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Preparation and in vitro characterization of a eutectic based semisolid self-nanoemulsified drug delivery system (SNEDDS) of ubiqui,8esua1mu0esua1mu0esua1mu0esu16.91(.7(

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Abstract

The objectives of the present work were, first, to develop a self-nanoemulsified drug delivery system (SNEDDS) based on the eutectic properties of ubiquinone (CoQ_{10}); and second, to study the progress of emulsion formation and drug release mechanisms by turbidimetry and droplet size analysis. Binary phase diagrams of CoQ_{10} with menthol and essential oils were constructed and used to develop the self-nanoemulsified formulation. Pseudo ternary phase diagram was constructed to identify the efficient self-emulsification region. Release mechanisms of the resultant formulas were quantified using turbidimetry in combination with dissolution studies. Turbidity time profiles revealed three distinctive regions: lag phase, plateau, and the pseudolinear phase. Lag phase was attributed to the liquid crystalline properties of the formula. Plateau turbidity was correlated with droplet size. Laser diffraction analysis revealed an average droplet diameter of 100 nm. Emulsification rate was obtained from the corrected slope of the pseudolinear phase of the profile. Stability of the formula was further evaluated using Fourier transform-infrared (FT-IR) attached to an attenuated total reflectance (ATR) accessory. The present study revealed a eutectic based semisolid self-emulsified delivery system that can overcome the drawbacks of the traditional emulsified systems such as low solubility and irreversible precipitation of the active drug in the vehicle with time.

Keywords: Coenzyme Q_{10}; Eutectic mixture; Self-nanoemulsified drug delivery system; SNEDDS; Turbidimetry; Liquid crystals

1. Introduction

Ubiquinone, also known as CoenzymeQ_{10} (CoQ_{10}) (Fig. 1), is a physiologically important compound acting as an electron shuttle in mitochondrial respiratory chain and as a stabilizing

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agent in cellular membranes (Grossi et al., 1992).
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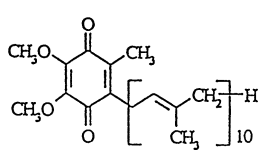

2.2. Methods

2.2.1. Differential scanning calorimetry (DSC) of CoQ_{10}-menthol and CoQ_{10}-essential oil binary systems.

CoQ_{10} and L-menthol were mixed at various ratios between 90:10 and 10:90 (w/w). Approximately 5 mg of the mixture was sealed in the aluminum pan and analyzed using a differential scanning calorimeter (DSC 7, Perkin–Elmer, Norwalk, CT). Thermal analysis was carried out between 25 and 60 °C under nitrogen gas flow against an empty reference pan at a heating rate of 10 °C min^{-1}. Similarly, different ratios of CoQ_{10} and the essential oil between 80:20 and 20:80 (w/w) were mixed and melted at 37 °C. Resulting oils were stored at 4 °C for 24 h to allow complete re-crystallization of CoQ_{10}. To avoid oil evaporation, approximately 10 mg of the mixture was weight onto a DSC sample pan and kept in an airtight container during storage prior to DSC analysis. For CoQ_{10}-essential oil mixtures at ratios between 80:20 and 60–40 (w/w), thermal analysis was carried out between 25 and 55 °C. Samples with higher essential oil concentration were analyzed between 10 and 40 °C. Heating rate used was 10 °C min^{-1}. Lower temperatures were maintained using refrigerated cooling accessory (Intracooler 2, Perkin–Elmer).

2.2.2. Determination of CoQ_{10} melting time

CoQ_{10} was accurately weighed and mixed with 50 and 60% w/w of peppermint oil, spearmint oil, anise oil or lemon oil in a screw-capped glass vials. Mixtures were allowed to melt at 37 °C in water bath (Ikamag® Ret-G, Terochem Scientific, Toronto, Canada). Cremophor EL was added to the melt at a concentration of 20, 40 and 60% w/w of the final weight using a positive displacement pipette (Microman®, Gilson Inc., Middleton, WI) and stirred with a magnetic bar. Vials were then capped and stored at ambient temperatures in tight containers protected from light. After 24 h sample vials containing the solidified preparations were immersed in water bath maintained at 37 °C. Samples were monitored for a change in their physical appearance and the time was recorded until a complete melt was obtained.

2.2.3. Formulation of the self-emulsified systems

A series of self-emulsifying systems were prepared with varying concentrations of the oily mix (37.5–60%), cremophor EL (0–62.5%), and capmul MCM-C8 (0–62.5%). The oily mix consisted of CoQ_{10} and lemon oil at a ratio of 50:50. CoQ_{10} and lemon oil were accurately weighed into screw-capped glass vial and melted in a water bath at 37 °C. Cremophor EL and capmul MCM-C8 were added to the oily mix using a positive displacement pipette and stirred with a magnetic bar. While molten, formulations with different concentrations of surfactant, co-surfactant, and the oil mix, each containing CoQ_{10} at a final loading of 30 mg, were filled into size 4 HPMC capsules. Filled capsules were stored at room temperature until their use in subsequent studies.

