Liposomal Formulation of Amphiphilic Fullerene Antioxidants

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General Experimental Procedures:
All reaction solvents and other reagents were commercially available, and used without further purification. HPLC grade ethanol was used for HPLC and TFA was filtered with 0.2μm before mixed with ethanol. C_{70} was purchased from BuckyUSA Inc. Compounds were purified by flash column chromatography or HPLC and characterized by NMR (300 MHz) and MALDI-TOF mass spectrometry. EPR studies were conducted in a contractor lab at UCSD by Dr. Laura L. Dugan.

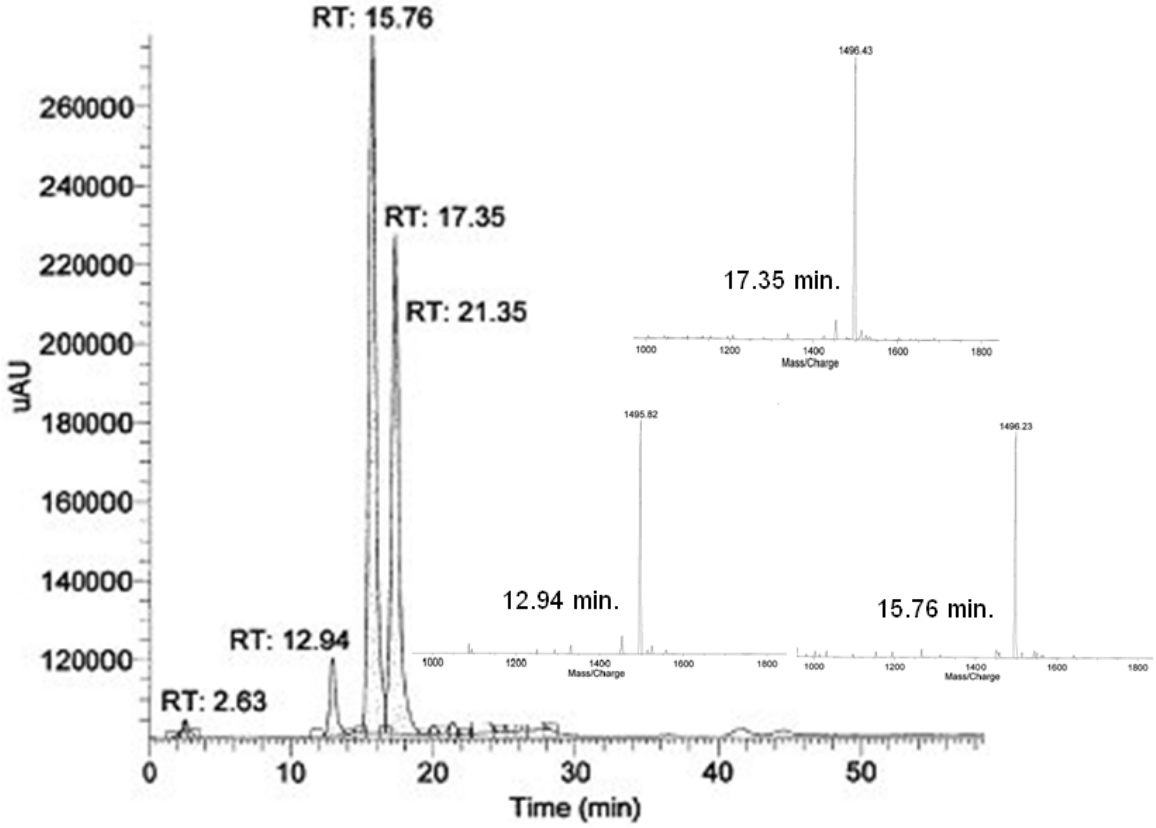

FIGURE S1: HPLC of ALM (C6-Phenyl column, 0.1% TFA-trifluoroacetic acid in ethanol). Three major bisadduct isomers were eluted at 12.94 min, 15.76 min., and 17.35 min. The minor peaks around 26-29 min. and 42-45 min. correspond to the mono ethyl ester and diethyl esters of ALM formed in the analytical process as confirmed by MALDI-MS (TFA catalyzed ester formation between COOH of ALM and ethanol when the fractions were concentrated). Insets show the MALDI-Mass spectra of the three ALM isomers at 1495.82, 1496.23 and 1496.43 respectively (calculated MW of ALM: 1496). HPLC grade ethanol was used and TFA was filtered with 0.2μm before mixed with ethanol. HPLC conditions: Gemini 3μ C6-Phenyl 110A column (4.6 mm x 150 mm) with 0.5% TFA in ethanol as the mobile phase. See MALDI-MS spectrum of ALM prior to HPLC separation in FIGURE S6.

