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Influence of lipolysis and droplet size on tocotrienol absorption from self-emulsifying formulations

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Abstract

A single dose comparative bioavailability study was conducted to evaluate the bioavailability of tocotrienols from two self-emulsifying formulations, one of which produced an emulsion that readily lipolysed under in vitro condition (SES-A), while the other produced a finer dispersion with negligible lipolysis (SES-B) in comparison with that of a non-self-emulsifying formulation in soya oil. The study was conducted according to a three-way crossover design using six healthy human volunteers. Statistically significant differences were observed between the logarithmic transformed peak plasma concentration (C_{max}) and total area under the plasma concentration–time curve ($AUC_{0-\alpha}$) values of both SES-A and -B compared to NSES-C indicating that SES-A and -B achieved a higher extent of absorption compared to NSES-C. Moreover, the 90% confidence interval of the $AUC_{0-\alpha}$ values of both SES-A and -B over those of NSES-C were between 2–3 suggesting an increase in bioavailability of about two–three times compared to NSES-C. Both SES-A and -B also achieved a faster onset of absorption. However, both SES-A and -B had comparable bioavailability, despite the fact that SES-B was able to form emulsions with smaller droplet size. Thus, it appeared that both droplet sizes as well as the rate and extent of lipolysis of the emulsion products formed were important for enhancing the bioavailability of the tocotrienols from the self-emulsifying systems.

Keywords: Tocotrienols; Self-emulsifying systems; Lipolysis; Droplet sizes

1. Introduction

Tocotrienols, which belong to the Vitamin E family, share similar structural features of a chroman head and a 16-carbon phytyl chain with tocopherols. The main difference between the tocopherols and tocotrienols lies in the former having a saturated phytyl chain, while that of the latter is unsaturated, with three double bonds at 3′, 7′ and 11′ positions (Kamal-Eldin and Appelqvist, 1996). In recent years, tocotrienols have generated much interest, as they were reported to possess some biological activities that were not observed with the tocopherols and these include cholesterol lowering activities (Qureshi et al., 1991; Qureshi et al., 1995), anticancer and tumour suppressive activities (Goh et al., 1994; Nesaretnam et al., 1998), antiaggregation of blood platelets (Mahadevappa et al., 1991) and neuroprotective properties (Sen et al., 2000).

We have previously shown that the bioavailability of the tocotrienols was poor and erratic and was markedly increased when taken with food, while their

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biological half-life values were relatively short, being almost four–five-fold shorter compared to that of α-tocopherol (Yap et al., 2001). In studies using rats, it was also discovered that the absorption of tocotrienols was low and incomplete via the oral route (Yap et al., 2003). There appeared to be biodiscrimination in the oral absorption and disposition among the three tocotrienols. While the absolute bioavailability of α-tocotrienol was approximately 28%, those of δ- and γ-tocotrienol were comparable with a value of approximately 9%. Such poor and erratic bioavailability, which is also influenced by food for enhanced bioavailability makes the tocotrienols suitable candidates for lipid-based formulations, such as self-emulsifying systems to improve their oral bioavailability (Charman, 2000).

Various formulation aspects in the development of self-emulsifying systems have been well discussed and reviewed by Pouton (1997) and more recently by Gershanik and Benita (2000). It was suggested that the droplet sizes of the emulsion products formed by the self-emulsifying systems should be as fine as possible, preferably in colloidal dimensions to facilitate enhanced drug bioavailability. On the other hand, if a lipid formulation is non-self-emulsifying, then its susceptibility to digestion and/or solubilization by mixed micelles of bile salts and phospholipids in the gastrointestinal tract is important to produce the required colloidal dispersion for absorption, on provision that the contained drug is not precipitated during the digestion process (Macgregor et al., 1997; Pouton, 2000). As such, for self-emulsifying systems that produce rather coarse emulsions, subsequent digestion or lipolysis of the oil droplets might be important in their utilization for improving drug bioavailability (Pouton, 2000).

In view of the potential role of digestion/lipolysis in enhancing drug absorption from self-emulsifying systems, the present study was conducted to evaluate the in vivo bioavailability of tocotrienols from two self-emulsifying formulations, one of which produced an emulsion product that readily lipolyzed under in vitro conditions, while the other produced a finer dispersion with negligible lipolysis under the same conditions. Both preparations were evaluated in comparison with a non-self-emulsifying lipid formulation of the tocotrienols using six healthy human volunteers.

2. Materials and methods

2.1. Materials

Tocomin® 50%, containing 21.6, 6.4, 10.7 and 10.9% of γ-, δ-, α-tocotrienol and α-tocopherol, respectively, was obtained from Carotech Pte. Ltd. (Ipoh, Malaysia). The rest of the Tocomin® 50% consisted mainly of palm olein, plant squalene and phyto–sterol complex, with trace amounts of phyto carotenoid complex and co-enzyme Q10. Tocotrienol standard kit was purchased from Merck (Darmstadt, Germany). Soya oil was purchased from Yee Lee Edible Oils Pte. Ltd. (Ipoh, Malaysia) while Labrasol (a C_8/C_{10} polyglycolyzed glycerides from coconut oil) and Tween 80 (polyoxyethylene (20) sorbitan oleate) were obtained from Gattefossé (Cedex, France) and Sigma Chemical Co. (St. Louis, Mo, USA), respectively. All of the other solvents used were either of analytical reagent grade or HPLC grade and were purchased from either Merck or Ajax Chemicals (Auburn, Australia).

