Phospholipid-assisted formation and dispersion of aqueous nano-C$_{60}$

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Introduction

Water-soluble carbon fullerenes have been widely explored as candidates in the areas of medicine, materials and cosmetics [1, 2]. Despite of their potential application, their instability in aqueous solution has limited their application [3, 4]. Recent efforts have attempted to overcome this. For example, Deguchi stabilized nano-C$_{60}$ by protein adsorption [5] and Govindaraj used cetyltrimethylammonium bromide (CTAB) to prepare nano-C$_{60}$ dispersion Water-soluble nano-C$_{60}$ is commonly prepared based on the method of Deguchi et al. [6]. This method is laborious, solvent intensive, and time consuming. In our study, DPPC vesicle assemblies were used to aid in the rapid formation of aqueous nano-C$_{60}$.

Dipalmityolphosphatidylcholine (DPPC, 734 MW) lipid dispersions form bilayer vesicles, or liposomes, spontaneously in aqueous solution because of their hydrophilic choline headgroups and their hydrophobic dual hexadecane (C$_{16}$) tails. DPPC is a main component of mammalian cell membranes and a primary lung surfactant. In addition to their use as drug delivery vehicles, DPPC liposomes have been used as nanoscale reactors and templating agents for synthesizing inorganic materials.

In the area of lipid-C$_{60}$ complexes, Ikeda et al. [3] used a C$_{60}$ fullerene exchange method to prepare lipid membrane-incorporated C$_{60}$ fullerenes and Szymanska [7] utilized fullerene-modified supported lipid membranes as sensors. These reports suggest that C$_{60}$ fullerenes are able to partition into liposome bilayers and enhance electron flow across the bilayer [8, 9]. However, the effect of C$_{60}$ and nano-C$_{60}$ aggregate on the stability of the bilayer, and how bilayer stability relates to the degree of dispersion, is unknown.

In this study, we examine the extent as which C$_{60}$ nanoparticles can be adsorbed or entrapped in the DPPC liposome bilayers, and if DPPC liposomes can assist in the formation and dispersion of aqueous nano-C$_{60}$. In this study, DPPC/C$_{60}$ vesicle assemblies were prepared by the reverse phase evaporation (REV) method [10]. We attempted to elucidate (i) the preference of molecular C$_{60}$ to partition into the vesicle bilayer or form nano-C$_{60}$ in solution, (ii) the formation of small nano-C$_{60}$ in the bilayer due to aggregation, and (iii) the ability to back extract the lipids and isolate nano-C$_{60}$ as a function of the DPPC/C$_{60}$ molar ratio and presence of magnesium perchlorate (Mg(ClO$_4$)$_2$). Effects of nanoparticle-lipid interactions on bilayer phase behavior investigated were by differential scanning calorimetry (DSC). Structure and morphology of the liposomes and nano-C$_{60}$ were imaged by cryogenic transmission electron microscopy (cryo-TEM). In addition to aiding the formation of small, potentially water-soluble nano-C$_{60}$, this work also provides insight into the ability of C$_{60}$ to partition into biological membranes and its effect on membrane structure.

Experimental

A modified reverse phase evaporation (REV) method for liposome formation was used to prepare the DPPC/C_{60} assemblies. A set of samples were prepared at DPPC concentrations of 20 mM with DPPC/C_{60} molar ratios of 500:1, 200:1, and 100:1. DPPC dissolved in chloroform and C_{60} dissolved in carbon disulfide where placed in round-bottom flask. Deionized water was then added to form an emulsion. The solvents were removed rotary evaporation at 50℃ (above the DPPC melting temperature of ~42℃) and 300 mbar for 15 minutes, at which point the pressure was reduced to 100 mbar for 10 minutes. The procedure is described in Figure 1, from A to C.

To observe the morphologies and sizes of DPPC/C_{60} assemblies, artifact-free images were taken by cryo-TEM. Samples were vitrified from room temperature in liquid ethane. Lipid bilayer phase behavior in the DPPC/C_{60} assemblies was analyzed by differential scanning calorimetry (DSC) where the samples were equilibrated at 25 and sequential heat/cool cycles were performed from 25-50℃ at scan rate of 1℃ min^{-1}.

