

# Encapsulation of Metal Nanoparticles With Thermoresponsive Triblock Copolymers

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## Abstract

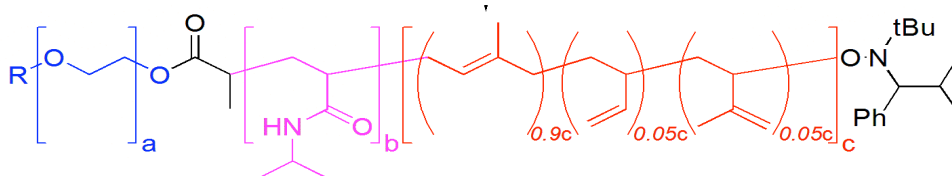
Amphiphilic molecules are known to self assemble into micelle and vesicle structures in water, and to encapsulate hydrophobic particles in them. Such encapsulations are of interest as potential medical treatments. In this study, two amphiphilic triblock copolymers and two hydrophobic metallic nanoparticles, iron oxide and cobalt, were used to make encapsulations using two methods. In the first encapsulation method, the polymer and nanoparticles were dissolved in tetrahydrofuran (THF) and water, after which the THF was allowed to evaporate. This appears to be a poor method for encapsulation as shown by a lack of increase of copolymer assembly size with Light Scattering Measurements (LSM). The second method, emulsion evaporation, was used only with iron oxide nanoparticles and the two polymers because the cobalt particles showed such poor solubility in water and THF. The iron oxide was dissolved in methylene chloride and the polymers in water. The two solutions were added to make different concentrations of each. The emulsions were evaporated under atmospheric pressure at methylene chloride's boiling point,  $\sim 40^{\circ}\text{C}$ . Once dry, water was added and the solution was sonicated for 2 days. LSM was done after sonication and after centrifugation. LSM after sonication shows greater increase in size by than by THF evaporation, indicating better encapsulation, especially with 1.0mg/ml of the lower molecular weight polymer and with a concentration of 0.5mg/ml of either polymer and 0.25 or 0.5mg/ml of iron oxide. Because interest in encapsulations is focused on potential biological uses, preliminary cytotoxicity testing was performed on the polymers and found them to be non-toxic at low concentrations.

## Introduction

Thermoresponsive polymers have different characteristics at different temperatures, usually changing reversibly from one condition to another as the polymer is heated (or cooled) above (or below) a certain temperature. One such polymer is poly(N-isopropylacrylamide) (PNIPAM), which reversibly transitions from hydrophilic to hydrophobic when heated above  $32^{\circ}\text{C}$  and the opposite if cooled [1].

Block copolymers can combine the characteristics of each block, creating a new polymer with unique capabilities. The hope for block copolymers is to create multifunctional macromolecules. One potential application is specified drug delivery systems. Utilizing a thermoresponsive component could lead to heat activated medical treatments. Human body temperature averages around  $37^{\circ}\text{C}$ , so PNIPAM, which transitions from hydrophilic to hydrophobic when heated above  $32^{\circ}\text{C}$  makes an ideal activation component for a drug delivery system that would activate when taken from a cooler room temperature setting and injected into the body.

The polymers in this study, AS91 and AS95, contain different lengths of a hydrophobic poly(isoprene) (PI) end. Both have a water soluble poly(ethylene oxide) (PEO) end and a thermoresponsive PNIPAM middle block.



### Triblock copolymer design

Poly(ethylene oxide) – PEO water soluble

Poly(N-isopropylacrylamide) – PNIPAM temperature sensitive

AS91: PEO(2k)PNIPAM(4.5k)PI(0.8k)

AS95: PEO(2k)PNIPAM(4.5k)PI(2.5k)

Amphiphilic molecules have a hydrophobic end and a hydrophilic end, such as AS91 and AS95. In water, they self-assemble into structures that allow the hydrophilic ends to touch the water and that collect the hydrophobic ends together, away from the water. The most common such structures are single layer micelles and bilayer vesicles.