2.2. Preparations studied

The composition of the preparations studied is listed in Table 1. The two formulations, SES-A and -B, which contained surfactants (Tween 80 and Labrasol) are self-emulsifying systems, while the non-self-emulsifying lipid preparation (NSES-C) consisted of Tocomin® 50% dissolved in soybean oil. SES-A was formulated to produce an emulsion product that lipolyzed under in-vitro conditions, while SES-B produced a finer dispersion with negligible lipolysis. All the formulations were accurately

Table 1
Composition of the two self-emulsifying formulations (SES-A and -B) and the non-self-emulsifying lipid formulation (NSES-C)

Ingredients	Weight (mg)		
	SES-A	SES-B	NSES-C
Tocomin 50%	148.7	148.7	148.7
Soybean oil	351.3	31.3	51.3
Tween 80	12.5	367.5	–
Labrasol	87.5	52.5	–
Total weight	600.0	600.0	200.0

weighed (±1% of the desired dose) into size '0' hard gelatin capsules for administration to the study subjects. Since the desired dose was 200 mg of mixed tocotienols (approximately 33.1, 111.6 and 55.3 mg of δ-, γ-, α-tocotrienol), the weight of SES-A and -B was about 2.1 g while that for NSES-C was 0.7 g. For NSES-C, 2 hard gelatin capsules were used while five capsules each were needed for both SES-A and -B.

2.3. Assessment of self-emulsifying properties and droplet sizes

To assess the self-emulsifying properties of SES-A and -B, 0.50 ml of each formulation was injected via a one-ml syringe into 500 ml of distilled water in a USP 24 (2000) Apparatus 2 dissolution test apparatus (Type PTWSIII/S12/D6-PTWS3C, Pharma Test Dissolution Tester, Hainburg, Germany) equipped with a paddle stirrer, stirring at 100 rpm and a stopwatch. The preparations were deemed to self-emulsify if an emulsion was formed within 3 min when viewed visually. The droplet sizes of the emulsions formed were measured using a Malvern Mastersizer S laser diffraction particle analyzer from Malvern Instrument (Worcestershire, UK). The volume median diameter, *D*50 was determined for each preparation. The Span value that gave an indication of the polydispersity of the emulsion system was calculated from the *D*10, *D*50 and *D*90 of the particle size distribution curve using the following equation:

$$\text{Span} = \frac{D90 - D10}{D50}$$

where *D*90 and *D*10 represent the size below which 90 and 10% of the volume lie. For each formulation, three emulsion products were prepared for the droplet size analysis and five measurements were taken for each sample.

2.4. In vitro assessment of lipolysis

The rate and extent of lipolysis of the emulsion products formed from the three formulations was determined in the presence of pancreatic enzymes based on the method described by Macgregor et al. (1997). A Mettler Toledo DL-50 autotitrator (Schwerzenbach, Germany) was used as the titration unit. These samples were weighed into the reaction vessel of the autotitrator and made up to 25 g with the lipolysis medium and maintained at 37 °C in a water bath. The lipolysis medium consisted of 50 mM Tris-maleate buffer, 5 mM calcium chloride, 150 mM sodium chloride, 8 mM sodium taurocholate and 1.5 mM Lecithin and was adjusted to pH 8.5 with 1.0 M sodium hydroxide (NaOH). The mixtures were then stirred at a prefixed speed of the instrument (denoted by 50%) for half an hour. A well-formed emulsion product was obtained with both SES formulations. A pancreatin solution was separately incubated at 37 °C in the water bath for the same length of time. At the end of the incubation period, the mixture in the reaction vessel was pretitrated to pH 8.5 with 0.1 M NaOH. When the pH reached 8.5, 1 ml of pancreatin solution was then added and the titration system was immediately activated with the end-point set at pH 8.5. As the lipolysis reaction proceeded, the liberated fatty acids would cause the pH to decline. However, the autotitrator was programmed to maintain the pH at 8.5 by autotitrating 0.1 M NaOH into the mixture. Titration readings were noted every 5 min. Thus, from the rate and amount of NaOH added to maintain the pH, the rate and extent of lipolysis could then be estimated. All experiments were conducted in triplicates. A negative control experiment containing no substrate was also carried out.

Prior to the experiment, the pH electrode of the autotitrator (DG115, Mettler Toledo, Schwerzenbach, Germany) was calibrated with standard pH 4.0, 7.0 and 9.2 buffers (Mettler Toledo, Schwerzenbach, Germany). The 0.1 M NaOH used was standardized using potassium hydrogen pthalate.

The pancreatin solution was prepared by adding 5.0 ml of distilled water to 1.0 g of pancreatin powder. The suspension was vortexed for 10 min and centrifuged for 15 min at 2000 × *g*. The supernatant was transferred to another tube and stored at 4 °C until used. Fresh pancreatin solution was prepared daily as it was reported that there was a gradual loss of lipase activity when the solutions were assayed for activity after more than a day (Alvarez and Stella, 1989).

To study the lipolysis of the individual excipients in the SES, 0.25 g of each of Tween 80, Labrasol and Tocomin® 50% were weighed into the reaction vessels. Samples of the three preparations, each containing 27.5 mg of mixed tocotrienols were used in the study. The weight of each sample was 0.30 g for

SES-A and -B and 0.10 g for the non-self-emulsifying lipid formulation (NSES-C).

