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International Journal of Pharmaceutics 285 (2004) 109–119
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Preparation and in vitro characterization of self-nanoemulsified drug delivery system (SNEDDS) of all-trans-retinol acetate

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Received 22 August 2003; received in revised form 18 March 2004; accepted 18 March 2004
Available online 11 September 2004

Abstract

Purpose: To prepare a self-nanoemulsified drug delivery system (SNEDDS) of all-trans-retinol acetate, with enhanced dissolution and better chance of oral absorption.

Method: All-trans-retinol acetate SNEDDS was prepared using different concentrations of soybean oil (solvent) Cremophor EL (surfactant) and Capmul MCM-C8 (co-surfactant). Particle size and turbidity of the SNEDDS were determined after adding water to the oily solution. Dissolution profile of SNEDDS filled in hydroxyl propyl methyl cellulose (HPMC) capsules was determined by using water in USP apparatus 2. Ternary phase diagrams were constructed to identify the self-nanoemulsified region. The SNEDDS were evaluated by the visual observation, turbidity in nephrometric turbidity units (NTU), mean particle size (μm) and Fourier transformed-infrared spectroscopy (FT-IR). SNEDDS were thermally characterized using differential scanning calorimetry (DSC) to ensure the compatibility of the SNEDDS ingredient.

Results: From the data obtained in this work, it was clear that surfactant to co-surfactant ratio has the main impact on the physical characteristics of the emulsion formed. The optimum surfactant to co-surfactant ratio was found to be 2:1 (37.5–50% for Cremophor EL, and 18.75–25% for Capmul MCM-C8). With this ratio, the resultant nanoemulsions obtained have a particle size range of 0.103–0.051 μm, turbidity range of 18.12–2.18 NTU and t_{30} values (cumulative% all-trans-retinol acetate dissolved in 30 min) of 90.42–99.5. Also the thermograms obtained from DSC experiments showed that there is no incompatibility or interaction between the SNEDDS ingredients (soybean oil, Cremophor EL, and Capmul MCM-C8) and all-trans-retinol acetate.

Conclusion: The present study revealed that the self-nanoemulsified drug delivery system of all-trans-retinol acetate increased its dissolution rate and has the potential to enhance its bioavailability without interaction or incompatibility between the ingredients.

Keywords: Preparation; In vitro characterization; Self-nanoemulsified drug delivery system (SNEDDS); All-trans-retinol acetate

1. Introduction

Vitamin A is fat-soluble vitamin, which is an essential nutrient for humans. It is involved in several important biological functions including vision, growth, reproduction, and the differentiation and

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doi:10.1016/j.ijpharm.2004.03.034

maintenance of epithelial tissue (Sporn et al., 1994; Olson, 1994). All-trans retinoic acid has recently been reported to improve treatment results in patients with some malignant cancers (Lanvers et al., 1996), and epithelial tumors (Stich et al., 1988; Baranowwitz, 1994). Vitamin A deficiency is common in conditions such as inflammatory bowel diseases and celiac sprue (Krasinski et al., 1984). Also patients with pancreatic and intestinal diseases develop fat malabsorption, which is an important factor in causing vitamin A deficiency (Smith and Goodman, 1971).

The lipophilicity of all-trans-retinol acetate provides a challenge for its delivery by the oral route. Various attempts have been made for the preparation and bioavailability enhancement of vitamin A or vitamin A acetate (Lin et al., 2000; Kirilenko and Gregoriadis, 1993; Shelley et al., 1982). Shelley et al. (1982) reported that all-trans retinoic acid microcapsules were found to be approximately 34% as bioavailable as the solution dosage form and the micro fine suspension 93% as bioavailable. The bioavailability of all-trans retinoic acid in oral solution dosage form was approximately 40% of the intravenous dose. Lin et al. (2000) reported that inclusion of all-trans retinoic acid in 2-hydroxypropyl-beta-cyclodextrin increases its the aqueous solubility suggesting that it might enhance its bioavailability. Kirilenko and Gregoriadis (1993) investigated the possible role of liposomes in facilitating the absorption of vitamin A after oral administration in situations where vitamin A absorption is impaired.

The absorption of vitamin A is governed by factors that determine the absorption of lipids. During digestion, vitamin A ester is hydrolyzed to vitamin A alcohol (retinol) by pancreatic and intestinal enzymes and then emulsified by bile acids. Therefore, any disturbance of gastrointestinal enzymes or pancreatic enzymes will lead to disturbance of vitamin A absorption (Elizabeth et al., 1992). Therefore it is conceivable that a SNEDD preparation, which relies on its surfactant/co-surfactant for emulsification rather than the bile acids, has a much greater chance of absorption.

Therefore in the present study, SNEDD formulations from which all-trans-retinol acetate is released in a soluble form based on self-nanoemulsified system are prepared and characterized.

2. Materials and methods

All-trans-retinol acetate, soybean oil, corn oil, olive oil and isopropyl alcohol were purchased from Sigma Chemicals Co. (NJ). Polyoxyl 35 castor oil (Cremophor EL) was obtained from BASF Corp. (Mount Olive, NJ). Capmul MCM-C8 was obtained from Abitec Corp. (Jamesvills, WI). HPMC capsules were supplied by Shionogi Qualicaps (Whitsett, NC). All chemicals were used as received.

