Synthesis and Characterization Gold Nanoparticles using polymeric micelles to Induce Block Copolymer Composition

Ikram Ullah Khan [1*]

Abstract

Background: Gold nanoparticles (AuNPs) have shown significant potential in biomedicine, particularly for applications in photothermal therapy and drug delivery. Conventional AuNP synthesis methods are often complex and involve multiple steps, driving interest in simpler, cost-effective alternatives. This study explores a novel approach using amine-containing polymeric micelles for the synthesis of AuNPs, aiming to streamline the process and enable precise control over nanoparticle properties. Methods: Seven block copolymers were synthesized via group transfer polymerization (GTP), including poly(ethylene glycol) methyl ether methacrylate (PEGMA), 2-(diethylamino)ethyl methacrylate (DEAEMA), and propargyl methacrylate (PMA). These copolymers varied in composition and architecture to investigate their self-assembly behavior and potential for AuNP formation. The structural properties were characterized using gel permeation chromatography (GPC) and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. Solution properties, such as pKa values, cloud points, and hydrodynamic diameters, were assessed using potentiometric titration, visual tests, and dynamic light scattering (DLS). Results: All synthesized polymers successfully formed micelles, with polymer 2 (PEGMA12-b-DEAEMA26-b-PEGMA12-b-PMA2) showing optimal properties for AuNP formation at pH 7 and 8. Ultraviolet-visible spectroscopy (UV-vis) and DLS analyses confirmed AuNP synthesis, with particle size varying depending on the pH. Conclusion: The study demonstrates the feasibility of using amine-containing polymeric micelles to synthesize AuNPs in a cost-effective manner. The results highlight the potential for optimizing polymer composition and architecture to tailor micellar behavior and AuNP properties, providing a promising approach for future biomedical applications.

Keywords: Gold nanoparticles (AuNPs), Polymeric micelles, Block copolymers, Group transfer polymerization (GTP), Biomedical applications.

Significance | This study showed cost-effective methods for synthesizing gold nanoparticles using polymeric micelles, enhancing their potential for biomedical applications.

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Introduction

Gold nanoparticles (AuNPs) have garnered significant attention in recent years due to their remarkable optical, electronic, and chemical properties, which render them highly suitable for various applications in biomedicine, including photothermal therapy and targeted drug delivery (Jain et al., 2006; Yang et al., 2009). Their unique features, such as localized surface plasmon resonance (LSPR), make them effective agents for imaging, diagnostics, and therapeutic interventions (Dreaden et al., 2012). The synthesis of AuNPs can be achieved through various methods, each with its own set of advantages and limitations. Among these, the use of

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polymeric micelles for the formation of AuNPs has emerged as a promising approach due to its simplicity and cost-effectiveness compared to conventional methods (Kwon et al., 2012).

Polymeric micelles are nanometer-sized aggregates formed by self-assembly of amphiphilic block copolymers in aqueous solutions. These micelles are characterized by a hydrophobic core and a hydrophilic shell, which allow them to encapsulate hydrophobic substances and stabilize them in aqueous environments (Discher & Eisenberg, 2002). The ability of polymeric micelles to encapsulate and reduce metal precursors makes them ideal candidates for the synthesis of metal nanoparticles, including AuNPs (Soppimath et al., 2001). The micellar system can be tailored by varying the composition and architecture of the block copolymers, which in turn affects their self-assembly behavior and the properties of the formed nanoparticles.

In this study, a series of block copolymers were synthesized using poly(ethylene glycol) methyl ether methacrylate (PEGMA), 2-(diethylamino)ethyl methacrylate (DEAEMA), and propargyl methacrylate (PMA) as monomers. PEGMA is a hydrophilic monomer based on polyethylene glycol (PEG), a biocompatible material approved by the FDA (Huang et al., 2010). DEAEMA, on the other hand, is a hydrophobic monomer with an amine group, which provides sites for gold incorporation and allows for pH-responsive behavior (Lee et al., 2011). PMA was included to enable further functionalization of the micelles due to its reactive triple bond (Tang et al., 2014).