2.2.4. Visual observations

To assess the self-emulsification properties, formulation (50 mg) pre-melted at 37 °C was introduced into 100 ml of water in a glass Erlenmeyer flask at 25 °C and the contents were gently stirred manually. The tendency to spontaneously form a transparent emulsion was judged as 'good', and it was judged 'bad' when there was poor or no emulsion formation (Craig et al., 1995; Kommuru et al., 2001). Phase diagrams were constructed identifying the good self-emulsifying region. All studies were repeated in triplicates with similar observations being made between repeats.

2.2.5. Emulsion droplet size analysis and turbidity measurements

Formulation (50 mg) melted at 37 °C was diluted with water, pre-equilibrated at 37 °C, to 100 ml in an Erlenmeyer flask and gently mixed with hand. The resultant emulsions was evaluated for its droplet size and turbidity as follow.

2.2.5.1. Droplet size analysis. The droplet size distribution of the resultant emulsions was determined by laser diffraction analysis using Coulter particle size analyzer (Model LS230, Miami, FL), which has a particle size measurement range of 0.04–2000 μm. The sizing of the emulsions was determined in a small volume module. Samples were directly placed into the module and the data

were collected for 6u(U(gl286.s(6u.U(gl286.P)087.areutlere)-7(sizere)-286.wasre)-286.9alculaecte7)]T0 -1.2 0 TDfrom

fringence of the mixtures was examined under a microscope with crossed polarizers (Nikon Eclipse E600 Pol, Nikon Instech Co., Japan) equipped with a CCD camera (Hitachi HV-C20, Hitachi America, Ltd., San Diego, CA). The mixtures were prepared by first mixing the co-surfactant and water and then adding the surfactant. The compositions in glass tubes were mixed acoustically and then placed into a centrifuge (Centra MP4R, International Equipment Company, Needham Heights, MA) for 0.5–1 h at 3000 rpm (RCF equivalent to $500 \times g$). Ultrapure water with specific resistance of better than or equal to 16 MΩ cm and pH around 4 was used. The test samples for polarizing microscopy studies were prepared in rectangular glass capillaries (gap thickness 100 μm and width 1 mm, length 5 cm). Both ends were sealed with epoxy resin.

2.2.

