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Regular Article

Effect of fullerene on the dispersibility of nanostructured lipid particles and encapsulation in sterically stabilized emulsions

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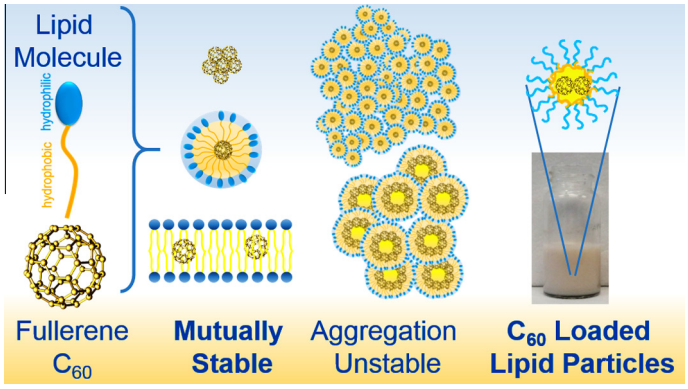
GRAPHICAL ABSTRACT



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ABSTRACT

We report on the effect of fullerenes (C_{60}) on the stability of nanostructured lipid emulsions. These (oil-in-water) emulsions are essentially aqueous dispersions of lipid particles exhibiting self-assembled nanostructures at their cores. The majority of previous studies on fullerenes were focused on planar and spherical lipid bilayer systems including pure lipids and liposomes. In this work, fullerenes were interacted with a lipid that forms nanostructured dispersions of non-lamellar self-assemblies. A range of parameters including the composition of emulsions and sonication parameters were examined to determine the influence of fullerenes on *in-situ* and *pre-stabilized* lipid emulsions. We found that fullerenes mutually stabilize very low concentrations of lipid molecules, while other concentration emulsions struggle to stay stable or even to form at first instance; we provide hypotheses to support these observations. Interestingly though, we were able to encapsulate varying amounts of fullerenes in sterically stabilized emulsions. This step has a significant positive impact, as we could effectively control an inherent aggregation tendency of fullerenes in aqueous environments. These novel hybrid nanomaterials may open a range of avenues for biotechnological and biomedical applications exploiting properties of both lipid and fullerene nanostructures.

1. Introduction

Fullerenes, since their discovery [1,2] have been considered as exciting materials for research and for the exploration of their

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novel applications [3–5]. However, they are sparingly soluble to almost insoluble in water (solubility < 10^{-9} mg/L) [6] and toxic to cells [3]. These aspects turn into major bottlenecks for employing fullerenes in biotechnological applications. Some synthetic chemists, working in this direction, have been able to functionalize fullerenes and obtain their water-soluble derivatives [4,7]. However, in order to understand the biological activity of fullerenes and their potential therapeutic role, it is important to investigate their interactions with cellular membranes [8] than just solubility studies.

Considerable theoretical (simulation-based) [9–13] and experimental [8,14] efforts have been taken to investigate fullerene interactions with biological as well as artificial membrane systems. These reports mainly involved studies with planar lipid bilayers, spherical micelles or vesicles. In this work we have taken a different approach and exploited dispersions of a lipid that forms non-lamellar self-assemblies. The main motivation behind this was twofold, (1) lyotropic liquid crystalline self-assemblies of non-lamellar (hexagonal and cubic) types, are highly attractive for biotechnological applications [15,16], hence studying their interaction with fullerenes may outline the possibilities of developing novel hybrid nanomaterials similar to carbon nanotube-lipid hybrids we developed recently [17–19], and (2) due to their unique structure fullerenes could act as stabilizers for nanostructured lipid emulsions, by which an elegant properties of both – fullerenes and lipid nanostructures could be utilized for novel applications.

Non-lamellar self-assemblies employed in this work were prepared from a mixture of monoglyceride lipids. Due to their amphiphilic (*amphi* means two; i.e. both hydrophilic and hydrophobic) character, these lipids undergo spontaneous self-assembling in presence of aqueous medium [15]. The self-assemblies take forms of 0, 1, 2, or 3 dimensional structures like, for example, inverse micelles, lamellar phase, hexagonal phase and cubic phases, respectively [15]. However, due to viscoelastic nature and variable domain consistency, some of these phases especially cubic ones, are hard to manipulate for certain applications. One of the best solutions researchers have found over this is to fragment these self-assemblies into a particle form using high energy input such as ultra-sonication [20–22]. This procedure is usually performed in presence of external stabilizers which shield fragmented particles and prevent their subsequent aggregation. Resulting dispersions are analogous to classical oil-in-water (o/w) emulsions but the oil phase, in this case, is replaced by a lipid self-assembly (Fig. 1). Structural organization of lipid self-assemblies in the bulk (non-dispersed form) and emulsions (dispersed form) is usually determined using small angle X-ray scattering (SAXS) and transmission electron microscopy (TEM) techniques [23–25]. Depending on the type of internal self-assembly, the nanostructured emulsions are recognized as *cubosomes* for cubic nanostructures, *hexosomes* for hexagonal nanostructures and so on [15,26–28]. The general term '*isasomes*' meaning *internally self-assembled particles* (*somes*) is also often used in this context [26,27,29]. These emulsions exhibit a water-like fluidity desired for a range of high-throughput application including carrier systems for pharmaceutical and biomedical technologies [26,27,30,31].