The synthesis of these block copolymers was achieved via group transfer polymerization (GTP), a method known for its control over molecular weight and polydispersity (Kumaki et al., 2004). The resulting copolymers were characterized by gel permeation chromatography (GPC) and proton nuclear magnetic resonance (1H NMR) spectroscopy to confirm their composition and architecture (Zhu et al., 2009). The self-assembly of these copolymers into micelles was evaluated by dynamic light scattering (DLS) and potentiometric titration, which provided insights into their size, stability, and pKa values (Gao et al., 2015).

The ability of these micelles to form AuNPs was tested at different pH values, with particular emphasis on the polymer PEGMA12-b-DEAEMA26-b-PEGMA12-b-PMA2. The successful formation of AuNPs was confirmed through ultraviolet-visible (UV-vis) spectroscopy and DLS, which showed changes in nanoparticle size with varying pH levels (Pissuwan et al., 2006). This approach allows for the tailoring of micellar behavior and AuNP properties based on the copolymer composition and environmental conditions.

The integration of functionalized block copolymers into micellar systems offers a versatile and efficient method for the synthesis of AuNPs. By optimizing the polymer composition and architecture, it is possible to achieve desirable properties in the resulting nanoparticles, which may enhance their applicability in various biomedical fields. The aim of this study is to optimize the synthesis of block copolymers and their micellar properties to enhance the formation of gold nanoparticles.

2. Materials and Methods

2.1 Materials

DEAEMA (MM=185.26 g/mol), PEGMA (MM=300 g/mol) and PMA (MM=124.14 g/mol) were the monomers used to synthesise triblock and tetrablock copolymers. Calcium hydride (CaH2, ≥90%), aluminum oxide activated basic (Al2O3·KOH) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were used for monomer purifications. THF (HPLC grade, polymerisation solvent, ≥99.9%, Aldrich, UK), MTS and TBABB were used as solvent, initiator and catalyst, respectively. Solvents used include methanol, n-hexane, deuterated chloroform (CDCl3, 99.8%) and THF (GPC grade, mobile phase in chromatography). The first two were solvents for polymer recovering and the latter two were solvents for proton nuclear magnetic resonance spectroscopy (1H NMR) and gel permeation chromatography (GPC). Sodium hydroxide (NaOH, 97%) and hydrochloric acid solution (volumetric, 1M) were the basic and acidic solutions used for titration. Gold(III) chloride trihydrate (MM=393.83 g/mol) was the gold source used to form gold nanoparticles. Acetone was needed for washing during the whole experimental process.

Most materials were used as received except from the monomers and the THF polymerisation solvent for which purifications were needed prior to the polymerisation. Each monomer was passed through basic alumina twice to remove any inhibitor and other acidic impurities. Calcium hydride was then added to eliminate moisture in the monomers. As a free-radical inhibitor, DPPH was also needed for DEAEMA and PMA to prevent any free-radical polymerisation unwanted. Finally, a distillation was conducted under vacuum to ensure that no moisture existed in the DEAEMA and PMA. As a high molar mass monomer, distillation was not required for PEGMA. Because of PEGMA's inability to be distilled, no DPPH was added after the passage through basic alumina. To ensure the polymerisation solvent was moisture-free as well, the sodium-potassium alloy was used to reflux the THF for three days.

2.2 Polymer Synthesis

2.2.1 Group transfer polymerisation (GTP)

Triblock and tetrablock copolymers with target MM at 12350 g/mole but differ in composition were synthesised via sequential GTP. The polymerising procedure followed as descripted by T.K. Georgiou et al. Around 10 mg TBABB was added in a 250 ml round bottom flask which was immediately sealed with a rubber septum, followed by injection of 60 ml freshly distilled THF and 2 ml MTS via syringes. Then, the first monomer was added. The monomers used during the polymerisation were PEGMA, DEAEMA and

PMA. As listed in the Table 1, seven copolymers with different weight percentage of each monomer were synthesised.