In each case, the mean extent of lipolysis in percent (%) and mean reaction rate (MRR) in terms of volume of NaOH consumed over a period of time ($\mu l\,min^{-1}$) were then estimated. The extent of lipolysis (% lipolysis) was calculated with the assumption that one mole of triglyceride yielded one mole of 2-monoglyceride and 2 mol of fatty acids. Typical refined soya oil has the following fatty acid composition, 50–57% linoleic acids, 5–10% linolenic acids, 17–26% oleic acids, 9–13% palmitic acids and 3–6% stearic acids. Based on the above composition, the theoretical amount of fatty acids liberated per gram of soya oil if complete lipolysis occurred would be approximately 2.3 mmol. Assuming that the other components of the SES (Tween 80, Labrasol and Tocomin® 50%) did not undergo lipolysis, the amount of NaOH consumed per gram of soya oil when lipolysis was completed, would be 2.3 mmol. The extent of lipolysis could then be calculated by dividing the amount of NaOH consumed per gram of soya oil in the lipolysis experiments with this value. However, the amount of NaOH consumed in the lipolysis experiments was corrected for background noise by subtracting for the amount of NaOH consumed when the experiment was run with the lipolysis medium alone.

In the estimation of the mean reaction rate (MRR), the rate of lipolysis after each time interval was first calculated by dividing the amount of NaOH consumed with the time interval (for example 5, 10, 15 min). All these rates were then averaged to give the mean reaction rate.

2.5. In vivo study protocol

The protocol for the study was approved by a joint School of Pharmaceutical Sciences, USM-General Hospital Penang Committee on Bioavailability Studies. Volunteers were given information of tocotrienols and nature of the study in advance of the trial. Six healthy adult male volunteers between 26 and 41 years old (mean = 34 ± 6) and weighing from 55 to 75 kg (mean = 65 ± 8), participated in a standard three period, three sequence crossover study after providing written informed consent. All were judged to be healthy and were not receiving any medication or Vitamin E supplementation 2 weeks before and during the study period. The volunteers were randomly divided into three groups of two each, and administered the preparations according to the schedule shown in Table 2 with a washout period of 1 week between phases of study.

Table 2
Sequence of administration of the three preparations

Group (two volunteers/group)	Period		
	I	II	III
1	SES-A	SES-B	NSES-C
2	SES-B	NSES-C	SES-A
3	NSES-C	SES-A	SES-B

The dose administered for all three formulations was 200 mg mixed tocotrienols (33.1, 111.6 and 55.3 mg δ-, γ- and α-tocotrienols, respectively). All products were administered in the morning (9:00 am) after a 12 h fast with 240 ml of water. Food and drinks was withheld for at least 4 h after dosing and plain water was given ad libitum. Lunch and dinner comprising chicken with rice were served at 4 and 10 h after dosing. Blood samples of 5 ml volume were collected in vacutainers (containing sodium heparin as anticoagulant) at 0 (before dosing), 1–8, 10, 12, 14, 18, 24 h after dosing via an in-dwelling cannula placed in the forearm. The blood samples were centrifuged for 15 min at 2000 × *g* and the plasma transferred to separate glass containers to be kept frozen until analysis.

2.6. Analysis α-, δ- and γ-tocotrienols in plasma

Plasma α-, δ- and γ-tocotrienols were determined using a high-performance liquid chromatographic (HPLC) method reported by Yap et al. (1999).

2.7. Data and pharmacokinetic analysis

The bioavailability of the tocotrienols from the different formulations were compared using the pharmacokinetic parameters, peak plasma concentration (C_{max}), time to reach peak plasma concentration (T_{max}) and total area under the plasma concentration–time curve ($AUC_{0-\infty}$), which were estimated from the plasma concentration-time data. Both C_{max} and T_{max} were obtained directly from the plasma concentration values (Weiner, 1981), while the

$AUC_{0-\infty}$ was calculated by adding the area from time zero to the last detectable sampling time t (AUC_{0-t}) and the area from time t to infinity ($AUC_{t-\infty}$). The former was calculated using the trapezoidal formula and the latter by dividing the last measurable plasma drug concentration with the elimination rate constant (k_e). In all cases, the $AUC_{t-\infty}$ was found to be <20% of the $AUC_{0-\infty}$. The k_e was estimated from the terminal slope of the individual plasma concentration–time curves after logarithmic transformation of the plasma concentration and application of linear regression (Gibaldi and Perrier, 1982). The elimination half-life ($t_{1/2}$) was calculated from the quotient $\ln 2/k_e$. The lag time of absorption (t_{lag}) was determined by extrapolating the initial ascending portion of the plasma concentration–time curves to the time axis.

2.8. Statistical analysis

The Student's t-test was used to determine the difference between the two SES formulations with regard to the mean droplet sizes and span values. In the case of the mean reaction rate (MRR) and the extent of lipolysis (%), an analysis of variance procedure (one-way ANOVA) appropriate for a parallel group design was used to compare the values of the three formulations. This is followed by Tukey's test if a statistically significant difference was detected.

