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# Nanoparticles Stabilized with MPEG-Polylysine Carbamate: Synthesis and Characterization

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WORCESTER POLYTECHNIC INSTITUTE

# Nanoparticles Stabilized With MPEG-Polylysine Carbamate:

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## Synthesis and Characterization

**A Major Qualifying Project Submitted to the Faculty of**

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirements for the

Degree of Bachelor of Science

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William Elmore

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Date:

Approved

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Drew Brodeur, Advisor

## Abstract

Biocompatible polymer-coated nanoparticles are at the forefront of research into the ever expanding field of drug delivery. We synthesized a reactive O-imidazolidine precursor of methoxy polyethylene glycol (MPEG) and linked it to poly-L-lysine of two separate molecular masses to synthesize biocompatible protected graft copolymers (MPEG-gPLL). The synthesis of the reactive precursor was confirmed by NMR, and the synthesis of the protected graft copolymers was confirmed by HPLC and TNBS testing. MPEG-gPLLs were then used for coating gold nanoparticles obtained in the presence of citrate as a capping/reducing reagent. We also performed the assessment of stabilization using surface plasmon changes and fluorescence spectroscopy and determined the appropriate concentrations of PGC to be between 0.0156 and 0.004 mg/ml and nanoparticles to be 0.99 mg/ml for optimal binding to the surface of the nanoparticles.

# Acknowledgments

I would like to offer special thanks to Professor Alexei Bogdanov, Jr. and the University of Massachusetts Medical School for their guidance and the use of their laboratory facilities for the duration of this project. I would also like to thank Professor Drew Brodeur for a great deal of advice and guidance in putting together my paper and presentation. I would also like to thank my friends and family for their continued support.

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# Introduction

A challenge that the pharmaceutical industry has been facing since its inception is the ability to produce a form of drug that is fast acting, effective, and easily distributable. Thus far, the industry has been dominated by medicines that are either orally dispensed or injected directly into the body. The fundamental purpose of a medication is to deliver a concentrated dose of a pharmaceutical to a specific area of the body to both relieve symptoms and to treat the malady at hand; however, the aforementioned techniques of drug delivery haven't been able to achieve the desired efficiency thus far (Vogelson, 2001). Orally taken medications must be taken in higher than necessary doses due to their tendency to degrade en route to the area of responsibility, to treat continuously as opposed to when needed, and their likelihood of dissociating in areas of high pH or adverse tissue conditions (Vogelson, 2001). For this reason, the pharmaceutical industry is turning to other methods, such as nanoparticles and polymer science, to be a key part of the future of drug delivery systems.

Methoxy polyethylene glycol, or MPEG as it will henceforth be referred to, is an addition polymer synthesized from ethylene oxide and methanol. Its general formula is given thusly, with  $n$  representing the number of oxyethylene monomer units present.

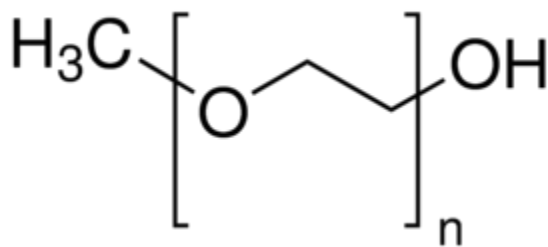


Figure 1: Structure of n-Methoxy Polyethylene Glycol



The presence of a terminal hydroxyl group gives MPEG the ability to undergo a number of modifications including carboxylation, tosylation, mesylation, and various other substitutions with the formation of a mono-functional activated derivative. MPEG can be supplied in a variety of average molecular weights ranging from 350-5000 Da. The molecular weight determines the phase of the polymer at room temperature; the procedures called for in this project use 5000 weight MPEG, which presents as a white solid at RTP.

The properties and applications of MPEG derivatives have been studied at length, particularly those applications in the field of drug delivery (Vogelsohn, 2001). The ability of MPEG to easily undergo substitution to gain functional groups as well as its ability of the resultant activated analogs to covalently modify amino, aldehyde and thio groups is a clear choice for attempts to create a delivery system for both covalently and non-covalently bonding drugs. One primary focus is the creation of protected graft copolymers, or PGCs. These highly versatile compounds consist of a conjugated backbone composed of polylysine and randomly distributed MPEG polymeric chains that provide multiple binding and linking sites (N-  $\epsilon$ -amino groups of polylysine) for drugs and/or adaptors (Bullock, Chowdhury, Severdia, Sweeney, Johnston, and Pachla, 1997). PGCs have historically been used as carrier molecules for use in MR imaging, but their biodegradability, non-toxic nature and abundance of positive charges at physiological pH are making them a popular choice for the delivery of drugs and DNA plasmids (Vogelsohn, 2001). Biodegradability and non-toxicity ensure that the carrier system can last for a long time in physiological conditions without causing adverse effects to the patient, and the positive charges give PGCs the ability to form polyionic complexes with a variety of plasmids (Bogdanov and Mazzanti, 2012). This makes PGCs a system of interest to the field of gene therapy.

# Background

## Modification of Methoxy Polyethylene Glycol

Several different modifications of MPEG have provided a basis for the research and syntheses discussed in this project. Methods for preparing tosylate, bromide, amine, and fatty acid ester derivatives have been established since the 1980's and are constantly being reexamined to raise efficiency, produce higher yields, and form increasingly stable polymers (Harris and Yelpani, 1984).

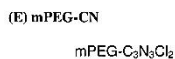
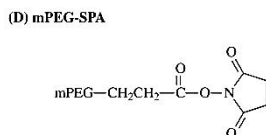
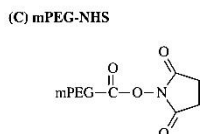
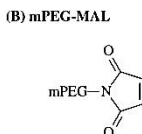


Figure 2: MPEG Derivatives (Tsui, 2009)

The formation of MPEG-Imidazolide that is one of the focuses of this experiment employs a modified version of the previously accepted procedure for tosylation of MPEG.