2.2.2. Polymer Recovery

After the completion of the polymerisation, all the polymerisation solutions were poured either into n-hexane or methanol for precipitation in order to remove any low MM molecules and catalyst left. The solvents were then removed and the precipitated polymers were dried in a vacuum oven at room temperature for few days. Once the solvents evaporated completely, the polymers were collected to vials with the usage of liquid nitrogen.

2.3 Polymer Characterisation

2.3.1 Gel Permeation Chromatography (GPC)

To measure the MM, degree of polymerisation (DP) and dispersity index of the polymers and their precursors, GPC analysis was conducted. The analysis was performed by an Agilent, SECurity GPC system with a Polymer Standard Service (PSS) SDV analytical linear M column (SDA083005LIM). The mobile phase of the system was trimethylamine contained THF (5% v/v Et3N in THF), which was pumped by a ''1260 Iso'' isocratic pump at the rate of 1ml/min. Importantly, triethylamine was added in the solvent as a mild base to avoid absorption of DEAEMA in the column which cloud eventually block the pores and the column. Refractive index (RI) signal was measured by an Agilent 1260 RI detector to indicate the intensity of each polymer chain. Six poly(methyl methacrylate) (PMMA) standard samples (2000, 4000, 8000, 20000, 50000 and 100000 g/mol) were used for calibration before using the instrument. Samples were prepared by dissolving 7-10 mg polymers in 2 ml THF and the solution was then filtered through a 0.45 µm non-sterile PTFE syringe filter purchased from Phenomenex.

2.3.2 Proton Nuclear Magnetic Resonance (1H NMR)

The chemical structures and compositions of all polymers were identified and calculated from the NMR spectra which were obtained by using a 400 MHz Avance Bruker NMR spectrometer instrument. Around 7-10 mg polymers were dissolved in CDCl3 as samples.

2.3.3 Potentiometric titration

Since the monomer, DEAEMA, has an amine group in its structure, which can be protonated at low pH making the hydrophobic block become hydrophilic, it is crucial to characterise this property. The dissociation constant (pKa), defined as the pH at which 50% of amine groups are protonated, can be determined by titration. 1wt% polymer aqueous solutions with pH adjusted to 2 were prepared as samples. The solution was titrated from pH 2 to 12, as the polymer solutions are more soluble at relatively low pH. 0.25 M NaOH and 1 M HCl aqueous solutions were used for the pH adjustment and titration during which the pH was monitored by a portable HI98103 pH checker from Hanna instruments. The pH checker was calibrated by using standard samples at both pH 4 and 7 each time before use.

2.3.4 Dynamic Light Scattering (DLS)

DLS is a useful technique which can detect the size and dispersity of polymer molecules in solutions. Samples were prepared by dissolving polymer in deionised (DI) water at 1wt% in concentration. Prior to the measurement, filtration was needed using nylon 0.45 µm PTFE syringe filters and then the samples left to settle in order to remove any bubbles formed during filtration. The measurements were conducted by using a Zetasizer Nano ZSP (Malvern, UK) instrument at room temperature at a backscatter angle of 173°. Each sample was transferred to a glass cuvette (PCS 1115) and it was run for three times and the results are given as the mean value.

