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Comparison between lipolysis and compendial dissolution as alternative techniques for the *in vitro* characterization of α-tocopherol self-emulsified drug delivery systems (SEDDS)

Hazem Ali [a], Mahmoud Nazzal [b], Abdel-Azim A. Zaghloul [c], Sami Nazzal [a,*]

[a] *Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of Louisiana at Monroe, Monroe, LA 71209-0497, United States*
[b] *Department of Allied Medical Sciences, Faculty of Applied Medical Sciences, Jordan University of Science and Technology, Irbid, Jordan*
[c] *Department of Pharmaceutics, Faculty of Pharmacy, Kuwait University, Safat, Kuwait*

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Abstract

In vitro characterization of α-tocopherol SEDDS formulations was performed by (1) lipolysis in bio-relevant media, and (2) physical assessment by dissolution, particle size, and turbidity analyses. Both methods were statistically correlated using a 25-run, five-factor multiple-level D-optimal mixture design. Independent variables were SEDDS composition [vitamin E (12.5–25%), Tween® 80 (10–40%), labrasol (0–10%), alcohol (0–10%), and captex 355 (20–50%)]. Measured responses were percent lipolysis, percent vitamin E retained in the aqueous layer of the digestion medium, and percent vitamin E dissolved in the dissolution medium. Percent lipolysis ranged from 0% to 66.3%. Percent vitamin E retrieved in the aqueous layer of the digestion and dissolution media ranged from 3% to 29.3% and from 25.9% to 101.7%, respectively. Turbidity ranged from 28 to 403 JTU and the average droplet size was >1.0 μm. All formulation ingredients had significant ($p < 0.05$) effect on percent lipolysis. Only two factors, Tween® and vitamin E had significant effect on vitamin retention in the aqueous layer post-lipolysis. Tween®, labrasol, and captex 355 had significant effect on vitamin E dissolution. Poor correlation was observed between the responses. Formulation ingredients influenced each response differently; and therefore, each method can only reveal distinctive characteristics of the SEDDS formulation and may not be used interchangeably.
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1. Introduction

Large proportions of new drug candidates have poor aqueous solubility (Gursoy and Benita, 2004). To overcome this problem, various formulation strategies such as micronization, complexation with cyclodextrins, and formation of solid dispersions were reported in the literature (Nazzal et al., 2002a). In recent years, however, much attention has been focused on lipid-based formulations, with particular emphasis on self-emulsifying drug delivery systems or SEDDS, which were shown to improve the oral bio-availability of many drugs, viz. halofantrine (Khoo et al., 1998), ontazolest (Hauss et al., 1998), cyclosporine (Klauser et al., 1997), and progesterone (MacGregor and Embleton, 1997).

SEDDS are isotropic mixtures of oil, surfactant, co-surfactant, co-solvent, and drug that form fine oil-in-water emulsion when introduced into an aqueous medium under gentle agitation (Charman et al., 1992; Craig et al., 1993; Constantinides, 1995; Gao et al., 1998; Gershanik and Benita, 2000; Pouton, 2000; Nazzal et al., 2002b). Among the critical factors that are frequently considered for the optimal *in vitro* performance of SEDDS are the surfactant concentration, oil to surfactant ratio, triglyceride and co-solvent content, polarity of the emulsion, and droplet size. Therefore, many characterization techniques such as droplet size, dissolution, zeta potential, and surface tension analyses, low frequency dielectric spectroscopy, and turbidimetry were used to predict the effect of these critical formulation parameters on the physical performance of SEDDS *in vitro* (Nazzal et al., 2002c). Recent studies, however, suggested that not only is the oral performance of SEDDS influenced by their physical properties such as the dissolution and droplet size of the microemulsion, but also it is affected by the chemical

* Corresponding author. Tel.: +1 318 342 1726; fax: +1 318 342 1737.
E-mail address: nazzal@ulm.edu (S. Nazzal).

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nature and digestion dynamics of its lipid ingredients (Devani et al., 2005; Fatouros et al., 2007). Therefore, lipolysis studies, using bio-relevant dissolution media containing enzymes and naturally occurring surfactants; such as bile salts and lecithin, were employed as an alternative method to assess the *in vitro* performance of SEDDS (Porter et al., 2004a; Sek et al., 2006; Shen and Zhong, 2006). While physical characterization techniques and lipolysis experiments provided wealth of data on the *in vitro* performance of SEDDS, no study attempted to correlate between the data generated using these two alternative methods (physical characterization as opposed to lipolysis) and their usefulness in optimizing SEDDS formulations in the absence of *in vivo* data. Therefore, the overall objective of the present study was to address this issue using α-tocopherol-(vitamin E) based SEDDS as model lipid formulations. Since aqueous solubilization of many drugs is critical for their absorption (Kaukonen et al., 2004a,b), we hypothesized that one approach by which the two methods could be correlated is by contrasting the role played by each ingredient of the lipid formulations on either dissolution of vitamin E in the aqueous dissolution medium or partition of the vitamin in the aqueous phase of the digestion medium. In essence, α-tocopherol was used as a liquid marker for the *in vitro* performance of SEDDS. In light of the aforementioned discussion, the specific objectives of the present study were (1) to explore the effect of the formulation ingredients on the dissolution performance of α-tocopherol-based lipid formulations using standard characterization techniques, including dissolution, particle size analysis, and turbidimetry; (2) to perform *in vitro* lipolysis experiments on these formulations using bio-relevant media; and (3) to correlate the *in vitro* lipolysis data with those obtained from the physical characterization studies. To achieve these goals, a five factor D-optimal mixture design was employed to facilitate data analysis and establish statistical correlations between the factors and the observed responses.

2. Materials and methods

2.1. Materials

Bile salts, calcium chloride dihydrate, pancreatin, sodium chloride, sodium hydroxide, Trizma® maleate, and vitamin E [(±)-α-tocopherol] were purchased from Sigma–Aldrich Co. (St. Louis, MO); Tween® 80 (polyoxyethylene sorbitan mono oleate) was provided by Uniqema (New Castle, DE); captex 355 (triglycerides of caprylic/capric acid) was provided by Abitec Corporation (Janesville, WI); labrasol (C_8/C_{10} polyglycolyzed glycerides from coconut oil) was provided by Gattefossé (Saint-Priest, Cedex, France); ethyl alcohol USP was purchased from AAPER Alcohol and Chemical Co. (Shelbyville, Kentucky); lecithin (approximately 24% pure phosphatidylcholine having a trade name Alcolec® FF100) was provided by American Lecithin Company (Oxford, CT); empty hard gelatin capsules size 0 were provided by Capsugel (Greenwood, SC); water was obtained from NanoPure purification system. All chemicals were used as supplied without further modification.

