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AB-Block Copolymer Self-Assembly and Nanoparticle Encapsulation: Final Product Size and How it is Determined from Chain-Length

by

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Abstract:

AB-block copolymers, which consisted of a hydrophilic A-block of polyethylene glycol and hydrophobic B-block of poly(L-Leucine), were used to study their self-assembly and their ability to incorporate metallic nanoparticles. The self-assembled polymeric aggregates were investigated for their size using a Zetasizer. Several experiments were carried out to determine the relation between changing the length of either the A or B-block and the resulting size of the polymeric aggregate carrying the encapsulated nanoparticle. The results indicate that increasing the polyethylene glycol chain length resulted in a larger aggregate but increasing the p(L-Leucine) chain length resulted in a smaller aggregate. Experiments were also conducted to determine the effect of changing the size of the metallic nanoparticle, and it was found that the size of the particle and the size of the aggregate were directly correlated.

Introduction and Theory:

The ability for a living organism to utilize amphihilic lipids to form bilayer cellular membranes is one of the most important defenses of the internal cellular environment (*1*). This membrane is the primary obstacle for the administration of chemicals to the cellular organelles for medicine or research in the field of chemistry. In order to bypass this obstacle, the research being done with AB-block copolymers Dr. Scholz's lab focuses on simulating a similar membrane whilst not being recognized as a foreign object. This is done to take advantage of the organism's own intercellular transport system.

Many different medical treatments and research rely on delivery of certain ions or chemicals into the cells of a living organism. This delivery involves either ingesting or injecting the substance into the organism for the cells to absorb, but there is research being conducted to determine alternate delivery

methods from pills and needles. Much of this research is devoted to bioengineering bacteria and viruses as possible delivery vectors for their targets (*2*) (*3*).

However, there is research being conducted at the University of Alabama in Huntsville towards the use of AB-block copolymers and their ability to self-assemble around nanoparticles as a delivery vector, and the results have been promising. In order for these copolymers to be truly useful as delivery vectors, they must be able avoid triggering an immune response, they must be of an appropriately small size similar to viruses, and they must be able to actually encapsulate the nanoparticles to be delivered. This paper will primarily focus on the second criteria of size, but the other criteria have also been addressed in the course of this research.

The first of these requirements was addressed by having the block copolymers consist of nontoxic amphiphilic molecules that will not trigger immune responses in the organism. The first part of these molecules is a chain made up of ethylene glycol subunits with a methoxy group on the end, which is abbreviated to mPEG. Polyethylene glycol has little toxicity and does not trigger an immune response in very small doses (*4*). Some polymers utilized in this lab contained an alcohol group instead of a methyl group at the end of this chain, which changes the above abbreviation to HO-PEG. The second part of the AB-block copolymer is a hydrophobic polyleucine amino acid chain. The abbreviation for this chain is p(Leu), so the full abbreviation for the full complex is PEG_x-b-$p(Leu)_y$, where x and y are the number of repeat units for each chain.

The second requirement is that the AB-block copolymer actually forms a capsule around the nanoparticle, and this necessitates an explanation of block copolymer self-assembly. Studies have been done in the interest of determining the supramolecular structures known as block copolymers that form from amphiphilic molecules (*5*)(*6*). One such study discovered a factor in block copolymer formation known as the packing parameter, which is calculated in the following formula $p=v/a_0l_c$, where p is the parameter, v

is the hydrophobic chain's volume, a_0 is the head group's optimal area, and l_c is the length of the hydrophobic chain (*7*). Figure 1 demonstrates how this parameter will affect the final shape of the copolymer structure.