2.1. Determination of all-trans-retinol acetate solubility in fixed oils

Solubility of all-trans-retinol acetate in three different fixed oils namely olive oil, corn oil and soybean oil was initially investigated. Excess all-trans-retinol acetate was added to 2 g of each of the oils and the mixtures were stirred using magnetic stirrer and covered with aluminum foil to protect all-trans-retinol acetate from light. Aliquots were taken at different time points to determine the amount of all-trans-retinol acetate until it reached the equilibrium solubility. As shown in Table 1, all-trans-retinol acetate was found to be very soluble in all the investigated fixed oils, but its solubility in soybean oil (1.55 g/1 g) was higher than corn oil (1.2 g/1 g) and olive oil (1.05 g/1 g). Therefore, soybean oil was chosen to formulate all-trans-retinol acetate SNEDDS.

2.2. Preparation of self-emulsified systems

A series of self-emulsifying systems were prepared with fixed concentrations of the drug (25% W/W) and varying concentrations of soybean oil (0–67.5%), Cremophor El (3.75–56.25%) and Capmul MCM-C8 (1.5–37.5%). The ingredients were accurately weighed and mixed using magnetic stirrer until a clear solution was obtained. Sixty milligrams of the preparation obtained (equivalent to 15 mg all-trans-retinol acetate)

Table 1
Solubility of vitamin A acetate in fixed oils

Oil	Solubility of vitamin A acetate g/1 g of oil
Olive oil	1.05
Corn oil	1.2
Soybean oil	1.55

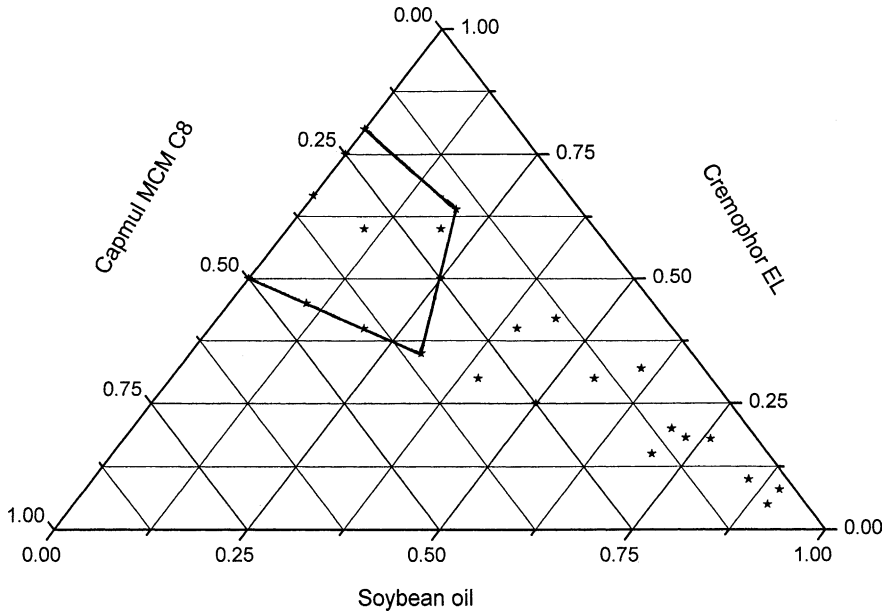

Fig. 1. Ternary phase diagram shows the efficient self-emulsification region.

were filled into HPMC size 3 capsules. The filled capsules were stored in a refrigerator until their use in subsequent studies.

2.2.1. *In vitro characterization of the prepared self-nanoemulsified formulations*

The prepared formulations were subjected to the following characterization studies.

2.2.1.1. Visual observations. To assess the self-emulsification properties, formulation (60 mg) 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; Nazzal et al., 2002). Phase diagram was constructed identifying the good self-emulsifying region. All studies were repeated in triplicates and the mean values are shown in Fig. 1. Table 2 shows all the formulation composition.

2.2.1.2. Emulsion droplet size analysis. To assess the self-emulsification properties, each formulation (60 mg) was introduced into 100 ml of water at 25 °C and the contents were gently stirred manually. The

Table 2
Vitamin A acetate SNEDDS composition (%W/W)

Formulation no.	Vitamin A acetate	Soybean oil	Cremophor EL	Capmul MCM-C8
1	25	67.5	3.75	3.75
2	25	60	7.5	7.5
3	25	52.5	11.25	11.25
4	25	37.5	18.75	18.75
5	25	30	22.5	22.5
6	25	22.5	26.25	26.25
7	25	15	30	30
8	25	7.5	33.75	33.75
9	25	0	37.5	37.5
10	25	63.75	7.5	3.75
11	25	52.5	15	7.5
12	25	41.25	22.5	11.25
13	25	30	30	15
14	25	18.75	37.5	18.75
15	25	7.5	45	22.5
16	25	0	50	25
17	25	57	13.5	4.5
18	25	33	31.5	10.5
19	25	15	45	15
20	25	0	56.25	18.75
21	25	67.5	6	1.5
22	25	45	24	6
23	25	15	48	12
24	25	0	60	15