The Folch Method was used to extract DPPC from the DPPC/C_{60} assemblies and recover the nano-C_{60}. We added a chloroform-methanol (2:1 by volume) mixture into C to extract the lipids. The bottom phase was composed of chloroform-methanol- water at 86:14:1 (by volume) and contained virtually all of the lipids, while the top phase was composed of chloroform-methanol-water at 3:48:47. Nano-C_{60} in the top phase (aqueous) was dried and imaged by TEM.
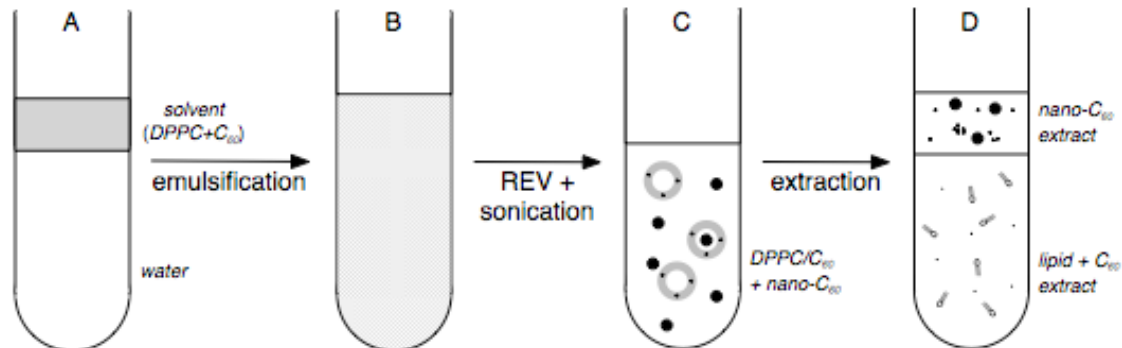
A	B	C	D

solvent (DPPC+C_{60})

emulsification

water

REV + sonication

DPPC/C_{60} + nano-C_{60}

extraction

nano-C_{60} extract

lipid + C_{60} extract

Figure 1. Schematic illustration for preparation of C_{60}/DPPC assembly and extraction of lipids from DPPC/C_{60} assemblies.

Results and Discussion

The size, morphology, and location of nano-C_{60}, relative to DPPC vesicles, that were formed during the REV process were observed by cryo-TEM. In Figures 2A-C, the C_{60} nanoparticles are observed in DPPC/C_{60} assemblies. From the observation, the size of C_{60} nanoparticles ranges from approximately 20 to 80 nm, which is similar to the average size of C_{60} nanoparticles reported previously (50 nm) in aqueous solution [8]. Moreover, the images show the location of nano-C_{60} that some C_{60} nanoparticles existed within liposomes, and others existed outside liposomes.
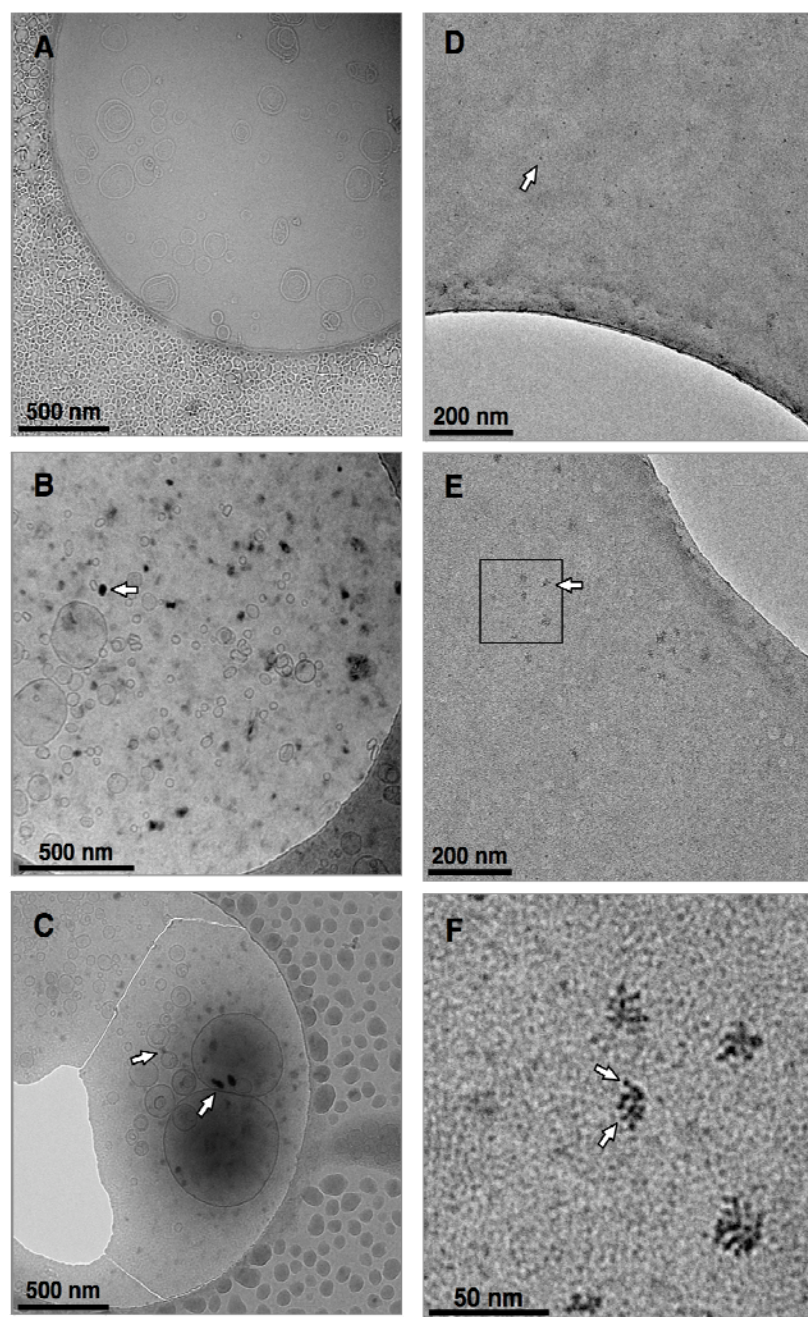
Figure 2. Cryo-TEM images of the DPPC vesicle control (A, no C_{60}) and samples at a DPPC/C_{60} ratio of 200:1 containing DPPC vesicles and nano-C_{60} (B and C). TEM images of the aqueous phase after extracting lipids from DPPC/C_{60} at a ratio of 200:1 (D-F; F is a magnification of the selected region in E).