2.2. Experimental design

A 25-run, five factor multiple-level D-optimal mixture design was used in this study to provide empirical mathematical models to describe the effect of formulation variables (α-tocopherol, Tween® 80, labrasol, captex 355, and alcohol USP) on the dependent responses (percentage lipolysis, percentage vitamin E retained in the aqueous phase post-lipolysis, and cumulative percent of vitamin E dissolved in the dissolution medium). The Design-Expert software (version 5.07; Stat-Ease, Inc., Minneapolis, MN) was used to construct the model and select the set of candidate points. These included factorial points (high and low level from the constraints on each factor), centers of edges (points midway between adjacent factorial points), constrains plane centroids, axial check points, and an overall center point. For completely randomized design with five factors at multiple levels, second order polynomial equations were generated using response surface methodology (RSM), which included quadratic terms and two factor interaction that explained the non-linear nature of the response. Results of statistical analysis were considered significant if their corresponding p-values were less than 0.05.

The independent and dependent design variables are listed in Table 1. The levels of each variable are based on preliminary experiments. Within these limits a homogenous transparent microemulsion pre-concentrate is produced. Outside these limits a non-homogenous product or a turbid microemulsion pre-concentrate is formed. Experimental runs and the observed responses are given in Table 2.

2.3. Preparation of the lipid formulation

Ten grams of each of the 25 formulations were prepared by blending the formulation ingredients at ratios pre-determined by the statistical model (Table 2). Briefly, in a borosilicate vial, the ingredients of each formulation were accurately weighed and thoroughly mixed at 20,000 rpm for 5 min using IKA® Ultra-Turrax T8 mixer (IKA® Works Inc., NC, USA).

Table 1
The D-optimal design summary: independent and dependent design variables

Independent variables	Units	Low	High
X_1: vitamin E	%, w/w	12.5	25.0
X_2: Tween® 80	%, w/w	20.0	50.0
X_3: labrasol	%, w/w	10.0	40.0
X_4: captex 355	%, w/w	0.0	10.0
X_5: alcohol USP	%, w/w	0.0	10.0
Dependent variables (responses)	**Units**	**Observed minimum**	**Observed maximum**
Y_1: free fatty acids released	$\times 10^{-3}$ mmole	0.15	238.50
Y_2: percent lipolysis	%	0.00	66.25
Y_3: vitamin E retained in the aqueous phase of the digestion medium	%, w/w	2.77	29.27
Y_4: vitamin E dissolved in the dissolution medium	%, w/w	25.91	101.72

Table 2
Experimental runs and the observed responses of the D-optimal mixture design

Run no.	Vitamin E (%, w/w)	Tween® 80 (%, w/w)	Labrasol (%, w/w)	Captex 355 (%, w/w)	Alcohol (%, w/w)	Y_1	Y_2	Y_3	Y_4	Turbidity (JTU)	Particle diameter	Visual grading
1	12.50	50.00	17.50	10.00	10.00	214.00	59.44	29.27	95.95	54.06	12.3 nm	−
2	25.00	32.50	22.50	10.00	10.00	139.00	38.61	11.79	38.04	399.36	>1 μm	+++
3	12.50	37.50	40.00	10.00	0.00	95.50	26.53	19.81	101.72	28.01	10.4 nm	−
4	25.00	37.50	27.50	0.00	10.00	0.15	0.00	7.18	25.91	306.32	>1 μm	++
5	25.00	25.00	40.00	10.00	0.00	197.00	54.72	5.11	42.03	217.26	>1 μm	++
6	25.00	37.50	27.50	10.00	0.00	142.50	39.58	12.96	41.36	207.91	>1 μm	++
7	25.00	20.00	40.00	5.00	10.00	63.50	35.28	8.31	35.87	232.05	>1 μm	++
8	20.00	20.00	40.00	10.00	10.00	108.50	30.14	2.77	91.85	171.22	>1 μm	+++
9	12.50	50.00	27.50	0.00	10.00	21.00	0.00	18.21	75.03	136.58	>1 μm	+
10	25.00	50.00	10.00	10.00	5.00	130.50	36.25	8.54	41.89	228.75	>1 μm	+++
11	22.50	50.00	10.00	7.50	10.00	82.00	30.37	8.60	43.83	170.98	>1 μm	+++
12	25.00	50.00	10.00	10.00	5.00	111.50	30.97	6.37	47.87	236.79	>1 μm	+++
13	16.25	40.80	34.55	5.45	2.95	94.00	47.91	14.81	75.04	121.35	>1 μm	+++
14	12.50	37.50	40.00	0.00	10.00	10.50	0.00	18.79	75.15	169.83	>1 μm	++
15	22.50	50.00	10.00	7.50	10.00	126.00	46.67	9.91	47.46	213.22	>1 μm	+++
16	25.00	35.00	40.00	0.00	0.00	0.50	0.00	6.42	43.17	357.14	>1 μm	+++
17	12.50	50.00	37.50	0.00	0.00	0.50	0.00	17.28	77.66	134.20	>1 μm	+
18	25.00	20.00	40.00	5.00	10.00	7.00	3.89	3.01	42.96	403.46	>1 μm	+++
19	12.50	38.75	28.75	10.00	10.00	107.50	29.86	16.03	97.35	40.91	12.00 nm	−
20	22.50	29.55	33.30	6.70	7.95	20.50	8.50	7.05	52.98	232.71	>1 μm	++
21	18.75	50.00	21.25	10.00	0.00	238.50	66.25	15.88	63.28	189.08	>1 μm	+++
22	25.00	50.00	15.00	0.00	10.00	7.50	0.00	13.01	37.83	250.32	>1 μm	+++
23	25.00	50.00	20.00	5.00	0.00	32.50	18.06	12.28	47.98	257.05	>1 μm	+++
24	25.00	35.00	40.00	0.00	0.00	2.50	0.00	12.49	38.93	303.80	>1 μm	+++
25	20.00	20.00	40.00	10.00	10.00	112.00	31.11	12.64	94.34	217.67	>1 μm	+

Visual grading: "−" denotes transparent, "+" denotes translucent, "++" denotes turbid, "+++" denotes milky".