2.3.5 Visual test

DMAEMA has been widely studied because of its attractive thermoresponsive property. As an analogous of it, DEAEMA only differs in the type of alkyl substituent at the amino group in its structure. Therefore, it should be thermoresponsive as well according to some studies reported in the literature Because of the thermoresponsiveness, a clear polymer solution becomes cloudy when the temperature is increased. Visual tests, which can identify the cloud point of each polymer, was conducted by immersing glass vials that contained 1 wt% aqueous polymer solutions in a water bath. These solutions were prepared by dissolving around 20 mg of polymer in DI water in order to produce 2 g of aqueous solution. Visual observations were recorded at each degree when the temperature of the bath was increased from 40℃ to 70℃. The water bath was continuously stirred by a stirrer hotplate (IKA RCT basic stirrer hotplate) beneath and the temperature was controlled by an IKA ETS-D5 temperature controller. As the cloud point can be affected by the pH of the polymer solution, it is important to adjust all the solutions to same pH prior to the experiment if the initial pHs are different.

2.3.6 Gold nanoparticle Synthesis

To form gold nanoparticles, aqueous solutions of Polymer 2 were prepared at concentration of 1 wt%, split in four vials, 3 ml of solution in each vial, and the pH was adjusted to 5, 6, 7, and 8, individually. DLS tests have been run for all four pHs in order to find out the optimum pH at which more monodispersed micelles were formed; these monodispersed micelles were used for the following experiment. The DLS was conducted following the procedure descripted in section 3.3.4. Once the optimum pH was chosen, a stock solution of gold(III) chloride trihydrate was prepared at the concentration of 10 mg/ml using a disposable plastic spatula and a volumetric flask. The solution was split and the pH was adjusted corresponds to the polymer solution individually. Mixing the gold solution and the corresponding polymer solutions in a 48 well-plate at the ratio of gold to amine group is 0.7. The well-plate was covered by foil to avoid lighting and left on a swirler for at least 48 hours.

Table 1. Theoretical Composition of each polymer and their weight percentage of each monomer

No.	Theoretical Composition	Theoretical Weight Percentage (%)
Polymer 1	$PEGMA_{14}$-*b*-$DEAEMA_{19}$-*b*-$PEGMA_{14}$-*b*-PMA_2	35-30-35
Polymer 2	$PEGMA_{12}$-*b*-$DEAEMA_{26}$-*b*-$PEGMA_{12}$-*b*-PMA_2	30-40-30
Polymer 3	$PEGMA_{10}$-*b*-$DEAEMA_{32}$-*b*-$PEGMA_{10}$-*b*-PMA_2	25-50-25
Polymer 4	$DEAEMA_{19}$-*b*-$PEGMA_{28}$-*b*-PMA_2	30-70
Polymer 5	$DEAEMA_{26}$-*b*-$PEGMA_{24}$-*b*-PMA_2	40-60
Polymer 6	$DEAEMA_{32}$-*b*-$PEGMA_{20}$-*b*-PMA_2	50-50
Polymer 7	$DEAEMA_{10}$-*b*-($PEGMA_{28}$-*co*-PMA_2)-*b*-$DEAEMA_{10}$	15-70-15

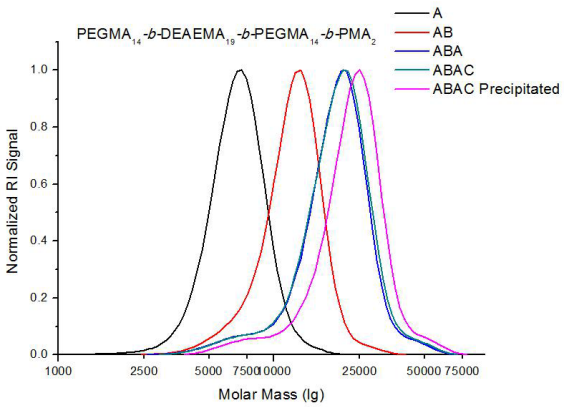

Figure 1. GPC chromatogram of Polymer 1: PEGMA14-b-DEAEMA19-b-PEGMA14 before and after precipitation (shown in turquoise and pink, respectively) and its precursors. The GPC traces of the first block, diblock and triblock copolymers are coloured in black, red, and blue, respectively.

