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Synthesis of Poly(methyl methacrylate)-*Block*-Poly(*L*-histidine) and Its Use as a Hybrid Silver Nanoparticle Conjugate

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Poly[(methyl methacrylate)-*block*-poly(*L*-histidine)] (PMMA-*b*-PHIS) was synthesized by combining atom transfer radical polymerization and living ring-opening polymerization of α-amino acid-*N*-carboxyanhydride. The resulting hybrid block copolymer forms reverse micelles in the mixture solution of water and *N*,*N*-dimethylformamide (DMF) and self-assembles into PHIS/PMMA core/shell spheres with controllable size in the range of 80 to 250 nm depending on the micellization temperature. The self-assembly of PMMA-*b*-PHIS was carried out in H_2O/DMF (3/7) mixture in the presence of $AgNO_3$. Reduction of the resulting Ag ions encapsulated inside of the reverse micelles yielded an attractive Ag nanoparticle core/polymer shell conjugate system.

Keywords: Amphiphilic Block Copolymer, Histidine-*N*-Carboxyanhydride, ATRP, Ring-Opening Polymerization, Polypeptide, Self-Assembly.

1. INTRODUCTION

Amphiphilic block copolymers have received widespread attention over the past few decades owing to the well-known fact that they exhibit many different types of morphologies in the bulk phase and in solution when placed in a selective solvent.[1–2] They give rise to diverse morphologies due to their interesting supramolecular self-assembling behavior which imparts a number of unique properties to them.[3–5] Especially amphiphilic hybrid materials that contain both peptide and synthetic polymer elements could synergistically combine the properties of the individual blocks and overcome several of their limitations.[6]

Even though peptides are attractive since their synthesis is very straightforward, the preparation of long sequences that are essential to achieve hierarchical supramolecular interactions is both tedious and very expensive. The preparation of well-defined materials from synthetic polypeptides is now feasible due to the invention of Co and Ni initiators that allow the living ring-opening polymerization (ROP) of α-amino acid-N-carboxyanhydrides (NCAs).[6] The versatility of this ROP, in particular the ability to incorporate a large variety of amino acid monomers, both natural and synthetic, results in unprecedented flexibility of molecular design. Monomer species are formed by converting α-amino acids into NCAs, usually in a single step, followed by solution polymerization where chain length is controlled simply by monomer to initiator stoichiometry. Subsequent batch wise addition of different NCA monomers to the active chain-ends leads to multiblock architectures with well-defined block lengths and low polydispersity.

Recently developed controlled radical polymerization (CRP) techniques such as atom transfer radical polymerization (ATRP) and reversible addition–fragmentation chain transfer (RAFT) polymerization can be used to produce a number of end-functionalized polymers for chemoselective reaction with nonnatural polypeptides.[6–8] The combination of CRP with living ROP of NCAs nonnatural protein engineering could result in unprecedented control over polymer conjugation, resulting in precise synthetic polymer bioconjugates for a variety of applications. The integration of bio-segments, which exhibit often highly specific bio-functions, into the world of synthetic polymers offers enormous possibilities for materials science and biomedical science. Indeed, the resulting bioconjugates are tools for the direct translation of bio-inspired science, leading already to interesting materials but also contributing to the understanding of functions and systemic behavior of biomacromolecules, biomaterials, and

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more complex bio-systems. In order to demonstrate a versatility of synthetic polymer polypeptide hybrid materials, we have combined ATRP and living ROP of NCA using nickel initiator and utilized them for the stable encapsulation of silver nanoparticle (AgNP).

