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Distribution of a model bioactive within solid lipid nanoparticles and nanostructured lipid carriers influences its loading efficiency and oxidative stability

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ABSTRACT

The overall goal of this study was to characterize the distribution of a model bioactive encapsulant in the lipid domain of SLNs and NLCs and its relationship with loading efficiency and reactivity of the model encapsulant with oxidative stress agents. Distribution of a model bioactive (beta-carotene) was compared to that of a fluorescent dye (Nile red) in SLNs, 10% NLC, 30% NLC, 50% NLC, 70% NLC (the number represents the percentage of liquid lipid within the total lipid amount) and emulsions. Fluorescence imaging shows that the distribution of Nile red in the lipid domain of colloidal carriers was similar to that of beta-carotene in all formulations. Based on the combination of imaging observations and loading efficiency measurements, the results demonstrate that beta-carotene was excluded from the lipid domain in both SLNs and NLCs. The extent of exclusion decreased, while uniformity in the distribution of encapsulant in the lipid domain of colloidal carrier increased with an increase in percentage of liquid lipid content of NLCs. Oxidative stability of the encapsulated beta-carotene in SLN and NLCs (at least until 30% liquid lipid composition) was significantly lower compared to that in emulsion. Only for the NLCs with 50 and 70% liquid lipid content, oxidative stability of the encapsulated compound was significantly higher than that in emulsions. Overall, the results demonstrate that differences in loading efficiency and oxidative stability of beta-carotene in SLNs and NLCs may be explained by the differences in the distribution of beta-carotene.

1. Introduction

Lipid based carriers have been widely used for encapsulation and delivery of hydrophobic compounds in food, pharmaceutical and cosmetic industries (Fathi et al., 2012; Muller et al., 2002b; Sagalowicz and Leser, 2010; Tamjidi et al., 2013). Among the various approaches used to classify these carriers, one approach is based on the physical state of the lipid phase. Based on this approach, lipid carriers that are commonly used in diverse applications can be classified as solid lipid nanoparticles (SLNs) with a solid lipid phase, emulsions with a fluid lipid phase and nanostructured lipid carriers with a combination of solid and liquid lipids, respectively (Muller et al., 2002a; Pardeike et al., 2009). Identification of optimal formulations among these diverse lipid carriers has been of significant research interest. Previous studies have indicated that the SLN and NLC based lipid carriers show improved stability against coalescence compared to emulsions (Mehnert and Mader, 2012; Muller et al., 2002b). In addition, prior studies have compared these formulations based on a variety of factors, including (a) encapsulation efficiency; (b) chemical stability; and (c) controlled and triggered release of the encapsulated ingredients (Fathi et al., 2012; Mehnert and Mader, 2012; Muller et al., 2002b; Tamjidi et al., 2013; Teeranachaideekul et al., 2007a; Tikekar and Nitin, 2011).

Based on these criteria, prior studies have indicated significant differences in the encapsulation efficiency (Mehnert and Mader, 2012; Muller et al., 2002a,b); oxidative stability (Muller et al., 2002b; Tikekar and Nitin, 2011) and controlled release (Mehnert and Mader, 2012; Muller et al., 2002a,b; Teeranachaideekul et al., 2007b) among emulsions, SLNs and NLCs. Distribution of encapsulants within the lipid domain has been proposed as one of the leading factors influencing these differences among lipid

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based colloidal carriers (Dan, 2014; Pardeike et al., 2009). Despite potential role of the distribution of encapsulants within the lipid phase on their encapsulation efficiency, oxidative stability and release, there is limited direct evidence of the variations in the distribution of the encapsulants within these lipid-based particles. Furthermore, there is limited understanding of differences in the oxidative barrier properties among SLNs, NLCs and emulsions. Recent evidence of variations in the distribution of the encapsulants among SLNs and selected compositions of NLCs and their impact on the reactivity of these encapsulants was presented using surrogate dyes such as Nile red and peroxyl radical sensitive BODIPY dyes or EPR probes such as PTMIO (Tikekar and Nitin, 2011, 2012; Yucel et al., 2013). It is widely recognized that the properties of the surrogate dyes such as their partition coefficient, solubility in lipid domains, and the ability to form crystalline states may not match the properties of the target encapsulants such as bioactive compounds. For example, the log P_{ow} value of Nile red dye (a lipid imaging dye used in a prior study) is ~4 (Turner and Guy, 1997), while the log P_{ow} value of beta-carotene (provitamin A pigment) is ~17 (Decker et al., 2005). In addition, bioactive compounds such as beta-carotene can form crystalline structures (Zhou et al., 1996) that can have a limited solubility in either lipid or aqueous phase. This complex behavior cannot be easily mimicked by the surrogate dyes. Furthermore, there is significant interest in optimization of NLC compositions for improving encapsulation efficiency, controlled release and/or improved oxidative stability of the bioactives (Tamjidi et al., 2013), but there is limited understanding of the differences in the distribution of encapsulants within these NLCs and its impact on encapsulation efficiency and oxidative stability of the encapsulant. Thus, measuring the distribution of encapsulants can be critical for understanding the differences in the functional properties of these formulations, such as controlled release and oxidative stability of the encapsulants.

