Effects of Solvents and Structures of Amphiphilic Polymers on the Formation of Nanoparticles

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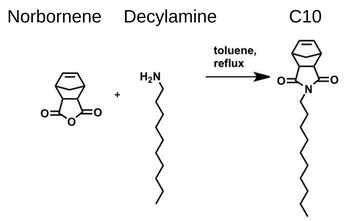
ABSTRACT

Nanoparticles (micelles) can be used for drug delivery and diagnosis when prepared under specific conditions. The structure of the nanoparticles is important for the delivery of drugs *in vivo*. There are many factors that can contribute to nanoparticle formation: we were interested in investigating the effect of the chemical structure of the consisting amphiphiles , and certain solvents on nanoparticle formation.

The nanoparticles in this project consisted of amphiphilic block copolymers with varying amounts of hydrophobic/hydrophilic monomer units The hydrophobic monomers used were norbornene-cyclohexane (Cy), -hexane (C6), and –decane (C10). Norbornene-polyethyleneglycol (PEG) was used as the hydrophilic block of each coblock polymer. The constituents (monomers) of each polymer were reacted with a ruthenium catalyst (Grubb's 2nd Generation-modified catalyst); the resulting ROMP (Ring Opening Metathesis Polymerization) amphiphiles were characterized and purified. These polymers were then dialyzed into water from various solvents to form micelles.

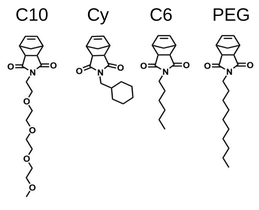
We predict that both the co-solvent for dialysis and chemical structure of the hydrophobic block will have an effect on the morphology of the nanoparticles.

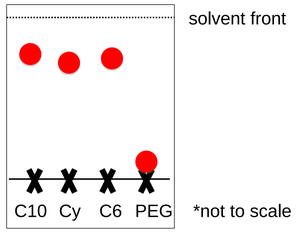
Hydrophobic Monomer Synthesis



Norbornene dicarboxylic anhydride and decylamine were refluxed in toluene with a Dean Stark Trap in place, to produce one of three hydrophobic monomers: Norbornene-decyl (C10). PEG, C6 and Cy monomers were produced previously in a similar fashion.

TLC to Predict Relative Polarities of Monomers

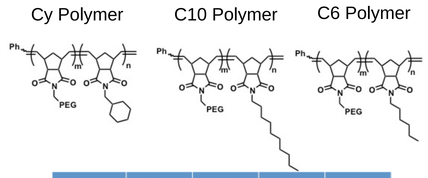




After the production of C10, C6 and Cyclohexane were selected as the two other hydrophobic monomers, and PEG was selected as the sole hydrophilic monomer. TLC (Thin Layer Chromatography) was conducted on each monomer to test their polarities. The solvents used were 30% ethyl acetate 70% hexane, 90% Hexane 10% ethyl acetate, and 30% hexane 70% ethyl acetate; the polarity of each solvent varied for hexane is nonpolar, while ethyl acetate is polar. When the TLC was conducted , Cy, C10, and C6 moved the furthest and none of them interacted with the polar silica gel unlike the PEG monomer. The Rf values of the nonpolar monomers exceeded that of PEG in all solvents and were almost equivalent to each others' ,consequently suggesting that the monomers were in fact nonpolar. PEG's Rf value stayed at 0 in the primarily nonpolar solvents (no affinity for nonpolar solvents) but it shifted to 0.197 when placed in the 70% ethyl acetate solvent, indicating that it is in fact polar.

Polymerization Process

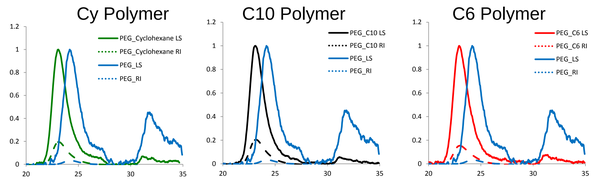
Grubb's Catalyst along with PEG was placed in DCM to allow for ROMP to take place. After 15 minutes, 3 aliquots of this mixture were taken and added to previously produced hydrophilic monomers. The polymers were quenched (the reaction was stopped) with an addition of ethyl vinyl ether.



Polymer	Block A: DP	Block B: DP*	MW (g/mol)	M_w/M_n
PEG	18.1	-	6,403	1.046
PEG-C10	18.1	24	13,690	1.012
PEG-C6	18.1	18.5	10,970	1.021
PEG-Cy	18.1	21.6	12,000	1.031

SLS

Static light scattering was used to characterize the number of units of monomers in each polymer block. After SLS, the polymers were purified by precipitation from ether.