Previously, MPEG tosylation was accomplished by the addition of tosyl chloride to MPEG in a dichloromethane in the presence of pyridine (Harris and Yalpani, 1984). However, this was found to result in an extensive level of polyethylene oxide chain cleavage, as well as forming a weakly reactive tosylate that was not able to be stored for longer periods of time without experiencing a gradual decomposition (1984). Further research by Harris and Yalpani revealed that during mesylation and bromination, the procedure for tosylation benefited from the use of a 60% excess of triethylamine as opposed to pyridine (1984). MPEG tosylates are good starting compounds for synthesizing other derivatives because of the tosyl group's ability to act as a leaving group while undergoing nucleophilic displacement. Synthesizing MPEG tosylate enabled the further reaction with mercaptoacetic acid, producing a thioacetate that has been extensively researched at a later time as a precursor for MPEG-thiol, a single-chain stabilizer of gold nanoparticles.

Instead of using tosylation or mesylation we investigated an alternative activated precursor for PEGylation, i.e. MPEG-O-imidazolide which has been previously suggested as a non-toxic, weakly reactive analog of MPEG that has been used for covalent modification of many proteins carrying primary amino groups. It was found that a procedure based upon the addition of 1,1'-carbonyl diimidazole (CDI) to MPEG could produce a relatively hydrolysis resistant, stable MPEG Imidazolide that is still sufficiently reactive in the presence of primary amino groups in the presence of water, especially if the nucleophilicity of amines is increased by using high pH. After the activation in the presence of CDI, the imidazolide is easily purified by hydrolyzing the excess of CDI and removing imidazole by water washes. The final purification can be easily performed, if required, by precipitating with a mixture of ethyl acetate and diethyl ether due to a lack of solubility of MPEG in diethyl ether and ethyl acetate. All these reactions

were found to be most effective when done in a closed system under argon with completely anhydrous solvents and reagents, as MPEG on its own can suffer from oxidative complications from air and is highly soluble in water.

### Protected Graft Co-Polymers

Synthesis of protected graft co-polymers is chiefly accomplished through the covalent grafting of modified MPEG chains to a polyamino acid. A number of syntheses exist that yield the protected graft copolymer, but these are all highly dependent on the functional groups of the amino acids and the level of reactivity of the modified MPEG used (Bogdanov and Mazzanti, 2012). Research has shown that it is also possible to synthesize PGCs through the use of a polycarboxylic acid as opposed to a polyamino acid, but not all polymers of this nature are both biocompatible and biodegradable. The synthesis performed by our laboratory was done using poly-L-Lysine and MPEG Imidazolide in alkaline environment, as past research has shown that poly-L-lysine achieves the highest degree of modification at a pH of 9.5 (Beauchamp, Gonias, Menapace, and Pizzo, 1983). The resultant polymer can be tested for remaining free amine groups; these groups can be further modified by using other amine-reactive compounds producing polymers with several types of molecules linked to a single amino acid backbone.

The presence of free amine groups on the poly-L-lysine backbone is an indicator of the completeness and the level of modification achieved in the PGC synthesis. Our laboratory used the 2,4,6-Trinitrobenzene Sulfonic Acid (TNBS) which is convenient to use in the case of water-soluble macromolecules. TNBS testing accomplishes this by mixing the amino-containing component (e.g. PGC) with 2,4,6-Trinitrobenzene Sulfonic Acid, a detergent, and water

(Schalkhammer, 2002). The reaction between 2,4,6-Trinitrobenzene Sulfonic Acid and each free amine group results in a chromogenic derivative that can be analyzed qualitatively through a differences in absorbance values measured in the range 330-430 nm between the modified PGC and pure poly-L-lysine (2002).

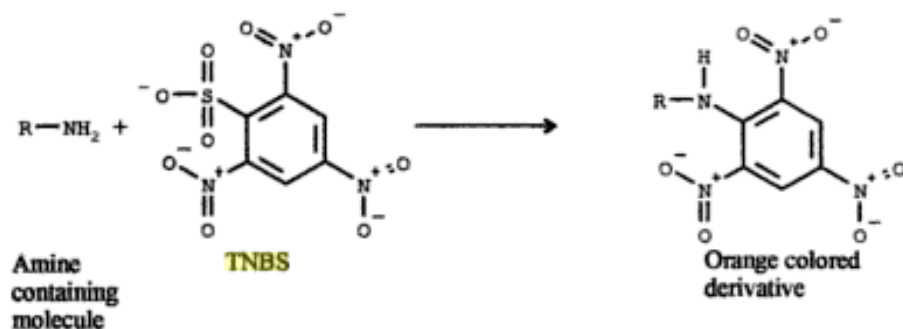


Figure 3: Reaction Scheme for Trinitrobenzene Sulfonic Acid Test (Schalkhammer, 2002)

An assay of modified PGC can be run simultaneously with a batch of pure poly-L-lysine and should show a decrease in absorbance due to the decrease in free amine groups associated with the successful synthesis of a PGC (2002). Alternatively, our laboratory also used High Performance Liquid Chromatography (HPLC) to confirm synthesis. The comparison of our products' spectra with a general reference elution profile of the modified poly-L-lysine was used as an additional proof of an acceptable degree of modification.

Z-average hydrodynamic diameter; absorbance at $\lambda_{max}$ ; $\lambda_{max}$ peak position.			
1	2	3	4
159 nm; 0.232; 530 nm	122 nm; 0.336; 530 nm	108 nm; 0.523; 535 nm	116 nm; 0.616; 535 nm
149 nm; 0.261; 530 nm	109 nm; 0.372 535 nm	76 nm; 0.560 535 nm	43 nm; 0.672 535 nm
34 nm; 0.292; 530 nm	52 nm; 0.385 535 nm	49 nm; 0.593 535 nm	43 nm; 0.756 535 nm
33 nm; 0.303; 530 nm	35 nm; 0.437 535 nm	36 nm; 0.653; 535 nm	41 nm; 0.760 535 nm
40 nm; 0.281; 530 nm	37 nm; 0.398 535 nm	39 nm; 0.561 535 nm	41 nm; 0.608 540 nm

Table 1: Characterization of GNPs Showing Scattering Intensity Hydrodynamic Diameter (nm), Plasmon Intensity and Plasmon Peak Maxima (Bogdanov and Gupta, in preparation)

## Nanoparticle Synthesis and Modification

Nanomaterials have been a primary focus in the field of drug delivery for the last decade based on their ability to increase longevity, solubility, and accumulation in target areas. The particular focus on gold nanoparticles (GNPs) was based on a variety of factors, including past history of human use in anti-arthritis formulations, not least of which is the ease with which they can be used for a plurality of novel applications, including bioelectronics and sensor development. The uniqueness of gold nanoparticles is in the presence of strong surface Plasmon effect that results in very efficient excitation of surface Plasmon-polariton wave in the visible range of light, suggesting the applicability of GNPs in imaging. Research by Li and Guo suggests that gold engenders a stable environment for synthesis, as well as offering controlled degradation in physiological conditions and a chance for resonance imaging (Li and Guo, 2011). Coating these nanoparticles in polymers, or theoretically our PGC, should ensure biocompatibility and a “biological stealthiness” (2011). GNPs covered in a layer of polymer

offer an opportunity to carry a variety of functional groups, such as contrast agents and tissue specific ligands (2011). Purification of these nanoparticles, once modified, can be accomplished by size-specific columns that utilize fiber filters with pores of a diameter that exclude the bulky nanoparticles and employ a centrifuge to move out all other solvents and reagents associated with synthesis.