As for the in vivo study, the values of C_{max}, $AUC_{0-\infty}$, k_e and t_{lag} obtained from the three formulations were analyzed using an analysis of variance (ANOVA) procedure appropriate for a crossover design. The C_{max} and $AUC_{0-\infty}$ values were logarithmic transformed prior to analysis. The Tukey's test was used to compare the means between groups if a statistically significant difference was detected. On the other hand, the T_{max} values were analyzed using the Friedman test for multiple comparisons. A statistically significant difference was considered at $P < 0.05$.

3. Results

3.1. In vitro assessment of self-emulsifying properties and droplet sizes of SES-A and -B and lipolysis of SES-A, -B and NSES-C

Results obtained from the lipolysis experiment conducted on the individual components used in the preparation of the SES are shown in Fig. 1 while the nu-

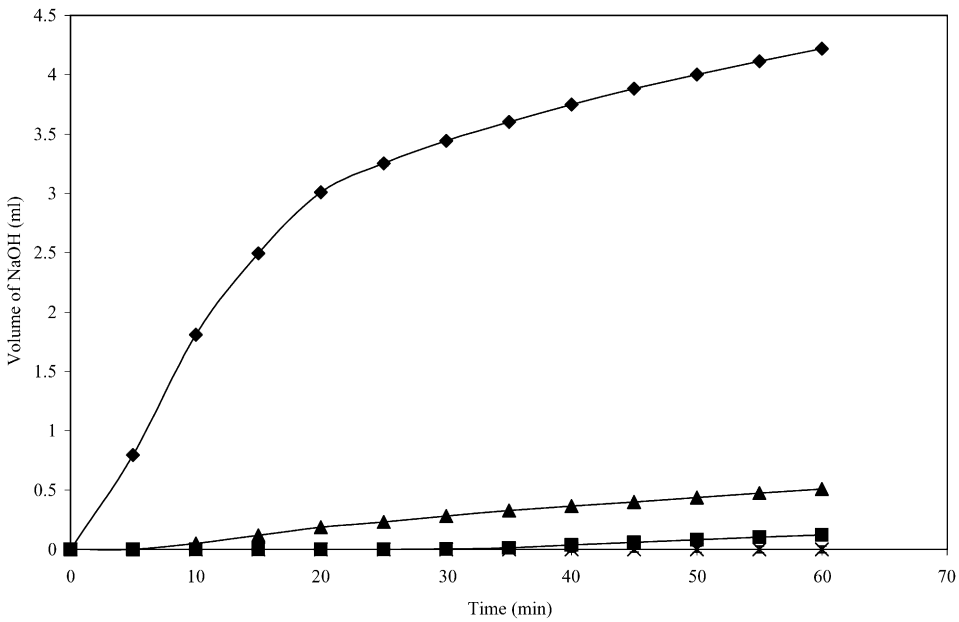


Fig. 1. The lipolysis of excipients: (×) Tween 80; (▲) Labrasol; (■) Tocomin® 50%, and (◆) soya oil, used in the formulation of self-emulsifying systems (mean ± S.E.M., $n = 3$) (the standard error of mean was within the size of the symbols).

Table 3
In vitro characterisation of the three formulations investigated in this study

Formulation	Visual observation	$D50$[a] (μm)	Span	lipolysis	
				% lipolysis[b] (%)	MRR[c] (μl/min)
SES-A	Cloudy emulsion	10.6 ± 0.1*	6.3 ± 0.1*	76.6 ± 1.1**	68.6 ± 5.3**
SES-B	Slightly cloudy, solubilised system	1.5 ± 0.0	2.8 ± 0.1	3.6 ± 0.5	0.7 ± 0.3
NSES-C	–	–	–	9.1 ± 0.4***	5.2 ± 0.6****
Excipients					
Tween 80				–	–
Labrasol				3.0 ± 0.1	7.9 ± 0.8
Tocomin® 50%				4.1 ± 0.5	0.7 ± 0.2
Soya oil				73.7 ± 0.1	117.5 ± 11.2

[a] Volume median diameter, values are mean ± standard error of mean.
[b] Extent of lipolysis (%), values are mean ± standard error of mean.
[c] Mean reaction rate, values are mean ± standard error of mean.
* $P < 0.001$ when compared to SES-B.
** $P < 0.01$ when compared to SES-B and NSES-C.
*** $P < 0.01$ when compared to SES-B.
**** $P < 0.05$ when compared to SES-B.

merical values of the rate and extent of lipolysis calculated are summarized in Table 3. From Fig. 1, it was found that apart from soya oil, the other components in the SES underwent negligible lipolysis. Tween 80 did not lipolyse at all while the extent of lipolysis for both Labrasol and Tocomin® 50% were <5.0%.

Table 3 also shows the visual observation of the emulsions formed, the volume median diameter, $D50$, the span values as well as the extent of lipolysis (% lipolysis) and the mean reaction rate (MRR) of SES-A and -B. The % lipolysis and MRR for NSES-C are also shown in Table 3. SES-A formed a very cloudy emulsion product while that of SES-B was a slightly cloudy, solubilised system. SES-A produced emulsions of bigger droplet size compared to SES-B ($P < 0.001$), but was better lipolysed compared to either SES-B or NSES-C, as shown in Fig. 2, where the volume of NaOH consumed was the highest for the former compared to that of the latter two formulations. As shown in Table 3, SES-A also had the highest extent of lipolysis and MRR among the three formulations, followed by NSES-C, while SES-B underwent negligible lipolysis.