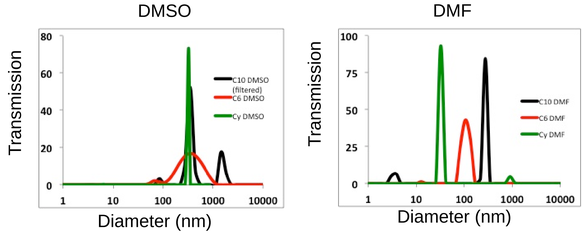


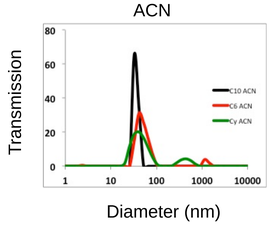
Micelle Formation

The polymers underwent dialysis in order to form micelles with 3 different co-solvents (DMSO, DMF, and ACN), and were dialyzed in to water for 3 days in order to replace organic solvent with water.

DLS

Following this, the particles were characterized by DLS (Dynamic Light Scattering) to approximate their sizes.





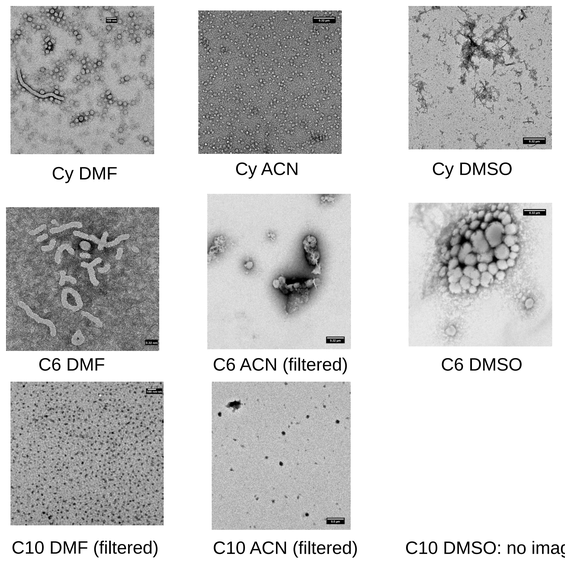
DMF: Each polymer's diameters seem to be of an exclusive range of sizes.

DMSO: Most particles seem to be similar in diameter, but C6 has a population with a variety of sizes ranging from 100-1000 nm

ACN: Most particles are of the same diameter. C6 and Cy seem to have slightly larger ranges of diameters, but do not stray from the 10-100 nm range.

TEM

TEM (transmission electron microscopy) allowed us to examine the particles more closely, and evaluate differences in morphology. TEM, unlike DLS describes the morphology (whether the nanoparticles have chain/spherical/network structures etc.)



DMF: Both Cy and C6 produced junction structures, but they are not as common as spherical structures in the Cy sample given the DLS range of diameters and the TEM imaging. C10 only produced spherical structures.

ACN: both Cy and C10 produced spherical structures, though they are not as common in the C10 sample which also appears to have a few small aggregates. This could be a result of the filtration. The C6 sample solely contains aggregates according to the imaging, which explains the broad range of diameter by DLS. Cy though the nanoparticle distribution does not seem to correspond to the DLS data, the discrepancy in techniques is not of much concern.

DMSO: The C6 sample appears to have defined aggregates (clusters of micelles) which would mean that individual micelles are also present in the sample. This explains the very broad range of diameters by DLS. It appears that the Cy sample primarily consists of aggregates, and although it does not correspond to the DLS data the discrepancy in techniques is not of much concern.

CONCLUSIONS AND FUTURE DIRECTIONS

DLS and TEM data indicate that amphiphile polymeric structure and organic co-solvent used in dialysis effect particle formation. According to this data, each polymer acted differently in certain solvents. (Ex. DMF – Cy produced both junction and spherical nanoparticles, C6 just produced junction nanoparticles, and C10 just produced spherical nanoparticles). The DMF solvent is the only solvent of the three that produced junction nanoparticles. It can be inferred that the morphology of a nanoparticle (determining factor of whether a nanoparticle is capable of drug delivery/diagnosis) is dependent on both the solvent and the consisting polymer structures.

Future Directions:

- Repeat Experiments to see if the nanoparticles assume the same shape/structure
- Repeat process with different co-solvents for micellization
- Repeat process with different combinations, types, and amounts of hydrophilic/hydrophobic monomers

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