2.4. Preparation of lipolysis reagents

In this study, a pancreatin extract with a lipase activity of eight Tributyrene Units (TBUs) per milligram of the dry powder was used. Five hundred milligrams of the dry powder were thoroughly mixed with 2 ml deionized water at 20,000 rpm for 2 min using IKA® Ultra-Turrax T8 mixer to produce a 250 mg/ml suspension. Extract suspensions were then incubated for 20 min at 37 °C after which 1 ml of the suspension was added to the bio-relevant medium exactly 10 min after the beginning of each lipolysis experiment. A pH 6.5 buffer was prepared by adding the following ingredients to sufficient deionized water to prepare 1 l of the buffer; $CaCl_2{\cdot}2H_2O$ (5 mM), NaCl (150 mM), tri-maleate (50 mM), and NaOH (39.75 mM).

2.5. In vitro lipolysis experiments using bio-relevant media

Lipolysis medium was prepared by dissolving 0.42 g of bile salts (5 mM) and 0.12 g of lecithin (1.25 mM) in 100 ml of pH 6.5 buffer at 50 °C with the aid of a magnetic stirrer/hot plate unit (Corning Stirrer/Hot Plate PC-351 plate, NY, USA). The heat and agitation was maintained for approximately 30 min until lecithin was fully dissolved. The pH of the buffer was re-adjusted to 6.5 using 1.0 M NaOH. Lipolysis experiments were performed in a water-jacketed reaction vessel connected to a thermostatically controlled water bath (HAAKE, Germany) which maintained the temperature of the medium at 37 ± 0.5 °C throughout the experiment. Prior to each experiment, the pH electrode of the autotitrator (Radiometer analytical SAS, France) was calibrated using standard buffers of pH values 4 and 10. The tip of the pH electrode was positioned 1 cm below the surface of the bio-relevant medium. The reaction vessel was also fitted with an ultra-fast fiber-optic probe. During each experiment, the media was scanned from 900 to 250 nm for 40 min at an approximate rate of 2 scans/min. At the beginning of each experiment, 1 g of the formulation was added to the bio-relevant medium. The pH stat autotitrator was set to maintain the pH of the reaction medium at 6.5 with an accuracy of ±0.05 pH units using 0.05 M NaOH as the titrant. After 10 min from the beginning of each experiment, 1 ml of the pancreatin suspension was added to the bioreaction vessel and the experiments were allowed to proceed for an additional 30 min, for a total duration of 40 min. The volume of sodium hydroxide consumed during the experiment was recorded and analyzed by the autotitrator using a TitraMaster 85 software version 3.1.0 (Radiometer analytical SAS, France). Titration data were used to calculate the mmole fatty acids released and subsequently percentage lipolysis. Blank experiments were performed using the bio-relevant medium alone with and without pancreatin suspension to measure the volume of sodium hydroxide consumed by the reaction media in the absence of the formulation. This value was subtracted from the volume that was consumed in the lipolysis experiments.

2.6. Calculation of mmole fatty acids released and percentage lipolysis

The mmole fatty acids released were calculated by multiplying the actual volume of sodium hydroxide consumed during the lipolysis experiment by its molarity. Percentage lipolysis (lipolysis extent) was calculated with the assumption that captex® 355 is the sole source of fatty acids and that one mole of triglyceride yielded one mole of 2-monoglyceride and two moles of free fatty acids. The fatty acid composition in captex® 355 is 6% caproic acid, 50–75% caprylic acid, 22–45% capric acid, and 4% lauric acid (Abitec Corporation, Janesville, WI, USA). Accordingly, the theoretical amount of the free fatty acids liberated from 1 g of captex® 355 after complete lipolysis was approximately 3.6 mmol. Therefore, the extent of lipolysis was determined from the following equation:

$$\%\,\text{lipolysis} = \left(\frac{\text{mmole free fatty acids}}{3.6 \times \text{amount of captex 355}}\right) \times 100$$

In preliminary studies none of the other formulation ingredients (Tween® 80, Labrasol, Alcohol USP, and α-tocopherol) underwent lipolysis in the presence of sodium hydroxide. Therefore they were not considered a source of free fatty acids. Similar observation was reported in the literature (Yap and Yuen, 2004).

2.7. Determination of vitamin E content in the aqueous phase post-lipolysis

To determine the concentration of vitamin E retained in the aqueous phase of the digestion medium post-lipolysis, the medium from each experiment was centrifuged at 10 °C using rotor type 90 Ti at 734,000 rpm (approximately 334,000 × *g*) for 35 min (Beckman Optima® LE-80K ultracentrifuge). Centrifugation was performed to separate the aqueous phase from the pellet and undigested phases (Porter et al., 2004b). After separation, the aqueous fraction was filtered through 0.2 μm membrane filter (Supor®-200, Pall corporation, MI, USA) and analyzed spectrophotometrically at 294 nm for α-tocopherol using Cary 50 probe UV spectrophotometer (Varian Inc., Cary, NC).

2.8. In vitro dissolution and physical characterization studies

Dissolution experiments were performed using USP Type II dissolution apparatus at a paddle speed of 50 rpm and bath temperature of 37 ± 0.5 °C (Vender Kamp® 600, Van-Kel Inc., Cary, NC). Dissolution vessels were fitted with an ultra-fast fiber-optic probe. At the beginning of each experiment, size 0 hard gelatin capsule was filled with 500 mg of the formulation. The capsule was then released into 500 ml of distilled water. Capsules were held at the bottom of the vessel using stainless-steel sinkers. Dissolution experiments were performed for 15 min during which the dissolution medium was scanned spectrophotometrically from 900 to 200 nm at a gradient rate of 1.5–5 scans/min (Cary 50 probe-UV spectrophotometer, Varian Inc., Cary, NC). At the end of each experiment, the dissolution medium was visually examined for signs of turbidity or sedimentation and was judged as transparent, translucent, turbid, or milky. The percentage of vitamin E dissolved was measured from the UV scans at 294 nm whereas the turbidity of the medium was estimated by converting the UV absorbance data at 420 nm into Jackson Turbidity Units (JTU). The mean particle diameter of the resultant emulsion/microemulsion was estimated by dynamic light scattering using a Nicomp® 380 particle size analyzer (PCC Nicomp, Santa Barbara, CA). The resulting histograms were weighed according to particle volume.