3.2. In vivo comparative bioavailability study

The mean plasma δ-, γ- and α-tocotrienol concentration versus time profiles of the three formulations are shown in Fig. 3a-c). It is apparent from the plots that tocotrienols administered as self-emulsifying preparations (SES-A and -B) had markedly higher plasma levels compared to that given as oily solution (NSES-C). The self-emulsifying preparations also appeared to have a faster rate of drug absorption as indicated by the more rapid onset of absorption and a shorter time to reach peak plasma levels.

Table 4 shows the mean values of $AUC_{0-\infty}$, C_{max}, T_{max}, k_e, $t_{1/2}$, and t_{lag} values estimated from the individual plasma drug concentration profiles of δ-, γ- and α-tocotrienol for the three preparations. There was a statistically significant difference among the logarithmic transformed $AUC_{0-\infty}$ ($P < 0.01$), as well as the logarithmic transformed C_{max} ($P < 0.01$) values of the three preparations for all three tocotrienols. Further analysis of the data using Tukey's test showed a statistically significant difference between SES-A and NSES-C ($P < 0.01$) as well as SES-B and NSES-C ($P < 0.01$) with regard to the values of $AUC_{0-\infty}$ and C_{max} for all three tocotrienols but not between those of SES-A and -B ($P > 0.05$). These findings indicate that the extent of bioavailability of the tocotrienols from both self-emulsifying formulations was comparable but both being significantly higher than that of the oily solution (NSES-C).

The 90% confidence intervals for the ratio of the $AUC_{0-\infty}$ as well as C_{max} values of SES-A and -B

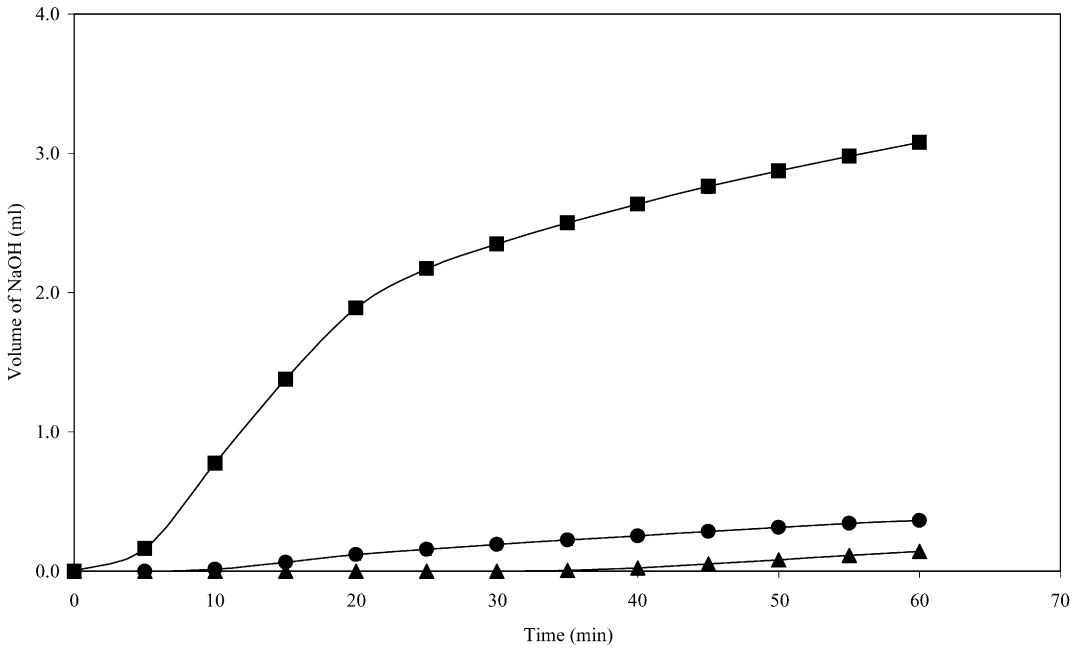


Fig. 2. The lipolysis profiles of: (■) SES-A, (▲) SES-B, and (●) NSES-C (mean ± S.E.M., $n = 3$) (the standard error of mean was within the size of the symbols).

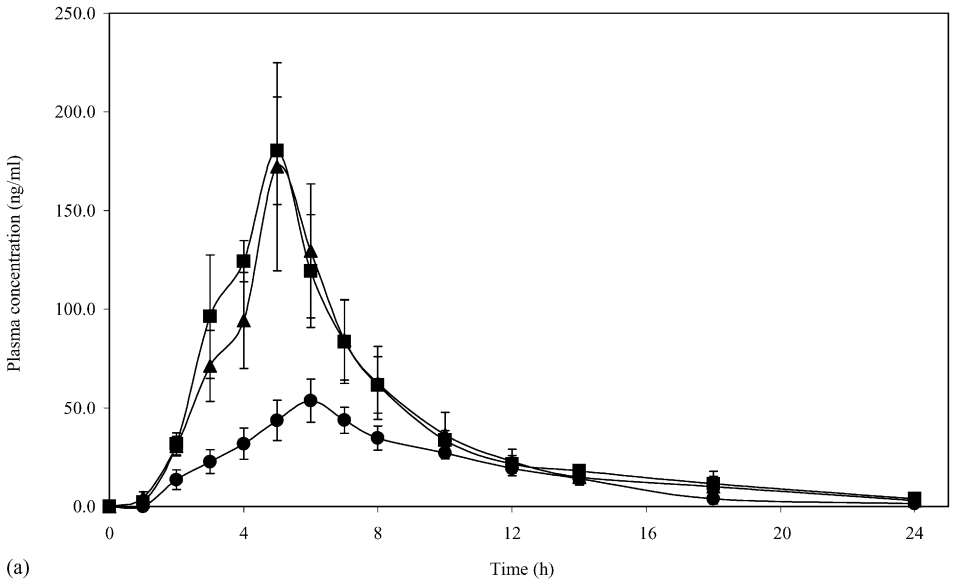
over those of the NSES-C with respect to δ-, γ- and α-tocotrienols are also listed in Table 4. For $AUC_{0-\infty}$, all the ratios were between 2.0–3.7, suggesting that the extent of bioavailability of the three tocotrienols were increased approximately three-fold when administered as SES-A or -B. As for C_{max}, the values were increased approximately four-fold (range: 2.6–5.4). Thus, both self-emulsifying formulations produced a similar increase in the extent of bioavailability of the three tocotrienols compared to that of the oily formulation.

