Colloids and Surfaces A: Physicochem. Eng. Aspects 353 (2010) 43–51

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

Preparation and *in vitro* antiproliferative effect of tocotrienol loaded lipid nanoparticles

Hazem Ali, Amit B. Shirode, Paul W. Sylvester, Sami Nazzal*

Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of Louisiana at Monroe, Monroe, LA 71209-0497, United States

ARTICLE INFO

Article history:
Received 27 August 2009
Received in revised form 14 October 2009
Accepted 18 October 2009
Available online 30 October 2009

Keywords:
Solid lipid nanoparticles
Nanostructured lipid carriers
Tocotrienol
Antiproliferation
Factorial design
Stability

ABSTRACT

The primary objective of this study was to prepare nanostructured lipid carriers loaded with tocotrienol-rich-fraction of palm oil (TRF-NLCs) and to evaluate their antiproliferative effects against neoplastic +SA mammary epithelial cells. This necessitated optimizing the ultrasonic homogenization process parameters and the surfactant to lipid ratio within the NLCs. Therefore, sonication time and pulsar rate were initially evaluated for their effect on the size and polydispersity of the nanoparticles using a full factorial design. Also, varying the surfactant to lipid ratio from 0.25:1 to 3:1 was evaluated for its effect on the same responses. Optimal nanoparticles were obtained when dispersions containing a surfactant to lipid ratio of 0.5:1, with a total lipid concentration of 0.25 (w/v), were sonicated at 60% pulsar rate for 10 min. These parameters were subsequently used to prepare TRF-NLCs. TRF was loaded into the nanoparticles by substituting 10% (TRF-10-NLC) or 50% (TRF-50-NLC) of the lipid phase with TRF. In an extended stability study, no significant change in particle size of the TRF-NLCs was observed over 6 months of storage. In the cell culture studies, TRF-NLCs were shown to exhibit potent antiproliferative effect against neoplastic +SA mammary epithelial cells. The IC_{50} values of TRF-10-NLCs were 2-fold lower than the IC_{50} value of the reference TRF/BSA solution. In contrast, TRF-50-NLCs had comparable IC_{50} values as the TRF/BSA solution, which signified the importance of TRF encapsulation within NLCs on their activity. Furthermore, these findings suggested that TRF-NLCs may have potential value in the treatment of breast cancer.

1. Introduction

The vitamin E extract from palm oil, which is commonly referred to as tocotrienol-rich-fraction or TRF (Fig. 1), is a mixture of individual tocopherols (20–30%) and tocotrienols (70–80%) [1]. Both tocopherols and tocotrienols have similar chemical structure characterized by a phytyl side chain attached to a chromane ring. The difference between individual isoforms of tocopherols and tocotrienols, however, lays in the degree of methylation of their chromane ring and the saturation of the phytyl chain.

In recent studies, TRF was shown to display potent antiproliferative and apoptotic activity against breast cancer cells through blockade of mevalonate synthesis [2,3]. Therefore, systemic delivery of TRF to the mammary tumor cells via the aid of colloidal drug carriers (CDCs) such as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) was undertaken to improve its potency and overcome its poor oral bioavailability [4].

SLNs are aqueous colloidal dispersions with a size in the range of 50–1000 nm, the matrix of which is composed of biodegradable and biocompatible solid lipids [5]. Unlike SLNs, however, the cores of the NLCs are composed of blends of lipids in which liquid low melting-point lipids are entrapped in the form of oily nanocompartments within a solid matrix [6,7].

Several techniques have been used for the preparation of SLNs and NLCs such as high pressure homogenization [8], solvent emulsification/evaporation [9], and melt emulsification [10]. The melt emulsification method is commonly employed for the production of liposomes and polymeric nanoparticles [11–13]. This method is suitable for dilute samples and for small-scale production without the need for highly sophisticated equipment [14,15]. Nonetheless, the quality of SLN and NLC dispersions prepared by melt emulsification might be compromised by the presence of large particles (microparticles), which may result in batch polydispersity. Therefore, subsequent homogenization of dispersions by ultrasound is often employed to overcome this problem [16–18]. During ultrasonic homogenization, several parameters, such as sonication time, power and pulsar rate may have an impact on the physical properties of SLNs and NLCs. While many studies documented the preparation and characterization of SLNs as described above, including those containing vitamin E [19], which has similar physiochemical properties as TRF, the impact of sonication parameters

* Corresponding author at: Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of Louisiana at Monroe, 1800 Bienville Drive, Monroe, LA 71201, United States. Tel.: +1 318 342 1726; fax: +1 318 342 1737.
E-mail address: nazzal@ulm.edu (S. Nazzal).