3. Results and discussion

3.1. Experimental design

A 25-run, five-factor, D-optimal design was utilized in this study to correlate the effect of formulation ingredients with the observed responses. The independent and dependent design variables are listed in Table 1. Experimental runs and the observed responses are given in Table 2. The four primary responses that were investigated in this study were (1) the amount of free fatty acids released, denoted as Y_1, (2) percentage lipolysis, denoted as Y_2, (3) percentage of vitamin E retained in the aqueous phase post-lipolysis, denoted as Y_3, and (4) percentage of vitamin E dissolved in the dissolution medium at the end of a standard *in vitro* dissolution experiment, denoted as Y_4. As reported in Table 2, formulation No. 21 released the maximum amount of free fatty acids and had the highest percent lipolysis, whereas formulation No. 4 had the minimum value for both responses. Formulation No. 1 resulted in the maximum percentage of vitamin E retained in the aqueous phase post-lipolysis while the minimum percentage of vitamin E retained in the aqueous phase was observed with formulation No. 8. The maximum and minimum percentage of vitamin E dissolved in water at the end of standard dissolution experiments were observed with formulations 3 and 4, respectively.

Experimental runs were analyzed by the sequential model comparison and lack of fit tests to select the model that best describes and fits the data. Results of the sequential model comparison, which indicate whether a model could describe a response, are given in Table 3. The probability value (α) for the test of significance was set at a standard level of 0.05, whereby p-values lower than 0.05 are considered significant. As seen from the table, the linear model was statistically significant ($p < 0.0001$) for Y_1, Y_2, and Y_3, which indicates that the model adequately describes the responses. Statistical significance was not improved by adding either quadratic or cubic terms. For the response Y_4, both the linear and the quadratic models were statistically significant ($p < 0.0001$ and 0.0013, respectively), that is, both models adequately describe the response. The lack of fit test was subsequently performed to further demonstrate the suitability of a given model. As seen in Table 3, large p-values of the linear model (0.0923, 0.3937, and 0.6585 for Y_1, Y_2, and Y_3, respectively) indicate that there is no lack of fit and that the linear model adequately fits the data. For Y_4 however, only the quadratic model had a $p > 0.05$, and thereby it was the only model that could fit the data for this response. Detailed anal-

Table 3
Sequential model comparison and the corresponding lack of fit tests for Y_1–Y_4

Type of model	Sequential comparison (p-value)	Lack of fit tests (p-value)
(A) Statistical model for the free fatty acids released (Y_1)		
Linear	<0.0001	0.0923
Quadratic	0.1592	0.1403
Special cubic	0.1403	N/A[a]
Cubic	N/A	N/A
(B) Statistical model for the percentage lipolysis (Y_2)		
Linear	<0.0001	0.3937
Quadratic	0.6312	0.2662
Special cubic	0.2662	N/A
Cubic	N/A	N/A
(C) Statistical model for the percentage vitamin E retained in the aqueous phase of the digestion medium (Y_3)		
Linear	<0.0001	0.6585
Quadratic	0.8689	0.3844
Special cubic	0.3844	N/A
Cubic	N/A	N/A
(D) Statistical model for the percentage vitamin E dissolved in the dissolution medium (Y_4)		
Linear	<0.0001	0.0124
Quadratic	0.0013	0.2344
Special cubic	0.2344	N/A
Cubic	N/A	N/A

[a] Non-applicable.

ysis of variance (ANOVA) for the responses is summarized in Table 4.

Based on the preceding discussion, the following linear and quadratic multiple regression equations were constructed and used in subsequent discussion to demonstrate the relationship between the formulation ingredients and the responses Y_1–Y_4.

$$Y_1 = -65.25X_1 + 51.89X_2 - 9.69X_3 + 820.96X_4 - 83.14X_5,$$

$$Y_2 = -7.241X_1 + 14.175X_2 - 2.433X_3 + 235.181X_4 - 22.036X_5, \quad Y_3 = -30.3668X_1 + 25.021X_2,$$

$$Y_4 = 152.6X_2 + 103.36X_3 - 141.84X_2X_3 - 756.57\,X_2X_4.$$

where X_1: Vitamin E; X_2: Tween® 80; X_3: Labrasol; X_4: captex 355; and X_5: alcohol. Terms with insignificant p-values were excluded from the equation. The significant effect of each

Table 4
Analysis of variance (ANOVA) for linear model of the responses Y_1–Y_3 and the quadratic model for Y_4

Source	Sum of squares	d.f.[a]	Mean square	Calculated F value	p-Value	
(A) ANOVA for Y_1						
Model	92008.26	4	23002.06	15.01	<0.0001	Significant
Residual	30658.33	20	1532.92			
Lack of fit	27905.58	15	1860.37	3.38	0.0923	Not significant
Pure error	2752.75	5	550.55			
Cor total	0.00001	24				
(B) ANOVA for Y_2						
Model	7448.87	4	1862.22	11.50	<0.0001	Significant
Residual	3239.05	20	161.95			
Lack of fit	2599.23	15	173.28	1.35	0.3937	Not significant
Pure error	639.82	5	127.96			
Cor total	10687.92	24				
(C) ANOVA for Y_3						
Model	589.85	4	147.46	10.20	<0.0001	Significant
Residual	289.24	20	14.46			
Lack of fit	204.94	15	13.66	0.81	0.6585	Not significant
Pure error	84.31	5	16.86			
Cor total	879.09	24				
(D) ANOVA for Y_4						
Model	12911.28	14	922.23	50.06	<0.0001	Significant
Residual	184.22	10	18.42			
Lack of fit	122.57	5	24.51	1.99	0.2344	Not significant
Pure error	61.64	5	12.33			
Cor total	13095.50	24				

[a] Degrees of freedom.

Table 5
Coefficient estimates and significance of formulation ingredients on responses Y_1–Y_4

Component	Coefficient estimate	p-value
(A) Coefficient estimates for Y_1		
Vitamin E	−65.25	0.0120
Tween 80	51.89	0.0413
Labrasol	−9.69	0.0011
Captex 355	820.96	<0.0001
Alcohol	−83.14	0.0133
(B) Coefficient estimates for Y_2		
Vitamin E	−7.241	0.0514
Tween 80	14.175	0.0428
Labrasol	−2.433	0.0022
Captex 355	235.181	<0.0001
Alcohol	−22.036	0.0250
(C) Coefficient estimates for Y_3		
Vitamin E	−30.3668	<0.0001
Tween 80	25.0210	0.0004
Labrasol	15.6445	0.0630
Captex 355	17.7592	0.2827
Alcohol	11.7536	0.6442
(D) Coefficient estimates for Y_4		
Vitamin E	−30.3668	<0.0001
Tween 80	25.0210	0.0004
Labrasol	15.6445	0.0630
Captex 355	17.7592	0.2827
Alcohol	11.7536	0.6442

coefficient in the linear and quadratic model is given in Table 5 and is discussed in greater detail in the subsequent sections.

