhanced the surfactants' ability to partition at higher levels into the oil–water interface;

both of which stabilized oil-in-water microemulsion formed (Huibers and Shah, 1997; Engels et al., 1995; Weingarten et al., 1991; Moreno et al., 2003; Ninham et al., 1984; Warisnoicharoen et al., 2000). Similar reports were seen in pharmaceutical applications. For example, Moreno et al. (2003) reported that the combined use of Tween 80 and soybean lecithin was found to greatly increase the oil content in the microemulsions (by three folds). Huibers and Shah (1997) also observed synergistic effects of surfactant combinations for water-in-oil microemulsions. Currently, the investigation is still on-going. This includes the study on the mechanism of surfactants' synergism, the Tween 20-Cremophor EL combination on oil-surfactant-water phase behavior, the formation of microemulsions from preconcentrates loaded with other drugs, and the ability of microemulsions to retain (or solubilize) the drug without phase separation.

5. Conclusions

The study indicates that, as compared to a single surfactant, combined use of surfactants (Tween 20 and Cremophor EL in this study) in the preconcentrate greatly improved the microemulsions generated upon dilution with aqueous medium. This shows the promise for future SMEDDS development for poorly water-soluble drugs such as flurbiprofen in generating desired self-emulsifying microemulsions with small particle size, increased drug loading, and increased physical stability.

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