Preparation of cochleates from soybean phosphatidylserine

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Introduction: Cochleates are particles composed of tightly packed and dehydrated phospholipid bilayers. They are prepared by mixing a calcium ion solution and negatively-charged liposomes (e.g. phosphatidylserine vesicles). Cochleates have a much higher chemical and mechanical stability compared to liposomes, which makes the platform very interesting for drug delivery purposes. However, cochleates have typically been prepared using highly purified or synthetic phospholipids, which are not the first choice for industrial drug development due to high costs. Therefore, the use of less purified phospholipids (e.g. phosphatidylserine from soybean lecithin) is of great importance towards development of cochleate formulations for drug delivery.

Aims: Preparation of cochleates from sodium salts of soybean phosphatidylserine of varying purity and preparation of cochleates directly from calcium salts of soybean phosphatidylserine were evaluated. Additionally, the cochleate formulations were extensively characterize particle structure and morphology.

Methods: Cochleate formulations were prepared from sodium and calcium salts of soybean phosphatidylserines of varying purity (53-94% PS) by mixing ultrasonicated liposomes with a calcium chloride solution in a 1:1 volumetric ratio to obtain formulations with a final lipid concentration of 10 mg/ml and a 1:1 Ca^{2+}/lipid molar ratio. For the calcium salts, 10 or 30 mM EDTA was added to form liposomes. Cochleates were also prepared from pure DOPS as control. The formulations were characterized using small-angle X-ray scattering (SAXS), scanning electron microscopy (SEM), and a fluorescence spectroscopy assay (Laurdan).

Results: The cochleate particles have a tightly packed lamellar structure, where the lamellar repeat distance in the soybean cochleates (4.9 nm) are smaller compared to DOPS cochleates (5.1 nm). The reflections were broader for the soybean cochleates compared to DOPS cochleates, especially for the lower purity lipids. Both rounded and cylindrical particles were observed using SEM and the cochleates formed from soybean phosphatidylserine of highest purity (>94% PS) are smaller compared to those prepared from DOPS. The fluorescence assay indicates that bilayer structure of cochleates prepared from phosphatidylserine with lower purity is less perfect compared to cochleates prepared from the highly purified soybean phosphatidylserine (>94% PS) and DOPS.

Conclusions: Cochleate formulations can be prepared from highly purified soybean phosphatidylserine. The cochleates are smaller, have a shorter lamellar repeat distance, and a similarly dehydrated structure to DOPS cochleates. From the less purified phosphatidylserines, cochleates with a highly ordered structure were obtained, however the structure was less ordered and possibly not completely dehydrated. Optimization of preparation from less purified lipids (changing pH and calcium ion concentration) is currently under investigation.

Keywords: Cochleates, natural phospholipids, drug delivery formulations, formulation characterization.