3.2. Lipolysis studies

The importance of screening lipids as vehicles for drug delivery was demonstrated in recent years with a surge in studies dealing with the mechanisms of fat digestion and absorption (Kaukonen et al., 2004a,b; Devani et al., 2005). Fats and oils are primarily digested by pancreatin; a mixture of lipase and colipase, into free fatty acids and monoglycerides at the physiological pH (Porter and Charman, 2001; Ljusberg-Wahren et al., 2005). In this study the *in vitro* lipolysis experiments were performed to investigate the effect of formulation ingredients on the digestion of α-tocopherol SEDDS formulation. Representative lipolysis data, presented as the mmole free fatty acids released from each formulation after digestion, are given in Fig. 1. The observed "lag time" of approximately 10 min represents the time elapsed between the moment the formulations were added to the digestion medium (time 0) and the moment pancreatin suspension was added to the medium. The effect pancreatin addition on triglyceride hydrolysis is apparent by the sudden surge in free fatty acids released.

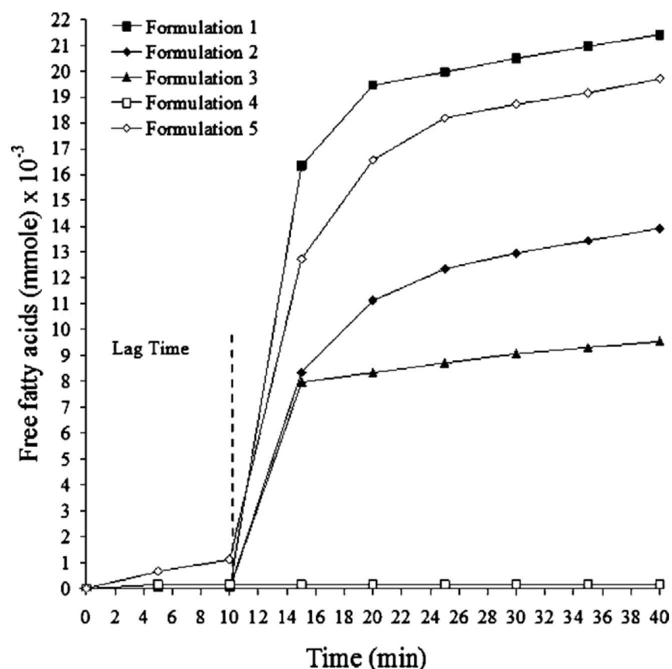


Fig. 1. A representative graph showing the *in vitro* lipolysis of formulations 1–5.

3.2.1. Effect of formulation ingredients on the extent of lipolysis

All formulation ingredients had a significant ($p < 0.05$) effect on lipolysis, whereby Tween® 80, captex 355 had a positive effect; whereas, vitamin E, labrasol, and alcohol had a negative effect (Table 5B). The "extent of" or "% lipolysis" of each formulation is given in Table 2. As given in the table, formulations containing low levels of vitamin E (<25%, w/w), maximum captex 355 content (i.e. 10%, w/w), and maximum amount of Tween® 80 (50%, w/w) exhibited higher % lipolysis than the other formulations. Since captex 355 is the source of triglycerides, its concentration in the formulation had the expected significant positive effect on lipolysis. The significant positive effect of Tween® 80, a non-ionic hydrophilic emulsifier, on lipolysis is probably due to its ability to capture captex 355 into the micelle interface and promote its retention in the aqueous phase for sufficient time that would allow hydrolysis of the triglyceride to proceed (Julianto et al., 2000).

The relationship between Tween® 80, labrasol, and vitamin E – at mid levels of captex 355 and alcohol – and their effect on lipolysis is given as a twodimensional contour plot in Fig. 2. As the levels of labrasol and vitamin E in the formulations increased, there was a pronounced decrease in lipolysis from 24.68% to 17.55%. The negative influence of the vitamin on lipolysis suggests that the vitamin suppressed the lipolysis of the triglyceride (captex 355). Since captex 355 and vitamin E are miscible, it is probable that the vitamin, which does not undergo lipolysis, acted as a shield against triglyceride lipolysis. A similar observation was reported by Yap and Yuen (2004), where Tocomin® 50% (which is a mixture of α-tocopherol and α-, β-, and γ-tocotrienols) exhibited negligible lipolysis (∼4%) when compared to soya oil (73.7%).

Labrasol, a caprylocaproyl macrogolglycerides, was added to the formulations as a coemulsifier to prevent the separation of the oily vitamin and to promote the formation of fine dispersions of low particle size (Gursoy and Benita, 2004). As with vitamin E, it had a significant negative effect on lipolysis. For example, at low level of vitamin E (12.5%, w/w) and maximum level of captex 355 (10%, w/w), labrasol diminished the extent of lipolysis from 46.95% to 39.73%, when its content increased

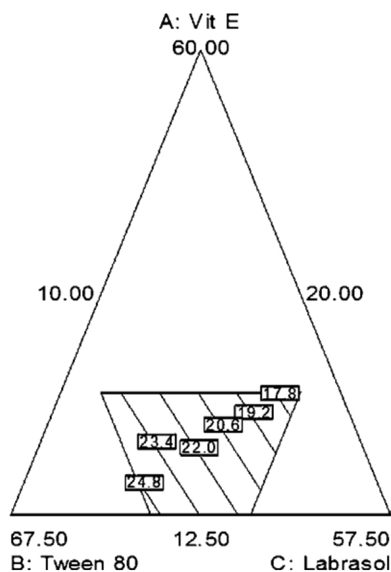


Fig. 2. Contour plot showing the effect of vitamin E, labrasol, and labrasol on the extent of lipolysis (% lipolysis).

from 28.75% to 39.65% by weight (Fig. 3). The effect of labrasol on lipolysis is not clear. Since labrasol has a lower solubilizing capacity than Tween® 80 (Nazzal et al., 2002a), it is possible that the incorporation of labrasol in the formulation on the expense of Tween® 80 diminished the positive effect of Tween® 80 on lipolysis.