The more hydrophobic the hydrophobic to hydrophilic ratio of an amphiphilic molecule, the more likely it is to make a bilayered vesicle. The less hydrophobic the ratio, the more likely it is to make a single layer micelle. For example, as shown on TEM, AS91, with less hydrophobic PI, tends to form micelles and AS95, with more hydrophobic PI, tends to make vesicles [1]. Hydrophobic particles can become encapsulated in these self-assembled structures.

Encapsulations, especially of magnetic particles, are of interest because of the potential of targeted hypothermia medical treatments. Once the encapsulations are in a certain region, such as a tumor, a rapidly reversing magnetic field could be applied, causing magnetic materials like iron oxide to heat and kill the surrounding tumor without affecting the rest of the body. Other drug delivery systems and targeted treatments are also of interest. Challenges include undesirable decomposition, biological incompatibility and toxicity.

The two nanoparticles in this study are Fe<sub>3</sub>O<sub>4</sub> coated in oleic acid resulting and cobalt with a PEO-PES coating, in the form of PEO-b-PES-Co. Previously conducted TEM shows the iron oxide nanoparticles to be about 10nm and the Co to be 12nm.

## Experiments & Results

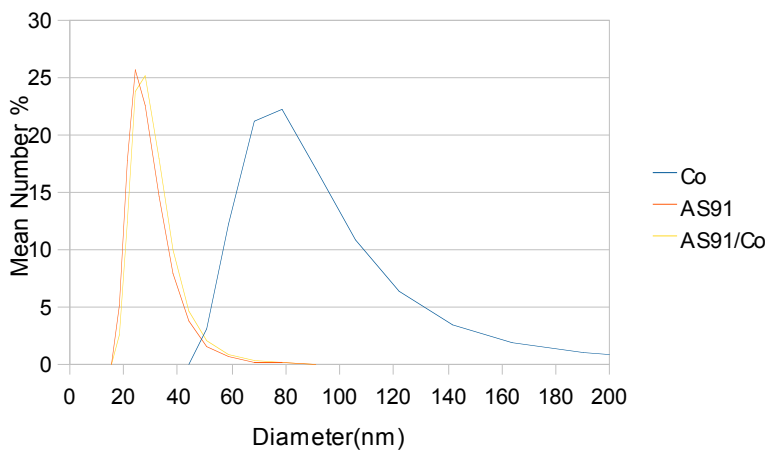
### I. THF Evaporation

0.5 mg of polymer or nanoparticle was dissolved in 1ml THF. THF solutions with cobalt were sonicated to try to dissolve the cobalt aggregates. Then, 0.5ml of a polymer-THF solution and 0.5ml of a nanoparticle-THF solution were added to 9ml of water and allowed to evaporate while stirring in atmosphere. The aqueous solution was allowed to evaporate for several days until it reached 7-8ml and then it was sealed and kept stirring, with a resulting final concentration of 0.031-0.036 mg/ml of polymer and of nanoparticles. This same procedure was used with 1ml

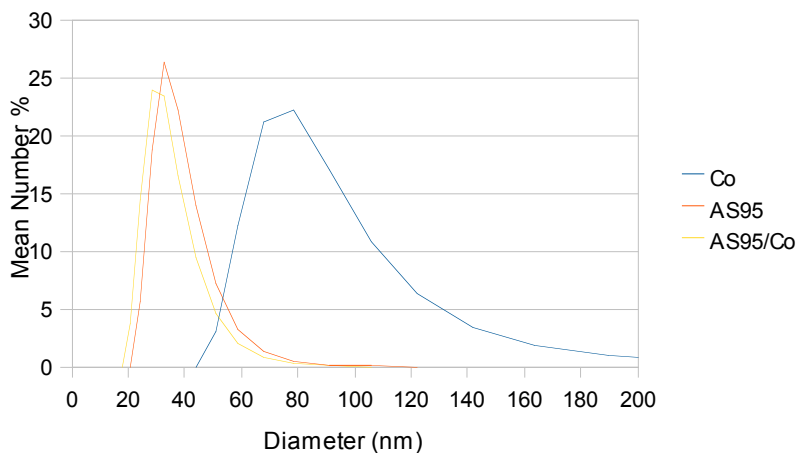
of polymer- or nanoparticle-THF solution to make polymer and nanoparticle aqueous solutions for comparison. All solutions were filtered before LSM with non-sterile 0.45 $\mu\text{m}$  syringe filters. LSM of assembly diameters were taken over a period of several weeks. Measurements over time show that the sizes were stable, so they are not shown. Some solution was also heated to 65 $^{\circ}\text{C}$  for LSM to measure assembly size changes indicating a change in assembly structure from micelles to vesicles. Previous research found that it took about 2 weeks for the assemblies to change structure [1]. The polymer and nanoparticles precipitated out and stuck to the vial, leaving too little in solution to make reliable LSM data, before enough time could pass for the assembly structure to change. Data taken before 2 weeks did not show any significant size change, so that data is not included.