The stability of PGC-coated GNPs is shown through their exposure to several different physiological conditions. In order to ensure their appropriateness as a drug delivery system, their ability to aggregate must be confirmed; this is accomplished through a battery of tests simulating saline levels, phosphate levels, and its ability to aggregate in serum. By again utilizing UV/Vis absorbance spectroscopy, we investigated the relative absorbance over time in order to ascertain the ability of the system to aggregate and stay stabilized in near-physiological conditions.

# Methodology

## Drying Polyethylene Glycol

- a. 50g of polyethylene glycol and 200 mL of >99.5% toluene were added to a 1 liter flask, stirred, and heated to 170 °C. The mixture was then slowly heated to 180°C until 180 mL of distillate were collected. The flask was cooled to room temperature, and a few milliliters of distillate were subsequently collected. The reaction flask was then sealed and stored in a freezer. This procedure was repeated twice more to ensure complete drying of the MPEG for subsequent syntheses.

## Synthesis of MPEG Imidazolidine

- b. 8.8 g of dried MPEG 5000 were dissolved in 45 mL of dried toluene and stirred under moderate heat to encourage dissolution. 139g of carbonyldiimidazole were dissolved in 20.5 mL of dioxane. This was immediately added to the MPEG solution, which remained colorless. This solution was placed over heat of 50°C with stirring. The flask was flushed with argon, sealed, and allowed to sit overnight stirring with constant heat. This solution was rotary evaporated at 60°C until a viscous yellow liquid remained. 100 mL of dichloromethane was added and the flask was placed in an ice bath, where the solution once again became colorless. 30 mL of 1 M sodium chloride was added; the flask was removed from the ice bath, and then stirred. Stirring produced effervescence, so the solution was



stirred until evolution of bubbles ceased. At this point, the solution split into a bilayer, with a colorless organic layer on bottom and a cloudy aqueous layer on top. This was separated using a separatory funnel, washed with 30 mL of water and re-separated. The clean organic layer was placed in another flask, where additional dichloromethane and anhydrous sodium sulfate were added and left over night. The solution was once more rotary evaporated at 60°C, and the product was lyophilized and stored for later use.

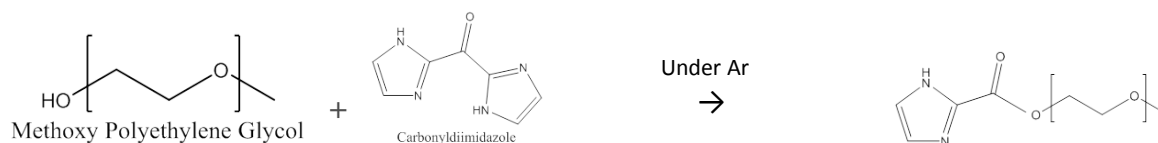


Figure 4: Reaction of MPEG 5000 with Carbonyldiimidazole

### Synthesis of MPEG Tosylate

- c. 8.06g of MPEG in 150 mL of toluene was rotary evaporated to dryness over the course of 20 minutes, resulting in a white crystalline product. 30 mL of dry dichloromethane was added to the flask and stirred until all crystals had dissolved. A septum was placed on the flask and the flask was flushed with argon. 2 mL of dry triethylamine were added. A solution of 40 mL of dry dichloromethane and 1.4g tosyl chloride was made during redissolution. This mixture was added dropwise through the septum, after which the mixture turned a light yellow in color and was allowed to stir for 48 hours at RTP. The light yellow mixture was then rotary evaporated until only a white solid remained. This solid was

redissolved in 30 mL of dichloromethane, then added to 175 mL of diethyl ether. Recrystallization began immediately, yielding a white crystalline solid. This was allowed to precipitate at RTP overnight, then precipitated in a freezer over the course of 48 hours. White crystals were vacuum filtered out of the mixture and washed with diethyl ether. After completely drying, the crystals were redissolved in 25 mL of dichloromethane, forming a cloudy yellow mixture, then added to 175 mL of diethyl ether. This was allowed to precipitate briefly at room temperature, then precipitated in a freezer overnight. The product was washed once more with dichloromethane and diethyl ether, then lyophilized and stored for later use.

#### Synthesis of MPEG Thioacetate

- d. 2g of MPEG tosylate were solubilized in 7.5 mL of dry dioxane and heated to 55°C until solubilized completely. This was filtered through 1 cm of celite by vacuum and transferred to a 50 mL flask that was then stoppered and flushed with argon. 0.25 mL of mercaptoacetic acid and 0.3 mL of pyridine were added. The mixture remained colorless, was flushed with argon, and allowed to mix at RTP for 48 hours. The mixture was once again vacuum filtered through 1 cm of celite and collected in an Erlenmeyer flask. The filtrate was added drop-wise to a mixture of 30 mL of diethyl ether and 10 mL of ethyl acetate and mixed for 30 minutes. The product was a white crystalline solid that was vacuum filtered with a glass filter and washed with diethyl ether.

## First Synthesis of MPEG Imidazolid-Poly-L-Lysine PGC

- e. The following procedure was followed for both High Molecular Weight and Low Molecular Weight Poly-L-Lysine. 50mg of PLL hydrobromide were added to 10mL of triethylammonium acetate buffer and adjusted to pH 9.5. 40  $\mu$ l were removed for zero time point control. PLL solutions were cooled on ice for 30 minutes. 265mg of MPEG Imidazolid were added in three equivalent portions to the PLL solutions and vortex mixed. pH was adjusted back to 9.5 using the triethylammonium acetate buffer. The samples were prepared for TNBS testing as shown in Table 1. All samples were vortexed before the addition of 2mM trinitrobenzene sulfonic acid, then incubated for 30 minutes. Absorbance of samples was then measured to ensure modification had occurred.