The negative influence of alcohol USP on lipolysis could be due to its low dielectric constant, which might have lowered the polarity of sodium hydroxide aqueous solution (D'Aprano et al., 1979). This decrease in the polarity may have had a negative impact on the release of protons from the free fatty acids and thereby suppressing the reaction between sodium hydroxide and the triglyceride in the formulation (Bianchi et al., 2005).

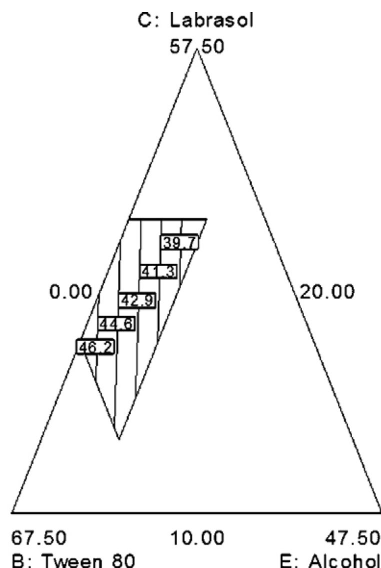


Fig. 3. Contour plot showing the effect of labrasol, Tween 80, and alcohol on the extent of lipolysis (% lipolysis).

3.2.2. *Effect of formulation ingredients on retention of vitamin E in the aqueous phase post-lipolysis*

The cumulative percent of vitamin E retrieved in the aqueous phase of the digestion medium after lipolysis, for each formulation, is given in Table 2. While all formulation ingredients had varying degree of influence on lipolysis, only two factors had a significant effect on the retention of vitamin E in the aqueous phase (Table 5C). These factors are the concentration of vitamin E (negative effect) and the concentration of Tween® 80 (positive effect). As the concentration of vitamin E in the formulations increased, the solubilization and retention of the vitamin in the aqueous phase decreased. This is probably because of the oily nature of the vitamin which enables it at low concentrations to partition into the micellar phase in presence of the surfactant. However, as the concentration of the vitamin increases beyond the solubilizing capacity of the surfactant, its affinity to the non-lipolysate oil (oil phase) of the digestion medium increases with a consequent decrease in its partitioning into the aqueous layer (micellar phase). Similar observations were reported by Borgström (1967) where cholesterol, which is insoluble and resembles α-tocopherol, partitioning into the aqueous layer was dependent on the concentration of the emulsifier available to solubilize it.

Formulations containing higher levels of Tween® 80 were able to retain higher levels of vitamin E in the aqueous phase. This is due to the ability of Tween® 80 to produce water-soluble micelles that contain vitamin E within its core. As reported in the literature, surfactants of high HLB (hyrophilic) are amphiphilic in nature and have the ability to dissolve large amounts of lipophilic drugs in aqueous fluids (Gursoy and Benita, 2004). Therefore Tween® 80 was able to increase vitamin E partitioning and transfer into the aqueous layer of the digestion medium. This effect however was independent of the lipolysis of triglycerides as evident by the insignificant effect of captex 355 concentration on vitamin E retention in the aqueous phase. The influence of lipolysis on the solubilization of vitamin E is further illustrated in Fig. 4, which demonstrates the correlation between the two parameters. As shown in the figure, no correlation was observed between the two responses, i.e. as lipolysis proceeds, the retention of vitamin E in the aqueous phase does not increase in a proportional manner.

3.3. *Physical characterization studies*

3.3.1. *Visual examination, turbidity, and particle size analysis*

During dissolution studies, it was observed that the formulations began to disperse after 2–4 min, which is the approximate time required to rupture the hard gelatin capsules (El-Malah and Nazzal, 2007). The volume-weighed Guassian particle size distribution of the resultant microemulsions or dispersions was determined as described in Section 2.2. Only three formulations – No. 1, 3, and 19 – resulted in transparent microemulsion with an average particle size of 12.3, 10.4, and 12.0 nm, respectively. The remaining formulations were judged as translucent, turbid or milky (Table 2). Even though some of these formulations contained up to 50% Tween® 80 their dispersions had

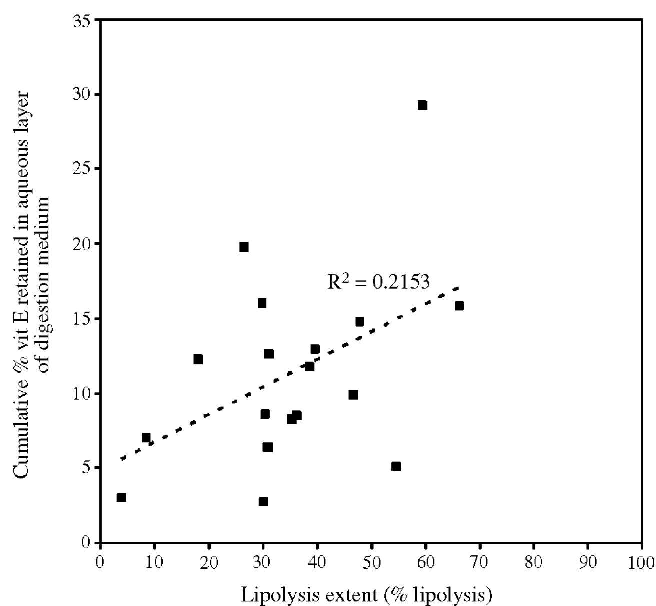


Fig. 4. Correlation between the extent of lipolysis (% lipolysis) and the cumulative percent of vitamin E retained in the aqueous layer of the digestion medium at the end of the lipolysis experiments.