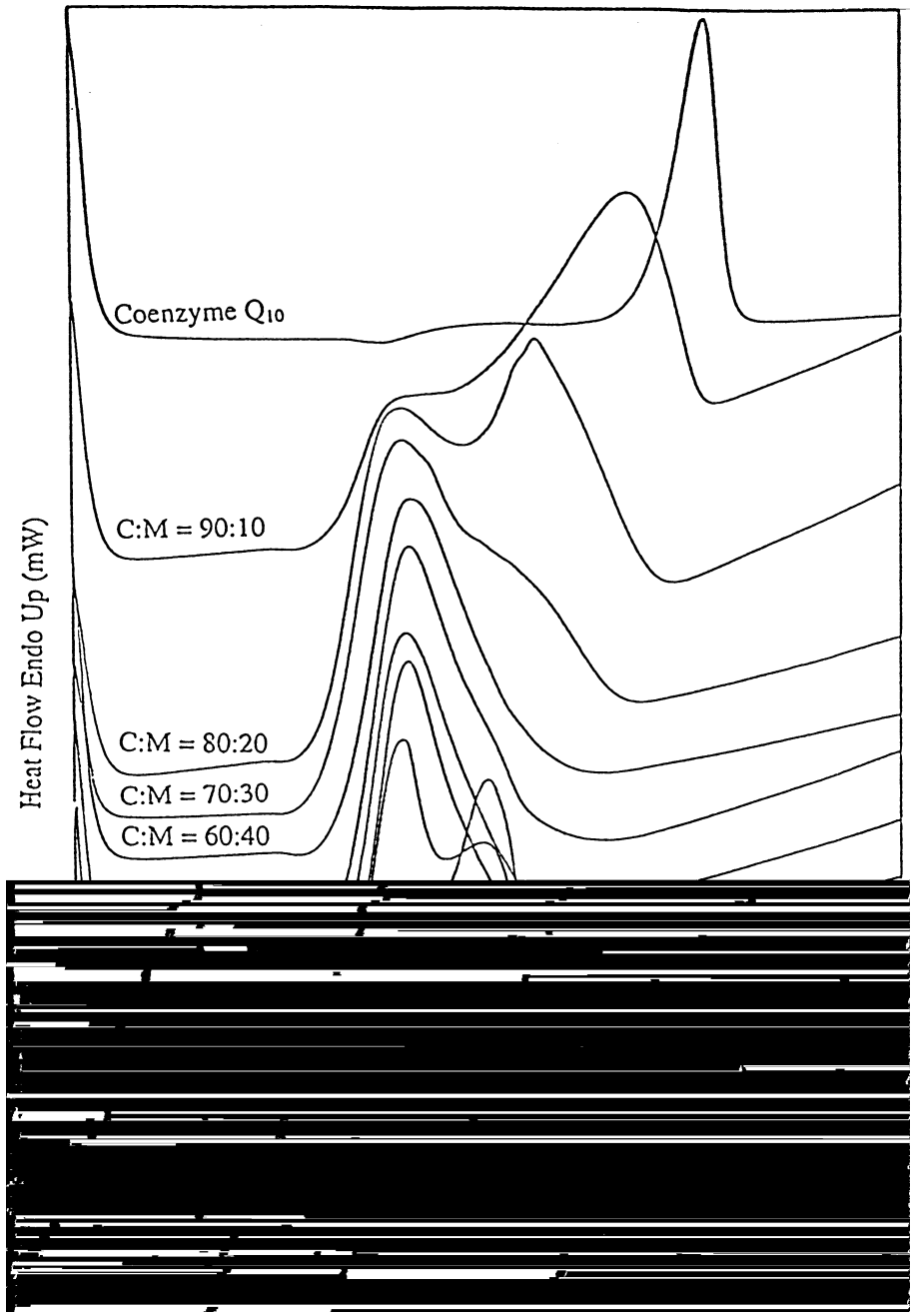

Fig. 2. DSC thermograms of CoQ_{10}, L-menthol, and their binary mixtures. Ratios by weight.

inhibiting cytochrome P450 (Benet et al., 1998). A preparation could be made at which body temperature is used to melt a system comprising essential oil, CoQ_{10} and an emulsifier when the essential oil is added in an amount sufficient to lower the melting temperature of CoQ_{10} to or below 37 °C. Essential oils, however, should be effective as eutectic agents in the presence of other liquid

excipients. Table 1 demonstrates the feasibility of the described approach. Four essential oils, spearmint oil, peppermint oil, lemon oil, and anise oil, were evaluated for their eutectic efficacy in the presence of other formulation excipients. Due to limited solubility of CoQ_{10} in surfactants (Kommuru et al., 2001) the use of cremophor EL as a model emulsifier not only induces crystallization of CoQ_{10} in the cooled supersaturated mixture but also may delay or retard re-melting the system at higher temperatures. The time necessary to melt different combinations of CoQ_{10}, essential oil and cremophor EL at 37 °C was recorded. When 60% w/w of cremophor EL was added, preparations made with 50 and 60% w/w lemon oil to CoQ_{10} melted within 5.3 and 1.8 min, respectively. Precipitation of CoQ_{10} at higher cremophor EL concentration for the formulas made with anise oil, peppermint oil and spearmint oils was however irreversible rendering them less effective for the preparation of emulsified systems. The use of lemon oil appears reasonable and attractive. At 50% w/w of lemon oil to CoQ_{10}, formulas would melt within 5 min from initial exposure to body temperatures. In this case, recrystallization of CoQ_{10} becomes advantageous in the production of a stable semisolid product compared with the