Table 3
Visual observation, mean particle size, turbidity and cumulative percent release after 30 min for vitamin A acetate SNEDDS formulations

Formulation no.	% Release after 30 min	Mean particle size (μm)	Turbidity (NTU)	Visual observation
1	3.91	9.604	>20	Bad
2	5.01	8.455	>20	Bad
3	13.9	4.013	>20	Bad
4	39.62	1.104	>20	Bad
5	55.99	0.311	>20	Bad
6	84.52	0.046	9.97	Good
7	84.68	0.043	7.53	Good
8	86.1	0.039	5.58	Good
9	86.23	0.035	3.33	Good
10	11.95	5.964	>20	Bad
11	28.82	2.891	>20	Bad
12	40.09	1.77	>20	Bad
13	42.74	1.54	>20	Bad
14	90.42	0.103	18.12	Good
15	94.41	0.034	2.64	Good
16	99.5	0.051	2.18	Good
17	32.01	3.052	>20	Bad
18	66.1	1.279	>20	Bad
19	75.92	0.109	11.82	Good
20	64.94	0.77	2.58	Good
21	50.08	1.603	>20	Bad
22	62.17	1.017	>20	Bad
23	80.34	0.129	2.03	Good
24	44.55	0.1	2.24	Good

mean particle size distribution of the resultant emulsions was determined by laser diffraction analysis (NiComp Particle Size system ZW380 Version-2 Santa Barbara, California, USA) and is given in Table 3. 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 10 min. Particle size was calculated from the volume size distribution. All measurements were done in duplicate and the mean values are reported in Table 2.

2.2.1.3. Turbidity measurements. Turbidity of the resultant emulsions given in nephlometric turbidity unit (NTU) was measured using Orbeco-Hellige model 966, Orbico Analytical System Inc., Farmingdale, NY, USA.

2.2.1.4. Fourier transform-infrared spectroscopy. FT-IR spectroscopy was performed using Nexus 470 FT-IR: Thermo Nicolet Corporation, Madison, WI, USA. An attenuated total reflectance (ATR) accessory was attached to the machine. ATR was fitted with single bounce diamond at 45° internally reflected incident lights providing a sampling area of 1 mm in diameter with a sample depth of several microns. Sample analyzed were all-trans-retinol acetate, soybean, all-trans-retinol acetate and soybean oil mixture in a ratio of 1:1, and all-trans-retinol acetate, soybean, Cremophor EL and Capmul MCM-C8 in a ratio of 1:1:1:1. A small amount of the samples was directly placed on the diamond disk and scanned for absorbance over the range from 4000 to 400 wave numbers (cm^{-1}) at a resolution of cm^{-1}.

2.2.1.5. Dissolution studies. Dissolution profiles of the capsules filled with the self-nanoemulsified formulations were determined using USP24 rotating paddle apparatus (VK7000) at 37 ± 0.5 °C and a rotating speed of 50 rpm in 900 ml of water. Capsules were held to the bottom of the vessel using copper sinkers. Samples (3 ml) withdrawn after 5, 10, 15, 20, and 30 min were filtered using a 10 μm VanKel filter and assayed for all-trans-retinol acetate using

spectrophotometric method at 326 nm (Varaporn et al., 2001). The dissolution experiments were carried out in triplicates.

2.2.1.6. Differential scanning calorimetry. Samples of 2–8 mg of the individual substances and 1:1 physical mixture of all-trans-retinol acetate and additives (Soybean oil, Cremophor EL, and Capmul MCM-C8) were accurately weighed, encapsulated and hermetically sealed in flat bottomed aluminum pan with crimped on lid. The pans were positioned on sample pan holder of a Perkin–Elmer DSC7. The samples were heated in an atmosphere of nitrogen over a temperature range from 30 to 250 °C with a constant heating rate of 10 °C/min. Thermograms were obtained by the DSC7 thermal analyzer program and recorded at constant chart speed of 1 in./min. The thermogram, transition temperature range, the onset of peak transition and the maximum peak of transition were recorded using Okidata Microline 320 and 9 Pin Printer. At least two replicates were made for each DSC thermogram using an empty sealed aluminum pan as reference and indium as instrument calibration standard. The results are shown in Fig. 10.