The THF evaporation method did not show indications of encapsulation. The solutions with polymers and Co nanoparticles were no bigger than solutions of just polymers, indicating that the self-assemblies did not encapsulate Co. The aqueous solution of Co shows the Co aggregates to be 87nm, AS91 to be 28nm and AS95 structures to be 37nm in diameter. Previous research measured the Co nanoparticles to be 12nm in diameter [1]. The Co aggregates were most likely too insoluble in water and THF to be encapsulated in the polymer structures. Therefore, further work was concentrated on iron oxide nanoparticles.

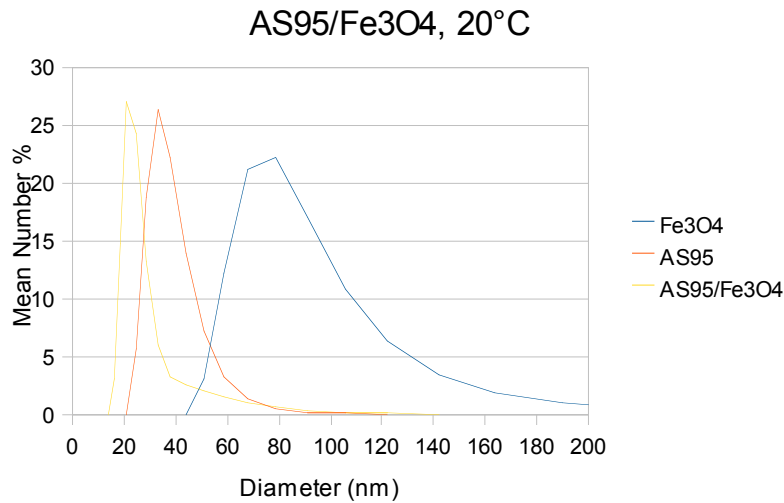
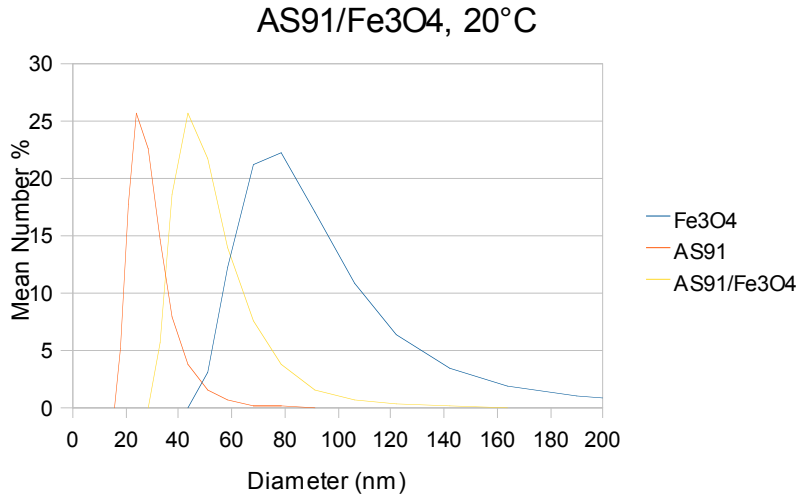
AS91/Co, 20 $^{\circ}\text{C}$



AS95/Co, 20 $^{\circ}\text{C}$



THF evaporation with iron indicated some encapsulation. Previous research measured the iron oxide nanoparticles to be about 10nm in diameter. LSM shows the iron oxide aggregates to be 87nm, AS91 to be 28nm and AS95 to be 37nm in. The AS91/ Fe<sub>3</sub>O<sub>4</sub> solution's structures increased to 50nm, indicating encapsulation, but AS95/ Fe<sub>3</sub>O<sub>4</sub> decreased to 26.5nm, indicating that AS95 did not encapsulated iron oxide nanoparticles.