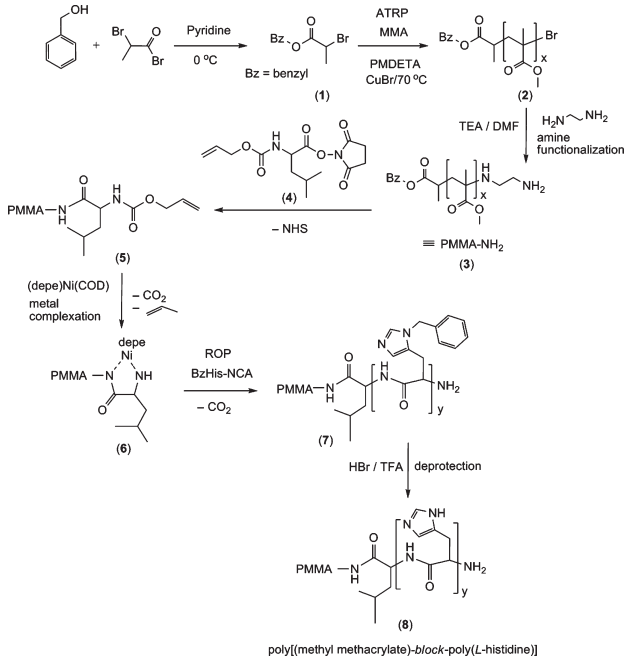
2. EXPERIMENTAL DETAILS

2.1. Materials and Measurements

Methyl methacrylate (MMA) and solvents purchased from Aldrich were purified according to the standard procedures and stored under nitrogen. *L*-histine monomer with blocking group, N_{α}-Boc-$N_{(im)}$-benzyl-*L*-histidine (Boc-His(Bzl)-OH), was obtained from Fluka. The cyclization of Boc-His(Bzl)-OH was carried out to get *N*-benzyl-*L*-histidine NCA · HCl.[9] All the reactions were performed under a purified nitrogen atmosphere using standard Schlenk techniques. ^{1}H NMR and ^{13}C NMR spectra of the compounds were recorded on a Varian Unity Plus-300 spectrometer in $CDCl_3$ or DMSO (d_6) as a solvent. Gel permeation chromatography (GPC) measurement was carried out using a Waters 515 in DMF with a refractive index detector calibrated by polystyrene standards. UV-vis analysis was performed by Shimadzu UV-1650PC. Micellization of block copolymers was performed using Biorad thermal cycler. The size of polymeric micelles was measured by dynamic light scattering using a Malvern's Zetasizer Nano. Transmission electron microscope (TEM) images were collected with a Philips EM400T TEM, at an accelerating voltage of 80 kV.

2.2. Synthesis of Poly(methyl methacrylate)-*Block*-Poly(*L*-histidine) (PMMA-*b*-PHIS)

The general procedures to prepare PMMA-*b*-PHIS were summarized in Scheme 1. The ATRP of MMA was performed at 70 °C by using CuBr/pentamethyldiethylenetriamine (PMDETA) system and benzyl 2-bromopropanoate (1)[10] as an initiator to give PMMA (2). The bromide groups in the chain end were converted to amine group by coupling reaction with ethylenediamine. The resulting amine terminated PMMA (3) was further reacted with alloc-*L*-leucine-*N*-hydroxy-succinimidyl ester (4) to give 5.[11, 12] The reaction of (depe)Ni(COD) (COD = 1,5-cyclooctadiene) complex, which has been *in situ* generated by reacting Ni(COD) and 1,2-bis(diethylphosphino)ethane in an equivalent ratio in THF (2 mL) for 15 min, with alloc-*L*-leucine terminated PMMA (5) yielded macro-initiator (6) bearing amido-amidate nickelacycle end groups.[12] In a typical ROP of NCA, (0.1 g, 0.013 mmol of macro-initiator (6) was added to *N*-benzyl-*L*-histidine NCA · HCl (0.34 g, 1.25 mmol) dissolved in 5 mL of THF, and then stirred



Scheme 1. Synthesis of poly(methyl methacrylate)-*block*-poly(*L*-histidine) by combining atom-transfer radical polymerization with living ring-opening polymerization of *L*-histidine-*N*-carboxyanhydride in the presence of (depe)Ni(COD) complex.