3. Results and discussion

3.1. Visual observations

For the development of a self-emulsified formulation, a right blend of low and high HLB (hydrophile lipophile balance) surfactant is necessary for the formation of a stable microemulsion (Craig et al., 1995; Pouton, 2000). Therefore, a high HLB surfactant (Cremophor EL with an average HLB of 13) and a low HLB co-surfactant (Capmul MCM-C8 with an average HLB of 3.5) were selected. It is important that the excipients used are listed as GRAS (generally regarded as safe) in the Handbook of Pharmaceutical Excipients (published by the American Pharmaceutical Association) or they have a history of use in marketed formulations. Cremophor EL has a history of use in several formulations that are commercially available (for example Norvir™ capsules by Abbott Laboratories, Retrovir® capsules by Glaxo Smith Kline, and Sandimmun® tablets by Novartis). The use of Capmul MCM C-8 has been reported in several formulations (Nazzal et al., 2002) and its drug master file (DMF) has been filed. Prior to the selection of Cremophor EL and Capmul MCM C-8 several other blends were attempted, but the results were unsatisfactory. Spontaneity of the formulations was evaluated as described in Section 2. The ternary phase diagram of the system comprising the Cremophor EL, Capmul MCM C-8 and soybean oil was constructed (Fig. 1). All the components were converted to percent weight per weight before constructing the phase diagram. The area enclosed within the solid line represents the region of self-emulsification. Within this area the SNEDDS form fine oil in water emulsion with only gentle agitation. This is possible as surfactant strongly localized to the surface of the emulsion droplet reduces interfacial free energy and provide a mechanical barrier to coalescence resulting in a thermomechanically spontaneous dispersion (Reiss, 1975). Furthermore, co-surfactant increases interfacial fluidity by penetrating into the surfactant film creating void space among surfactant molecules (Constantinides and Scalart, 1997). In the present case, Capmul MCM C-8 is likely to increase the interfacial fluidity of Cremophor EL boundaries in the micelles because of the entrapment of low HLB surfactant in the high HLB surfacatant.

3.2. Emulsion droplet size analysis

Nanoemulsions are characterized by the droplet size in nanometer size range. Therefore particle size analysis was performed to see whether the resultant emulsions are indeed nanoemulsions. As seen in Table 3, one half of all the formulations prepared (5–9, 14–16, 19, 20, 23, and 24) are in nanometer size range while others are in micrometer size range.

A careful observation in Table 3 shows that the amount of all-trans-retinol acetate dissolved in 30 min (t_{30} values) increases when the particle size decreases. For convenience, representative figure was plotted to show the inverse relationship of particle size with dissolution (Fig. 2). Similar trend was observed with all the other formulations.

Fig. 3 shows the effect of surfactant and co-surfactant on the particle size distribution of the formulations. From the figure it is clear that increasing the concentration of both surfactant and co-surfactant lead to decreasing particle size which means that the

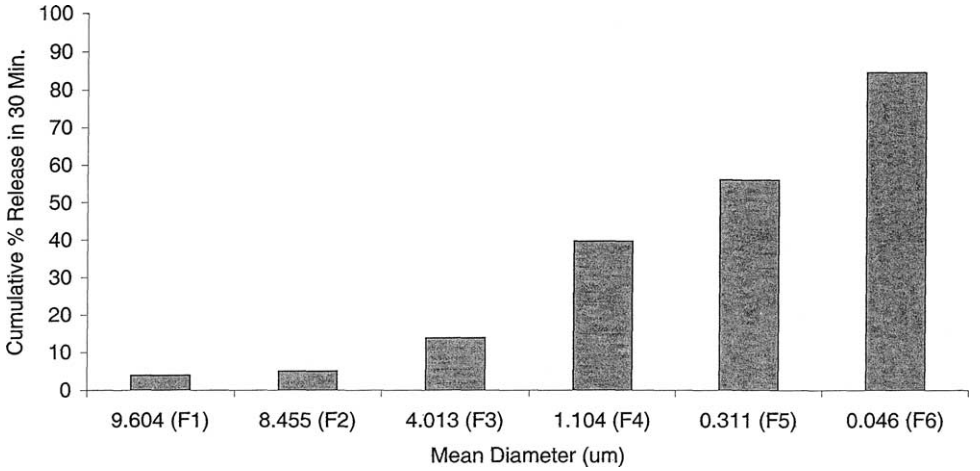

Fig. 2. Relationship between mean diameter of particle size and cumulative percent release of vitamin A acetate formulations (F1 to F6).

particle size distribution is inversely proportional to the concentration of surfactant and co-surfactant. From Tables 2 and 3 it can be seen that the surfactant and co-surfactant ratio of 26.25:26.25 (formulation 6) to 37.5:37.5 (formulation 8) results in the production of particle size ranging from 0.046 to 0.039 m, similarly surfactant to co-surfactant ratio of 45:22.5 (formulation 15) to 50:25 (formulation 16) gave particle size between 0.034 and 0.051 μm. Therefore using a proper ratio of surfactant and co-surfactant results in production of formulation in nano range particle size.

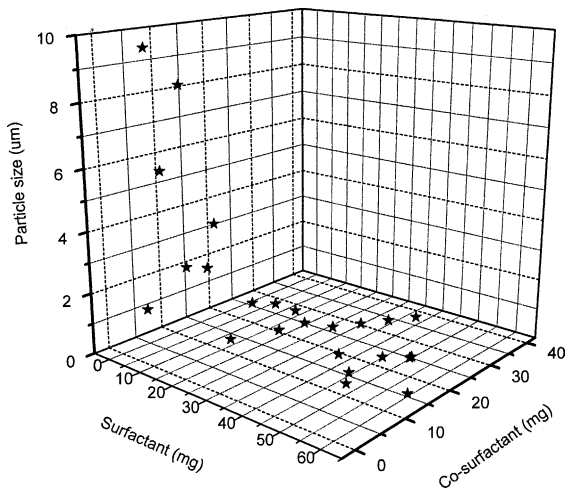

Fig. 3. Correlation between surfactant, co-surfactant and particle size diameter for vitamin A acetate formulations.