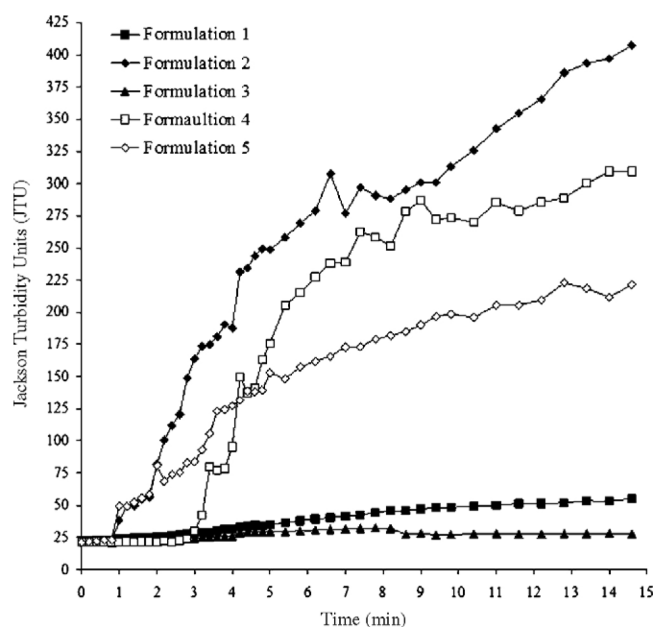


Fig. 5. A representative graph showing the real-time change in the turbidity of the dissolution medium with time for formulations 1–5.

a droplet size ≥1 μm. The turbid appearance of the formulations indicates incomplete micellar solubilization of the lipidic ingredients with a consequent increase in droplet diameter. As reported in the literature, the visual appearance of the formulation after its dispersion in water is a measure of the spontaneity of emulsification and an indirect measure of the particle size of the dispersed phase in the self-emulsifying system (Craig et al., 1993). Measurement of turbidity of the medium during the dissolution process was recently used as an alternative method to quantify the emulsification rate of SEDDS formulations and provide rapid real-time estimate of droplet size (Nazzal et al., 2002c). This method stemmed from the fact that light scattered by the particles in a medium provides an indication on size and number of scattered particles. 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 $\tau = Kn\upsilon^2$, where K is a machine constant, υ the particle volume and n is the number of particles (Groves and Mustafa, 1974). The turbidity of the medium during the dissolution study (Table 2) was measured in Jackson Turbidity Units (JTU) at 420 nm as illustrated in Section 2.2. JTU were in the range from 28.01 (formulation No. 3) to 403.46 (formulation No. 18). Formulation No. 18 contained the highest percentage vitamin E [25%, w/w] and the lowest Tween® 80 content [20%, w/w]. Measured turbidity supported the visual examination (i.e. the transparent formulations gave smaller JTU values). During the emulsification process, the measured turbidity and scattered-light relative intensity were correlated with time. A representative JTU-time plots are given in Fig. 5. The observed lag phase of the measured turbidity-time profiles corresponds to the time elapsed before rupture of the hard gelatin capsule and the release of its contents into the dissolution medium.

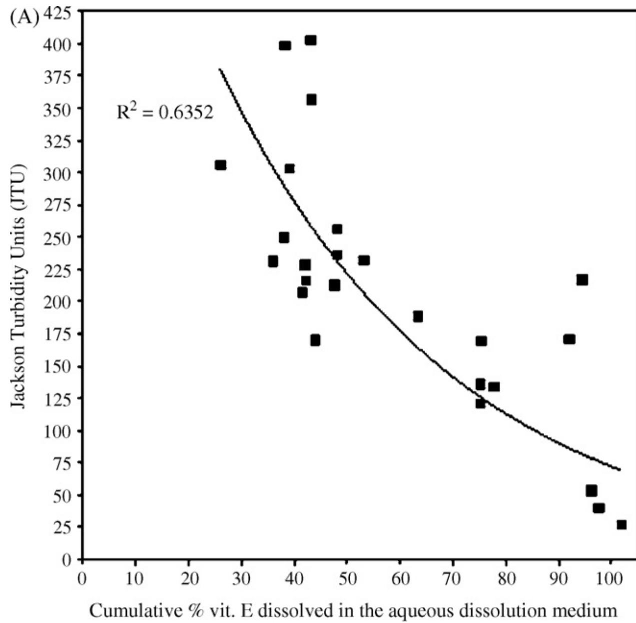
Formulations that produced transparent dispersions showed lower droplet size. These results are in agreement with those obtained by Nazzal et al. (2002c) who established a comparison between visual observation and particle size.

The preceding discussion illustrates that lipid formulations at different blend compositions impart different visual properties when dispersed in aqueous media. Such observations are frequently used to optimize SEDDS formulations (Nazzal et al., 2002c). The relationship between the turbidity of the dissolution medium and the cumulative percent of vitamin E dissolved in the dissolution medium or the cumulative percent of the vitamin retained in the aqueous layer of the digestion medium is given in Fig. 6. As shown in the figure, the exponential relationship between turbidity and dissolution of vitamin E implies that turbidity might be a beneficial parameter when developing SEDDS formulations based on *in vitro* characterization data. However, while turbidity shows a similar trend with both responses, there was no apparent correlation between turbidity and the cumulative percent of the vitamin retained in the aqueous layer of the digestion medium (Fig. 6B).

3.3.2. Measurement of the percentage vitamin E dissolved in the dissolution medium

The cumulative % of vitamin E dissolved in the aqueous dissolution medium at the end of the *in vitro* dissolution studies is given in Table 2. The maximum and minimum amount of the vitamin dissolved was 101.72% and 25.91%, respectively. The significance of the formulation ingredients and their interaction on vitamin E dissolution is given in Table 5D.

Only three formulation ingredients; Tween® 80, labrasol, and captex 355, had a positive and significant effect on vitamin E solubilization in the dissolution medium; whereas, the interaction effect between Tween® 80 with either labrasol or captex 355 had a negative significant effect ($p < 0.05$) on vitamin E dissolution (Table 5D).



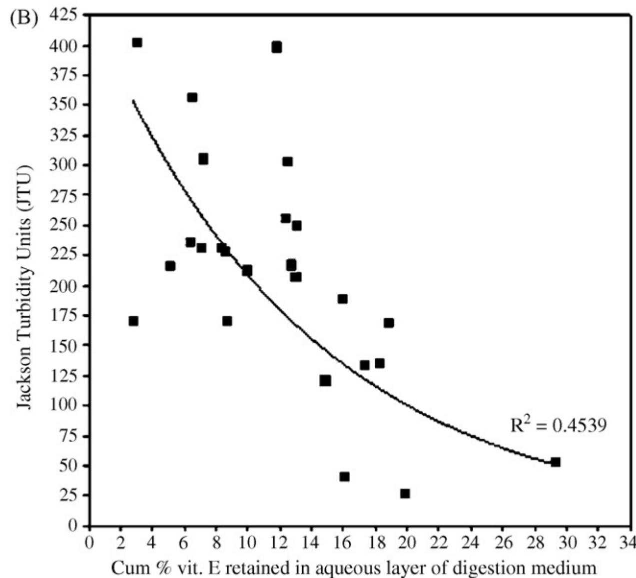


Fig. 6. Correlation between the turbidity of the dissolution medium (JTU) and (A) the cumulative % of vitamin E dissolved in the dissolution medium and (B) the cumulative percent of vitamin E retained in the aqueous layer of the digestion medium at the end of the lipolysis experiments.

