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Application Note: Use of PolyVivo AK01 (mPEG-PCL) to form poorly soluble drug loaded liposomes

The processes shown here are conventional and not claimed as intellectual property of Akina, Inc. Feel free to use this process for making liposomes in your own lab, preferably using Polyvivo brand polymers.

Background

A classic drug delivery problem is difficulty with IV injections of poorly soluble drugs. The requirement for large quantities of saline to dissolve such materials limits their clinical use. One common solution for this problem is to form drug-loaded liposomes by a variety of methods. Here "liposome" is used to indicate a micelle structure containing poorly soluble drug with a diameter <1µm though some may also refer to this as a "nanoparticle".

Drug Model: Coumarin-6 (C6)

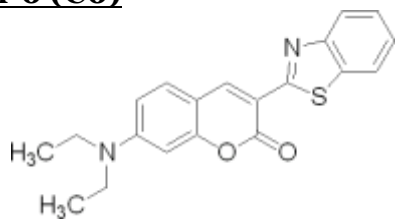

Figure 1. Coumarin-6 structure

Coumarin-6 (Fig 1) is traditionally used as a laser dye due to its highly fluorescent nature however it is commonly used as a model hydrophobic drug for studies involving release and tracking of localized delivery. The native solubility of Coumarin-6 in water is 0.25 µg/ml which is makes it a good model for hydrophobic drugs such as paclitaxel, everolimus, and others. Visibly this material is bright yellow making it easy to see even to the naked eye.

Polymer System: Polyvivo AK01 mPEG-PCL (AK01)

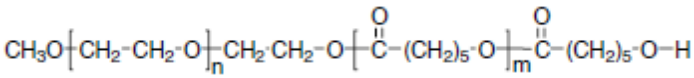

Figure 2. Generalized structure of mPEG-PCL

AK01 is the catalogue number for Methoxy Poly(ethylene glycol)-b-Polycaprolactone (2,000:5,200 Da) (Fig 2) it is a block copolymer of hydrophilic poly(ethylene glycol) and hydrophobic poly(caprolactone). This polymer does not dissolve in water when added directly it is soluble in organic solvents such as dichloromethane. In solid state it is white powder but when dissolved it is colorless.


Figure 3. Stirring paddle setup.

Method

Polymer-Drug Solution
The polymer was dissolved in dichloromethane (DCM) at a concentration of 5% w/v. Separately 2.5% w/v C6 was dissolved in DCM and added as 0.1 ml of C6 solution into 1 ml of polymer solution. This renders a solution such that the final ratio of drug/polymer was 5% w/w drug to polymer. As a negative control a pure DCM (no polymer) mixed with C6 was used.

Liposomes
A 22 ml scintillation vial had 20 ml of distilled water put into it. It was agitated using a VWR "lab-egg" stirrer equipped with a screwdriver at a rate of 2000 RPM (Fig 3). While being stirred, 1.1 ml of each polymer-drug solution was added to the stirring water 100 µL at a time, and the whole was left to stir for at least 1 hour to allow for DCM evaporation.

Screening
In order to remove a macroscopic scum layer each solution was first passed through a folded paper filter (qualitative, fast) by gravity. Following this each solution was loaded into a syringe and pushed through a 0.45 µm (450 nm) PVDF filter to eliminate any microparticulate manner. The filtered solution was considered to be the liposomal solution as anything larger than 450 nm had been excluded (Fig 4).

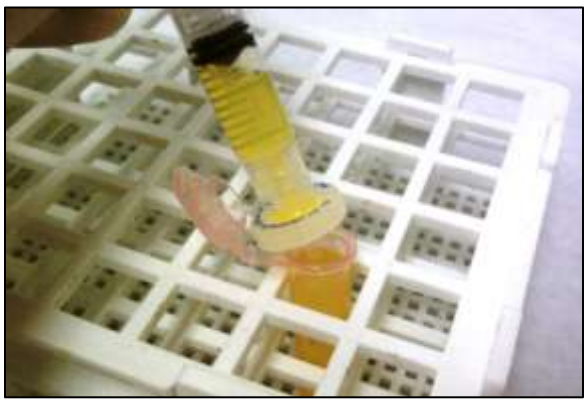
Figure 4. Screening to size.

After screening, solution stability was briefly tested by centrifuging at 2000 RPM for 1 min and then allowing the solution to sit still overnight.