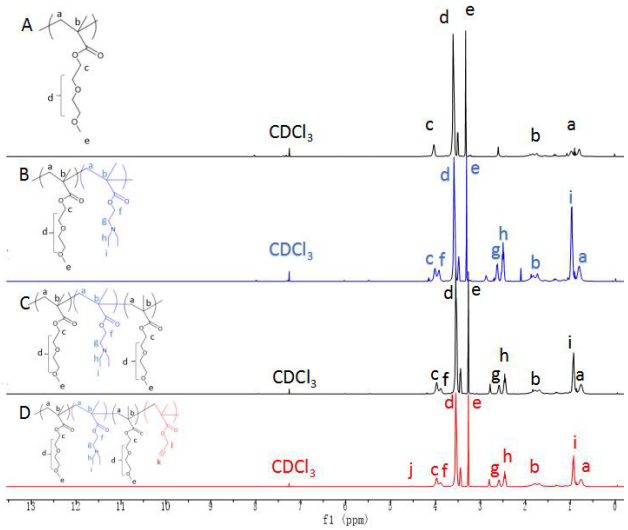

Figure 2. the 1H NMR spectrum of Polymer 1: PEGMA14-b-DEAEMA19-b-PEGMA14-b-PMA2 A) the first block PEGMA14 (black), B) the diblock copolymer PEGMA14-b-DEAEMA19 (blue), C) the triblock copolymer PEGMA14-b-DEAEMA19-b-PEGMA14 (black) and D) the tetrablock copolymer PEGMA14-b-DEAEMA19-b-PEGMA14-b-PMA2 (red).

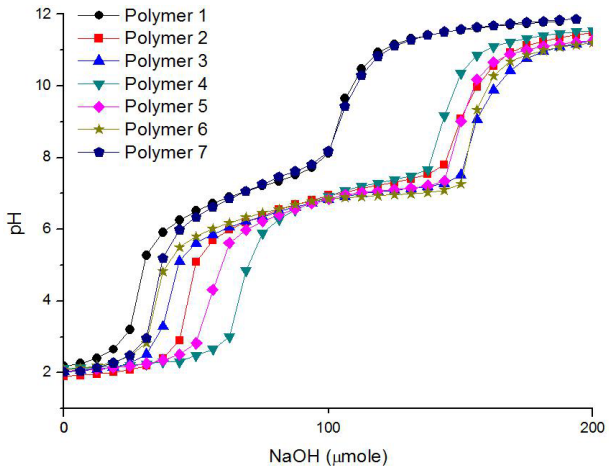

Figure 3 Titration curves of all polymers

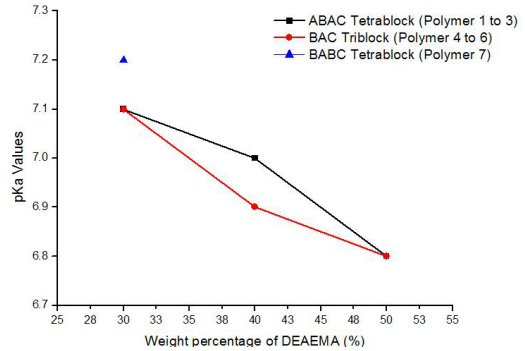

Figure 4. pKa values against weight percentage of DEAEMA.

