Lyotropic Lipids of the Endogenous, n-Acylethanolamide Variety for Drug Delivery to Ocular Tissue

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Statement of Purpose: Since amphiphiles self assemble in the presence of a polar solvent and are stable against dilution, sustained drug delivery is feasible from the lyotropic liquid crystalline phases of these materials. These materials form five main phases. The first is a fluid lamellar phase, consisting a one-dimensional stack of flat amphiphilic bilayers. The next is an inverse bicontinuous cubic phase, which are further characterized as Pn3m, Im3m and Ia3d depending on their crystallographic space group. These can then transition to a hexagonal phase, which appear as cylinders. Finally, a disordered packing leads to a L_2 inverse micellar phase. Two examples of these lipids are oleoyl ethanolamide and linoleoyl ethanolamide (Sagnella, 2010); however, both of these molecules form cubic phases at temperatures which are not ideal for ocular delivery. It is hypothesized that new materials, synthesized as mixtures of these pure lipids, would yield properties more amenable to drug delivery for the anterior and posterior of the eye. Specifically, atropine was examined for delivery to the anterior of the eye and roscovitine and R-547 were explored for posterior delivery as chemo-preventative agents for retinoblastoma.

Methods: The monoethanolamides were prepared by dissolving the fatty acids in dichloromethane (DCM) and reacting them with oxalyl chloride. The resulting fatty acid chloride was added to ethanolamide in DCM and the resulting product was filtered and rinsed. Purity of each monoethanolamide, oleoyl ethanolamide (OEA) and linoleoyl ethanolamide (LEA), was determined by HPLC and LC/MS. These materials were combined in set ratios after being dissolved in tert-butanol (TBA) to form novel compounds, identified according to the amount of LEA in the mixture. To characterize the materials, differential scanning calorimetry (DSC) was performed to determine the energies and peak temperatures of the endotherms. Water penetration into the amphiphiles was assessed using an inverted optical microscope via polarizing optical microscopy in the presence and absence of cross polarizing lenses. These samples were heated at a maximum rate of 1°C/min and allowed to equilibrate before analysis was completed. Small angle x-ray scattering (SAXS) was used to determine phase assignment and lattice parameters for all materials at various temperatures. Dispersions were created for various mixtures using a homogenizer for 5 minutes or more and stabilized using small amounts of poloxamer-407. The particle sizes were then measured using differential light scattering (DLS).

Results: Initial DSC results, shown in Figure 1, demonstrated that the mixture materials transitioned in between the pure OEA and LEA transitions shown in previous work (Sagnella, 2010). As the amount of LEA in the materials increased, the transition temperature decreased to that of pure LEA. Water penetration results showed that polarized phases developed for all materials as water content and temperature increased. Importantly, as the amount of LEA in the material increased, the temperature at which the polarized phases formed decreased. This was consistent with the lower cubic phase transition of LEA compared to OEA. This data was supported by SAXS analysis, performed with 10% and excess water, which showed that cubic phases, specifically Pn3m, formed by 35°C for higher amounts of LEA materials. However, by 45°C, the highest amounts of LEA had melted to an L_2 state. The presence of therapeutic agents alters the material's phase transitions.

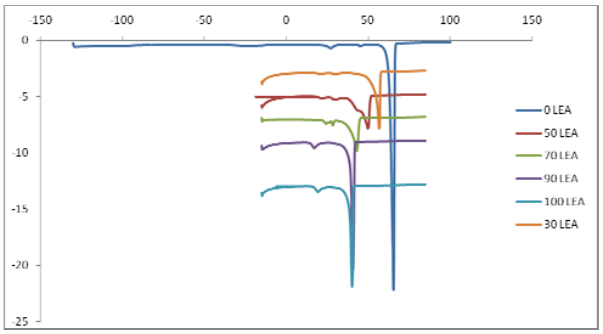

Figure 1. DSC results showing the phase transitions.

Dispersions were created for materials fluid at room temperature in excess water, namely those from 60% LEA to 100% LEA. DLS results for these showed the particle size to be around 160-170 nm with a polydispersity (PDI) generally less than 0.22. SAXS revealed the phase of the dispersions to be Im3m, which is different from bulk materials due to the poloxmer-407 stabilizer.

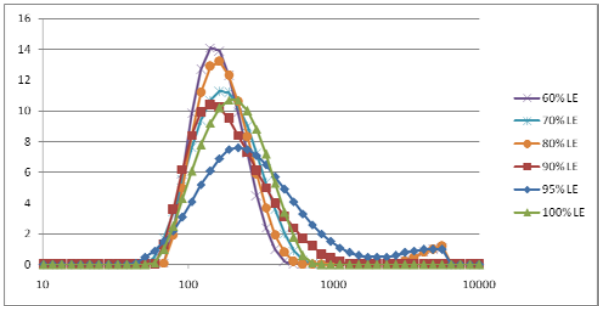

Figure 2. Size and PDI of dispersions from DLS.

Conclusions: Combinations of oleoyl ethanolamide and linoleoyl ethanolamide yield materials with cubic phase transition properties appropriate for ocular drug delivery, but these transitions vary according to drug amounts.

References: Sagnella SM. Langmuir. 2010;26:3084-94.