Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 352 (2008) 104–114
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

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*

Received 27 June 2007; received in revised form 11 September 2007; accepted 19 October 2007
Available online 26 October 2007

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.

Keywords: Lipolysis; SEDDS; Griseofulvin; Dissolution; Optimization

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).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.10.023

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