3.3. Turbidity

Turbidity values have been reported to be of use in SNEDDS characterization (Nazzal et al., 2002). In the turbidity measurement, the amount of scattered light (when an incident light is subjected to strike small particles) is measured and used in turbidity calculations as per the Rayleigh's theory (Pouton, 1985). Light scattering by colloids conforms to Raleigh theory, which predicts that light scattering or measured turbidity τ in a simplified equation can be given by

$$\tau = Knv^2$$

in which K is a machine constant, v is particle volume and n is the number of particles (Pouton, 1985). The turbidity measurements may be reasonable compromise when dissolution of a drug from SNEDDS cannot be measured because of low solubility of drugs.

The measured turbidity of the formulations is given in Table 3. From the table, it can be seen that there is a good correlation between the visual observation and turbidity of all formulations. From Table 3 we can also notice that the formulation that gives high turbidity (>20) gave a particle size diameter of more than 1 μm indicating a direct correlation between the particle size and the turbidity. Regarding the relation between the turbidity, surfactant and co-surfactant concentrations, from Tables 2 and 3, it is obvious that surfactant and co-surfactant ratio of 26.25:26.25 (formulation 6) to 37.5:37.5 (formulation 8) results in

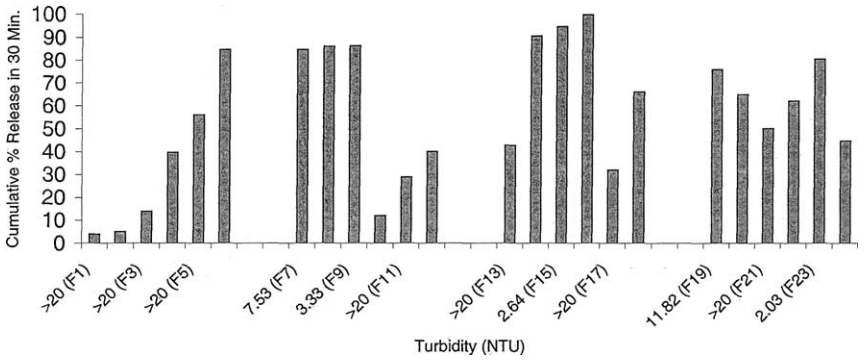

Fig. 4. Relationship between mean turbidity (NTU) and cumulative percent release of vitamin A acetate formulations (F1 to F24).

turbidity ranging from 9.97 to 5.58 NTU. Also, surfactant to co-surfactant ratio of 37.5:18.75 (formulation 15) to 50:25 (formulation 16) gave turbidity values between 18.12 and 2.18 NTU. Similarly the surfactant to co-surfactant ratio of 45:15 to 56.25:18.75 and 48:12 to 60:15 gave turbidity ranging from 11.82 to 2.58 and 2.03 to 2.24, respectively. From Fig. 4 it can be concluded that in most of the prepared formulations, there is a good correlation between turbidity and dissolution rate of all-trans-retinol acetate from the prepared formulations.

3.4. Fourier transform-infrared spectroscopy

FT-IR spectrums are mainly used to determine if there is any interaction between the drug and any of the excipient used (Nazzal et al., 2002). The presence of interaction is detected by the disappearance of important functional group of the drug (Fig. 5).

All-trans-retinol acetate compatibility with the ingredients of self-nanoemulsified formulations was tested using FT-IR (Fig. 6). The absorbance spectrums of all-trans-retinol acetate and soybean oil showed several characteristic peaks. The spectrum of all-trans-retinol acetate and soybean oil mixture had the feature of each of the components and did not change the infrared spectrum of all-trans-retinol acetate. This indicated that there is no chemical interaction in the binary system and that the molecular structure of all-trans-retinol acetate remained completely intact. Similarly, in the mixture of all-trans-retinol acetate, soybean oil, Cremophor EL and Capmul MCM C8, the resulting infrared spectrum had the characteristic of vitamin A acetate bands. Therefore all-trans-retinol acetate did not interact with any one of the self-nanoemulsified components.

3.5. Dissolution studies

The dissolution profiles obtained are shown in Fig. 7. The negative control without Cremophor EL or Capmul MCM-C8 did not show any release of all-trans-retinol acetate. The release of all-trans-retinol acetate from the investigated SNEDD formulations was markedly different from one formulation to another. Fig. 8 indicates that there is a good correlation between drug release rate and both the particle size distribution and the measured turbidity on most of the formulations, with the exception of formulation F20.