at room temperature for 3 days. The mixture was precipitated in methanol, filtered and vacuum dried at 50 °C. The resulting block copolymer (7) was deprotected by hydrolysis to give PMMA-*b*-PHIS (8).[13]

2.3. Fabrication of Polymeric Micelles and Ag-Polymer Conjugate

The stable micelle solution was prepared by thermal cycler in DMF/H_2O mixture solution. In a typical process, 1 mg of poly(MMA_{77}-*b*-HIS_{28}) was dissolved in 0.7 mL of DMF, and then 0.3 mL of deionized water was injected into the copolymer solution. The resulting mixture was heated to 110 °C, and then slowly cooled down to a desired temperature (1 °C/min) by using thermal cycler and kept for 5 h at this temperature.

For fabrication of AgNP-polymer conjugates, 1 μL of $AgNO_3$ solution (0.01 M) is mixed with 5 μL of poly(MMA_{77}-*b*-HIS_{28}) stock solution (20 mg/mL DMF), 35 μL of DMF and 15 μL of air-free water. The mixture was then heated to 110 °C for 2 h. It was then cool down to 50 °C at a rate of 1 °C/min. To remove excess $AgNO_3$, the 5 batch of this solution was diluted with 2 mL of water and centrifuged at 20,000 rpm for 30 min. This step was repeated two more times and a concentrated polymer-Ag ion conjugate solution was obtained. The same experiments were repeated by changing the amount of $AgNO_3$ solution to 5 μL. The Ag ions encapsulated inside of the micelles were further reduced to fine AgNPs by diffusing

the reducing agent ($NaBH_4$), resulting in the formation of PMMA-*b*-PHIS encapsulating AgNP composite cores.

3. RESULTS AND DISCUSSION

As a means of demonstrating a versatility of synthetic polymer polypeptide hybrid materials, PMMA-*b*-PHIS was synthesized by the combination of ATRP and living ROP of NCA using nickel initiator (Scheme 1). Figure 1 shows the ^{1}H-NMR spectrum of the resulting PMMA-*b*-PHIS. The disappearance of benzylic blocking groups in histidine moiety and appearance of characteristic peaks in histidine and MMA blocks clearly show that well-defined poly(MMA_{77}-*b*-HIS_{28}) is formed. The structure of the block copolymer could also be confirmed by ^{13}C-NMR spectrum (not shown). In addition molecular weight (MW) of PMMA increases from 8000 to 12000 after further polymerization of his-NCA (Fig. 2). The resulting polymer is characterized by low polydispersity index (1.25). Two more PMMA-*b*-PHISs, poly(MMA_{37}-*b*-HIS_{30}) (M_n = 8200; PDI = 1.27) and poly(MMA_{25}-*b*-HIS_{35}) (M_n = 7600; PDI = 1.24), were also synthesized by similar procedures using amido-amidate nickelacycle end groups functionalized PMMA with different MW (see Table I for detailed results).

One reason that has driven part of the current interest in synthetic polymer–polypeptide hybrids is related to the very specific self-assembly properties that can be programmed in the primary structure of the peptide segment. The self-assembly of common low MW amphiphiles or high MW block copolymers is generally driven by unspecific interactions. In the case of synthetic polymer–polypeptide hybrids, however, this process is mediated by the specific folding and organization properties of the peptide sequence, which allows access to complex, hierarchically organized, and potentially functional materials that are difficult to generate from purely synthetic building blocks.[15]