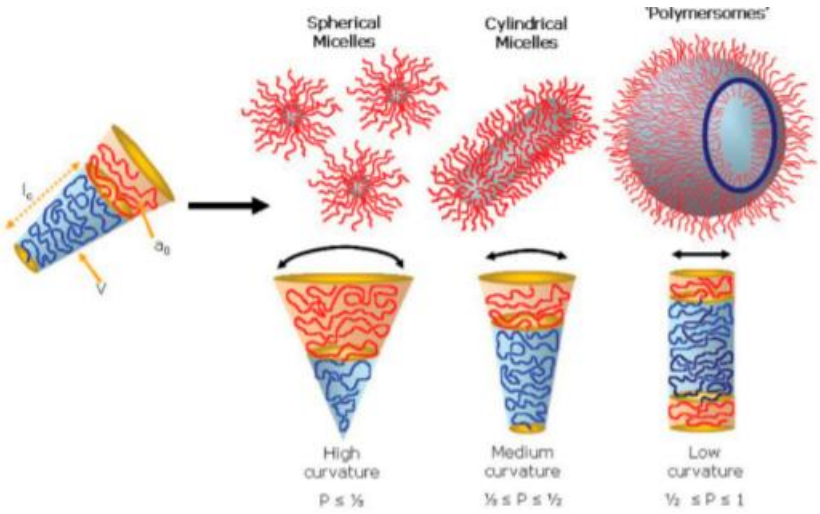

Figure 1: Various Structures that Form with Specific Values for the Packing Parameter as Said Parameter Increases from p<1/2 to p=1. Reprinted from Reference (*8*)

In this study, the polymers utilized had packing parameter values that would lead to spherical micelle formation. However, even if micelles are formed, the primary interest of this particular study was to determine if the micelles could be manipulated to an optimally small size for the transport of nanoparticles. The goal was to decrease the micelles' average diameter to less than 100nm, but diameters of less than 50nm were even more preferable. The factor selected for manipulation in the above formula was chain length, both in total length and in the ratio of chain A to chain B in the AB-block copolymer. The working hypothesis was that a higher ratio of the hydrophobic p(Leu) chain would produce micelles of smaller size so long as the volume of said chain did not increase.

Experimental:

Materials:

The chemicals utilized in the self-assembly procedure consisted of the AB-block copolymer synthesized in-lab, tetrahydrofuran, 5nm Fe^{3+} nanoparticles, and distilled water.

Instruments:

The instruments utilized in the course of the self-assembly process were an electronic scale accurate to 0.001mg, disposable vials made of plastic and glass, a glass syringe, filters for said syringe, a vortexer, disposable glass pipettes, a stir plate, and a zetasizer.

Procedure:

The self-assembly started by weighing out 1mg of the AB-block copolymer and transferring it to a disposable plastic vial. Then, 750mL of tetrahydrofuran was added to the vial to dissolve the copolymer. A filter was added to the glass syringe and the solution was then transferred to said syringe. After the solution was filtered through the syringe into either a fresh disposable plastic vial or a similar vial with 5nm nanoparticles, 1mL of distilled water was added to a disposable glass vial. The level of water was marked, and the filtered solution was added dropwise to the glass vial via a glass pipette whilst the glass vial's contents were being shaken by a vortexer. The vortexer was necessary to promote mixing between the tetrahydrofuran solution and the distilled water. This new mixture was then left overnight whilst being continuously shaken by a stir plate. After 24 hours, the vials were examined to see if the mixtures had evaporated back down to the original mark and then taken to the zetasizer to determine the average diameter of the resultant AB-block copolymer capsules. After five readings, the results were averaged, and the mass fraction of the L-Leucine was calculated by using results from NMR Spectroscopy for the copolymer in question.

Data Analysis:

The data presented here represent results gained over multiple runs of the procedure for multiple different AB-block copolymers. However, some data sets are incomplete owing to restrictions imposed on the UAH campus due to the COVID-19 virus outbreak.