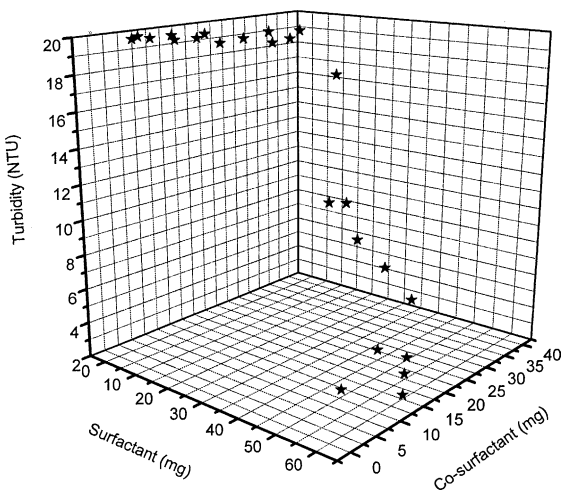

Fig. 5. Correlation between surfactant, co-surfactant and turbidity for vitamin A acetate formulations.

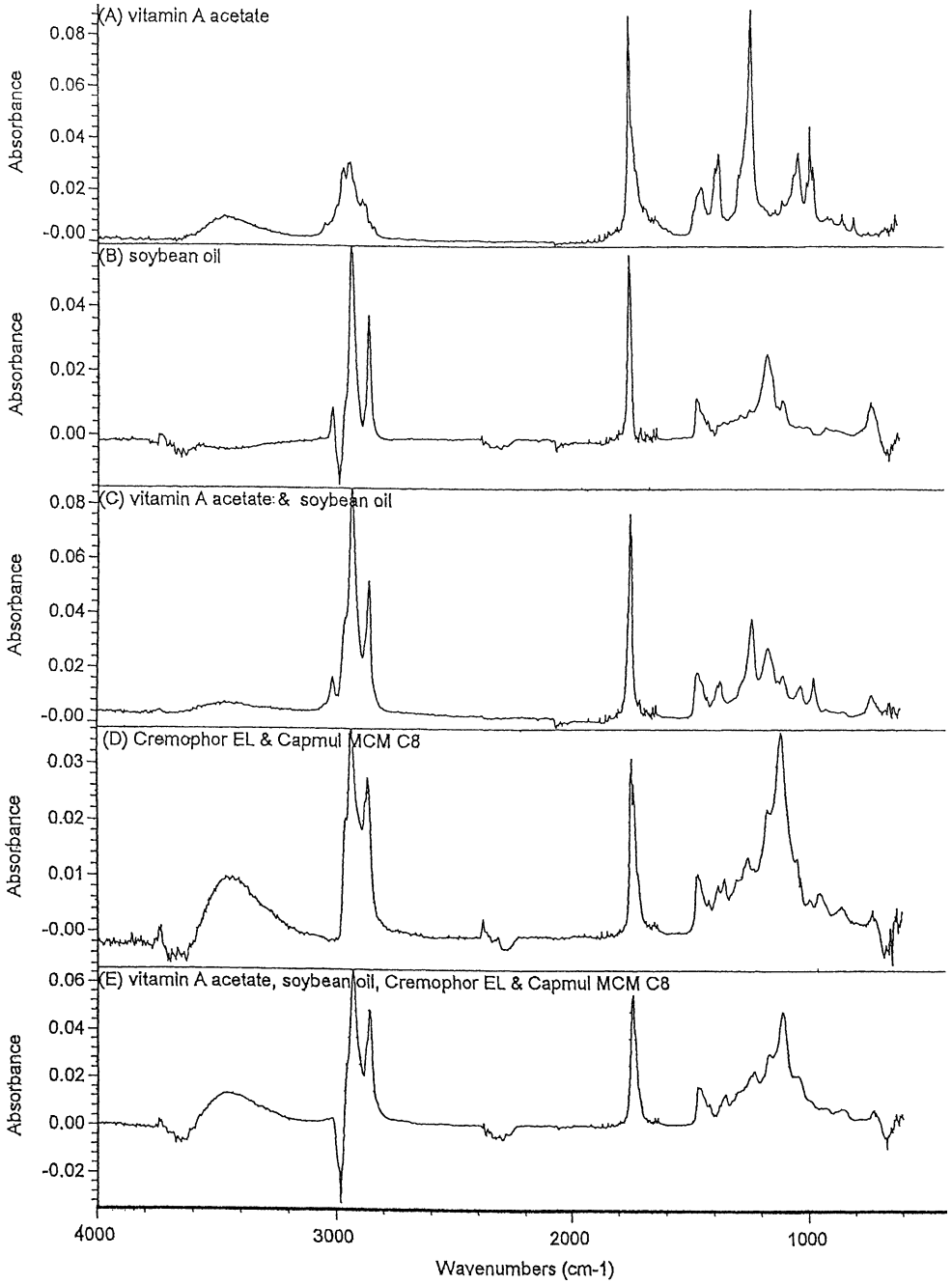

Fig. 6. FT-IR spectra of vitamin A acetate (A), soybean oil (B), vitamin A acetate and soybean oil mixture(C), Cremophor EL and Capmul MCM-C8 (D), and vitamin A acetate, soybean oil, Cremophor EL and Capmul MCM-C8 mixture (E).