Reagent	Tube Number					
	1	2	3	4	5	6
Sodium Tetraborate	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l
1% Triton X-100	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l
Deionized Water	97 $\mu$ l	97 $\mu$ l	97 $\mu$ l	97 $\mu$ l	100 $\mu$ l	800 $\mu$ l
MPEGI-PLL PGC	3 $\mu$ l	3 $\mu$ l	0 $\mu$ l	0 $\mu$ l	0 $\mu$ l	0 $\mu$ l
PLL	0 $\mu$ l	0 $\mu$ l	3 $\mu$ l	3 $\mu$ l	0 $\mu$ l	0 $\mu$ l
2 mM TNBS	700 $\mu$ l	700 $\mu$ l	700 $\mu$ l	700 $\mu$ l	700 $\mu$ l	0 $\mu$ l

Table 1: Test Tube Content Scheme for TNBS Testing

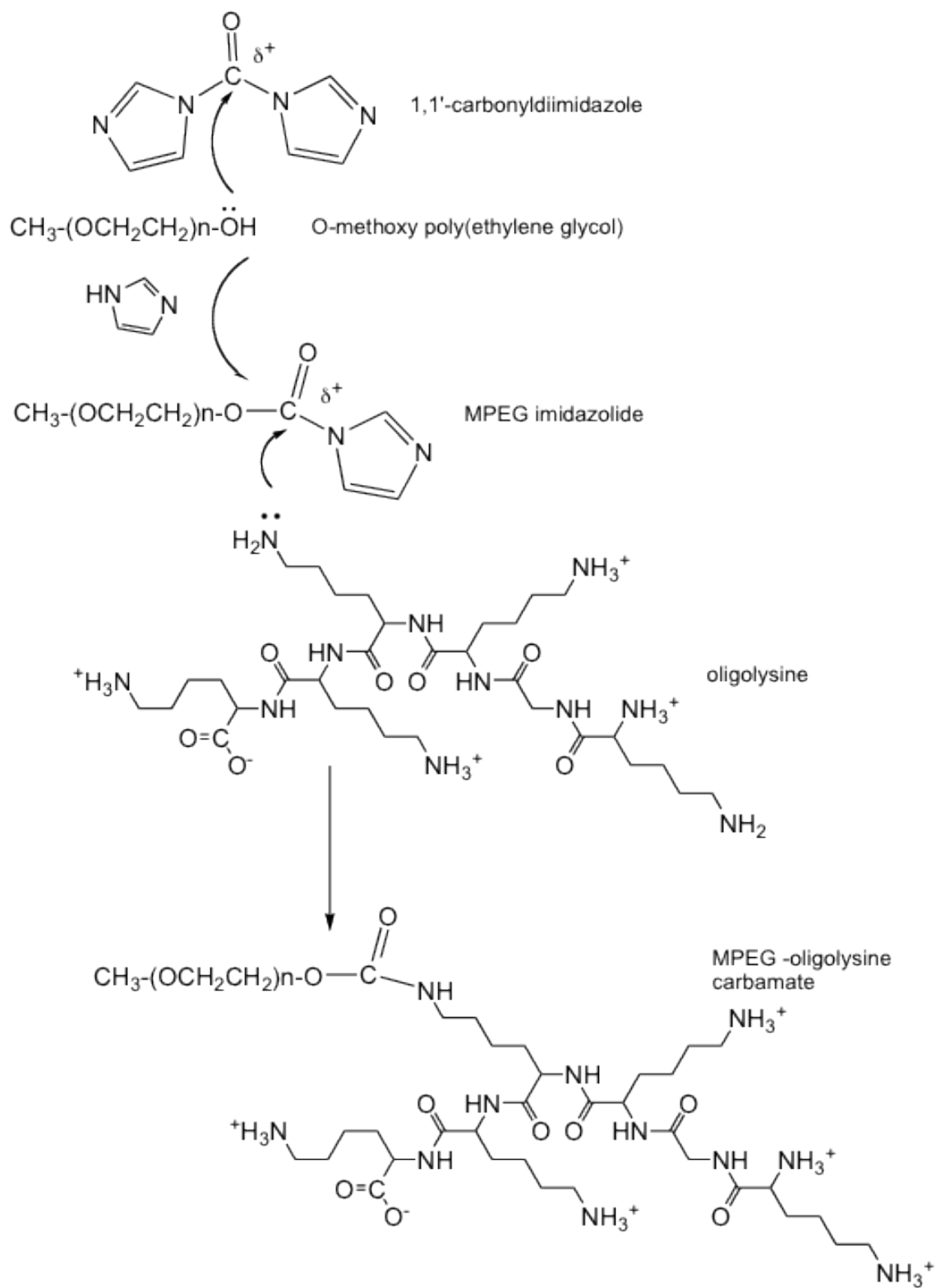


Figure 5: A scheme of MPEG-Imidazolide synthesis and its reaction with pentalysine (illustrating a fragment Poly-L-Lysine)

### Second Synthesis of MPEG Imidazolid-Poly-L-Lysine PGC

- f. The following procedure was only used for High Molecular Weight Poly-L-Lysine. 100mg of PLL hydrobromide was added to 20mL of triethylammonium acetate buffer and adjusted to pH 9.5. 80  $\mu$ l were removed for zero time point control. PLL solutions were cooled on ice for 30 minutes. 530mg of MPEG Imidazolid were added in three equivalent portions to the PLL solutions and vortex mixed. pH was adjusted back to 9.5 using the triethylammonium acetate buffer. The samples were prepared for TNBS testing using the same procedure described in Methodology Part E, except the Triton X-100 detergent was changed to a different detergent. Samples were incubated for 30 minutes, vortex mixed, and absorbance was measured.