In the case of the parameter T_{max}, no statistically significant difference was observed among the values of the three preparations except between those of SES-A and NSES-C for δ- and γ-tocotrienols ($P < 0.05$), indicating that the length of time in which drug absorption was occurring, was essentially comparable among the three formulations.

Referring to Table 4, it can also be observed that there was no statistically significant difference ($P > 0.05$) among the k_e values obtained from the three preparations for all the tocotrienols. An absorption lag time as represented by t_{lag} was observed for all preparations, with both self-emulsifying formulations having significantly shorter t_{lag} values ($P < 0.05$) compared to NSES-C (except between the values of SES-A and NSES-C for δ-tocotrienol), indicating a faster onset of absorption from the self-emulsifying formulations.

4. Discussion

α-Tocopherol, which is absorbed in the same pathway as other non-polar lipids such as triglycerides and cholesterol, requires the presence of bile salts for emulsification and micelle formation. After absorption it is transported through the lymphatic system, being too lipophilic to be absorbed via the hepatic portal vein (Kayden and Traber, 1993). From the results of our previous study on bioavailability of the tocotrienols under fed and fasted states (Yap et al., 2001), it appears that their absorption requires similar intraluminal processing involving the bile salts as evidenced by the increased bioavailability when given with food (which stimulates bile secretion). In addition, Ikeda et al. (1996) also observed that the tocotrienols were



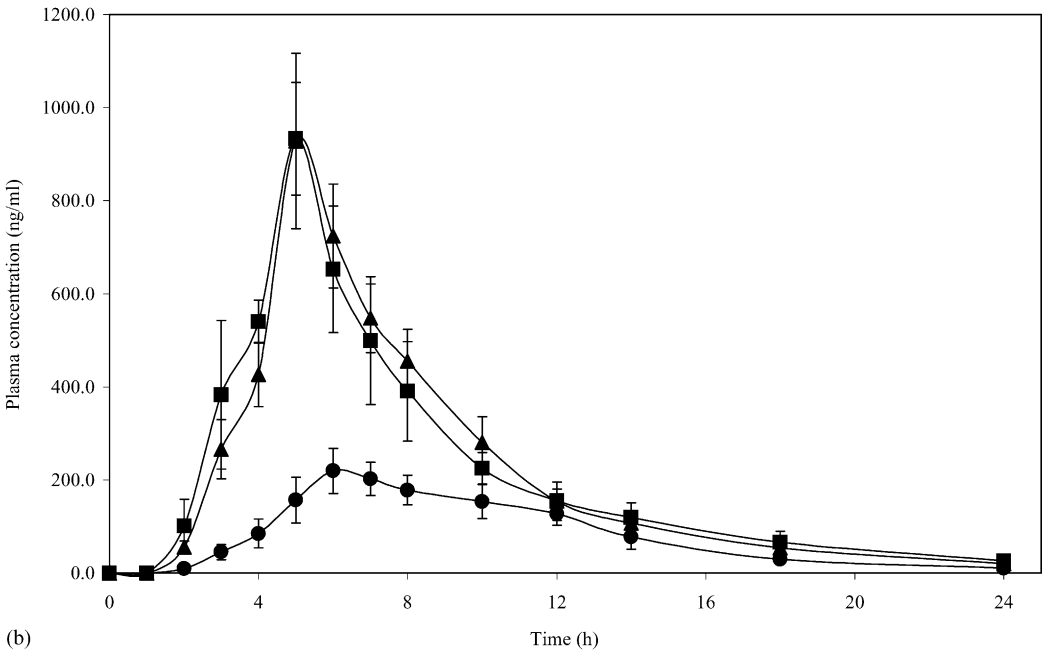


Fig. 3. (a) Mean plasma concentration (±S.E.M., $n = 6$) of: (a) δ-tocotrienol as a function of time following oral administration of 200 mg mixed tocotrienols in the form of (■) SES-A, (▲) SES-B, and (●) NSES-C; (b) γ-tocotrienol as a function of time following oral administration of 200 mg mixed tocotrienols in the form of (■) SES-A, (▲) SES-B, and (●) NSES-C; (c) α-tocotrienol as a function of time following oral administration of 200mg mixed tocotrienols in the form of (■) SES-A, (▲) SES-B, and (●) NSES-C.