## II. Emulsion Evaporation

Iron oxide was dissolved in methylene chloride and the polymers in water. A predetermined amount of each was added to the vials to make the following concentrations for 2ml of final aqueous solution. The sizes measured by LSM after sonication are also listed.

Filtered			
Solution	Constituents	Concentration (mg/ml)	D (nm)
ZA20	AS91	0.5	24.5
ZA21	AS91/Fe3O4	0.5/0.25	122.1
ZA22	AS91/Fe3O4	0.5/0.5**	
ZA23	AS91/Fe3O4	0.5/1.0	43.9
ZA24	AS91/Fe3O4	0.5/1.5	45.3
ZA25	AS91/Fe3O4	0.5/2.0	49.6
ZA30	AS95	0.5	25.0
ZA31	AS95/Fe3O4	0.5/0.25	88.3
ZA32	AS95/Fe3O4	0.5/0.5	52.3
ZA33	AS95/Fe3O4	0.5/1.0*	
ZA34	AS95/Fe3O4	0.5/1.5*	
ZA35	AS95/Fe3O4	0.5/2.0*	
ZA61	Fe3O4	0.25	27.6
ZA62	Fe3O4	0.5	25.7
ZA63	Fe3O4	1.0	26.1
ZA64	Fe3O4	1.5	19.9
ZA65	Fe3O4	2.0	22.9

Unfiltered			
Solution	Constituents	Concentration (mg/ml)	D (nm)
ZA40	AS91	1.0	18.5
ZA41	AS91/Fe3O4	1.0/0.25	125.2
ZA42	AS91/Fe3O4	1.0/0.5	131.5
ZA43	AS91/Fe3O4	1.0/1.0	92.4
ZA44	AS91/Fe3O4	1.0/1.5	134.6
ZA45	AS91/Fe3O4	1.0/2.0	93.9
ZA50	AS95	1.0	23.9
ZA51	AS95/Fe3O4	1.0/0.25**	
ZA52	AS95/Fe3O4	1.0/0.5	163.3
ZA53	AS95/Fe3O4	1.0/1.0	71.6
ZA54	AS95/Fe3O4	1.0/1.5*	
ZA55	AS95/Fe3O4	1.0/2.0*	

\*Solutions did not appear to make a suspension, so were not sonicated and no LSM data was taken.