Figure 2 D-F shows TEM images of C_{60} nanoparticles and nano-C_{60} aggregates that remained in the top aqueous phase after extracting the lipids. In Figure 2F, we observe large nano-C_{60} aggregates (ca. 20-40 nm) composed of small individual C_{60} nanoparticles (ca. 5 nm). The small C_{60} nanoparticles have diameters similar to that of

the thickness of a lipid bilayer (4-5 nm), which suggest that these small nanoparticles may have formed within the bilayer.

Based on cryo-TEM, the presence of lipid assisted in the rapid formation of aqueous nano-C_{60} during liposome formation; however, C_{60} is hydrophobic and has also been shown to partition into the acyl region of lipid bilayers. This may occur as molecular C_{60} or, as suggested by back-extraction studies, small nano-C_{60}. To detect the presence of C_{60} within the bilayers, we have examined the effects of C_{60} on the lipid bilayer phase behavior using DSC. Figure 3 shows the DSC transition curves of the samples at different DPPC/C_{60} molar ratios. The presence of C_{60} broadened the temperature range of main phase transition (i.e. bilayer melting) and lowered the melting (T_m). Meanwhile, the pre-transition of DPPC/C_{60} liposome assemblies is eliminated. This suggests that nano-C_{60} can be incorporated in lipid bilayers.
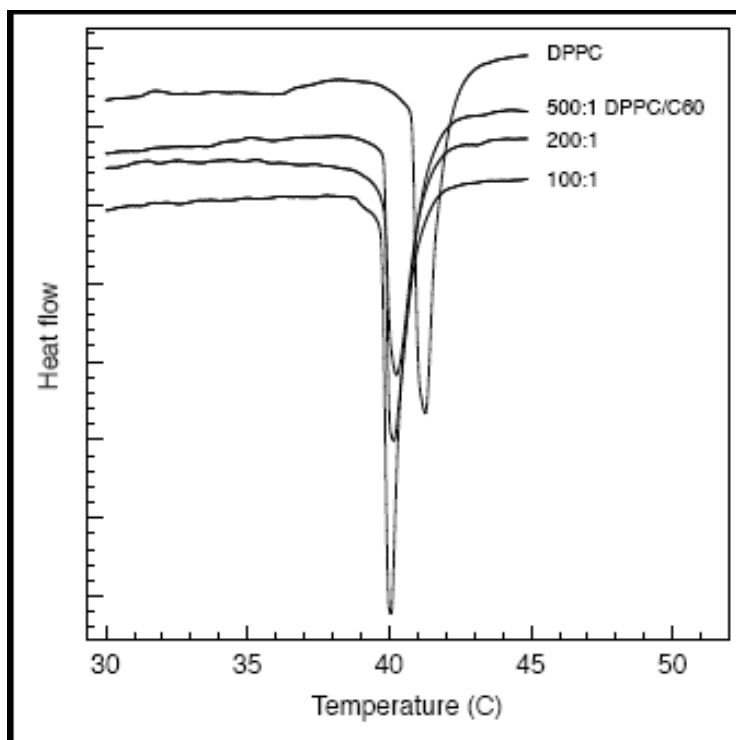

Figure 3. DSC transition curves of DPPC liposome and DPPC/C_{60} assemblies.

Conclusion

In this study, C_{60} nanoparticles were form in aqueous solution assisted with DPPC liposomes, which improved the stability of C_{60} and increased the rate of formation. From the cryo-TEM results, two kinds of C_{60} nanoparticles were observed: (i) large aqueous nano-C_{60} existing outside or encapsulated within the vesicles and (ii) small nano-C_{60} trapped within the bilayer. DSC results suggest that small nano-C_{60}, and likely molecular C_{60}, trapped within the bilayer disrupted the bilayer, decreasing the melting temperature and broadening the phase transition range. We propose that the DPPC/C_{60} assemblies provide a convenient means of rapidly forming nano-C_{60} in aqueous solution.

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