### Third Synthesis of MPEG Imidazolid-Poly-L-Lysine PGC

- g. The following procedure was only used for High Molecular Weight Poly-L-Lysine. 100 mg of PLL hydrobromide was added to 20mL of sodium borate buffer and adjusted to pH 9.5. 80  $\mu$ l were removed for zero time point control. PLL solutions were cooled on ice for 30 minutes. 795mg of MPEG Imidazolid were added in three equivalent portions to the PLL solutions and vortex mixed after the addition of each portion. pH was adjusted back to 9.5 using the sodium borate buffer. The samples were prepared for TNBS testing using the same procedure described in Methodology Part E. Absorbance was measured.

## Verification of Modification Using HPLC

- h. 50  $\mu$ l of each synthesized MPEG Imidazolide-Poly-L-Lysine PGC were added to 50  $\mu$ l of sodium bicarbonate and 3  $\mu$ l of 680 NHS radiolabeled dye. This produced a blue colored solution that was allowed to sit for 30 minutes at RTP. 15  $\mu$ l of NHS ester of acetic acid were added to each sample and allowed to incubate at RTP for a further 30 minutes. These samples were then run through P30 samples with 0.1 M Ammonium Acetate by spinning in a centrifuge for 4 minutes at 1000 rcf three times. These purified samples were run through a High Performance Liquid Chromatograph that served as a second verification of modification level of the poly-L-Lysine backbone.

## Synthesis and Stabilization of MPEGI/PLL coated Gold Nanoparticles

- i. This procedure was used for the Low Molecular Weight PGC synthesized in Methodology Part E and the High Molecular Weight PGC synthesized in Methodology Part G. 10  $\mu$ l of  $\text{HAuCl}_4$  were added to 10  $\mu$ l of water. These were heated to 95°C, then 1.75mL of 1% citrate solution were added to the solution. 25mg of MPEGI-PLL were dissolved in 500  $\mu$ l of water, then added to the GNP solutions. These gradually changed from a light yellow solution to a reddish purple solution in the case of Modified MPEGI-PLL samples. GNPs were also coated in pure poly-L-lysine using a similar procedure, and produced a reddish pink solution. These were stored in a refrigerator for later stabilization testing.

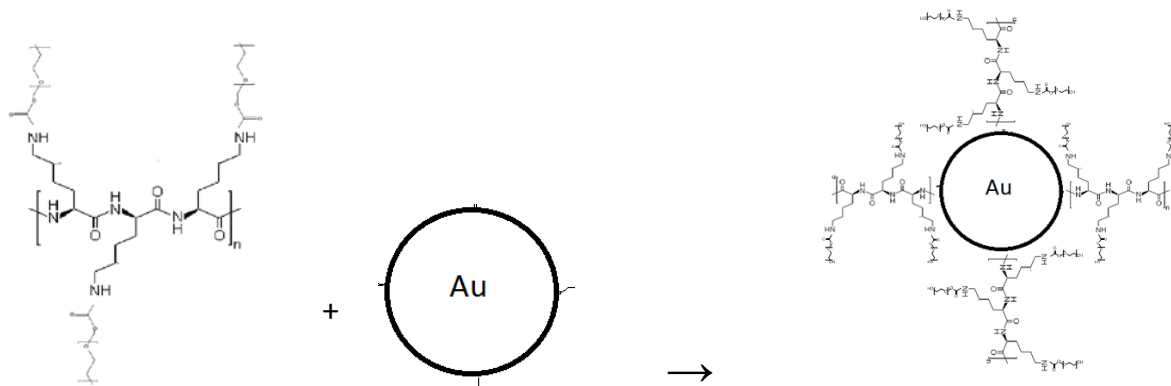


Figure 6: Reaction of MPEGI/PLL with GNP

### Stabilization Testing of MPEGI/PLL GNPs

- j. Serial dilutions of MPEG-gPLL from 4mg/ml to 0.01 mg/ml were prepared in a well plate in volumes of 50  $\mu$ l. These were added to 200 mcl of citrate stabilized GNPs using a multi-channel pipette and allowed to incubate for 30 minutes. Absorbance was measured by absorbance spectroscopy reading from 500nm to 550nm. The plasmon peak shift that was observed was used to determine the MPEG-gPLL concentrations necessary for fluorescence spectroscopy. Two rows of a well plate were prepared with concentrations of MPEG-gPLL-AF488 at 0.156 and 0.04 mg/ml, and 90mcl of water was added to one well as a 100% polymer control well. Citrate stabilized GNPs were diluted serially and 90 mcl of each well were added to the polymer wells using a multi-channel pipette. After 1 hour of incubation, this plate was tested for fluorescence intensity by exciting at 490 nm and measuring emission at 520 nm.

# Results and Discussion

## Confirmation of Syntheses

The first phase of this project was concerned with the successful synthesis of MPEG derivatives and their purification. First, synthesis of MPEG-Imidazolide was attempted twice, with success confirmed by the use of a 300 MHz Bruker Nuclear Magnetic Resonance Spectrometer as shown below to collect proton NMR spectra. The peaks observed on these spectra were consistent with the literature values for protons found in an Imidazolide.