A wide range of stabilizers are employed for stabilizing aforementioned lipid nanoparticle emulsions [26]. Surfactant based stabilizers are more popular but solid particles have also been exploited with a varying level of success [26]. Emulsions stabilized by solid particles are termed generally as Pickering [32] or Ramsden-Pickering [33] emulsions. Clay platelets [34], food hydrocolloids [35,36] and silica nanoparticles [37] are among such stabilizers, but in our recent work [18] we have employed carbon nanotubes (CNTs) for stabilizing nanostructured lipid particles. Pristine single-walled (SW) CNTs and functionalized multi-walled (MW) CNTs were employed successfully to stabilize internally

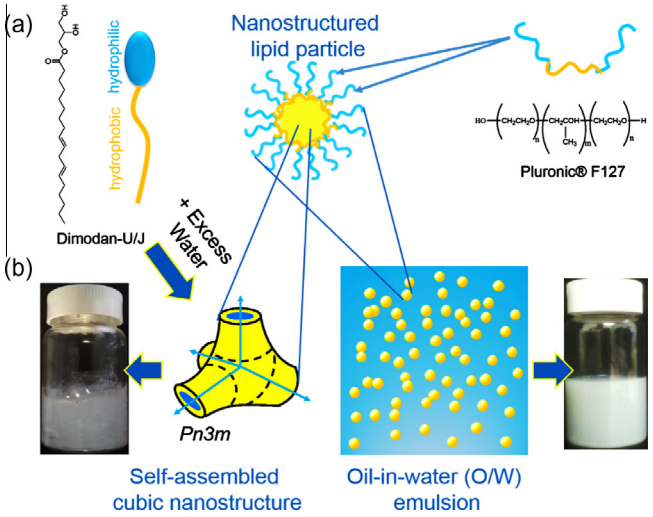

Fig. 1. Composition of nanostructured emulsions: (a) Chemical structures of Dimodan U/J (a lipid) and tri-block copolymer F127 (a stabilizer). Schematic diagrams shown near structures depict hydrophobic parts (yellow) and hydrophilic (blue) parts of molecules. (b) In presence of water, lipid molecules self-assemble into cubic nanostructure (*Pn3m* phase) which is fragmented into sub-micron sized particles that are stabilized using the F127 stabilizer [20]. Lipid cubic phase is usually highly viscous (shown on left) but the o/w emulsion has water-like fluidity (shown on right) improving their handling and applicability. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

self-assembled lipid particles [18]. More interestingly, concentrations of CNT-stabilizers were very low (<10 μg/ml). The lipid coating [18,19] onto CNTs is believed to reduce their potential cytotoxicity given the fact that lipids are already among essential biomolecules in cells. The 'fullerene' is another carbon allotrope like CNTs, hence it is worth examining for stabilizing properties of nanostructured emulsions similar to CNTs [18]. Moreover, this study is also important in terms of understanding the interaction of fullerenes with a different (non-lamellar) type of model lipid membranes, which, to our knowledge has never been studied before.

2. Materials and methods

2.1. Materials

Powdered buckminsterfullerene (C_{60}) and pluronic® F127 (PEO_{99}-PPO_{67}-PEO_{99}) were obtained from Sigma-Aldrich Co. Ltd (Dorset, UK) and used without further treatment. Dimodan U/J® (DU) was generously provided by Danisco, Denmark; it is a commercial lipid containing distilled monoglycerides. Water was purified using Barnstead Nanopure, Thermoscientific (USA).

2.2. Preparation of surfactant-stabilized (pre-stabilized) nanostructured lipid particles (Fig. 1)

Pre-stabilized lipid emulsions were prepared as follows: 500 mg of molten lipid (DU) was dispensed into 20 ml glass vial followed by addition of aqueous solution of 9.5 ml 0.5 wt.% F127 surfactant. Final emulsion had 5 wt.% lipid; this concentration was used throughout the current work, unless mentioned specifically; also wt. (weight)% is represented with only % sign. The above mixture was ultra-sonicated (Sonics & Materials Vibra-Cell VCX750, Jencons, UK) with 1 s pulses and 1 s delay times for (total) 20 min using 30% (of the maximum) power. Final liquid crystalline