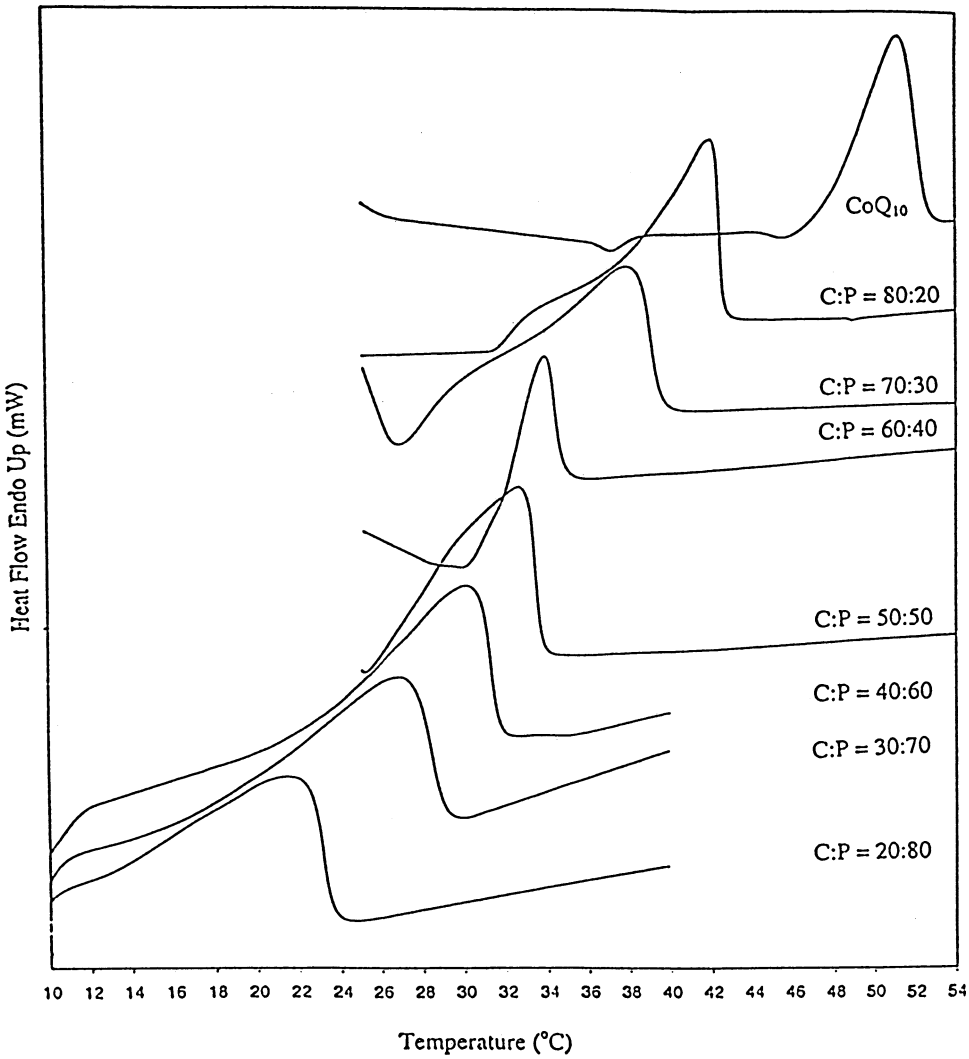

Fig. 4. DSC thermograms of CoQ_{10}, peppermint oil, and their binary mixtures. Ratios by weight.

tion. Within this area a ternary mixture forms a fine oil in water emulsion with only gentle agitation. This is possible as surfactants strongly localized to the surface of the emulsion droplet reduces interfacial free energy and provide a mechanical barrier to coalescence resulting in a thermodynamically spontaneous dispersion (Reiss, 1975). Furthermore, co-surfactants increase interfacial fluidity by penetrating into the surfactant film creating void space among surfactant molecules (Constantinides and Scalart, 1997). Constraints on the formulas were placed so that oil phase was not less than 37.5% to ensure melting of the crystallized product based on the early predictions given in Table 1, and did not exceed 63% to ensure efficient CoQ_{10} emulsification.

3.4. Droplet size analysis and turbidity measurements

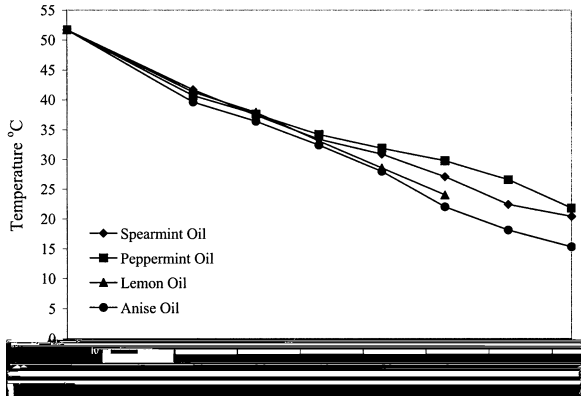

Fig. 5. The temperature/composition phase diagram of CoQ_{10}–essential oil binary systems determined by DSC.

The effect of surfactant to co-surfactant ratio on droplet size is given in Fig. 7. At ratios greater than 0.5, globule size was relatively constant at about 100 nm and independent on any component of the ternary system. It was only at ratios smaller than 0.5 when globule size increased and became greatly dependent on cremophor EL and capmul MCM-C8 concentrations yet independent

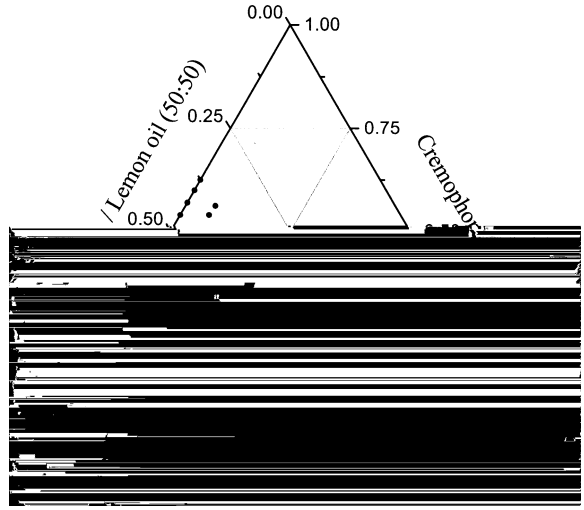

Fig. 6. Pseudo-ternary phase diagram indicating the efficient self-emulsification region (key, the region of efficient self-emulsification is bound by the solid line; and the filled circles represent the compositions which were evaluated).

on the added oil phase. Gao et al. (1998) have reported similar observations with the microemulsion containing captex-355, cremophor EL, transcutol and saline where the droplet size decreased with increasing surfactant to co-surfactant ratio and became constant at a ratio above 2:1. It was reported that the addition of surfactants to the microemulsion systems causes the interfacial film to stabilize and condense, while the addition of co-surfactant causes the film to expand (Constantinides and Scalart, 1997; Gao et al., 1998). Comparison of droplet size data with the visual observationsda.6(to)-3showa.6(to)4763.1(dal.,)-..8(was)]T6S

