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Effect of distribution of solid and liquid lipid domains on transport of free radicals in nanostructured lipid carriers

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ABSTRACT

Peroxyl radical sensitive fluorescent dye (BODIPY 665/676) encapsulated nanostructured lipid carriers (NLCs) were prepared by blending solid (eicosane) and liquid (glyceryl trioctanoate) lipid in various proportions (10:90, 30:70, 50:50, 100:0 liquid: solid). Peroxyl radical transport rate was measured by adding 20 mmol/l 2,2′-azobis-2-methyl-propanimidamide, dihydrochloride in the aqueous phase. Fluorescence imaging of NLCs revealed that fluorescent dye was distributed in the liquid lipid regions of 10 and 30% NLCs. However, 50% NLC resembled NLCs with 100% liquid lipid in that, little or no solid domains were observed and the dye was more or less uniformly distributed. Peroxyl radical transport was in the order, 100% < 50% <30% < 10% NLCs. More uniform distribution of the encapsulant possibly reduced the effective peroxyl radical transport rate, particularly when solid domains were present in the core of NLCs. Thus, 100% liquid lipid emulsions may be better than NLCs in improving the oxidative stability of the encapsulant.

1. Introduction

The inherent biocompatibility of lipid-based nano-carriers has led to extensive interest in their use in applications ranging from drug delivery to cosmetics (Pardeike, Hommoss, & Mueller, 2009; Wissing, Kayser, & Muller, 2004). Solid lipid nanoparticles (SLN) are composed of lipids whose meting point is higher than room or body temperature. However, potential expulsion of the encapsulated compounds may limit the encapsulation efficiency, stability of the encapsulant within SLNs and control over its release (Fang, Fang, Liu, & Su, 2008). To address some of these limitations, nanostructured lipid carriers (NLC) have been developed. NLCs are particles composed of different lipid types that are mixed to reduce the degree of crystallinity and control the core microstructure, with the goal of increasing loading efficiency and achieving better control over the release rates (Muller, Radtke, & Wissing, 2002). It is hypothesized that in some NLCs, mixing lipids disrupts the long-range order of the crystalline structure, thereby allowing encapsulants to intercalate in the interstitial spaces between the lipid crystal grains. In other types of NLCs, phase separation occurs between solid and liquid phases, where the encapsulant is typically solubilized in the liquid domains. In a third category, crystalline domain formation is completely suppressed resulting in a glass-like core (Muller, Petersen, Hornmoss, & Pardeike, 2007; Tamjidi, Shahedi, Varshosaz, & Nasirpour, 2013). However, there is limited understating of the lipid interactions that may result in these distinct structures within NLCs.

Prior studies have examined the release of encapsulated compounds from SLNs and NLCs (Pardeike et al., 2009; Wissing et al., 2004; Yang et al., 1999; zur Muhlen, Schwarz, & Mehnert, 1998). Some find that the release rate is the slowest in SLNs, and increases with increasing liquid phase content in NLCs (Hu et al., 2005), while others found the opposite (Shen, Sun, Ping, Ying, & Liu, 2010; Wang, Liu, Sung, Tsai, & Fang, 2009), where SLNs displayed the most rapid release rate, and the release rate from NLCs decreased with increasing liquid lipid content. Another study did not see a discernable effect of lipid composition on the release rate (Chinsriwongkul et al., 2012). Most unexpected are the results where NLCs—regardless of their composition—had an initial

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release rate that was faster than that of the 100% liquid particles over a shorter period and the trend reversed over longer durations (Teeranachaideekul, Souto, Junyaprasert, & Muller, 2007). We anticipate that these differences in release profile result from differences in intra-particle organization of lipids and the encapsulant molecules. Due to limited understanding of these differences in the current literature, the comparison among different formulations of SLNs and NLCs is expected to provide an inconsistent trend.

To develop a more consistent understanding of the effect of intra-particle organization of solid and liquid lipid domains on the transport through these particles, we examined the effect of the ratio of solid and liquid lipid fractions on the distribution of encapsulant within these particles. These differences in distribution of encapsulants within NLCs were then correlated with differences in their susceptibility to oxidation from peroxyl radicals transported into the NLCs. To achieve this, we used a previously developed approach, where we examined the intra-particle distribution of the encapsulated fluorescent dye molecule using fluorescence microscopy and measured its susceptibility to oxidation triggered by peroxyl radicals generated in the aqueous phase within SLN and two compositions of NLCs (Tikekar & Nitin, 2012).

2. Materials and methods

Eicosane, glyceryl trioctanoate (GT), 2,2′-azobis-2-methyl-propanimidamide dihydrochloride (AAPH), L-phosphatidylcholine and bile salts were purchased from Sigma–Aldrich Incorporated (St. Louis, MO). Peroxyl radical sensitive dye BODIPY 665/676 was purchased from Life Technologies Incorporated (Carlsbad, CA).