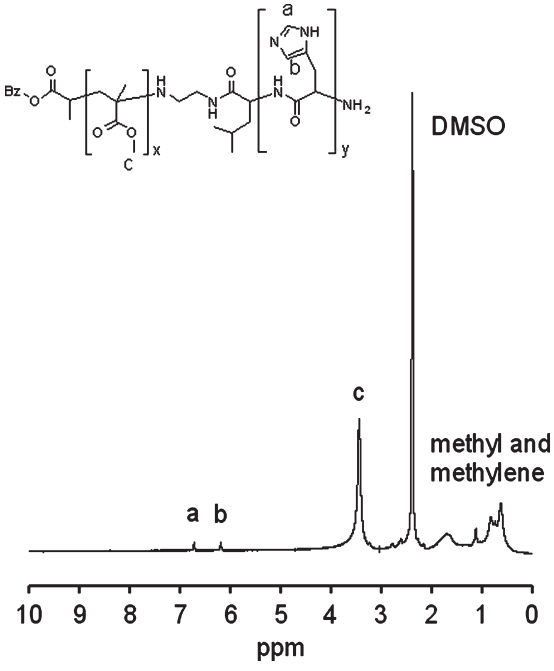


Fig. 1. ^{1}H-NMR spectrum of poly(MMA_{77}-*b*-HIS_{28}).

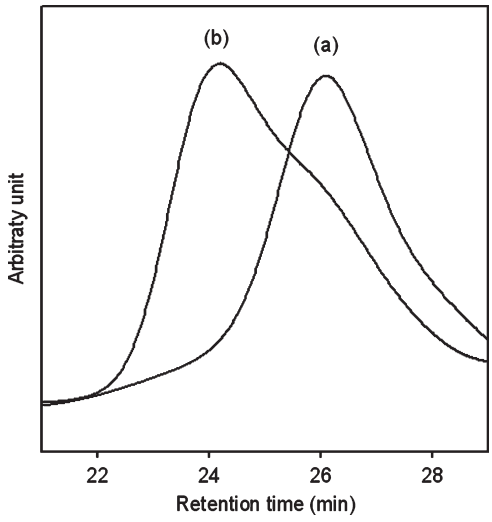


Fig. 2. GPC chromatograms of (a) PMMA (M_n = 8000) and (b) PMMA-*b*-PHIS [poly(MMA_{77}-*b*-HIS_{28})] (M_n = 12000).

We studied the self-assembly properties of poly(MMA_{77}-*b*-HIS_{28}) block copolymer. This hybrid block copolymer can be considered a giant amphiphile in which the PMMA chain represents the hydrophobic tail and the PHIS chain acts as the hydrophilic head group. The formation of micelles can be evidenced by means of TEM analysis (Fig. 3). The contrast in Figure 3(a) clearly indicates that stable core/shell spherical micellular structure is formed. The average diameter of the resulting micelles obtained at room temperature is around 250 nm. It is interesting to note that the size of micelles is tunable by changing the micellization temperature: i.e., the size of micelles decreases with the increase of the temperature, which can also be monitored by dynamics light scattering (DLS) analysis (Figs. 3(c, d)). As a result the average diameter decreases to 80 nm at 85 °C (Fig. 3(b)). The similar DLS analyses by changing the micellization temperature carried out with poly(MMA_{37}-*b*-HIS_{30}) and poly(MMA_{25}-*b*-HIS_{35}) yield similar results (Fig. 3(d)) and no conspicuous change of the size of the micelle has been observed according to the block size.

The core of the micelle consists of hydrophilic PHIS blocks containing imidazole groups in them. The imidazole moieties, which are the driving factor of polymer self-assembly, as well as, Ag-conjugation, make this a unique system. The high affinity of the imidazole groups for the Ag induces the self-organization of polymer molecules in a reverse fashion (Scheme 2). By means of these functional

Table I. Polymerization results of ROP of *N*-benzyl-*L*-histidine NCA · HCl (BzHIS-NCA) initiated by amine terminated PMMAs of with different molecular weights. Conditions: NCA/PMMA-NH_2/(depe)Ni(COD) = 100/1/1 (molar ratio), THF = 5 mL, temperature = 80 °C, and time = 3 days.