Table 2
Effect of SNEDDS composition on the emulsion droplet size distribution

Formulations	SNEDDS composition (% w/w)				SNEDDS emulsion droplet size (μm)						
	CoQ10	Lemon oil	Capmul	Cremophor	Mean	Span	*D*(0.1)	*D*(0.25)	*D*(0.5)	*D*(0.75)	*D*(0.9)
1	18.8	18.8	56.3	6.3	2.817	0.270	3.179	3.014	2.806	2.619	2.468
2	18.8	18.8	50.0	12.5	0.402	0.277	0.845	0.572	0.323	0.117	0.110
3	18.8	18.8	43.8	18.8	0.121	0.015	0.142	0.130	0.119	0.100	0.101
4	18.8	18.8	37.5	25.0	0.112	0.037	0.165	0.135	0.106	0.084	0.070
5	18.8	18.8	31.3	31.3	0.090	0.012	0.107	0.099	0.089	0.081	0.045
6	18.8	18.8	25.0	37.5	0.113	0.017	0.137	0.125	0.112	0.100	0.092
7	18.8	18.8	18.8	43.8	<0.040						
8	20.0	20.0	53.3	6.7	0.845	0.308	1.287	1.027	0.786	0.607	0.499
9	20.0	20.0	46.7	13.3	0.725	0.213	1.031	0.862	0.693	0.558	0.472
10	20.0	20.0	40.0	20.0	0.121	0.048	0.170	0.141	0.110	0.083	0.067
11	20.0	20.0	33.3	26.7	0.089	0.026	0.107	0.098	0.089	0.081	0.074
12	20.0	20.0	26.7	33.3							
13						0.025	0.117	0.107	0.099	0.091	

0.06781735 0 TD20.037.5 0.02.995 0 TD20.0

0.10158.8(3.179)-30002.8(0.862)-3292.4(0.119)-3101.3(0.084)]T10-5.024 0 TD[7130.1125

received at an angle normal to the concentric light beam provides indications on size and number of scattered particles (Groves and Mustafa, 1974). Light scattering by colloids conforms to Rayleigh theory, which predicts that light scattering or measured turbidity τ in a simplified equation can be given by

$$\tau = Knv^2$$

where K is a machine constant, v the particle volume and n is the number of particles (Groves and Mustafa, 1974; Pouton, 1985).

The effect of surfactant to co-surfactant ratio of the emulsified formulas on $NTU_{obsreved}$ and $NTU_{plateau}$ turbidity readings is given in Fig. 7. As seen in the plot, turbidity follows the same trend as droplet size. Pouton (1985) has reported a linear correlation between the intensity of the scattered light and the squared volume of the dispersed droplets. Hence, NTU could be directly used to predict relative droplet size of the emulsion. To give a sense about the clarity of the formulas, turbidity of drinking water ranges from 0 to 1 NTU (Hongve and Akesson, 1998).

3.5. Fourier transform-infrared spectroscopy (FT-IR)

The ease of handling aqueous solutions and semisolid preparations is one of the major advantages of ATR used in conjugation with FT-IR spectrometry (Yang and Her, 1999). CoQ_{10} compatibility with the excipients of self-nanoemulsified preparation can be tested with FT-IR. Absorbance spectrums of CoQ_{10} and lemon oil are given in Fig. 8. CoQ_{10} spectrum showed several (29) sharp characteristic peaks. The spectrum of the 50:50 melt of CoQ_{10} and lemon oil, given in Fig. 8, had features of each of the components with the expected peak broadening due to its amorphous character whereas a sample of the solidified mixture had sharp lines and resembled the CoQ_{10} spectrum in every detail. Lemon oil did not change the infrared spectrum of CoQ_{10} indi-

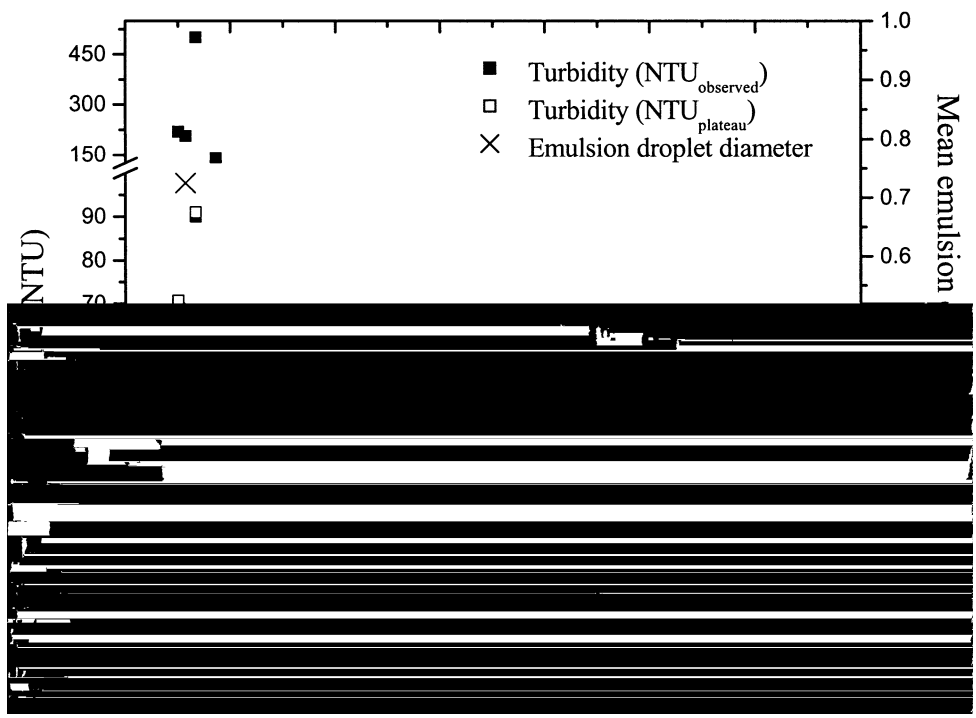

Fig. 7. Effect of surfactant (cremophor EL) to co-surfactant (capmul MCM-C8) ratios on mean droplet size diameter, and on $NTU_{observed}$ and $NTU_{plateau}$ turbidity values.