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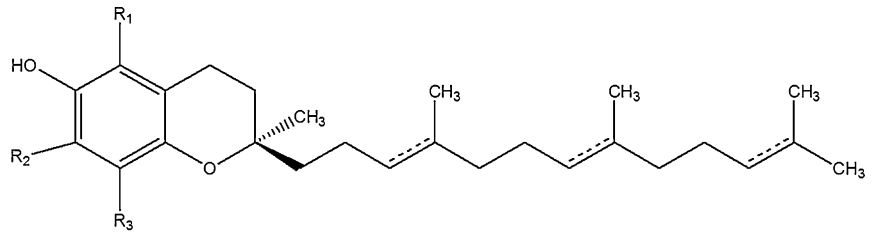
doi:10.1016/j.colsurfa.2009.10.020



Compound	R_1	R_2	R_3	Phytyl chain
α-tocopherol	CH_3	CH_3	CH_3	Saturated
γ-tocopherol	H	CH_3	CH_3	Saturated
δ-tocopherol	H	H	CH_3	Saturated
α-tocotrienol	CH_3	CH_3	CH_3	Unsaturated
γ-tocotrienol	H	CH_3	CH_3	Unsaturated
δ-tocotrienol	H	H	CH_3	Unsaturated

Fig. 1. Generalized chemical structure of vitamin E, which is a mixture of individual tocopherol and tocotrienols isoforms. Both tocopherols and tocotrienols have similar chemical structure characterized by a phytyl side chain attached to a chromane ring. The difference between individual isoforms of tocopherols and tocotrienols, however, lays in the degree of methylation of their chromane ring and the saturation of the phytyl chain.

on the size and polydispersity of the lipid nanoparticles, however, has not been extensively evaluated.

Therefore, the objective of the first part of this study was to evaluate the effect of sonication time and pulsar rate on the size and distribution of unloaded (blank) SLNs. These parameters were evaluated using a full factorial design to allow the estimation of the optimal conditions for the production of SLNs. Due to the effect of formulation composition on particle size, which is a critical quality attribute of nanoparticles, the effect of lipid composition and lipid to surfactant ratio on the size and polydispersity of the nanoparticles was evaluated as a secondary objective.

In the second part, TRF was incorporated into lipid nanoparticles using the optimal process conditions and formulation composition, which were identified in the first phase of this study. The objectives of the second part were therefore to evaluate the long-term stability of the TRF nanostructured lipid carriers (TRF-NLCs) with respect to size and to assess the cellular antiproliferative affect of TRF-NLCs against neoplastic +SA mammary epithelial cells.

2. Materials and methods

2.1. Materials

Cetyl palmitate (melting point: 45–55 °C) was purchased from TCI America (Portland, OR); Dynasan® 118 (glyceryl tristearate, melting point: 70–72 °C) was provided by Sasol Chemicals North America LLC (Houston, TX); Compritol® 888 ATO (glyceryl behenate, melting point: 71–74 °C), which is a mixture of ~15% mono-, 50% di- and 35% triglycerides of behenic acid, and Precirol® ATO 5 (glyceryl palmitostearate, melting point: 53–56 °C) were provided by Gattefossé (Saint-Priest, Cedex, France); Lutrol® F 68 NF (poloxamer 188) was obtained from BASF (Florham Park, NJ); tocotrienol-rich-fraction of palm oil (TRF), which contains 20.2% α-tocopherol, 16.8% α-tocotrienol, 44.9% γ-tocotrienol, 14.8% δ-tocotrienol, and 3.2% of a non-vitamin E lipid soluble contaminants, was a gift from the Malaysian Palm Oil Board (Selangor, Malaysia); Methylthiazolyldiphenyl tetrazolium bromide (MTT), bovine serum albumin (BSA), and other chemicals for cell culture experiments were purchased from Sigma Chemical Company (St. Louis, MO, USA); water was obtained from NanoPure purification system. All chemicals were used as supplied without further modification.

2.2. SLNs and TRF-NLCs preparation from o/w microemulsion precursors

For the process evaluation study, unloaded SLNs were prepared by the hot o/w microemulsion technique using high-shear homogenization as described by Ahlin et al. [20] with slight modifications. In brief, Compritol® 888 ATO (SLN-COMP) was allowed to melt at 80 °C, meanwhile in a separate vial, Lutrol® F68 was dissolved in purified water and the solution was heated to 80 °C. The hot surfactant solution was then added to the molten lipid under high-shear homogenization at 20,000 rpm using IKA® Ultra-Turrax T8 mixer (IKA® Works Inc., NC, USA). The concentration of compritol, as the lipid phase, in the dispersion was 0.25% (w/v) whereas the surfactant to lipid ratio was 0.5:1. After 5 min, the o/w microemulsion was further processed by probe sonication using an ultrasonic homogenizer (Model 150VT, Biologics, Inc., Manassas, VA). Sonication time and pulsar rate were adjusted according to the experimental design (Table 1). During sonication, the temperature of the dispersions was recorded in real-time every 10 s using a digital dip probe (Traceable® thermometer Type K Rs 232, Calibration Control Company, Houston, TX) connected to a computer preloaded with a DAS-3TM-data acquisition software. After homogenization, SLNs were formed by annealing the sonicated dispersions overnight at 4 °C. To study the effect of formulation composition, unloaded SLNs were prepared as described above with minor adjustments. Different SLNs were prepared using one of the following four lipids as their lipid core: cetyl palmitate (SLN-CET), Dynasan® 118 (SLN-DYN), Compritol® 888 ATO (SLN-COMP), and Precirol® ATO 5 (SLN-PREC). The concentration of the lipids

Table 1
The full factorial design summary: independent and dependent design variables.

Independent variables	Units	Levels		
		Low	Medium	High
X_1: Time	min	2.0	6.0	10.0
X_2: Pulsar rate	%	30.0	60.0	90.0

Dependent variables	Units	Observed responses	
		Minimum	Maximum
Y_1: Particle size	nm	113.6	578.9
Y_2: Polydispersity index (PI)	N/A	0.25	0.45
Y_3: Change in temperature (ΔT)	°C	1.6	13.0