Polymer	Measure 1 (nm)	Measure 2 (nm)	Measure 3 (nm)	Measure 4 (nm)	Measure 5 (nm)
$mPEG_{45}$-p(L-Leu)$_6$	772	872	797	817	960
$mPEG_{45}$-p(L-Leu)$_{22}$	617	657	770	762	626
$mPEG_{45}$-p(L-Leu)$_{53}$	321	370	505	318	325
$mPEG_{113}$-p(L-Leu)$_{22}$	50.65	55.27	49.77	50.07	51.81
$mPEG_{113}$-p(L-Leu)$_{45}$	28.55	23.48	51.94	49.15	44.73
$mPEG_{113}$-p(L-Leu)$_{65}$	27.04	30.98	17.15	25.55	

Table 1: Individual Zetasizer Measurements for the Diameters of mPEG-b-p(L-Leu) Copolymer Capsules without 5nm Nanoparticles

Polymer	Measure 1 (nm)	Measure 2 (nm)	Measure 3 (nm)	Measure 4 (nm)	Measure 5 (nm)
$mPEG_{113}$-p(L-Leu)$_{22}$	52.03	71.40	21.43	83.47	130.8
$mPEG_{113}$-p(L-Leu)$_{45}$	52.78	51.62	71.57	56.21	52.93
$mPEG_{113}$-p(L-Leu)$_{65}$	67.07	61.03	51.12	45.67	48.53

Table 2: Individual Zetasizer Measurements for the Diameters of mPEG-b-p(L-Leu) Copolymer Capsules with 10µL of 5nm Nanoparticles added to Solution (no data for $mPEG_{45}$ series)

Polymer	Diameter with 5nm Nanoparticles (nm)	Diameter without 5nm Nanoparticles (nm)	Mass Fraction of Leucine in Copolymer
$mPEG_{45}$-p(L-Leu)$_6$		843.6	25%
$mPEG_{45}$-p(L-Leu)$_{22}$		686.4	53%
$mPEG_{45}$-p(L-Leu)$_{53}$		367.8	78%
$mPEG_{113}$-p(L-Leu)$_{22}$	71.83	51.51	35%
$mPEG_{113}$-p(L-Leu)$_{45}$	57.02	39.57	53%
$mPEG_{113}$-p(L-Leu)$_{65}$	54.68	25.18	59%

Table 3: Average Zetasizer Measurements and Calculated Leucine Mass Fractions for mPEG-b-p(L-Leu) Copolymers

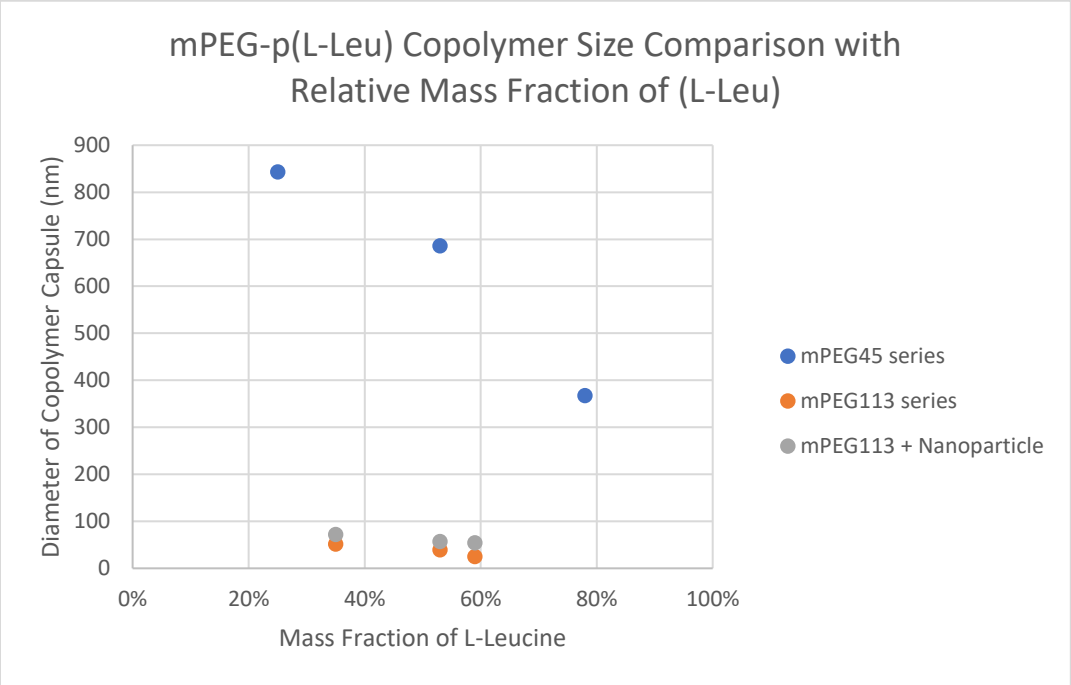

Figure 2: A Comparison of the Mass Fractions of Leucine per Copolymer in the mPEG series with the Mean Diameters of Capsules Formed