Table 3
Effect of SNEDDS composition on turbidity readings and the cumulative amount of CoQ10 released after 15 min

Formulations	SNEDDS composition (% w/w)				SNEDDS turbidity			CoQ10 released (15 min)	
	CoQ10	Lemon oil	Capmul	Cremophor	NTUobsrved	NTUexp	NTUplateau	Percent released	STD
1	18.8	18.8	56.3	6.3	605.5				
2	18.8	18.8	50.0	12.5	220.0	78.2	70.7	94.0	2.18
3	18.8	18.8	43.8	18.8	25.1	8.9	19.5	90.3	7.87
4	18.8	18.8	37.5	25.0	9.0	3.2	6.0	92.8	2.52
5	18.8	18.8	31.3	31.3	6.9	2.5	4.8	88.8	2.52
6	18.8	18.8	25.0	37.5	5.3	1.9	4.1	88.0	2.84
7	18.8	18.8	18.8	43.8	2.4	0.8	3.1	87.4	4.42
8	20.0	20.0	53.3	6.7	513.0				
9	20.0	20.0	46.7	13.3	207.0	69.0	51.7	85.0	1.14
10	20.0	20.0	40.0	20.0	32.7	10.9	13.8	87.3	1.14
11	20.0	20.0	33.3	26.7	12.0	4.0	5.7	91.0	5.35
12	20.0	20.0	26.7	33.3	7.0	2.3	3.5	96.3	1.28
13	20.0	20.0	20.0	40.0	4.5	1.5	3.0	99.5	0.64
14	21.4	21.4	50.0	7.1	510.5				
15	21.4	21.4	42.9	14.3	90.1	28.0	52.0	89.8	3.98
16	21.4	21.4	35.7	21.4	20.1	6.2	10.3	94.7	0.05
17	21.4	21.4	28.6	28.6	10.6	3.3	4.1	94.7	1.12
18		21.4	21.4	35.7	5.9	1.8	2.7		