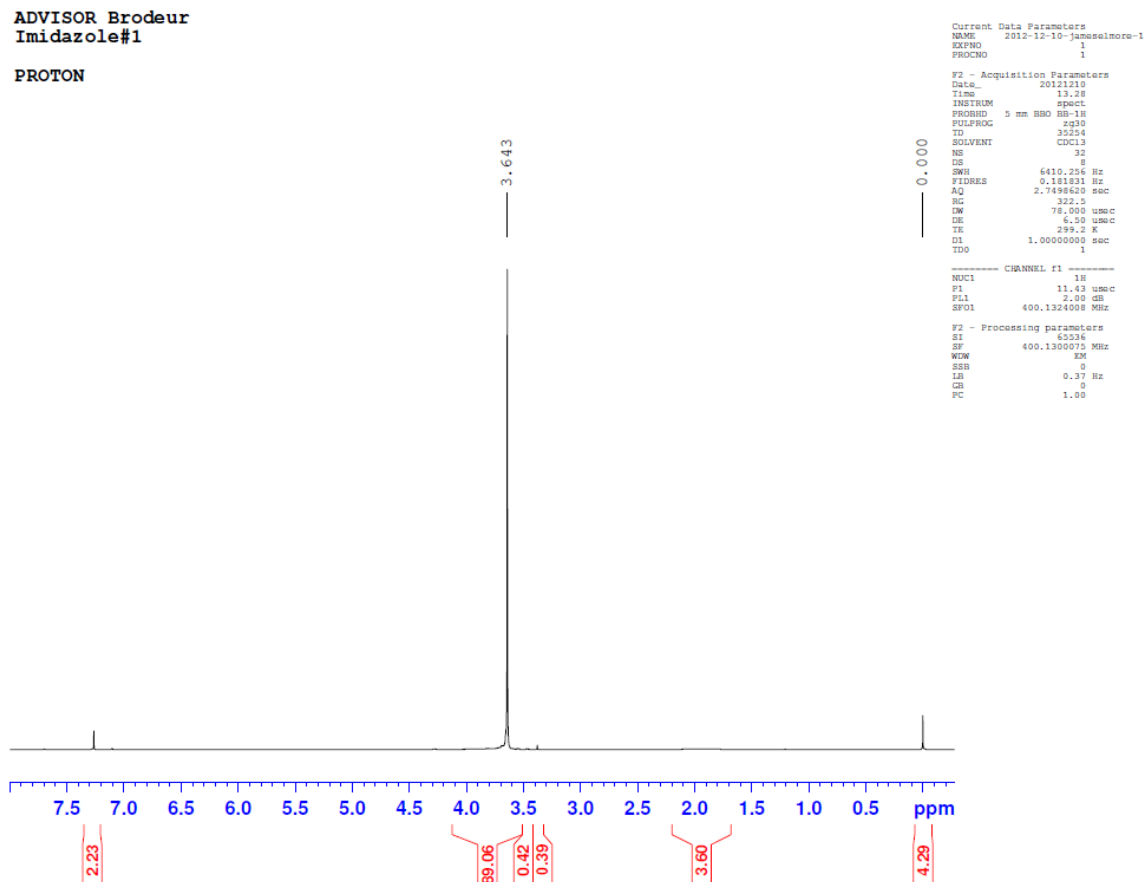


Figure 7: NMR Spectrum of MPEG-Imidazolide Sample 1



ADVISOR Brodeur  
Imidazole#2

PROTON

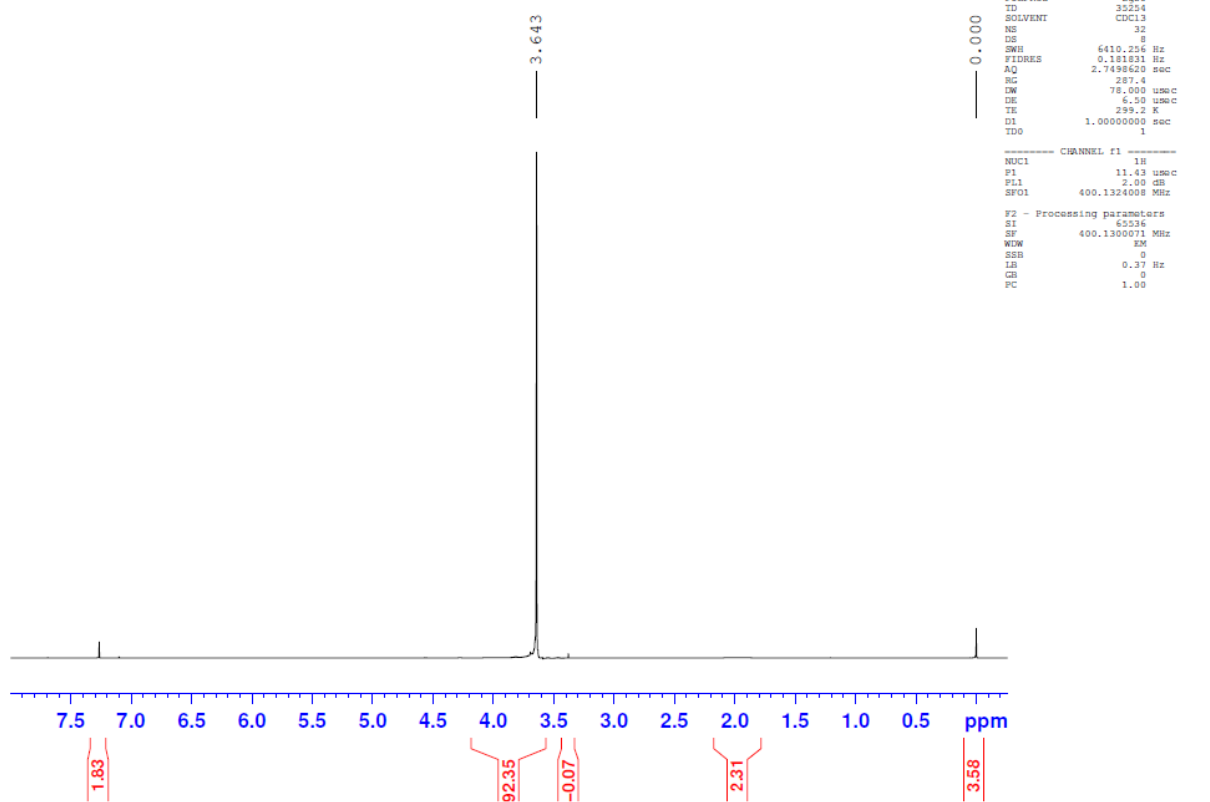


Figure 8: NMR Spectrum of MPEG-Imidazolidine Sample 2

The further synthesis of an MPEG-Thioacetate was also confirmed using the same NMR. The spectra shown below are also consistent with literature for formation of thioacetates.