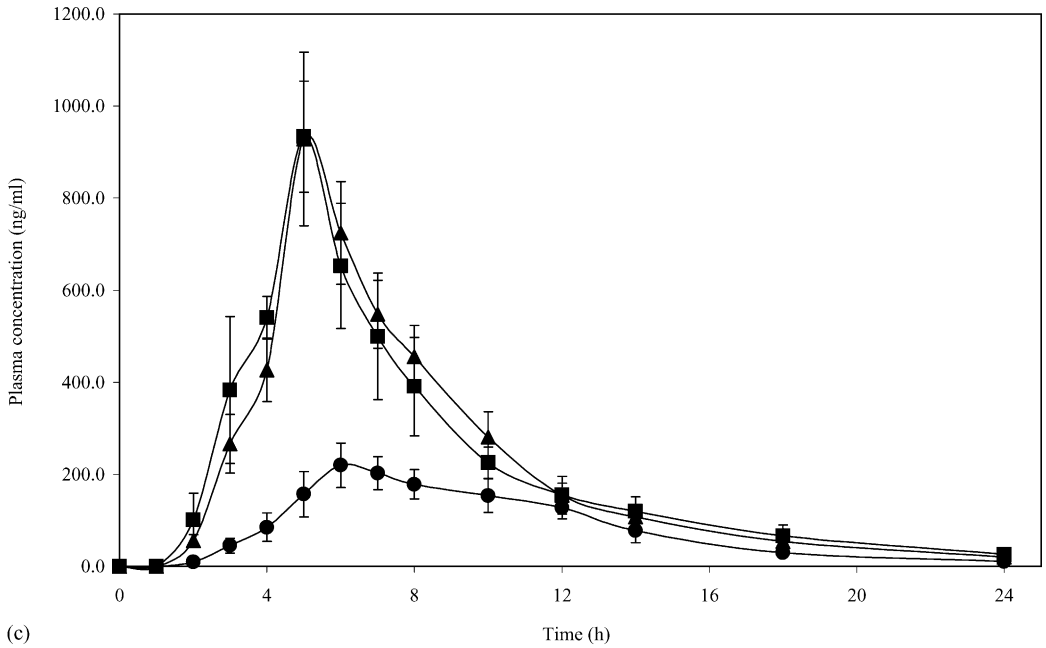


Fig. 3. (*Continued*).

transported via the intestinal lymphatic system after absorption.

As pointed out by Macgregor et al. (1997) and Pouton (2000), emulsification of lipid formulations by bile salts and/or lipolysis by pancreatic enzymes to form a disperision of colloidal dimension is crucial for enhancing the bioavailability of the contained drug. Thus, the poor bioavailability observed with NSES-C, a non-self-emulsifying lipid formulation, when given in the fasted state may be due to inadequate secretion of bile and pancreatic enzymes required for the intraluminal processes of emulsification and lipolysis. That being the case, the enhanced bioavailability of the tocotrienols when given as self-emulsifying formulations in the fasted state as observed in this study, could be attributed to their ability to form dispersions of sufficiently small droplet sizes for absorption. Thus, they were not dependent on the intraluminal processing by the bile salts or pancreatic enzymes to form the colloidal dispersion. Similar observations were reported by Smidt et al. (2004), where the bioavailability of penclomedine was higher when administered as emulsions compared to when it was administered as a crude oily formulation.

SES-B as mentioned earlier, could self-emulsify to produce a fine dispersion. While SES-A formed a coarser emulsion product, it was readily lipolysed to form, presumably a fine dispersion. Due to the presence of other components in the lipolysis medium, it was not possible to measure the emulsion droplet sizes of the three formulations before and after the lipolysis experiment. Nevertheless, it has been reported that lipolysis would lead to emulsion droplet size reduction (Palin, 1985; Macgregor et al., 1997). From the results of this study, it appears, therefore, that the self-emulsifying system should form a sufficiently fine dispersion to achieve enhanced bioavailability of the contained drug. Should a coarse emulsion product be formed, further lipolysis would be crucial to achieve finer submicron dimensions. The importance of droplet size is further supported by the in situ gut perfusion study of Tarr and Yalkowsky (1989) and the conscious rat study by Smidt et al. (2004), where both studies found that a finer emulsion helped to increase the bioavailability of the drugs studied.

Smidt et al. (2004) had reported that the bioavailability of penclomedine with and without co-administration of a lipolysis inhibitor was similar when given

Table 4
Pharmacokinetic parameters of δ-, γ- and α-tocotrienols after oral administration of 200 mg (33.1, 111.6 and 55.3 δ-, γ- and α-tocotrienol, respectively) of mixed tocotrienols