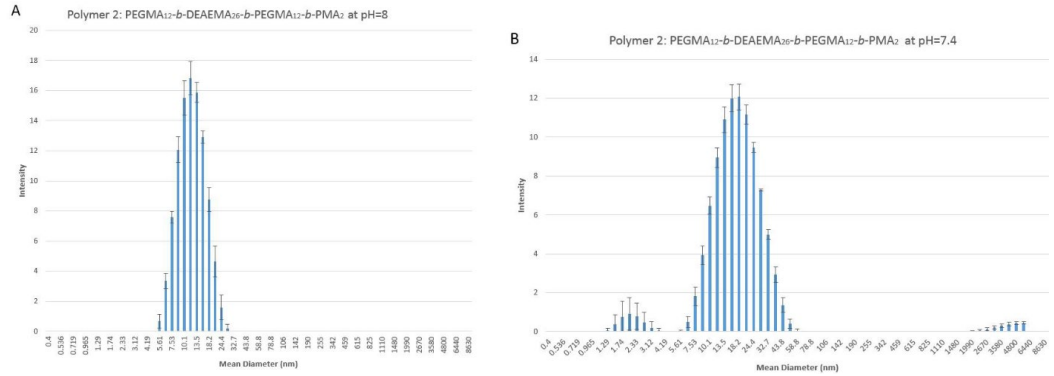

Figure 5. DLS histogram of PEGMA12-b-DEAEMA26-b-PEGMA12-b-PMA2 (Polymer 2) as intensity vs mean diameter (nm) at both pH=8 (A) and physiological pH (B).

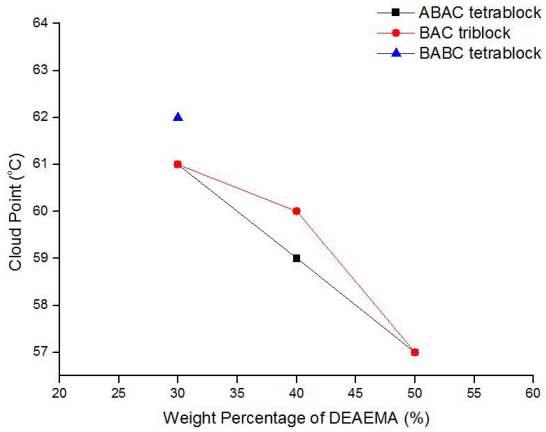

Figure 6. Cloud points against weight percentage of DEAEMA.

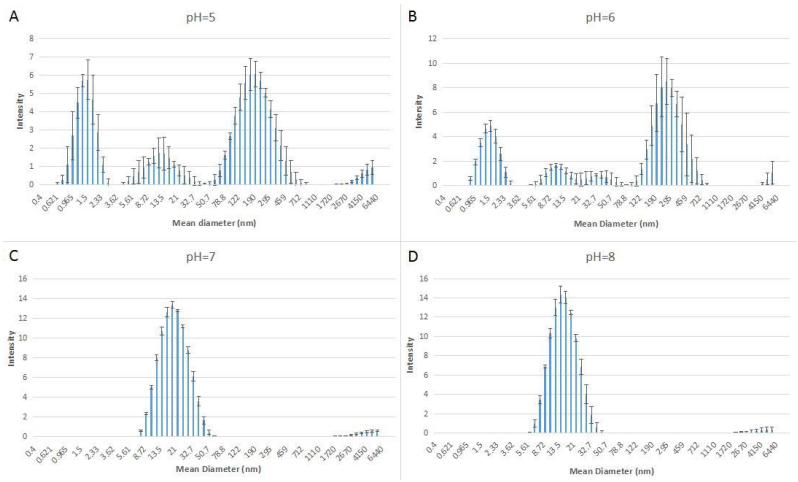

Figure 7. Histogram of DLS results of Polymer 2 at different pH, A: pH=5, B: pH=6, C: pH=7 and D: pH=8

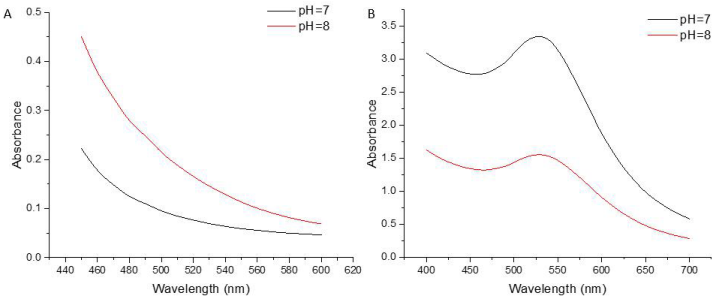

Figure 8 UV-vis results of (A) initial mixture of polymer solution and gold solution and (B) 4 hours after the mixture