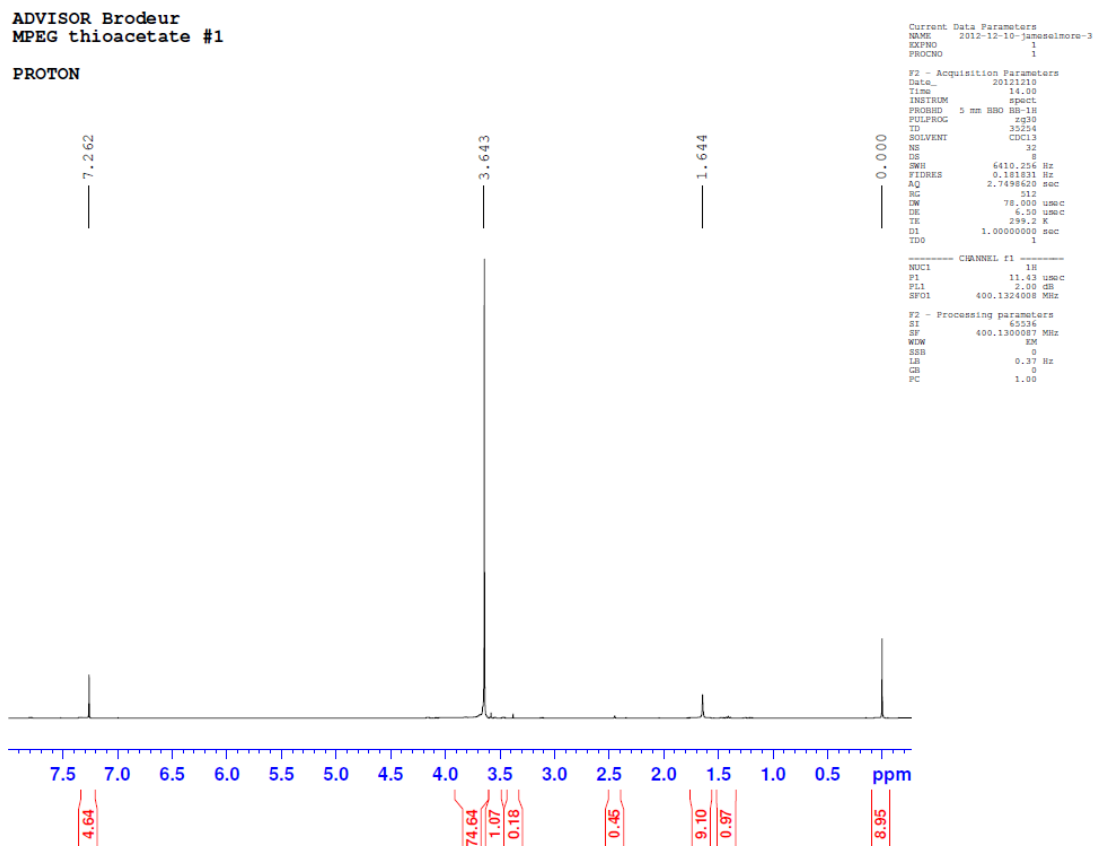


Figure 9: NMR Spectrum of MPEG-Thioacetate Sample 1

ADVISOR Brodeur  
MPEG thioacetate #2  
PROTON

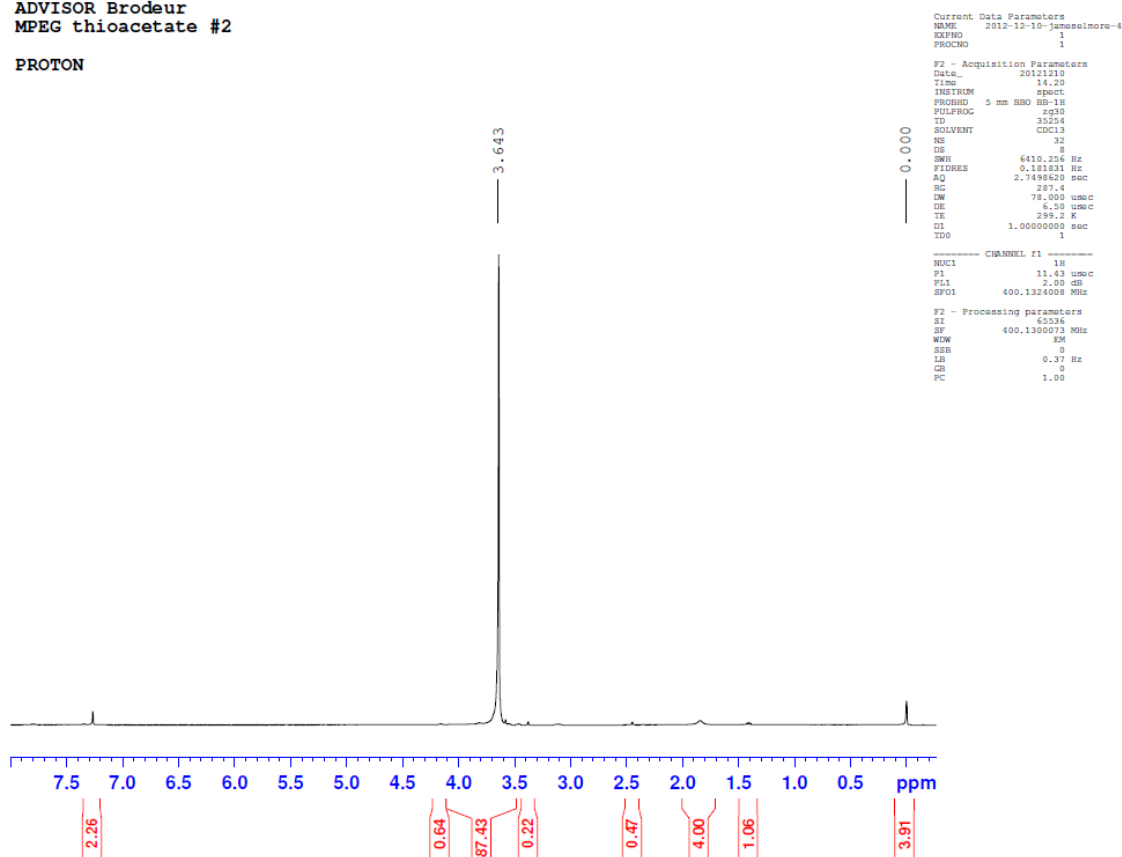


Figure 10: NMR Spectrum of MPEG-Thioacetate Sample 2

The next phase of the project, the synthesis of the protected graft co-polymers, was confirmed using TNBS testing and HPLC. The results of TNBS testing are shown in the tables below. Although the TNBS testing showed definite changes in the level of modification, the results were inconsistent with the expected values, leading us to believe that the concentrations of amine groups present were too high for the TNBS test to handle, leading to absorbance values that were inverse or inconsistent.

