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Molecular interaction and localization of tocotrienol-rich fraction (TRF) within the matrices of lipid nanoparticles: Evidence studies by Differential Scanning Calorimetry (DSC) and Proton Nuclear Magnetic Resonance spectroscopy (^{1}H NMR)

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ABSTRACT

Nanostructured lipid carriers (NLCs), made from mixtures of solid and liquid lipids, were postulated to have superior properties over solid lipid nanoparticles (SLNs). Nonetheless, the architecture of their inner cores remains elusive. The objective of this study was to elucidate the mode by which tocotrienol-rich fraction (TRF) is entrapped within NLCs and the impact of TRF interaction with solid lipids on the long-term stability of the nanoparticles. The mode of TRF localization was postulated from TEM image analysis and ^{1}H NMR signals' amplitude. The size, polydispersity, and fusion enthalpy were found to decrease with an increase in TRF loading, which implied a distortion in the crystallinity of the nanoparticles and the preferential entrapment of TRF within the cores of the NLCs. Nonetheless, ^{1}H NMR spectra of TRF-NLCs broadened as TRF load decreased from 100 to 10%, which was attributed to partial TRF mobility on the surface of the nanoparticles. This was confirmed by TEM images of NLCs at 50% TRF loads. These data led to the conclusion that NLCs have limited capacity to accommodate TRF with the excess being expelled to the surface of the nanoparticles. Such arrangement may have implication on future utility of the NLCs as drug delivery vehicles.

1. Introduction

A vitamin E rich extract of palm oil is commonly referred to as tocotrienol-rich-fraction or TRF (Fig. 1). TRF is an oily mixture of tocopherols and tocotrienols, in which tocotrienols constitutes 70–80% of the blend [1,2]. The fundamental structural difference between the two groups is the phytyl chain, which is unsaturated in tocotrienols (Fig. 1). The isoforms of tocopherols and tocotrienols differ from each other by the degree of methylation of the chromane ring.

In recent studies, TRF was shown to display potent antiproliferative and apoptotic activity against breast cancer cells [3]. Therefore, targeted delivery of TRF to the mammary cancer cells via the aid of colloidal drug carriers (CDCs) was considered a promising adjuvant or alternative to the currently used therapeutic regimens [4]. Among the CDCs, solid lipid nanoparticles (SLNs) have attracted increasing interest by many researchers [5,6]. SLNs were proposed as alternative carrier systems to other traditional colloidal systems such as emulsions, liposomes and polymeric microparticles [7].

SLNs are aqueous colloidal dispersions with a size in the range of 50–1000 nm [8]; the matrix of which is comprised of biodegradable and biocompatible solid lipids. The advantages of SLN include the provision of controlled drug release and drug targeting, protection of incorporated drugs against chemical degradation, biosafety of the carrier, and the feasibility of large scale production [9]. Furthermore, SLNs were shown to be ideal carriers for lipophilic as well as hydrophilic drugs, such as leuprolide [10], doxorubicin [11], methotrexate [12], 10-hydroxycamptothecin [13], chlorambucil [14], saquinavir [15], diazepam [16], ketoconazole [17], cisplatin [18], temozolomide [19], and bromocriptine [20].

Most often, SLNs are prepared by dispersing a molten blend of a high melting point lipid and a drug in a warm aqueous dispersion containing an emulsifier [19]. However, due to the tendency of the lipids to re-crystallize into more perfect β-modification with limited intermolecular distances after cooling, the incorporated drugs tend to leak or squeeze-out from the nanoparticles after days or months of storage leading to a low drug payload [21]. Therefore, a new generation of lipid nanoparticles, termed nanostructured lipid carriers or NLCs, were developed to overcome the limitations of SLNs and provide a better accommodation for the incorporated drugs [22]. Unlike SLNs, the cores of the NLCs are composed of blends of low (liquid) and high (solid) melting point lipids. Such

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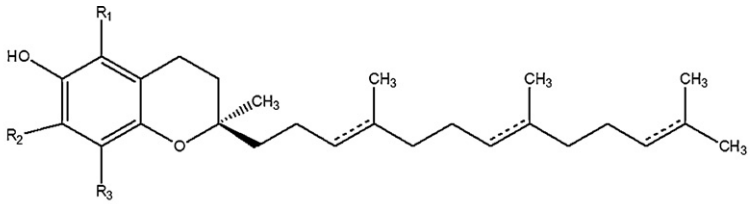

Compound	R_1	R_2	R_3	Phytyl chain
α-tocopherol	CH_3	CH_3	CH_3	Saturated
γ-tocopherol	H	CH_3	CH_3	Saturated
δ-tocopherol	H	H	CH_3	Saturated
α-tocotrienol	CH_3	CH_3	CH_3	Unsaturated
γ-tocotrienol	H	CH_3	CH_3	Unsaturated
δ-tocotrienol	H	H	CH_3	Unsaturated

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, lies in the degree of methylation of their chromane ring and the saturation of the phytyl chain.

blends create imperfections in the crystalline lipid core of the SLNs that increase particles' loading capacity [21] and allow the entrapment of the low melting point lipid.