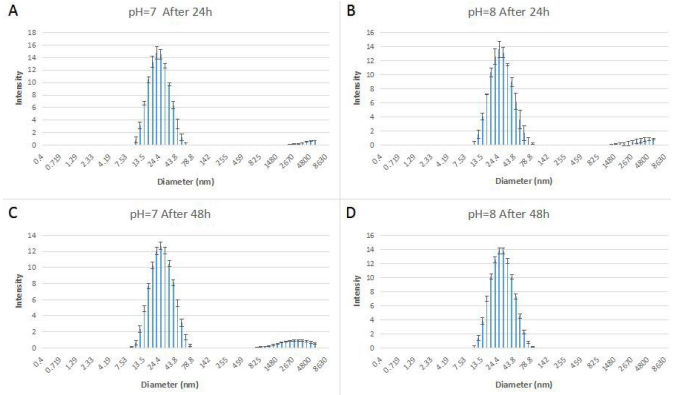

Figure .9 Histogram of DLS results A) 24 hours after addition of gold solution at pH 7; B) 24 hours after addition of gold solution at pH 8; C) 48 hours after addition of gold solution at pH 7 and D) 48 hours after addition of gold solution at pH 8.

2.4 Gold nanoparticles Characterisation *DLS*

DLS test was also conducted 24 h and 48 h after the mixture has been formed, but no filtration was required as it may destabilize the gold particles. A micropipette was used to extract 1 ml of solution from the well-plate and returned the solution back to the well-plate every time after finishing the measurement and the plate was left back on the swirler.

2.5.2 Ultraviolet-Visible Spectroscopy (UV-vis)

The surface plasmon resonance (SPR) of gold nanoparticles allow them to be analysed by light-based analytical techniques such as UV-vis in terms of size, concentration and aggregation level. An Aglient Technologies Cary 5000 UV-vis-NIR Spectrometer was used with a wavelength range of 450 nm to 700 nm at the data interval of 10 nm for UV-vis test. Measurements were taken immediately before and after the addition of the gold solution, and 24 h and 48 h after the addition as well. The sample was shaken for 30 seconds in the machine each time before the measurement for homogeneous mixture. Additionally, if the absorbance was too strong to be detected by the facility, 100 µl solution would be transferred to a 96-well plate for the measurement or diluted by 10 times.

4. Results and Discussion

Polymer Synthesis

GTP

The increase in temperature observed with each addition of monomer indicates successful polymerization reactions. For instance, in Polymer 1 (PEGMA14-b-DEAEMA19-b-PEGMA14-b-PMA2), the temperature rose from 25.2°C to 29.0°C within 4 minutes after PEGMA addition, from 26.4°C to 29.4°C in 8 minutes after DEAEMA addition, and from 28.2°C to 28.5°C in 2 minutes after PMA addition.

Polymer Recovery

Most polymers (except Polymer 6: DEAEMA32-b-PEGMA20-b-PMA2) were soluble in methanol but precipitated in n-hexane, which was used for their recovery. Approximately 10 g of each polymer was collected. Polymer 6, soluble in both solvents, was purified and recovered using benzoylated dialysis tubing, yielding 2.5 g from 3 g dialyzed.

Polymer Characterisation

Molar Mass

Figure 1 shows the GPC results for Polymer 1 and its precursors. The GPC traces indicate an increase in molecular mass as monomers were added, confirming successful polymer chain growth. The data suggest that while most polymers exhibited increased Mn and Mp with additional monomers, Polymers 4 and 5 showed decreased Mn due to the attachment of more PMA to shorter polymer chains. The dispersity (Đ) ranged from 1.06 to 1.29, indicating relatively homogeneous polymers. The experimental MMs were higher than theoretical due to moisture affecting the initiator, leading to longer polymer chains.