	Reactants					Poly(methyl methacrylate)-*block*-poly(*L*-histidine)			
	PMMA[a]					No. of repeating unit in block copolymer			
Sample code	M_n[b]	PDI[b]	Amount [g (mmol)]	BzHIS-NCA (g)	BzHIS-NCA/PMMA-NH_2[c]	MMA	HTD	M_n[b]	PDI[b]
Poly(MMA_{77}-*b*-HTD_{28})	8000	1.20	0.1 (0.013)	0.34	100	77	28	12000	1.25
Poly(MMA_{37}-*b*-HTD_{30})	4000	1.19	0.1 (0.025)	0.68	100	37	30	8200	1.27
Poly(MMA_{25}-*b*-HTD_{35})	2800	1.20	0.1 (0.036)	0.98	100	25	35	7600	1.24

[a]Amido-amidate nickelacycle end group functionalized PMMA to utilize as an initiator for ring-opening polymerization of BzHIS-NCA. [b]Number average molecular weight (M_n) and polydispersity (PDI) of polymers determined by gel permeation chromatography (GPC). [c]Molar feed ratio of *N*-benzyl-*L*-histidine NCA · HCl/amido-amidate nickelacycle end group functionalized PMMA macroinitiator.

groups, the Ag ions are expected to be easily coordinated and immobilized in the PHIS core matrix.

The formation of the polymer-Ag conjugate was monitored by using TEM (Fig. 4). In order to check the effect of Ag ion concentration on the formation of Ag-polymer conjugates, the amount of $AgNO_3$ (0.01 M) was changed from 1 to 5 μL. At low concentration the relative percentage to form the Ag-polymer conjugates is low, so that there are relatively large proportions of spherical micelles that contain no AgNPs in them. The white (polymer) and black (AgNP) contrast was achieved by oxidizing the organic portion with aqueous $(NH_4)_2MoO_4$ (2 wt%).

The relative proportions of free micelles containing no AgNPs decrease with the increase of $AgNO_3$ added. It is intersting to note from the TEM observation that (1) most of the spherical conjugates bear only one AgNPs in them and (2) the size of AgNPs (~38 nm) are independent of the added amount of $AgNO_3$. These results demonstrate that all nanoparticles formed inside of the micelle are aggregated to form one bigger nanoparticle and that

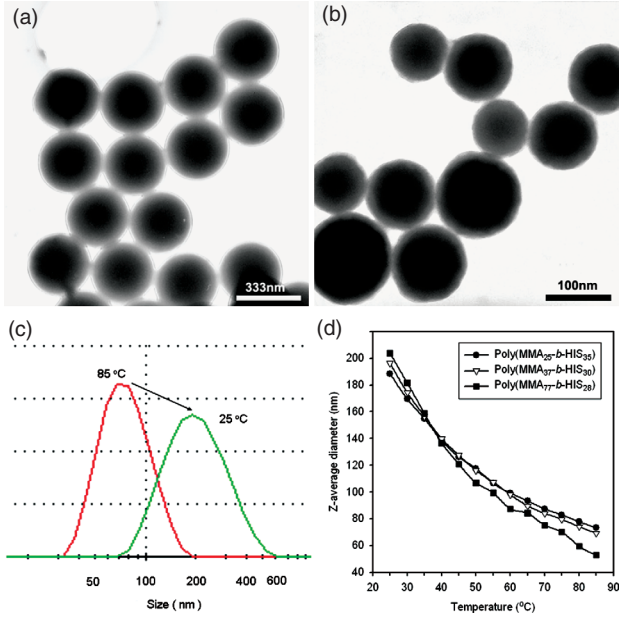
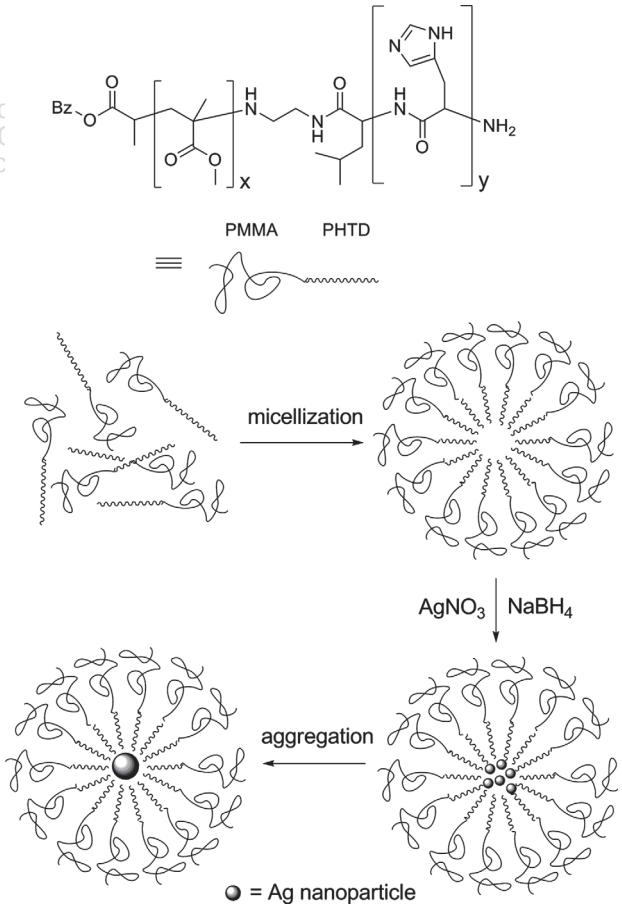


