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Structural Determinants of Divalent Cation-Induced Phosphatidylserine Cochleate Crystallization

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Cochleates, stable precipitates of phosphatidyserine (PS) and calcium (Ca^{2+}), having a unique multilayered structure consisting of a single, rolled bilayer sheet, are a safe, natural and effective platform technology for drug-delivery [1-5]. Using small-angle x-ray scattering (SAXS) and cold field emission scanning electron microscopy (SEM), we report on the physical parameters, particularly, cation, lipid, temperature and drug effects which directly influence cochleate ultrastructure with the goal of optimizing particle efficacy.

SAXS studies carried out at the Advanced Photon Source of Argonne National Laboratory to determine the structure of plain cochleates prepared 10mg/mL using phospholipids consisting of 99% pure1,2-dioleoyl-sn-Glycero-3-[Phospho-L-Serine] (DOPS) and 85% pure Soy PS, a mixture of phosphatidicacid and phosptidylcholine moieties in DOPS revealed a highly ordered lamellar structure with Bragg peak repeat unit diffraction spacings of 49.9-50.7Å and second and third order spacings of 25Å and 16Å (ratios ½, 1/3). Although no significant structural change occurred with method of formulation, variation in the lipid and cation used in encochleation caused a significant reduction in lamellar order. When palmitoyl-oleoyl phosphatidylserine (POPS) was substituted as lipid, lamellar structure was severely compromised to planar. Results confirmed on SEM showed multilayered rolled 1-5um cylinders in DOPS, spherical, indented rolled 200-300nm particles with Soy PS and planar 1um sheets in POPS. Addition of Amphotericin B (AmB), to the cochleates (5:1 w/w/, lipid/drug) and shown preclinically to reduce toxicity and increase bioavailability over current market formulations [6-8], resulted in a similar soy PS-like 200-300nm rolled particle for all lipids with no change in lamellar repeat distance (Fig. 1.). This represents an appreciable reduction in size but not lamellar shape in the DOPS but, more significantly, a POPS lamellar ordering not indicated previously. Use of magnesium, zinc and barium divalent cations to affect encochleation resulted in a successive, significant reduction in lamellar structure, indicative of an optimal atomic radius for cation insertion into the polar PS headgroup. As before, addition of AmB resulted in a reversion to higher order, particularly for zinc and barium both on SAXS and SEM (Fig. 2.). Assessment of AmB cochleate stability over time (0-6 mos) and temperature (26-70^{o}C) did not result in any lamellar changes. Maintenance and possible enhancement of highly ordered cochleate lamellar structure persists despite the drug encochleated. In addition to AmB, Ibuprofen and Green Fluorescent Protein (GFP) DNA provide evidence of encochleation as each drug produces narrowing of Bragg peak and lamellar width. There is evidence to suggest that cochleate-associated molecules are present in the inner layers of a solid, stable, water and oxygen impermeable structure ultimately capable of extending molecular shelf life [3]. Further, there appears to be little or no aqueous space between bilayers. Our results reliably point to a consistent aqueous space of approx.14-16Å for all cochleates. This would correspond to the *condensing effect* of PS over other diacyl phospholipids [9,11] and temperature independence [12] previously documented for plain DOPS cochleates. Taken together, these results point to the formation and maintenance of a thermodynamically stable nanoparticle which appears to result from the drug's ability to intercalate into the phosphoserine hydrophobic interior to equalize packing irregularities arising from differences in acyl chain length, PS impurities and heterogeneous drug properties.

The proven stability of the DOPS:Ca^{2+} cochleate makes it a suitable means of drug delivery. That SAXS and SEM cochleate profiles remain reliably consistent within a narrow range even with the addition of heterogeneous drug additives, may provide an indication of behavior and the necessary physiologic stability when used as a drug delivery platform.

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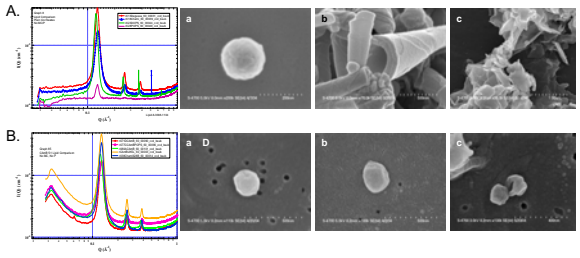

Fig. 1. SAXS and SEM lipid effect of addition of 2 mg/mL Amphotericin B (B) on cochleate structure for Soy PS (a), DOPS (b) and POPS (c) as compared with plain cochleates (A). AmB enhances lamellar structure particularly for POPS cochleates, and appears to standardize cochleate size and shape to a spherical 250nm particle for all lipids.

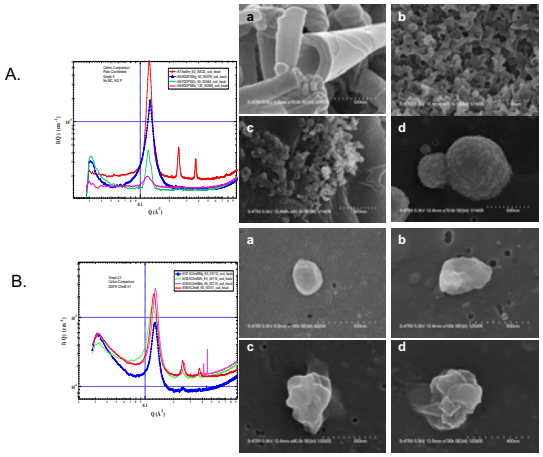

Fig. 2. SAXS and SEM divalent cation effect of addition of 2 mg/mL Amphotericin B (B) on DOPS cochleate structure for calcium (a), magnesium (b), zinc (c) and barium (d) as compared with plain DOPS cochleates (A). AmB enhances lamellar structure for magnesium, zinc and barium cochleates and reduces particle size from 1-5um to 250nm in calcium cochleates

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