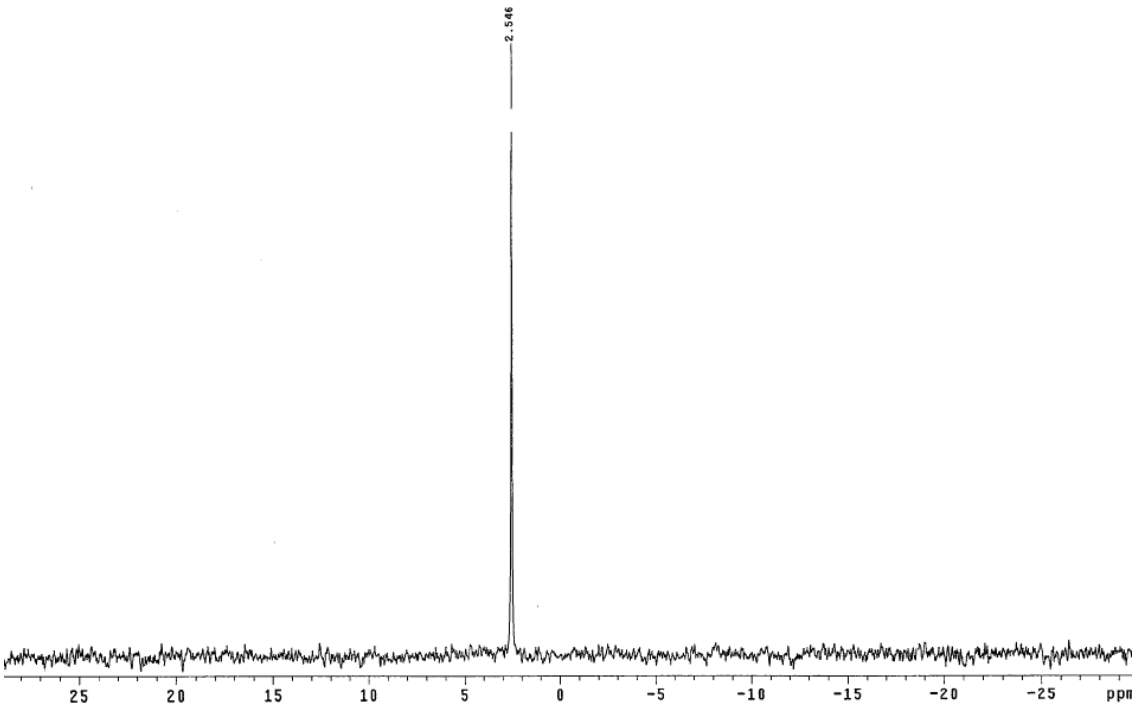

FIGURE S2: ^{31}P NMR of ALM-PC liposome in D_2O showing a very sharp signal at 2.55 ppm with a very small half-width $W_{1/2}$ of 2.8 Hz, which is comparable to that for liposomes made of phophatidylcholine only, indicating that ALM is structurally compatible with lipids and its presence does not interfere with the assembly of lipid molecules. The ALM content in the liposome sample is 50% by weight (1:1 molar ratio of ALM to lipids).

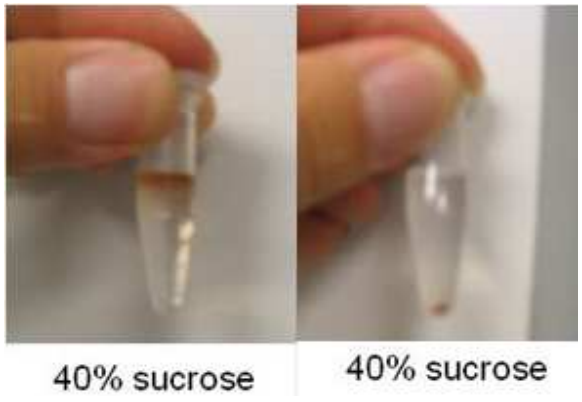

FIGURE S3: Buoyant density test under high-speed centrifugation at 18,000 G for 30 min. Left: ALM liposome sample (1:1 egg-PC to ALM), where fullerenes (brownish) stably associated in lipid bilayers stayed on the top of 40% sucrose cushion. Right: fullerene ALM itself didn't form stable liposomes and precipitated to the bottom of the sucrose solution.