Fig. 3. Characterizations of PMMA-*b*-PHIS block copolymer micelle solution. TEM image of stable spherical micellular structure obtained at (a) 25 °C and (b) 85 °C; (c) representative particle size distribution curves (by DLS) measured at 85 °C and 25 °C; and (d) average micelle diameter versus temperature plots obtained by poly(MMA_{37}-*b*-HIS_{30}), poly(MMA_{25}-*b*-HIS_{35}), and poly(MMA_{77}-*b*-HIS_{28}).



Scheme 2. The schematic representation of the self-assembly of poly(methyl methacrylate)-*block*-poly(*L*-histidine) and the formation of polymer-AgNP conjugate by the encapsulating of aggregated AgNPs inside of the polymer micelle.

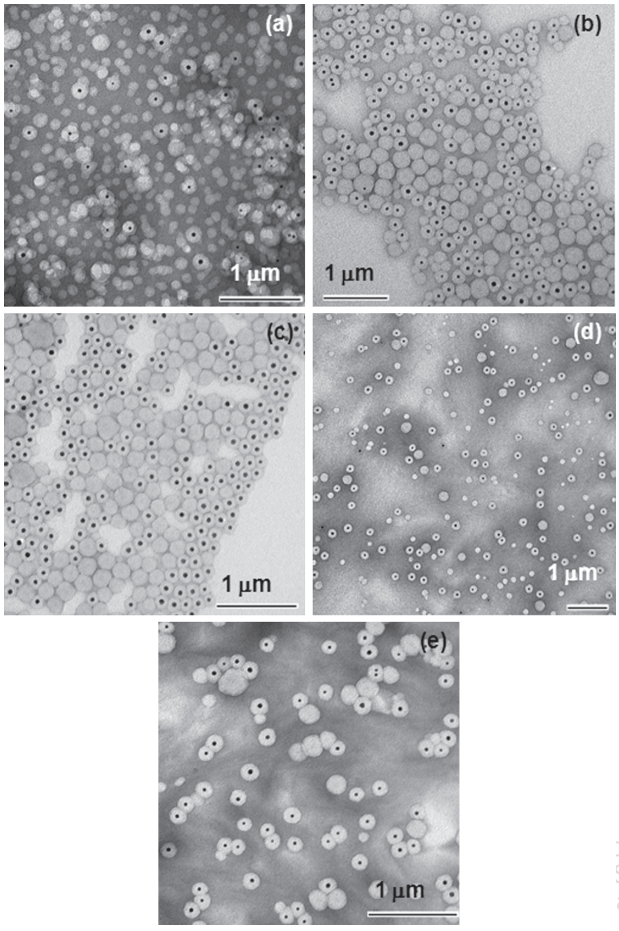


Fig. 4. TEM images of block copolymer-AgNP conjugate obtained by adding different amounts of $AgNO_3$ in the mixture consisting of 5 μL of poly(MMA_{77}-*b*-HIS_{28}) stock solution (20 mg/mL DMF), 35 μL of DMF and 15 μL of water: (a) $AgNO_3$ (0.01 M) = 1, (b) 2, (c) 3, (d) 4, and (e) 5 μL. The scale bar corresponds to 1 μm.