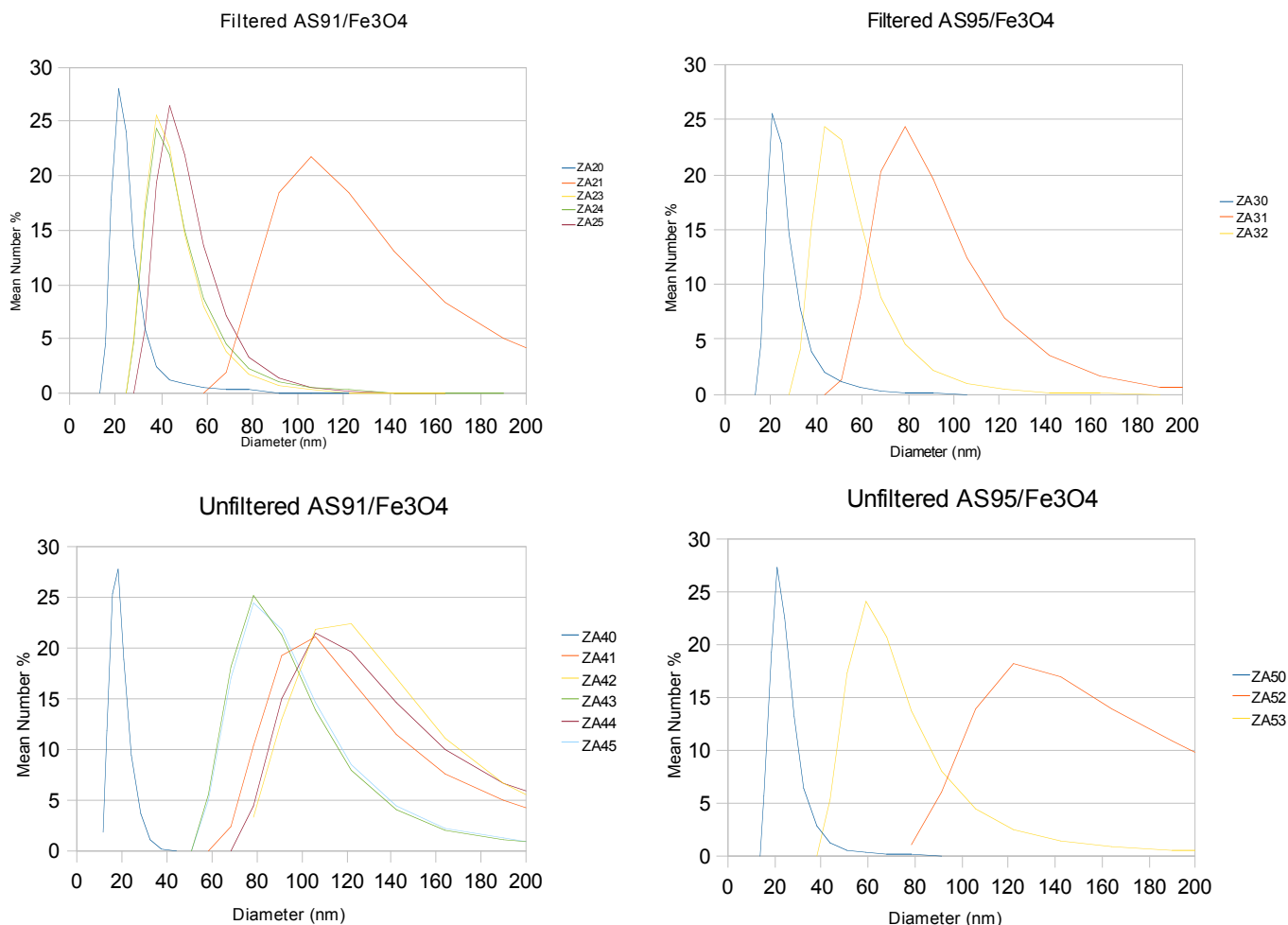
\*\*Solutions appeared to make suspensions and were of interest, but solutions were lost to accidents.

The solutions settled with the iron-methylene-chloride solution on the bottom and the aqueous polymer solution on top. A magnetic stir bar was added or was placed in the vial before addition of the solution. The presence of a magnetic stir bar resulted in the iron oxide visibly concentrating around the stir bar. The vials were set to stir at ~40°C for 16hrs. By that time, they were dry. Then 2ml of water was added to each vial and set to stir. Some stir bars had to be manually removed from the polymer-iron crust that had formed. After a day of stirring, the vials were sonicated for 2 days. Water in the sonicator heats up and evaporates, so every 8-16 hours the hot water was replaced with ice water. After sonication, the vials that appeared to have made a cloudy suspension were measured by LSM, as well as the solutions of just polymers and just iron (ZA20, 30, 40, 50 and 61-5). All vials that contained iron had a visible reddish brown deposit on the vial and the stir bar, so the fluid measured by LSM did not contain all the iron oxide added. The samples that had a high level of dust (particles above 1000nm, which are too large to be self-assemblies) were filtered with the same 0.45µm syringe filters used with the THF evaporation method solutions. After initial measurements, these samples were put in a centrifuge and decanted to remove excess unencapsulated iron and large polymer aggregates. LSM were repeated. Centrifugation removed some iron, but visibly removed polymer as well, often leaving the decanted fluid completely clear. LSM after centrifugation showed polymodal size distributions, reflected no discernible pattern and showed no correlation with LSM before centrifugation. LSM after sonication and before centrifugation showed unimodal size distributions, except for distinct dust peaks, which were easily accounted for, so analysis will focus on LSM before centrifugation.