Results

After filtration the solution was pictured and it was clear that without the stabilizing polymer almost all of the C6 had been screened out of the control. By itself C6 lacks the solubility to be retained in water however when co-dissolved with AK01 it had high solubility in water.

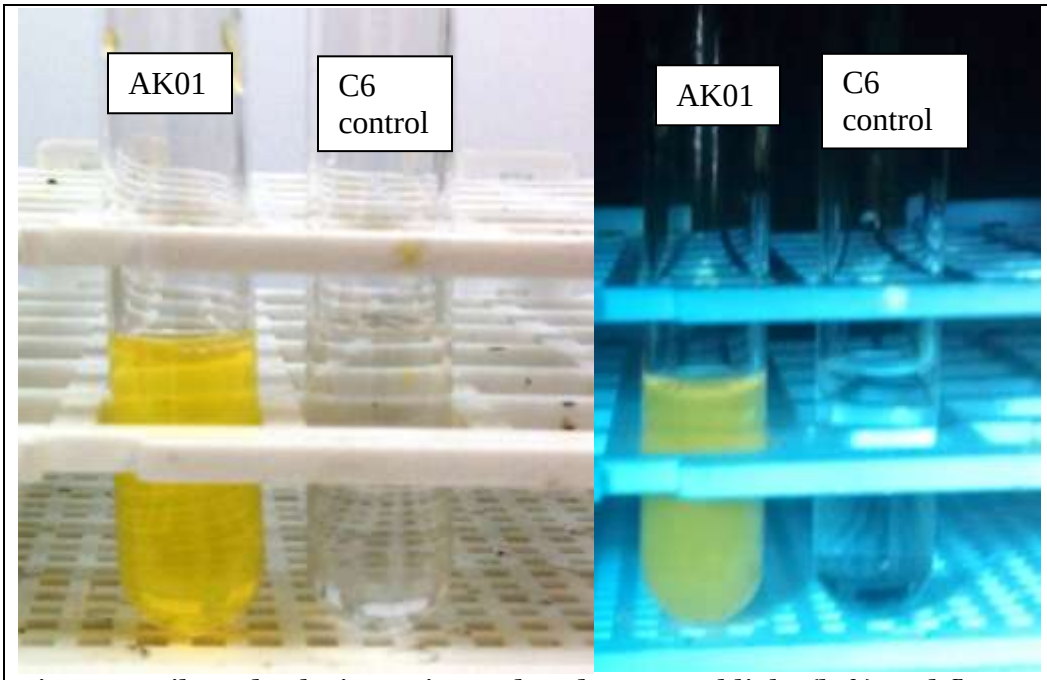

Figure 5. Filtered solutions pictured under normal light (left) and fluorescently using Fluorophone kit (right)

These were diluted 1:10 in ethanol and tested by UV/Vis spectroscopy for C6 content at 469 nm and compared to known standards to obtain C6 content. The C6 control without had no measurable C6 content. The C6 content in the AK01 liposomes was 118 ± 19 µg/ml (N=2, ±standard deviation) this is roughly 500X the maximum solubility of C6 in water. Stability was tested by centrifuging the material at 2000 RPM for 1 min and no settling was observed indicating that the C6 was evenly dispersed in the aqueous phase and stabilized against settling (Fig 6).

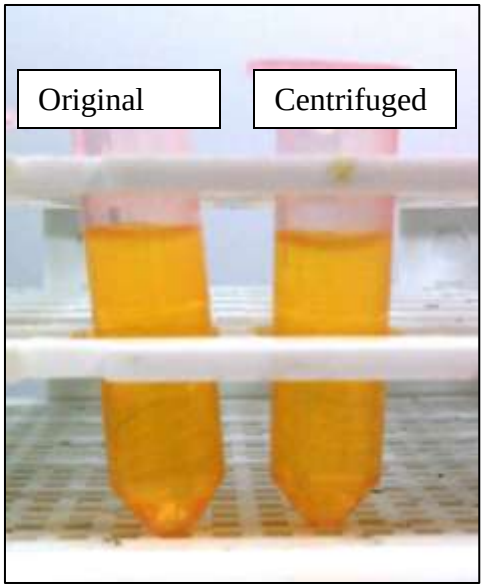

Figure 6. AK01-C6 liposomes before and after centrifugation.

Conclusion

Using this method AK01 diblock copolymer has good functionality for generating stable solutions of poorly soluble C6 at up to 500X its maximum solubility.

Want to learn more about liposome formation? Check out the Morissey Lab protocol for an alternate method.