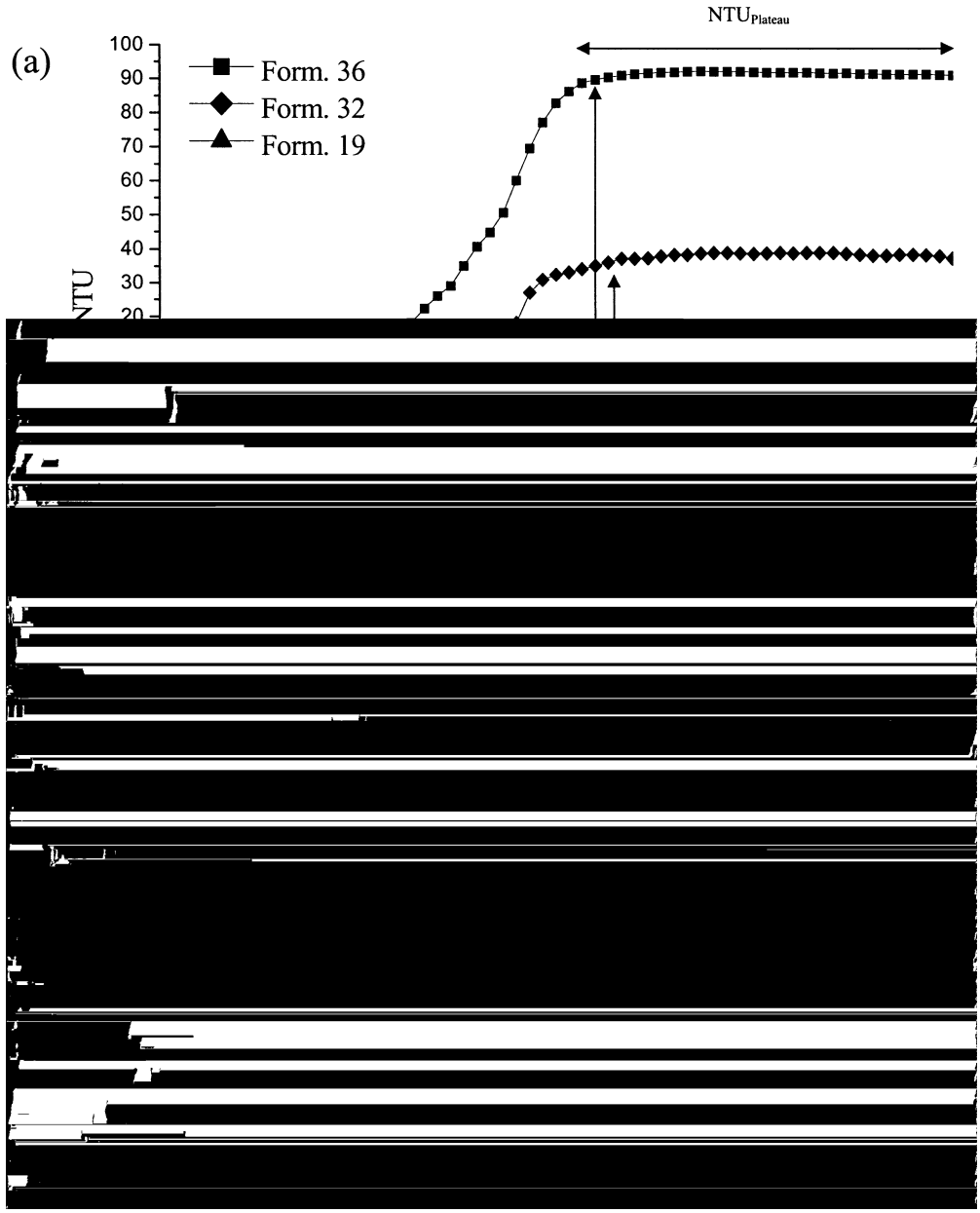

Fig. 9. (a) Turbidity–

At a ratio of 1:1:3 the mixture revealed distinctive liquid crystalline textures when observed under the polarizing microscope as shown in Fig. 11c–e. Fig. 11c shows a birefringent texture of the 1:1:3 mixture with defect features similar to the so-called parabolic focal conic domains in lamellar (smectic) liquid crystals (Kleman, 1983). Fig. 11d shows another typical defect of the lamellar phase: the so-called oily streaks that represent the bundles of linear defects- dislocations, sometimes decorated with chains of focal conic domains (Boltenhagen et al., 1991). Fig. 11e shows an air bubble trapped in the mixture as seen under a regular setting with two crossed polarizers. The liquid crystal material is aligned near the surface of the bubble. Polarizing microscope fitted with crossed polarizers, with a quartz wedge inserted between the sample and the analyzer, showed a change in the interference colors around the air bubble that is consistent with an idea that the mixture is a lamellar type of the lyotropic liquid crystal (Kurik and Lavrentovich, 1983). Hence, a delay in the spontaneity of emulsion formation might be due to the time required for the transformation from one liquid crystalline structure to another during the first stages of the disruption process (Groves and de Galindez, 1976).

A cumulative percent of the formulation emulsified with time could be obtained by plotting

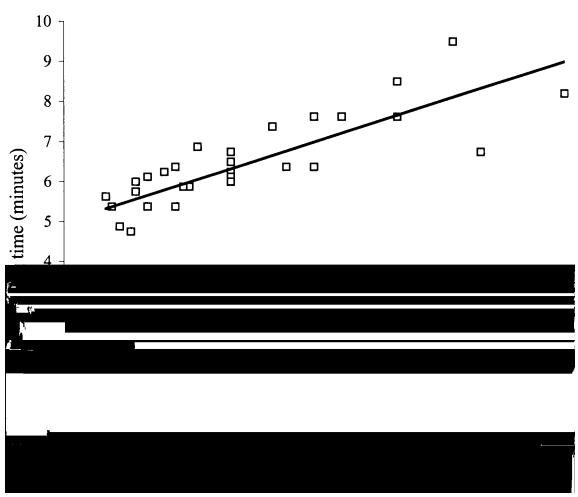

Fig. 10. Effect of surfactant (cremophor EL) to co-surfactant

cumulative turbidity given by $^{t}_{NTU} \times 100/NTU_{plateau}$ as a function of time, assuming that $NTU_{plateau}$ reflect 100% of the formula released from the capsules regardless of the actual amount of CoQ_{10} dissolved in the medium (Fig. 9b). $^{t}_{NTU}$ is the turbidity reading at any time *t*. Given re-arrangement allows directly correlating formulations with different turbidity–time profiles. As seen from Fig. 9b, plots of cumulative percent of the formulation released with time are identical to the original profiles correlating turbidity with time where curve characteristics mainly lag time, pseudo linear phase and plateau are preserved. Thus, slope of the pseudo linear phase for the line correlating cumulative percent emulsified with time could be regarded as the emulsification rate (E_{rate}) or emulsification efficacy. This value is very useful in comparing emulsification tendency of the self-emulsified preparation. Fig. 12 correlates emulsification rate with oil loading and surfactant to co-surfactant ratios. Maximum emulsification rate was obtained at a surfactant to co-surfactant ratio of 1 and oil loading of 42.6%. This might be due to an optimum HLB of the mixture. Bachynsky et al. (1997) showed that the HLB of the surfactant has a significant effect on the performance of the self-emulsifying system. However, optimum surfactant mixture should be obtained at an appropriate combination with the oily phase (Shah et al., 1994). At higher concentrations of the oily phase, proportion of the surfactant mix that facilitates water penetration decreases and the mixture becomes more lipophilic with increasing difficulty of emulsification (Halbaut et al., 1996). Thus, emulsion progress becomes less dependent on surfactant and co-surfactant concentrations.