Emulsion evaporation resulted in greater and more consistent size increases from polymer to polymer-nanoparticle solutions, indicating better encapsulation. Iron oxide solutions had aggregates of 20-27nm in size in solutions ZA61-65. The smaller size than was observed with the THF evaporation method indicates that the iron oxide was better dissolved with the emulsion evaporation method.

As shown by the following graphs, solutions of AS91 and nanoparticles made suspensions over a wider concentration range than AS95, indicating that AS91 was more efficient at encapsulating nanoparticles. Furthermore, ZA41-45 with 1.0 mg/ml of AS91, showed

the most consistent significant size increases, indicating that 1.0mg/ml of AS91 was the best concentration of polymer for encapsulation. For the other concentrations of polymer, the highest level of encapsulation observed occurred with 0.25 or 0.5mg/ml of nanoparticles.



### III. Cytotoxicity

A common test for any substance's toxicity is to expose cells to the substance for a certain amount of time, and compare how many cells are alive to a control without the experimental substance. MTG-B cells, a murine breast adenocarcinoma cell line, were plated in many small wells in a nutrient solution. The nutrient solution is a from a stock of 500 ml of minimum essential medium (MEM), 50 ml of fetal bovine serum (FBS), 5 ml L-glutamine, and 5 mL of 10,000 mcg/ml penicillin-streptomycin. A predetermined amount of aqueous polymer solution was added to 1.5ml of nutrient solution to make several concentrations of total solution that the cells were exposed to. The highest concentration was 3mg/ml of polymer in the final water-nutrient medium. AS91 and AS95 were expected to be non-toxic at low levels because PEO, PI and PNIPAM are known to be relatively non-toxic [1]. A control well of only nutrient solution was also made for comparison. After 2 days, the number of living cells in the experimental wells and the control were counted with a hemacytometer and trypan blue stain.

A second cytotoxicity test was done at much higher concentrations, 6 mg/ml and 8 mg/ml, but only using 1ml of nutrient solution.

The first cytotoxicity testing at concentrations up to 3 mg/ml showed no significant difference from the control, as expected. However, at 8 mg/ml of AS91 10% of the cells were still alive and for AS95 about 5% were still alive compared to the control. At 6 mg/ml, 28% of the cells were alive compared to the control for AS91 and AS95. This high level of cell death was unexpected, and may not be entirely due to the polymers. One possibility is that because our polymers were made and prepared under non-sterile conditions, they may have been contaminated with substance that was toxic to the cells or contaminated with a microorganism much smaller than the tumor cells, which were not affected by staining or visible under the optical microscope, that used the nutrients too, effectively starving the cells. At such high concentrations of polymer, the cells may not have had enough contact with the nutrients to live, another form of starvation. Also, with a higher ratio of aqueous solution, the pH and ion balance of the nutrient-polymer environment may have been outside of the cells' survival range. In the first test, the greatest volume of aqueous polymer solution added was 0.5ml to 1.5ml of nutrient solution. In the second test, it was 0.5ml of aqueous polymer solution to 1.0ml of nutrient solution. The decrease from 75% nutrient solution by volume to 67% nutrient solution may have been too low for cell survival. Thus, the second cytotoxicity testing was inconclusive, and the first test determined that 3mg/ml is within the non-toxic level of AS91 and AS95. Further cytotoxicity studies are necessary to determine the upper limit of non-toxicity of AS91 and AS95.

## Conclusions

The evaporation of THF solutions in water to form an aqueous solution of polymer and nanoparticles was not successful for encapsulations. However, the evaporation of the aqueous polymer and iron oxide nanoparticles-methylene-chloride emulsion indicated encapsulation, especially with 1.0 mg/ml of the lower molecular weight polymer, AS91 and with both polymers at 0.5mg/ml and AS95 at 1.0mg/ml when the concentration of iron oxide was 0.25 or 0.5mg/ml.

More cytotoxicity testing is needed to determine the safe concentration range of these polymers, especially the upper limit of the range. These preliminary tests show that low levels, up to 3 mg/ml, are non-toxic to MTG-B cells.

## References

[1] Sundararaman, A; Stephan, T.; Grubbs, R. B.; Submitted for publication 2008.