Several theories were postulated on the mode by which oil molecules or low melting point lipids are entrapped within the crystalline matrices of solid lipids and suggested that upon cooling the mixtures of liquid and solid lipids NLC particles become solid but solid lipids do not crystallize (i.e. remain in amorphous state) [21]. Being entrapped within solid matrices, the mobility of the liquid oil will then be diminished. Differential Scanning Calorimetry (DSC) measurements can confirm the amorphous state of the NLCs by the absence of the melting endotherm of the solid lipid. Complimentary to DSC, Proton Nuclear Magnetic Resonance (^{1}H NMR) spectra would show slightly broaden proton peaks corresponding to the oil molecules, which are now rendered immobile by the solid lipid matrix [21].

Alternatively, liquid molecules may be entrapped in the form of oily nanocompartments or nanodomains, which are completely surrounded by the solid lipid matrix [21]. These domains might be ejected from the molten lipid matrix during lipid crystallization at the cooling step and relocate into a more polar environment, which is the bulk surfactant solution [23]. NLCs were, therefore, described as having a "nanospoon" structure where the spherical liquid droplets are attached to the surface of thin platelet shaped crystalline solid lipid particles [23].

The objective of the present work was to provide additional insight into the nanostructure of the NLCs using TRF as the model low melting point lipid. The mode of TRF entrapment and/or affinity to the solid lipid matrices as well as its tendency to form supercooled melts was evaluated by complementing thermal analysis with ^{1}H NMR studies. Thermal analysis via DSC was found to be a suitable method to study the degree of crystallinity, the polymorphic state of lipids, and the presence or absence of separate solid lipid particles and liquid oil droplets [24,25], such as TRF. On the other hand, the mobility, arrangement, and the mode of liquid entrapment within solid matrices were evaluated by ^{1}H NMR [26]. The specific objectives of this study were therefore (I) to evaluate the effect of TRF on the crystallinity and supercooling of the TRF/solid lipid physical blends and NLCs by DSC and (II) to examine the mode of TRF entrapment/association within the NLCs by ^{1}H NMR. To further illustrate the effect of NLC composition on TRF entrapment, four high melting point lipids, namely glyceryl tristearate (Dynasan® 118, DYN), glyceryl behenate (Compritol® 888 ATO, COMP), glyceryl palmitostearate (Precirol® ATO 5, PREC), and cetyl palmitate (CET) that vary in their chemistry, were selected as the matrix forming solid lipids. A secondary objective of this work was to evaluate the long-term stability of the TRF-NLCs with respect to their size.

2. Materials and methods

2.1. Materials

Cetyl palmitate (CET, melting point: 45–55 °C) was purchased from TCI America (Portland, OR); deuterium oxide (Deuterated water) and deuterated chloroform were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA); Dynasan® 118 (DYN, glyceryl tristearate, melting point: 70–72 °C) was provided by Sasol Chemicals North America LLC (Houston, TX); Compritol® 888 ATO US/NF (COMP, glyceryl behenate, a mixture of ~15% monoglycerides, 50% diglycerides and 35% triglycerides of behenic acid, melting point: 71–74 °C) and Precirol® ATO 5 (PREC, glyceryl palmitostearate, melting point: 53–56 °C) was 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 provided by the Malaysian Palm Oil Board (Selangor, Malaysia).

2.2. Preparation of SLN, NLC, and TRF nanoemulsion (TRF-NEmu)

SLNs, NLCs, and TRF nanoemulsion (Table 1) were manufactured by hot o/w microemulsion using a high-shear homogenization technique [27]. Briefly, 10% (w/v) lipid phase was melted at 85 °C. Five percent (w/v) of Lutrol® F68 was dissolved in deuterium oxide (85%, w/v), and the aqueous surfactant solution was preheated to 85 °C. Then, the solution was added to the molten lipid. The premix was homogenized at 20,000 rpm for 5 min using IKA® Ultra-Turrax T8 mixer (IKA® Works Inc., NC, USA). The formed hot pre-emulsion was then ultrasonicated, at 60% pulsar rate, for 10 min using ultrasonic probe homogenizer (Biologics, Inc., VA). Nanoparticles were subsequently formed by cooling the sonicated nanoemulsion overnight at 4 °C. NLC formulations, containing TRF at 10, 30, and 50% (w/w) of the total lipid phase, were prepared by the same procedure as described above for the preparation of SLNs with the exception that TRF was first mixed with the molten lipid at 85 °C before the addition of the hot surfactant solution. Likewise, TRF-NEmu, containing 100% TRF of total lipid phase, was prepared by heating TRF to 85 °C before the addition of the hot sur-