2.1. Preparation of NLCs and emulsions

We used a previously developed protocol (Tikekar & Nitin, 2012). Eicosane was melted by heating at 70 °C on a stir plate. The lipid phase for each NLC sample was prepared by adding GT in various weight proportions expressed as the percentage of the total lipid content (10%, 30%, 50% NLCs and 100% emulsion). The total lipid (solid + liquid fractions) content remained constant at 5 g/100 ml of aqueous phase for all the NLC samples. BODIPY 665/676 dye was added to the oil phase at the level of 3 μg/g oil. Aqueous phase was prepared by mixing 1 g/100 ml L-phosphatidylcholine and 0.5 g/100 ml bile salt in distilled water. The solution was heated to 70 °C while under stirring. The lipid phase was mixed with the aqueous phase and homogenized using a hand-held disperser operating at 8000 rpm for 2 min. This coarse emulsion was sonicated at 60% amplitude for 3 min to obtain a stable NLC dispersion. These samples were then added with 0.1 g/100 ml sodium azide to prevent microbial growth and stored at 4 °C overnight to enable crystallization of the solid phase (eicosane). Particle size of these nanoparticles was measured using Malvern Zetasizer (Malvern Instruments Inc., Westborough, MA).

2.2. Fluorescence imaging

Fluorescence imaging was performed on the coarse particles to elucidate differences in the distribution of BODIPY 665/676 dye within these particles as a function of their liquid lipid content. Imaging was performed using a IX 71 fluorescence microscope with a 60× oil immersion objective with a numerical aperture of 1.35.

2.3. Measurement of peroxyl radical transport

We followed a procedure developed previously (Bricarello, Prada, & Nitin, 2012; Tikekar & Nitin, 2011, 2012): Peroxyl radicals were generated by dissolving 40 mmol/l AAPH in ultrapure water. It was further diluted to 20 mmol/l by mixing 1 mL of the sample with 1 mL of AAPH solution, immediately after which, the samples were placed in a plate-reader (Spectramax M5, Molecular 10 Devices, Carlsbad CA) to measure the fluorescence intensity. The values were obtained at an interval of 20 min for a period of 20 h. The excitation and emission wavelengths were 620 nm and 675 nm, respectively. The relative fluorescence intensity was calculated using the following equation:

$$\text{Relative fluorescence intensity} = (I_{t\ AAPH}/I_{max\ AAPH}) \times 100/(I_{t\ control}/I_{max\ control}) \quad (1)$$

where, $I_{t\ AAPH}$ is the fluorescence intensity of the sample after 't' min of exposure to AAPH, $I_{max\ AAPH}$ is the fluorescence intensity of the sample at the maximum value after the addition of AAPH, $I_{t\ control}$ is the fluorescence intensity of control after 't' min, $I_{max\ control}$ is the maximal fluorescence intensity of control. In some formulations, the maximal intensity is obtained immediately after adding the AAPH. However, in the ones discussed here, the maximal intensity occurs some time after that point. This is in agreement with previous studies of the same system, where the maximal intensity was observed approximately 100 min after the addition of AAPH (see (Tikekar & Nitin, 2012) for details).

3. Results and discussion

The average diameter for 100%, 50%, 30% and 10% NLC particles was 225 ± 5, 215 ± 10, 280 ± 10 and 160 ± 25 nm, respectively. Thus, despite some variations in sizes, all the NLC particles had approximately similar particle size range. In Fig. 1, we show characteristic fluorescence images obtained for these coarse NLC formulations. As may be expected, in particles composed of 100% liquid lipids (Fig. 1a) the dye was uniformly distributed throughout the particles, with no apparent internal structure. In contrast, NLCs containing 10% and 30% (Fig. 1b and c) clearly show dark and light domains interspaced throughout the nanoparticles. These suggest coexistence between solid-lipid dominated domains with low dye solubility, and liquid-lipid dominated domains where the majority of the dye is solubilized. In the 50% NLCs (Fig. 1d), the majority of the particle is composed of a dye-containing region, quite similar to the 100% ones (Fig. 1a). In some particles (see the centre one in Fig. 1d) there are some slightly darker domains, suggesting regions with somewhat less solubilized dye, but these domains are not as sharply defined as the domains observed in the 10% and 30% NLCs. This suggests that in this composition, phase separation between solid and liquid domains does not take place.

Next we examine the transport rate of free radicals into the NLCs, as indicated by the fluorescence intensity. As shown in Fig. 2, the decay rate in fluorescence as a function of time is most rapid for the 10% NLCs, namely, those with a high fraction of solid lipids, and therefore solid domains. The decay rate for 30% NLCs is intermediate, while that of 50% and 100% liquid lipid particles is similar, and the slowest.

Similar rates of fluorescence quenching for samples containing 100% and 50% liquid lipids were observed. This is in agreement with our results from particle imaging (Fig. 1d): The dye was uniformly distributed throughout the particle in both 50% and 100% NLC particles. Therefore, it is expected that dye encapsulated within these samples will have similar susceptibility to peroxyl radicals. Interestingly at lower liquid concentrations (10 and 30% liquid lipid) the rate of extinction was significantly higher, suggesting that free radicals can access the dye more rapidly in such cases, because of preferential localization of the dye molecules at the interface.