The overall motivation for this study was to characterize the distribution of a model bioactive encapsulant within the lipid domain of SLNs and NLCs and its relationship with loading efficiency and reactivity of the model encapsulant with oxidative stress agents. With this motivation, the specific goals of this study were to: (1) directly visualize, compare and correlate the distribution of a model dye and a model bioactive compound within the lipid domain of SLNs, 10% NLC, 30% NLC, 50% NLC, 70% NLC (the number represents the percentage of liquid lipid incorporated within the total lipid amount) and emulsion; and (2) evaluate the effect of variations in the distribution of the model bioactive compound within the lipid domain of the colloidal carriers on its loading efficiency and oxidative stability.

Eicosane and glyceryl trioctanoate (GT) were selected as the solid and liquid lipids, respectively. The % of solid and liquid lipids used for the NLC formulations selected for this study have been used in some prior studies to evaluate the physicochemical properties and release profiles of various encapsulants (Jenning et al., 2000b; Souto et al., 2004; Teeranachaideekul et al., 2008). A fluorescence imaging approach was used to visualize the distribution of a model dye (Nile red) and a model bioactive compound (beta-carotene) within the lipid domain of the colloidal carriers. Beta-carotene has antioxidant activity and has been proposed as a cancer prevention agent (Burton and Ingold, 1984; Omenn et al., 1996). However, limited water solubility and poor oxidative stability limits its broad application in diverse products. The unique and novel aspects of this study are: (1) imaging based evidence for the differences in the distribution of a selected bioactive and a dye molecule within the lipid domain of SLNs, NLCs and emulsion formulations. As discussed earlier, the prior studies including the recent studies have either postulated about differences in the distribution of encapsulants within the lipid domain of colloidal particles (Jenning and Gohla, 2001; Jenning et al., 2000a; Salminen et al., 2016; zur Muhlen et al., 1998) or provided an indirect evidence for it (Jores et al., 2003; Teeranachaideekul et al., 2008); (2) a systematic comparison among SLN, NLCs and emulsion for characterizing influence of the lipid composition on the loading efficiency and oxidative stability of the encapsulants. The observed effects on the loading efficiency and oxidative stability of the encapsulants in these colloidal formulations could be specifically attributed to the lipid composition since the colloidal carriers were prepared using identical methods and were stabilized using the same set of emulsifier molecules; and (3) a comparison between the distribution of a model hydrophobic bioactive compound and a fluorescent hydrophobic dye within the lipid domain of the colloidal carriers. The results of this analysis may validate and enable the use of low—cost, fluorescent dyes as surrogates to evaluate lipid particles for encapsulation applications. Success in this approach may enable evaluation of a broad set of bioactive compounds that may not have endogenous fluorescent properties.

2. Materials and methods

2.1. Materials

Bile salts, eicosane, glyceryl trioctanoate (GT), Nile red, beta-carotene, tetrahydrofuran (THF) and 2,2′-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) were purchased from Sigma-Aldrich Incorporated (St. Louis, MO). High-melting lecithin (Phospholipon® 80 H) was a gift from American Lecithin Inc. (Oxford, CT). Ultrapure water (16MΩ-cm) was obtained from an in-house water filtration system.

2.2. Preparation of SLNs, NLCs and emulsions

The aqueous phase was prepared by dispersing high-melting lecithin and bile salts in ultrapure water to achieve the final concentration of 2 w/v% and 1 w/v% of the aqueous phase, respectively. The total lipid content for all SLNs, NLCs and emulsions was set at 10 w/v% of the aqueous phase. For SLNs, the lipid phase consisted of 100% eicosane. NLCs were prepared by blending liquid (glyceryl trioctanoate) and solid (eicosane) lipids in various proportions (10:90, 30:70, 50:50, and 70:30 liquid:solid) to form 10%, 30%, 50%, and 70% NLCs, respectively. Emulsions contained only GT as the lipid phase. The lipid phase was heated to 70 °C to ensure a complete melting of eicosane. Nile red (12 μg/g total lipid) or beta-carotene (1 mg/g total lipid) was mixed with the lipid phase. The amount selected for beta-carotene was based on the solubility limit of beta-carotene in bulk triglyceride (Borel et al., 1996). The aqueous phase was also heated to 70 °C using a hot plate. The lipid phase was mixed with the aqueous phase and dispersed using a hand-held disperser (Ultra-Turrax model T25, IKA Works, Wilmington, NC) set at 9500 rpm for 2 min. The coarse emulsion was then sonicated for 30 s with a probe-tip sonicator (Qsonica Q55 ultrasonic processor, Newton, CT) at 50% of the maximum amplitude to form stable SLNs, NLCs and emulsion. Sodium azide (0.1 w/v%) was added to SLNs, NLCs and emulsion to prevent the microbial growth and the samples were stored at 4 °C overnight to enable solidification of eicosane.

2.3. Particle size measurements

Hydrodynamic diameters of SLN and NLC particles and emulsion droplets was measured using a particle size analyzer (Model: Malvern Nano Series, Malvern Instruments, Inc., Westborough, MA). The settings for the analyzer were- material type: oil, particle refractive index = 1.45, dispersant type: water, dispersant refractive index = 1.33, temperature: 25 °C. Particle size