FIGURE S4: Left - ALM liposome samples made of egg-PC and ALM at 1:1 weight ratio, at 4 different concentrations. Right – comparison of preparations made of 1:1 ALM/egg-PC and ALM only at 1mg/mL, showing that ALM only didn't form vesicles at 1mg/mL with most materials precipitated to the bottom of the vial 3 days after its preparation. Only 15-20% ALM self-assembled into vesicles in the absence of egg-PC, demonstrating the necessity of regular lipids to promote the formation of high-loading and high-content ALM-PC liposome. It also demonstrated the incorporation of ALM into the lipid bilayer.

Cytotoxicity: The effects of ALM liposome on U937 monocyte cell viability was examined at different concentrations in parallel with Vitamin E, a natural antioxidant. As seen in Figure S5, cells incubated with Vitamin C or ALM did not have toxic effects on the viability of serum-starved cells. No significant differences in cell viability were observed using up to 67 μM ALM (100 μg/mL) compared to control cells at days 3 and 6. The cell viability was evaluated with well established MTT assay.

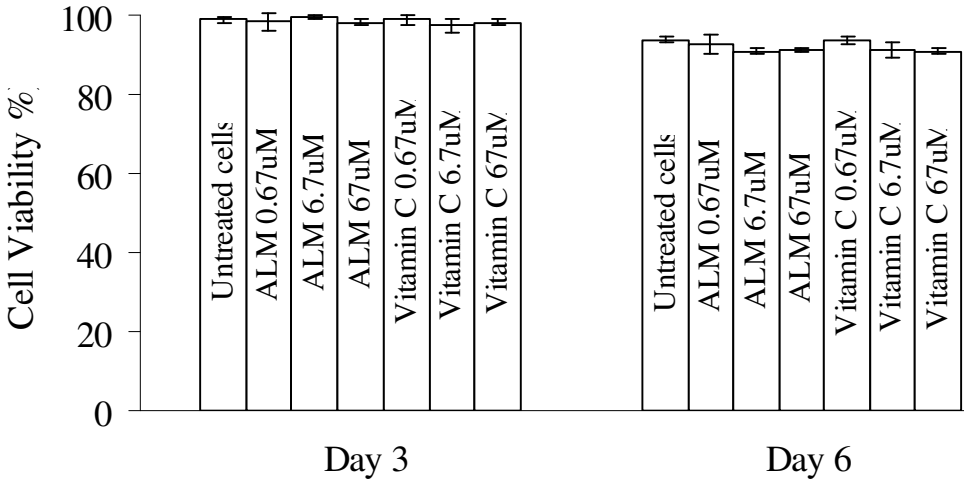

FIGURE S5: Toxic effects of ALM liposome and natural antioxidant Vitamin C on cell viability shown with error bars. Serum-starved U937 monocyte cells were treated with indicated concentrations of ALM or Vitamin C, or untreated through 6 days.

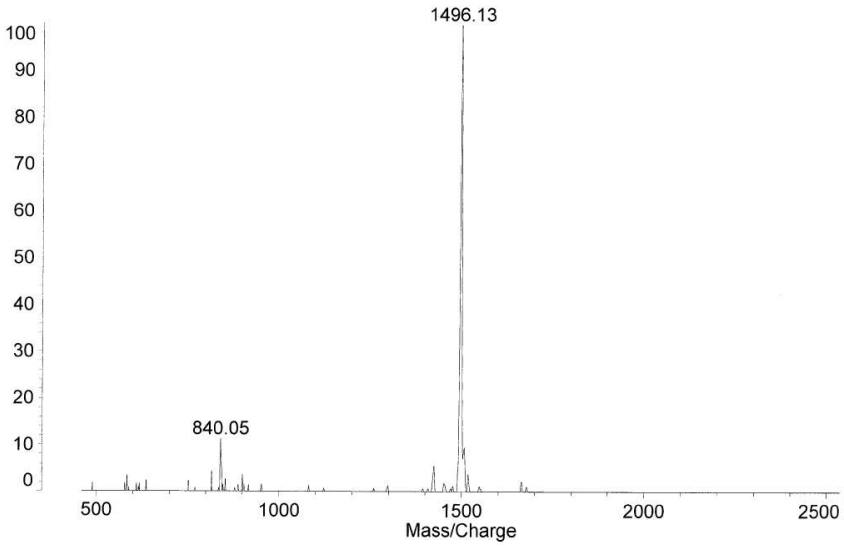

FIGURE S6: MALDI-MS spectrum of ALM prior to HPLC separation showing the molecular ion peak of ALM at 1496.13 and C_{70} fragment at 840.05 formed under laser desorption/ionization conditions (Calculated MW of ALM: 1496).