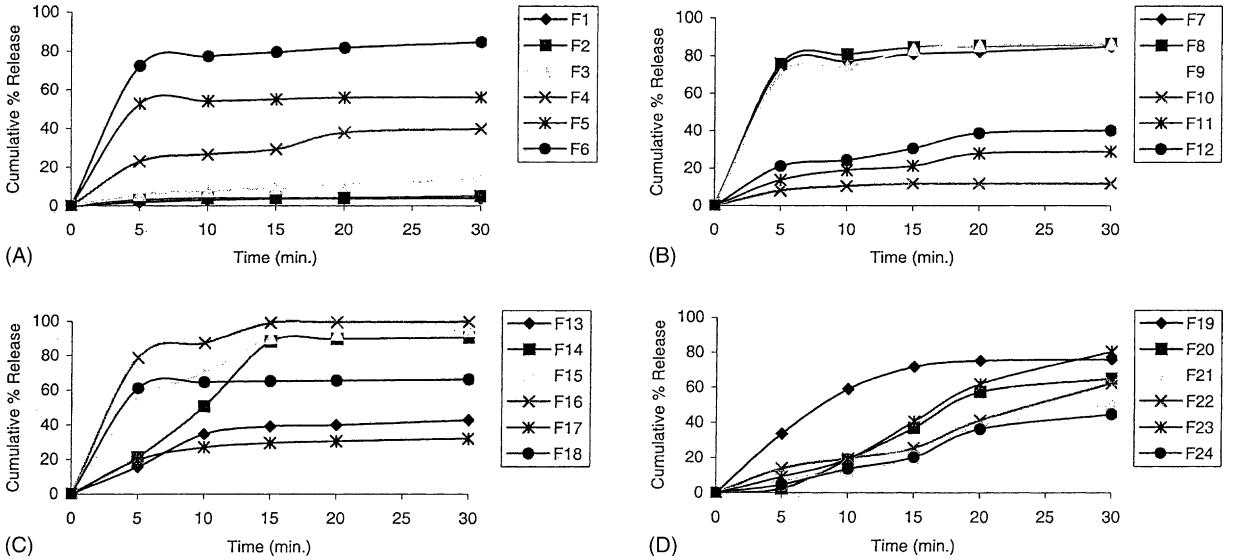

Fig. 7. Cumulative percent release of vitamin A acetate from (A) formulations F1 to F6, (B) formulations F7 to F12, (C) formulation F13 to F18, and (D) formulations F19 to F24.

The formulation F20 gave 64.94% of all-trans-retinol acetate release although its particle size and turbidity were 0.77 μm and 2.58 NTU, respectively. Similarly formulation F24 gave 44.55% all-trans-retinol acetate release when its particle size and turbidity were 0.1 μm and 2.24 NTU, respectively. There was also a good correlation between all-trans-retinol acetate release rate and the surfactant and co-surfactant concentration. It is obvious from Fig. 9 that increasing the concentration of co-surfactant led to increased dissolution rate of all-trans-retinol acetate on most of the formulations. From Table 3 one can conclude that surfactant to co-surfactant ratio of 2:1 was found to give the highest amount of all-trans-retinol acetate release – 90.42 (F14), 94.41 (F15) and 99.5 (F16) – followed by surfactant to co-surfactant ratio of 1:1

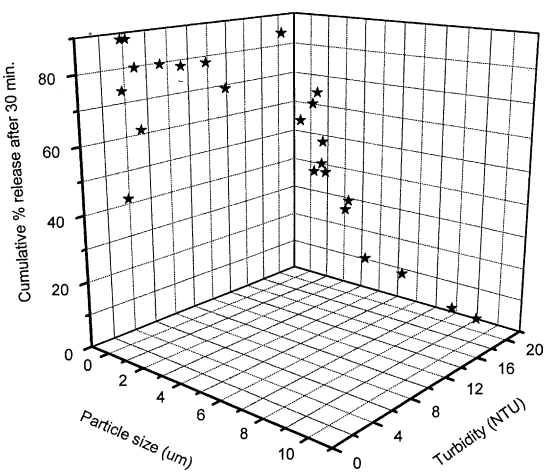

Fig. 8. Correlation between particle size diameter, turbidity and cumulative percent of vitamin A acetate formulations.

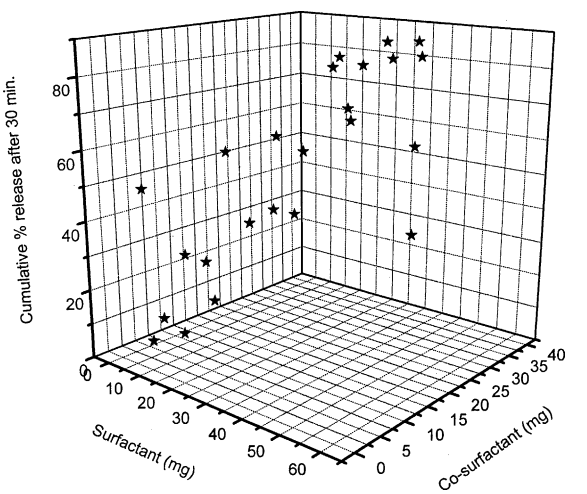

Fig. 9. Correlation between surfactant, co-surfactant and cumulative percent release for vitamin A acetate formulations.