The data in Figure 2 shows an overall decrease in the size of the capsule as the mass fraction of the L-Leucine chain increases. This is what was expected in the hypothesis. Addition of the

nanoparticles slightly increased the diameter of the capsule for the $mPEG_{113}$ series, but the overall sizes were still within acceptability. Overall, this data supports the hypothesis.

Polymer	Measure 1 (nm)	Measure 2 (nm)	Measure 3 (nm)	Measure 4 (nm)	Measure 5 (nm)
HO-PEG_{45}-p(L-Leu)$_9$	52.77	56.55	65.43	58.45	58.83
HO-PEG_{77}-p(L-Leu)$_{15}$	28.58	32.78	30.52	29.69	31.21
HO-PEG_{77}-p(L-Leu)$_{30}$					
HO-PEG_{77}-p(L-Leu)$_{60}$	43.10	45.08	42.42	39.74	45.21
HO-PEG_{113}-p(L-Leu)$_{15}$	990	902	1090	1365	1083
HO-PEG_{113}-p(L-Leu)$_{45}$	42.00	41.00	43.00	55.00	40.00

Table 4: Individual Zetasizer Measurements for the Diameters of HO-PEG-b-p(L-Leu) Copolymer Capsules without 5nm Nanoparticles

Polymer	Measure 1 (nm)	Measure 2 (nm)	Measure 3 (nm)	Measure 4 (nm)	Measure 5 (nm)
HO-PEG_{45}-p(L-Leu)$_9$					
HO-PEG_{77}-p(L-Leu)$_{15}$	105.4	83.89	87.89	74.31	78.76
HO-PEG_{77}-p(L-Leu)$_{30}$	93.88	81.15	58.87	87.00	58.07
HO-PEG_{77}-p(L-Leu)$_{60}$	64.45	65.66	64.41	65.79	69.41
HO-PEG_{113}-p(L-Leu)$_{15}$	990	902	1090	1365	1083
HO-PEG_{113}-p(L-Leu)$_{45}$	42.00	41.00	43.00	55.00	40.00

Table 5: Individual Zetasizer Measurements for the Diameters of HO-PEG-b-p(L-Leu) Copolymer Capsules with 10µL of 5nm Nanoparticles

Polymer	Diameter with 5nm Nanoparticles (nm)	Diameter without 5nm Nanoparticles (nm)	Mass Fraction of Leucine in Copolymer
HO-PEG_{45}-p(L-Leu)$_9$		58.41	67%
HO-PEG_{77}-p(L-Leu)$_{15}$	86.05	30.56	33%
HO-PEG_{77}-p(L-Leu)$_{30}$	75.79		51%
HO-PEG_{77}-p(L-Leu)$_{60}$	65.94	43.11	66%
HO-PEG_{113}-p(L-Leu)$_{15}$		1086	4%
HO-PEG_{113}-p(L-Leu)$_{45}$	55.53	44.2	59%

Table 6: Average Zetasizer Measurements and Calculated Leucine Mass Fractions for HO-PEG-b-p(L-Leu) Copolymers

