Endogenous Monoethanolamide Lipids for Successful Ocular Drug Delivery

Frances Lasowski[1], Kathleen Ingram[1], Minoo Moghaddam[2], Heather Sheardown[1].
[1]McMaster University, Hamilton, Ontario, Canada; [2]CSIRO, Sydney, New South Wales, Australia.

Statement of Purpose: Atropine and roscovitine have been suggested as pharmacologic agents to treat the ocular conditions of myopia and retinoblastoma respectively. While topical administration is the most prevalent delivery method, significant loss (Gaudana, 2008) and side effects necessitate a more effective method. Given that 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. Oleoyl ethanolamide and linoleoyl ethanolamide, two examples of these materials (Sagnella, 2010), form cubic phases at temperatures not ideal for ocular drug delivery. However, it is hypothesized that new materials synthesized as mixtures of these pure lipids will provide the appropriate properties for drug delivery from the anterior of the eye.

Methods: Oleoyl ethanolamide (OEA) and linoleoyl ethanolamide (LEA) were prepared by converting the fatty acid to an acid chloride using oxalyl chloride, then subsequently reacting it with ethanol amide. HPLC, LC/MS and NMR were used to confirm the purity of the ethanolamides. These materials were combined in set ratios 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. Small angle x-ray scattering (SAXS) was used to determine phase assignment and lattice parameters for materials at various temperatures. Dispersions were also created, stabilized by small amounts of poloxamer-407, and characterized using differential light scattering (DLS). Lipids were formed and added to phosphate-buffered saline (PBS) for drug delivery studies at 37°C. The PBS was replaced at regular intervals and the samples analyzed by UV spectrophotometry, HPLC or LC/MS.

Results: Initial DSC results 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 (Sagnella, 2010). This data was supported by SAXS analysis, performed with 10% water and excess water, which showed that cubic phases, specifically Pn3m, formed by 35°C for higher amounts of LEA materials. This is shown in Table 1, where Lat is the lattice parameter, the +/- shows the standard deviation in the lattice size and % represents the liklihood the phase assignment is incorrect. By 45°C, the highest amounts of LEA had melted to an L_2 state, which would not be suitable for drug delivery. The presence of therapeutic agents alters the material's phase transitions and thus compositions which form the correct phase at the proper temperature with appropriate drug content are optimized.

Table 1. Example SAXS phase and lattice paramter data.

Material	T	Phase	Lat.	+/-	%
90 LEA	25°C	Pn3m	10.2	0.07	0.74
	45°C	L_2	4.43	-	-
90 LEA, 10% roscovitine	25°C	Pn3m	7.83	0.11	1.45
	45°C	L_2	3.71	-	-

Dispersions were created in excess water for mixtures greater than 60% LEA. DLS 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 1 shows a sample drug release curve from 80 LEA loaded with 5% roscovitine. Drug release was shown to be sustained over at least 2 weeks, with the release rate dependent on lipid degradation and loading, though a significant initial burst is present. Physical incorporation of the drug is being examined to reduce this effect.

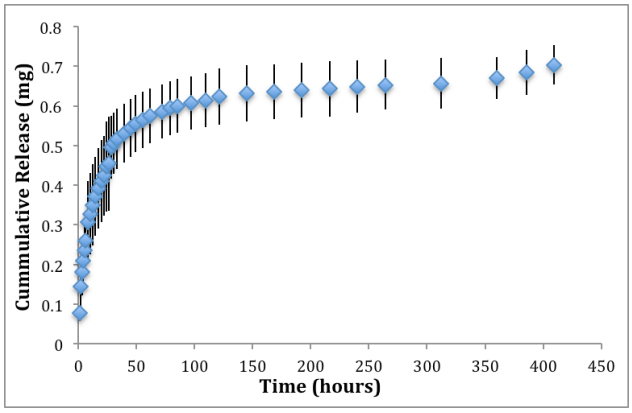

Figure 1. 80LEA, 5% roscovitine drug release profile.

Conclusions: Combinations of oleoyl ethanolamide and linoleoyl ethanolamide yield materials with cubic phase transition properties appropriate for ocular drug delivery. These transitions vary according to drug amounts, water content and lipid composition representing a tunable system for drug release. Release is possible over sustained periods of time, though an initial burst currently exists.

References: Gaudana R. Pharm Res. 2008;26:1197. Sagnella SM. Langmuir. 2010;26:3084-94.