4. Conclusion

The present investigation illustrated the potential use of eutectic mixtures with essential oils for the preparation of SNEDDS. Prepared SNEDDS improved the dissolution of CoQ_{10}. Recrystallization may add to the stability of the drug while providing attractive semisolid preparation that could be filled into hard HPMC capsules. Tur-

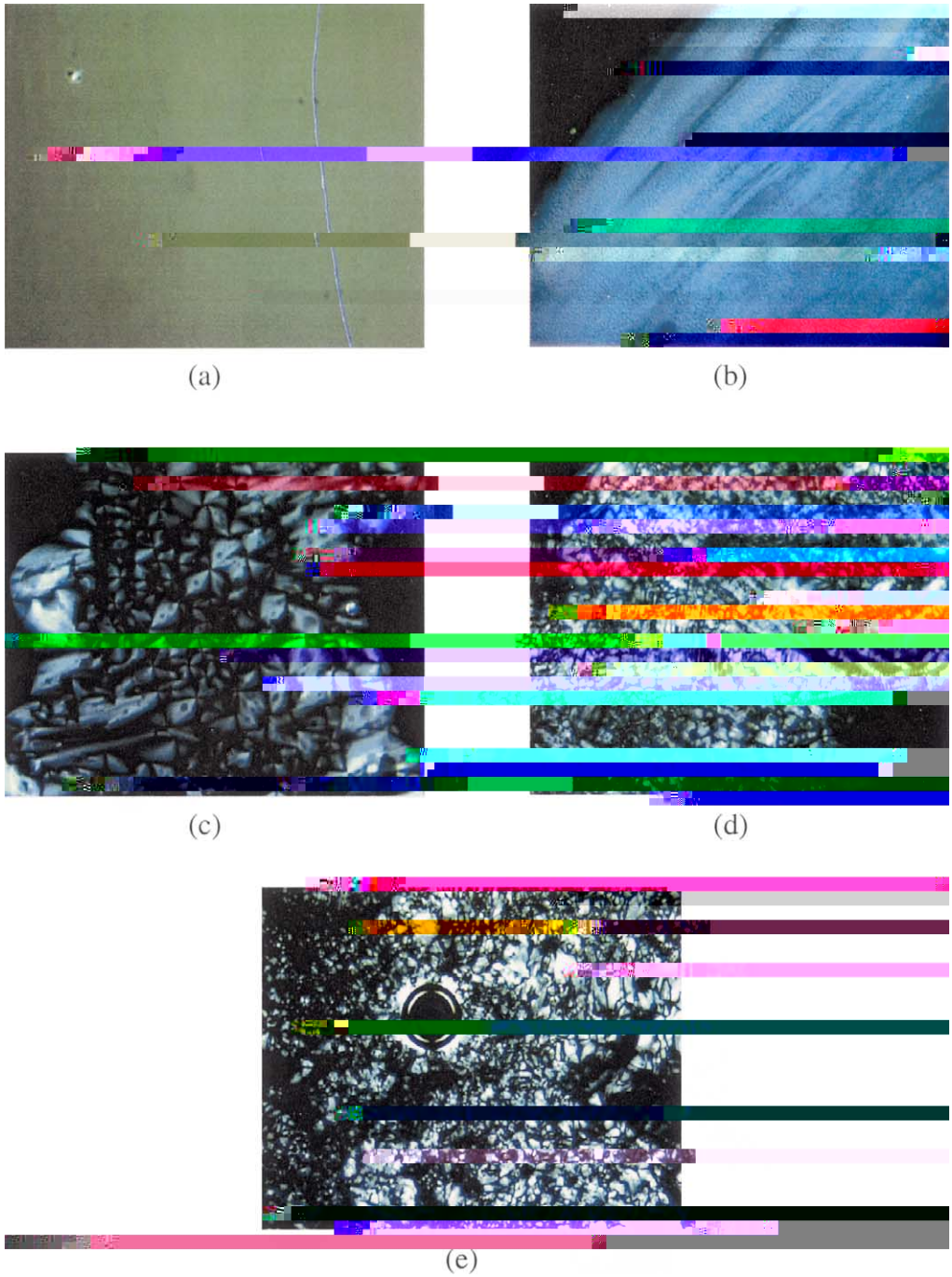

Fig. 11. (a) Optical microscope with no polarizers showing the texture of the surfactant, co-surfactant contact line; no birefringence. Polarizing microscope with crossed polarizers showing (b) birefringent texture of a gel formed in the mixture surfactant:water in weight proportion 1:3, (c) birefringent liquid crystalline texture of surfactant:co-surfactant:water mixture at a weight proportion of 1:1:3, (d) birefringent liquid crystalline texture with oily streaks at a mixture of 1:1:3, and (e) birefringent liquid crystalline texture of the mixture 1:1:3 with a trapped air.

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