Tube Designation	Absorbance (nm)		
	Test 1 ( $\lambda=325\text{nm}$ )	Test 2 ( $\lambda=420\text{nm}$ )	Test 3 ( $\lambda=420\text{nm}$ )
Blank (1)	0.329	0.004	0.001
Blank (2)	0.341	0.574	0.93
LMW (1)	0.571	N/A	N/A
LMW (2)	0.551	N/A	N/A
HMW (1)	0.606	1.208	1.216
HMW (2)	0.598	1.231	1.204
Modified LMW (1)	0.553	N/A	N/A
Modified LMW (2)	0.563	N/A	N/A
Modified HMW (1)	0.606	1.269	1.248
Modified HMW (2)	0.612	1.297	1.285

**Table 2: Results of TNBS Testing on Procedures 1, 2, and 3**

This inconsistency in results from TNBS testing led us to attempt confirmation of modification using High Performance Liquid Chromatography. Chromatograms were taken of each attempt at synthesis both before and after purification using YM100 membrane concentrators. The first set of HPLC spectra showed a set of peaks that confirmed some degree of modification, but were consistent with an incomplete modification due to an overabundance of polylysine. The second set of HPLC spectra were inconclusive and showed an extremely noisy and impure signal consistent with an overabundance of unmodified polylysine and free MPEG-Imidazolide. The third set of HPLC spectra showed a clean peak that is consistent with the literature value for modified polylysine, and showed only very small traces of free MPEG-Imidazolide. These spectra only improved after further purification was attempted.

## Stabilization Testing

After successful synthesis of the different molecular weights of PGC, the PGCs were adhered to citrate stabilized gold nanoparticles. This led to the testing and subsequent analysis of MPEG-gPLL coated GNPs to determine the optimum concentrations for binding and stability. The first cycle of tests involved varying the concentrations of MPEG-gPLL in a constant concentration of citrate stabilized GNPs and measuring their absorbance. This showed a huge spectral shift between the concentrations 0.0156 mg/ml and 0.004 mg/ml consistent with successful binding. These concentrations were then both increased 10 fold and used for fluorescence spectroscopy. This well plate varied the concentrations of citrate stabilized gold nanoparticles while keeping the 10 fold increased MPEG-gPLL concentrations constant. The resulting graph below shows fluorescence quenching occurring at an approximate concentration of 0.99 mg/ml of GNPs; this again denotes the concentration at which successful binding occurs. Both concentrations depict quenching at this concentration, and have curves of best fit that have a corresponding R-squared value of 0.97. The combined conclusions from these spectra suggest that the optimal concentrations for complete MPEG-gPLL and GNP binding occur at the following concentrations: MPEG-gPLL (0.0156 - 0.004 mg/ml) and GNP (0.99 mg/ml).

## Analysis of Hydrodynamic Diameter and Zeta Potential Values

In order to ascertain the viability of High Molecular Weight vs. Low Molecular Weight varieties of the protected graft copolymer for surface coating, our laboratory measured several properties of the coated GNPs that were synthesized. As a preliminary step, the Z average of the nanoparticles were measured and found to be nearly identical, due to the preparations involved for each being similarly carried out. Hydrodynamic diameters by number and volume were also

found to be similar, but hydrodynamic diameter by intensity yielded much different results. Low Molecular Weight MPEG-gPLL coated GNPs showed a nearly 20 nm increase in this case. The intensity distribution of a set of coated nanoparticles is affected greatly by the size of subfractions of particles, so it was concluded that the LMW version of our system showed much more aggregation in nanoparticles of larger size. Additionally, the Zeta potential of the LMW system showed a larger negative value which corresponds to an inability of the PGC to displace the citrate stabilizing the GNPs prior to synthesis of the coated nanoparticles. HMW populations showed only a small positive charge, suggesting that more efficient binding took place for this version of the PGC

	<b>LMW MPEG-gPLL coated GNPs</b>	<b>HMW MPEG-gPLL coated GNPs</b>	<b>Results</b>
Z average, nm	44.0	44.7	Both particle preparations are nearly identical
Hydrodynamic diameter, by Intensity nm	59.0±30.3	39.2±16.0	Since intensity distribution is a measure highly biased by the presence of a larger sub-fraction of particles, LMWs appear to have more larger aggregated NPs
Hydrodynamic diameter, by Number, nm	22.75±7.2	21.2±5.8	Nearly identical
Hydrodynamic diameter, by volume, nm	32.2±15.5	26.8±9.9	Nearly identical
Zeta potential (charge), average, mV	-7.84	+1.59 (two populations)	LMW MPEG-gPLL does not provide a complete displacement of citrate off the GNPs

**Table 3: Comparison of Zeta Potential Values and Hydrodynamic Diameter for LMW and HMW MPEG-gPLL Coated GNPs**

## Conclusions

A stable protected graft copolymer was synthesized utilizing both high and low molecular weight versions of poly-L-lysine. The procedure for synthesizing the biocompatible MPEG derivative used was determined to be a low-cost, high yield method capable of making a product that can be stored in reasonable conditions. Synthesizing the protected graft copolymer proved to be a more complicated procedure, but through several trials and revisions of the procedure, a viable product was procured.

The stabilization of MPEG-gPLL coated nanoparticles showed that concentrations of MPEG-gPLL can vary between 0.0156 mg/ml and 0.004 mg/ml and a concentration of 0.99 mg/ml of GNPs to promote optimal binding between the PGC and the GNPs. Measurement of the hydrodynamic diameters and Zeta potentials of these coated nanoparticles showed that HMW poly-lysine (which resulted in higher mass PGC) provides better stabilization and neutralization of GNPs; LMW polymers showed a higher degree of aggregation of resultant GNPs, and showed an incomplete displacement of citrate unlike the HMW polylysine based PGC.

It is my recommendation that further research is undertaken to pursue the incorporation of the MPEG-thioacetate into a protected graft copolymer in order to determine whether the biocompatible derivative is a factor that contributes to the degree of modification and eventual stabilization of the complex. The MPEG-Imidazolide PGC should also be the focus of further testing; changing the synthetic procedure managed to promote better modification of PLL, so it could follow that modifying the nanoparticle coating procedure could result in a more desirable stabilization for one or both versions of nanoparticle.



After the completion of the above research, the nanoparticles should be stabilization tested in conditions mimicking those encountered in the body. This research was begun during my project with the MPEG-Imidazolide PGC, however results were not able to be processed before the conclusion of the project. Investigating this stability would give valuable information on how such a drug delivery system could be adapted to target specific tissues and physiological conditions to increase overall effectiveness of pharmaceutical treatment.

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