the loaded amount of Ag ion in one micelle is similar (Scheme 2).

The encapsulation using PMMA-*b*-PHIS is very useful in that it does not require any initial surface modification of AgNPs with common aliphatic ligands or thiolipids. The formation of polymer Ag conjugates could also be evidenced by monitoring the UV-vis absorbance of the resulting micelles which are corresponding to the surface plasmon resonance energy of this polymer-AgNP conjugate. As shown in Figure 5, the resulting conjugates exhibit similar absorbance bands centred at 389 nm with increasing intensities as the employed amount of $AgNO_3$ (0.01 M) is increased from 1 μL to 5 μL. All the samples exhibit quite broad absorbance band, most probably due to the corona effect of polymer shell. Moreover, the UV-vis absorption of these nanoparticle conjugates after storage over a prolonged time resembled to the newly synthesized material. From all these experimental observations, it can be concluded that the AgNPs are resistant to aggregation upon conjugation with this new class of PMMA-*b*-PHIS hybrid polymeric system.

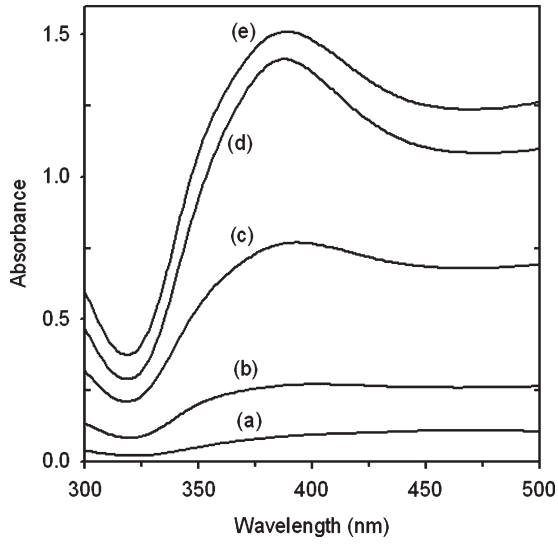


Fig. 5. UV-vis absorbance of block copolymer-AgNP conjugate obtained by adding different amounts of $AgNO_3$ (0.01 M) in the mixture consisting of 5 μL of poly(MMA_{77}-*b*-HIS_{28}) stock solution (20 mg/mL DMF), 35 μL of DMF and 15 μL of water: (a) $AgNO_3$ (0.01 M) = 1, (b) 2, (c) 3, (d) 4, and (e) 5 μL.

4. CONCLUSION

A series of synthetic polymer block polypeptide (PMMA-*b*-PHIS) copolymers were synthesized by the combination of atom transfer radical polymerization of MMA and living ring-opening polymerization of his-NCA in the presence of (depe)Ni(COD) complex. The resulting hybrid block copolymers are self-assembled to form spherical micelles with PHIS/PMMA core/shell structure due to their intrinsic amphiphilicity in DMF/water mixture. The size of micelles can be effectively controlled by varying micellization temperature. By utilizing the chemical affinity of the PHIS core to attract metal ions, we demonstrated PMMA-*b*-PHIS polymers are a good hybrid material to construct a facile and aggregation-free polymer AgNP conjugate. Only a few polymeric systems have been developed for the stabilization of nanoparticles and the successful application of this new class of block copolymer as surface stabilizers and a new preparative protocol should find wide applications.

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