	δ-tocotrienol			γ-tocotrienol			α-tocotrienol		
	NSES-C	SES-A	SES-B	NSES-C	SES-A	SES-B	NSES-C	SES-A	SES-B
C_{max} (ng/ml)	57.8 ± 25.4	205.7 ± 55.3*	198.2 ± 113.5*	254.2 ± 108.5	1037.9 ± 219.6*	996.8 ± 458.7*	192.0 ± 47.8	647.0 ± 174.2*	883.6 ± 522.9*
T_{max} (h)	6.7 ± 0.8	5.0 ± 1.0**	6.0 ± 0.8	7.3 ± 1.5	5.0 ± 1.0**	5.5 ± 0.8	6.8 ± 0.8	5.7 ± 0.8	5.8 ± 1.0
$AUC_{0-\infty}$ (ng h/ml)	447.5 ± 209.9	1028.5 ± 212.7*	1120.4 ± 446.3*	2080.2 ± 1078.1	5528.3 ± 1632.2*	5313.9 ± 1695.9*	1727.7 ± 465.1	3644.0 ± 518.5*	4790.0 ± 1967.3*
k_e (h^{-1})	0.175 ± 0.055	0.142 ± 0.046	0.160 ± 0.048	0.183 ± 0.063	0.201 ± 0.097	0.197 ± 0.083	0.165 ± 0.051	0.160 ± 0.049	0.162 ± 0.045
$t_{1/2}$ (h)	4.4 ± 1.7	5.3 ± 1.6	4.8 ± 2.0	4.1 ± 1.1	3.9 ± 1.2	4.0 ± 1.4	4.6 ± 1.4	4.7 ± 1.4	4.6 ± 1.2
t_{lag} (h)	1.7 ± 0.8	1.0 ± 0.4	0.6 ± 0.3**	2.4 ± 1.4	0.9 ± 0.6**	0.9 ± 0.3**	2.1 ± 0.5	1.1 ± 0.6*	1.1 ± 0.3*
C.I. C_{max}		3.1–4.5[a]	2.7–3.9[b]		3.8–4.9[a]	3.4–4.4[b]		2.6–4.4[a]	3.2–5.4[b]
C.I. $AUC_{0-\infty}$		2.0–3.1[a]	2.1–3.2[b]		2.2–3.7[a]	2.1–3.6[b]		1.7–2.7[a]	2.1–3.4[b]

All the values shown are mean ± S.D.
C.I. C_{max} is the 90% confidence interval of the C_{max} values of [a]SES-A or [b]SES-B over that of NSES-C.
C.I. $AUC_{0-\infty}$ is the 90% confidence interval of the $AUC_{0-\infty}$ values of [a]SES-A or [b]SES-B over that of NSES-C.
* $P < 0.01$ when compared to NSES-C.
** $P < 0.05$ when compared to NSES-C.

as prepared submicron emulsions. This was demonstrated with two emulsion formulations containing different surfactant to oil ratios with mean emulsion droplet sizes of 160 and 720 nm, respectively. However, when one of the formulations was given as an undispersed form (without prior emulsification), the bioavailability of penclomedine was reduced two-fold with co-administration of a lipolysis inhibitor. Thus, the results of Smidt et al. (2004) tended to suggest that if an emulsion product is in the submicron sizes, lipolysis would not be critical for the absorption of the contained drug. On the other hand, if given as an undispersed form or when only a crude emulsion product is obtained from a self-emulsifying formulation, then lipolysis might still be important in the drug absorption.

It was found in a separate study (Yap, 2003) that the phase I cytochrome P-450 3A4 metabolic enzyme (CYP3A4) and P-glycoprotein (Pgp) at the gastrointestinal epithelium might be involved in the oral absorption of the tocotrienols. The surfactant, Tween 80, used in the current study has been reported to inhibit Pgp, leading to enhanced uptake of substrates for Pgp (Woodcock et al., 1990; Nerurkar et al., 1996). However, it should be noted that the amount of Tween 80 in SES-B was approximately 30 times that of SES-A, but the bioavailability of tocotrienols from both formulations was essentially similar, suggesting that Tween 80 has no significant influence on the absorption of the tocotrienols. Nevertheless, a rapidly absorbing formulation of the tocotrienols as provided by the self-emulsifying systems, would be advantageous since both CYP3A4 and Pgp are saturable. Therefore, if more drug molecules were made available at the absorption site leading to saturation of the CYP3A4 and Pgp, a higher proportion of the drug would gain passage across the enterocytes. This might in part help to explain the increased bioavailability of the tocotrienols administered as self-emulsifying systems in the present study.

As noted earlier, the time to reach peak plasma concentrations (T_{max}) of the tocotrienols was quite similar for all three preparations, suggesting that the absorption of the tocotrienols occurred over a comparable period of time, albeit, absorption was occurring at a faster rate for both self-emulsifying products resulting in the increase in extent of bioavailability. Moreover, the t_{lag} for absorption of tocotrienols from the conven-

tional preparation was in accord to those observed in our previous study (Yap et al., 2001), being approximately 2 h. The lag time could be attributed to the time lapse taken for secretion of bile salts to emulsify and solubilize the tocotrienols prior to their absorption. With both self-emulsifying systems, a shorter lag time was observed (approximately, 1 h). This might be attributed to their ability to form a readily absorbable form without the need for bile secretion. Moreover, the emulsion product formed might be emptied more rapidly from the stomach to the absorption site in the small intestinal area. From a separate in vitro disintegration study, the capsules of all the three formulations were found to disintegrate and discharge their contents within 2 min, suggesting that the rate of disintegration of the capsules has no influence on the onset of absorption.

5. Conclusion

On the basis of the results obtained from the present study, it can be concluded that both SES formulations achieved a faster onset of absorption, with marked increase in the extent of bioavailability of the tocotrienols by two–three-fold compared to the non-self-emulsifying oily solution (NSES-C) under fasted condition. The droplet sizes of the emulsion product formed should be sufficiently fine, preferably in the submicron range to achieve enhanced oral absorption of the contained drug. However, if only a crude emulsion product is formed, lipolysis may be an important consideration.

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