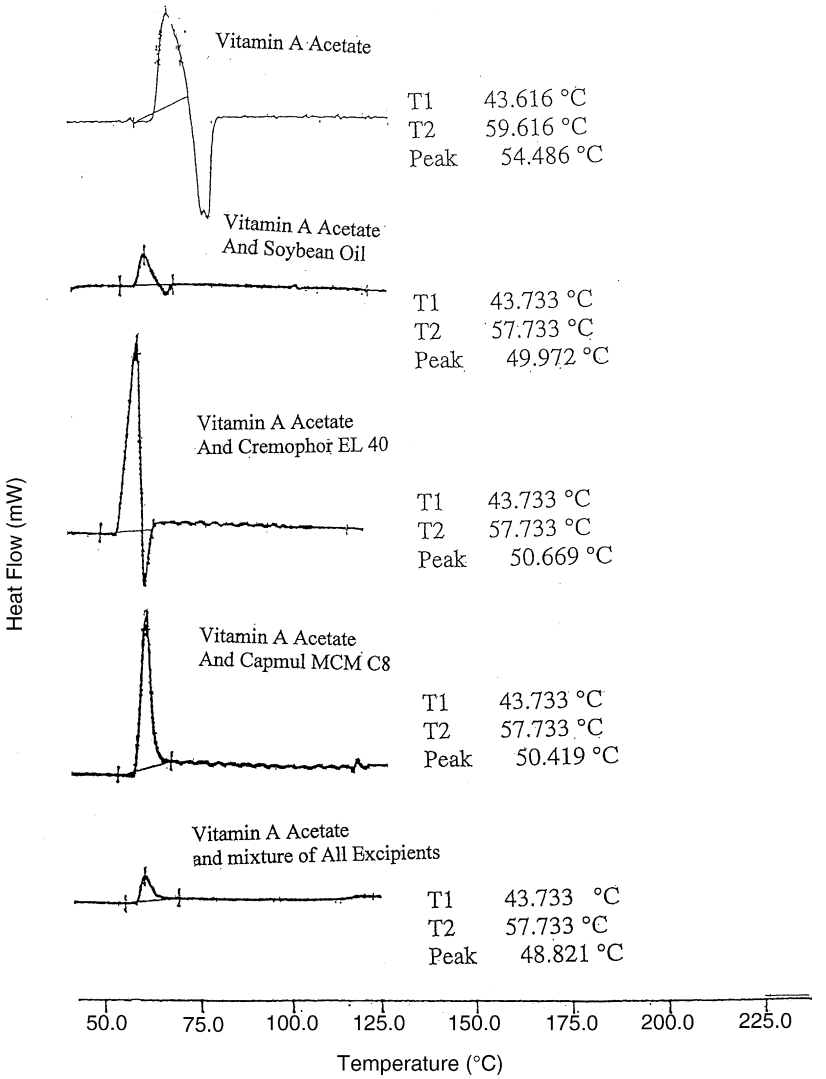

Fig. 10. DSC thermograms of vitamin A acetate and soybean oil mixture (1:1), vitamin A acetate and Cremophor EL mixture (1:1), vitamin A and Capmul MCM-C8 mixture (1:1) and vitamin A acetate, soybean oil, Cremophor EL and Capmul MCM-C8 mixture (1:1:1:1).

which gave drug release of 86.23 (F9), 86.1 (F8), 84.68 (F7) and 84.52 (F6). Surfactant to co-surfactant ratio of 3:1 and 4:1 was found to give moderate to poor all-trans-retinol acetate release, which may be due to the entrapment of drug inside the micelle structure due to an increase in the amount of the surfactant and co-surfactant amounts.

From the above data, it is obvious that any change in the particle size and/or the turbidity of the prepared formulations is reflecting dramatically on the dissolution rate of all-trans-retinol acetate. The particle size and measured turbidity of the formulations are inversely proportional to the dissolution rate of the drug. The HPMC is known to rupture and release the drug before the first dissolution time point of 5 min.

3.6. Differential scanning calorimetry

Fig. 10 shows the thermogram of physical mixture of all-trans-retinol acetate and soybean oil, all-trans-

retinol acetate and Cremophor EL, all-trans-retinol acetate and Capmul MCM-C8, and self-nanoemulsified drug delivery system of all-trans-retinol aCETATE mixture in a ratio of 1:1, respectively. All-trans-retinol acetate shows a characteristic peak at 54 °C. From the figure it is clear that the physical mixture of all-trans-retinol acetate and all the capsule ingredients at a ratio of 1:1 indicate no chemical interaction or incompatibility between all-trans-retinol acetate and the other ingredients. The reduction in peak intensity is a consequence of less overall all-trans-retinol acetate present in the mixture as compared to all-trans-retinol acetate alone.

4. Conclusion

The results obtained from this study revealed that by using the proper ratio and kind of surfactant and co-surfactant, all-trans-retinol acetate can be easily formulated into a SNEDDS with desired particle size range, turbidity and the amount of drug released. The surfactant to co-surfactant ratio of 1:1 and 2:1 was found to yield the desired SNEDD of all-trans-retinol acetate.

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