The relationship between Tween® 80, labrasol, and captex 355 and their interaction effect on the percent vitamin E dissolved in the dissolution medium is given in Fig. 7. As shown in the figure, the cumulative percent of vitamin E dissolved increased with an increase in the concentration of either Tween® 80 or labrasol. Also, the addition of captex 355 to the Tween® 80/labrasol blend increased vitamin E dissolution. It is probable that captex 355 stabilized the interfacial film formed at the oil/water interface, which improved the solubilization capacity of the blend. The combination of these three ingredients is a classic example of a microemulsion formulation, whereby primary and secondary surfactants are combined with an oil phase to produce stable microemulsion (Pouton, 2006).

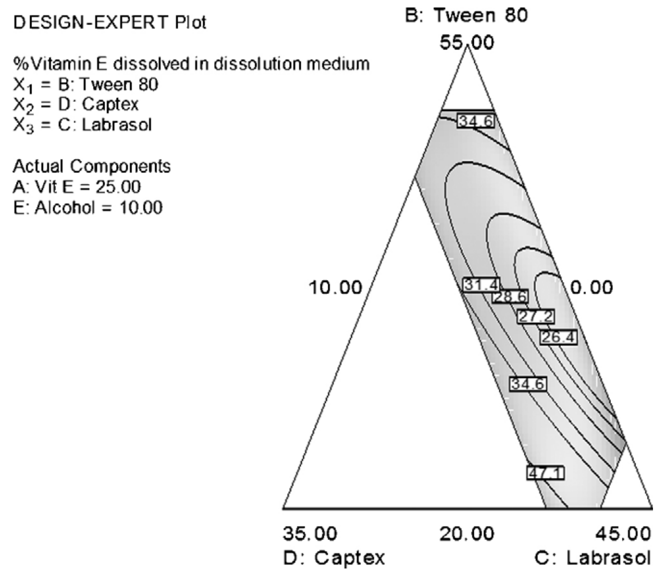


Fig. 7. Contour plot showing the effect of Tween® 80, captex 355, and labrasol on the percent vitamin E dissolved in the aqueous dissolution medium.

The correlation between the cumulative percentage of vitamin E partitioned into the aqueous layer of the digestion medium during lipolysis and the percentage of vitamin E dissolved in the aqueous dissolution medium is given in Fig. 8. The low correlation coefficient (0.344) suggests a weak relationship between the two parameters. Vitamin E solubilization in the aqueous layer of the digestion medium, which contains bile salts and enzymes was dependent only on Tween® 80. In the dissolution medium where deionized water was used, absence of bile salts and enzymes was compensated by the composition of the formulation.

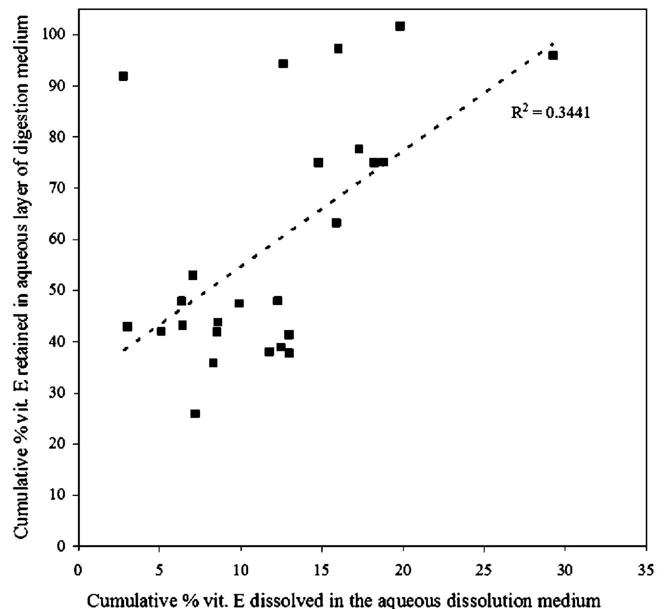


Fig. 8. Correlation between the cumulative percent of vitamin E retained in the aqueous layer of the digestion medium and the percent of vitamin E dissolved in the aqueous dissolution medium.

4. Conclusions

The two objectives that were evaluated in this study were to compare dissolution and lipolysis data and to demonstrate the different roles played by SEDDS ingredients on either dissolution or lipolysis of a poorly soluble drug. The extent of vitamin E solubilization in the aqueous dissolution medium or its retention in the aqueous phase of the digestion medium after lipolysis is directly related to the composition of the SEDDS formulation. The individual ingredients of a lipid formulation often play significant and in times unique role in lipolysis and dissolution. For example, this study demonstrated that the oil phase, exemplified by captex 355, is an essential component of a lipid formulation and is required to form stable microemulsions *in vitro*. This was observed in the dissolution experiments where solubilization and/or emulsification of vitamin E was a result of a complex interaction between the three primary components of the lipid formulation. Therefore, optimizing the ratio of primary and secondary surfactants, and oil are essential to produce SEDDS with desirable *in vitro* characteristics. In this study, however, no correlation was observed between dissolution and lipolysis data and between the extent of lipolysis and the percent of vitamin E partitioned to the aqueous phase of the digestion medium. Therefore, data generated from lipolysis experiments cannot be used to estimate the solubilization of vitamin E in the aqueous layer; instead they are merely a measure of triglyceride (captex 355) digestion. Captex 355 had a significant impact on lipolysis, yet it had a negligible effect on vitamin E partitioning into the aqueous phase during lipolysis. Retention of the vitamin in the aqueous phase was only influenced by the concentration of Tween 80® in the formulation. This implies that during lipolysis, the extent to which the vitamin partitions between the phases of the digestion medium is mediated by interactions between Tween 80® and bile salts rather than the composition of the lipid formulation or the lipolysis of triglycerides. Based on these data it could be concluded that standard dissolution studies are suitable for optimizing SEDDS formulations and for identifying critical formulation variables. While a weak correlation was observed between vitamin E dissolution and its retention in the aqueous phase of the digestion medium, dissolution studies are better suited to predict the performance of a drug in digestion medium than lipolysis data.

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