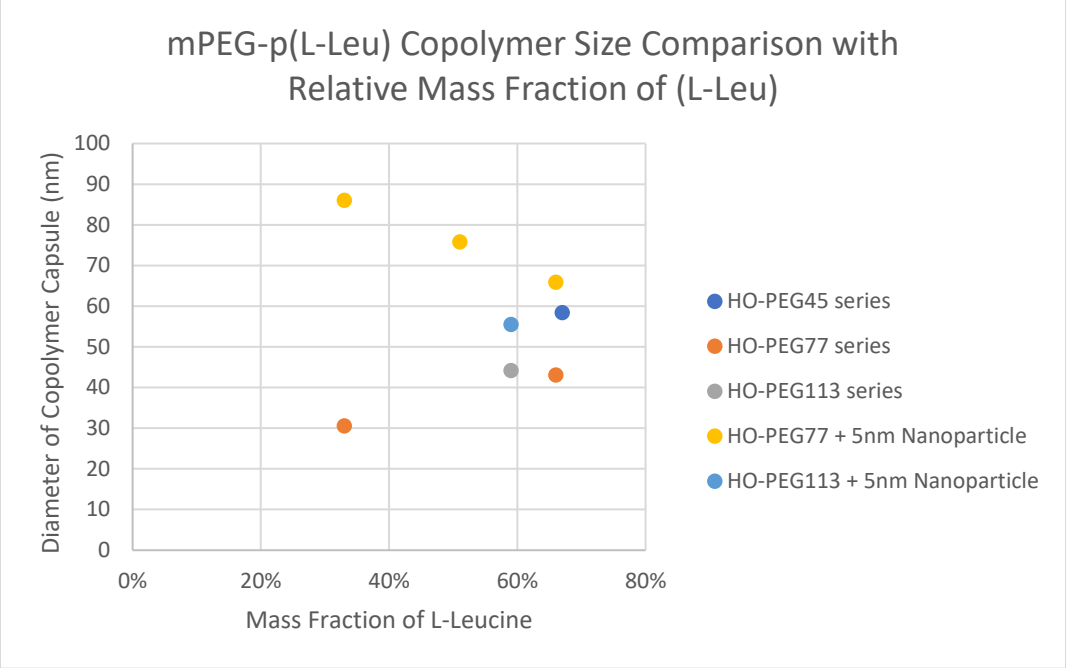

Figure 3: A Comparison of the Mass Fractions of Leucine per Copolymer in the HO-PEG series with the Mean Diameters of Capsules Formed

Unfortunately, even less of the HO-PEG data sets were able to be completed than the mPEG series due to the COVID-19 outbreak. However, aside from the individual data points, the HO-PEG_{77} series indicates an increase in size with the L-Leucine fraction. This is counter to the hypothesis, but when the nanoparticles were added, the trend reversed and demonstrated a decrease with increasing L-Leucine mass fraction. As for the HO-PEG_{113} series, the first data point was at 1083nm for the diameter, so the point could not be included on the graph and still show clear data. However, even if the data point was included, it would indicate a marked decrease in diameter with the increasing L-Leucine mass fraction.

Conclusion:

The data strongly supports the hypothesis that increasing the mass fraction of L-Leucine decreases the overall diameter of the capsule formed. Furthermore, one of the more complete data sets

was the $mPEG_{113}$ series, which demonstrated the exact sizes needed for the kind of work intended for the AB-block copolymer capsules and their encapsulated nanoparticles. This is a promising result for the research into these copolymers and I would personally encourage further research into this area of study given the implications for the medical industry.

Error Analysis:

As stated previously, the data sets used are incomplete due to the quarantine imposed on the campus of the University of Alabama in Huntsville during the COVID-19 virus outbreak. The incomplete data may have led to inaccuracies in any conclusions drawn from the data. Additionally, not all polymers originally proposed for use in the procedure are represented in the data sets.

Conflicts of Interest:

There are no conflicts of interest to cite here.

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Carl Amborski Honors Capstone

Carmen Scholz <scholzc@uah.edu> Tue, May 19, 2020 at 6:29 PM
To: David Cook <dac0010@uah.edu>

Dear David,
I think I forgot to send you my approval of the Honors Thesis written by Carl Amborski. Ok here we go:

I hereby approve of the thesis entitled "AB Block Copolymer Self-Assembly and Nanoparticle Encapsulation: Final Product Size and How it is determined from Chain Length" written by Carl Amborski in fulfillment of his Honors Studies.

Carmen

--
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