Polymer Composition

Experimental weight percentages and DPs of polymers, determined by 1H NMR. The 1H NMR spectrum for Polymer 1 (Figure 2) shows that the presence of PMA is confirmed by the peak around 4.89 ppm. The spectra for other polymers are provided in Appendix Fig. A2 to A7.

Effective pKa Values

Figure 3 shows titration curves indicating pKa values around 7.0 for DEAEMA units, consistent with previous studies. The experimental and theoretical pKa values, with a trend of decreasing pKa with increasing DEAEMA weight percentage. Polymer 7 (DEAEMA10-b-(PEGMA28-co-PMA2)-b-DEAEMA10) had a slightly higher pKa due to its hydrophobic block configuration.

Hydrodynamic Diameters

DLS measurements revealed that Polymer 2 (PEGMA12-b-DEAEMA26-b-PEGMA12-b-PMA2) formed monodispersed micelles at pH 8 (Fig. 5-A) but exhibited multiple peaks at physiological pH (Figure 4-B) due to changes in polymer amphiphilicity. Theoretical hydrodynamic diameters, assuming spherical micelles, were generally higher than experimental values, indicating coiled polymer chains. The data also suggest that polymers with higher hydrophobic content have narrower micelle size distribution.

Cloud Points

Cloud points for all polymers were around 60°C. A trend was observed where cloud points decreased with increasing DEAEMA weight percentage (Figure 6). Polymer 7 had the highest cloud point due to its low hydrophobic content and potential protonation at pH 8.

Gold Nanoparticle Synthesis and Characterisation

UV Absorbance

Figure 7 shows that Polymer 2 at pH 7 and 8 formed gold nanoparticles, evident from a peak between 525 nm and 550 nm in the UV-vis spectra (Figure 8 B). Absorbance increased over 48 hours, with pH 7 showing higher absorbance due to less accessible amine groups in smaller polymer micelles.

Hydrodynamic Diameters

Figure 9 show that gold nanoparticles formed by Polymer 2 had diameters of approximately 25 nm and 28 nm at pH 7 and 8, respectively. The diameter increased significantly after gold addition, indicating successful incorporation. Dispersity increased at pH 7 but decreased at pH 8, possibly due to limitations in incorporating gold ions at higher pH.

5. Conclusions

In this study, seven block copolymers based on PEGMA, DEAEMA, and PMA were successfully synthesized using GTP, and their

properties and micellar behavior were thoroughly investigated. The results indicate that triblock and tetrablock copolymers with well-controlled architecture and composition, and narrow molecular weight dispersity, can be effectively achieved through GTP. All the synthesized polymers were capable of forming micelles, with the size and dispersity of these micelles being influenced by the composition, architecture, and pH of the solution. Specifically, more monodispersed micelles were observed at slightly higher pH values, and an increase in hydrophobic content tended to narrow the size dispersity. The properties of the polymer solutions, such as pKa values and cloud points, were also affected by both composition and architecture, with both parameters decreasing as hydrophobic content increased. Notably, the copolymer with a hydrophilic block in the middle, which was assumed to form "flower-type" micelles, exhibited the highest pKa value and cloud point. Furthermore, gold nanoparticles were successfully formed using Polymer 2: PEGMA12-b-DEAEMA26-b-PEGMA12-b-PMA2 at both pH 7 and 8, with variations in diameter and UV absorbance, demonstrating the potential of using polymer micelles to tailor the size and properties of gold nanoparticles. Due to time constraints, not all polymers were tested for gold nanoparticle formation, which will be the next step in the project. Future work will also involve biocompatibility testing and functionalization of the nanoparticles before considering their application in biomedical fields.

Author Contribution

I.U.K. is the principal author, responsible for setting objectives, conducting data analysis, and finalizing the paper. All authors reviewed and approved the final manuscript.

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Competing financial interests

The authors have no conflict of interest.

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