

Poly(amino acid)-coated iron oxide nanoparticles as ultrasmall magnetic resonance probes

Hee-Man Yang,^a Hyun Jin Lee,^a Kwang-Suk Jang,^a Chan Woo Park,^a Hee Won Yang,^b
Won Do Heo^b and Jong-Duk Kim^{*a}

^a *Department of Chemical and Biomolecular Engineering (BK21 Graduate Program), Korea Advanced Institute of Science and Technology, Daejeon 305-701, Republic of Korea. E-mail: jdkim@kaist.ac.kr*

^b *Department of Biological Sciences and KI for the BioCentury, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Republic of Korea.*

^{*} *Corresponding author. Tel.: +82-42-350-3961. Fax: +82-42-350-3910. E-mail: jdkim@kaist.ac.kr.*

Materials

Iron (III) acetylacetonate, phenyl ether, benzyl ether, oleic acid (90%), oleylamine (> 70%), 1,2-hexadecanediol (90%), L-aspartic acid, mesitylene, sulfolane, ethylenediamine, aminoethanol, octadecylamine, sebacic acid, tetrahydrofuran, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, and N-hydroxy-succinimide were purchased from Sigma-Aldrich and were used as received. Phosphoric acid, dimethyl sulfoxide, and N,N-dimethylformamide were purchased from Junsei. Buffer solution standards at pH 4, pH 7, and pH 10 were purchased from DC Chemical Co., Ltd. Dimethyl sulfoxide-d₆ (DMSO-d₆) and deuterium oxide used in NMR experiments were Sigma-Aldrich products.

Synthesis of the PHEA-g-C₁₈-NH₂, and PHEA-g-C₁₈-C₈COOH polymer

The grafting mole % of C₁₈ and amine groups PHEA-g-C₁₈-NH₂ was controlled by feed mole ratio of octadecylamine and 2-aminoethanol. First, the precursor polymer, poly(succinimide) (PSI), was synthesized via acid-catalyzed polycondensation of L-aspartic acid using phosphoric acid as the catalyst, as has been previously reported.⁴⁰ Purified PSI (0.97 g, 10 mmol succinimide unit) was dissolved in water-free DMF (7 ml), followed by aminolysis with 8 mole % of octadecylamine at 70°C for 24 h. PHEA-g-C₁₈-NH₂, containing an amine group, was next prepared by aminolysis with 90 mole % of 2-amino-ethanol at room temperature for 6 h and remaining succinimide unit was treated with excess ethylenediamine to introduce amine groups in polymer side chain at 70°C for 12 h.^{38,41-43} The reaction mixture was precipitated twice from cold ether and

dried in vacuo at 50°C. The product was dissolved in DMSO, extensively dialyzed against DDI water, and freeze-dried. PHEA-g-C₁₈-NH₂ and sebacic acid were dissolved in water-free DMF, followed by addition of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxy-succinimide (NHS); reaction proceeded at room temperature for 24 h. The reaction mixture was precipitated twice from cold ether and dried in vacuo at 50°C. The product was dissolved in DMSO, extensively dialyzed against DDI water, and freeze-dried.

Synthesis of Fe₃O₄ nanoparticles

Fe₃O₄ magnetic nanoparticles of different diameters (4 nm, 6 nm, 8 nm, and 11 nm) were synthesized using the thermal decomposition method.¹ Briefly, to synthesize nanoparticles 6 nm in diameter, iron (III) acetylacetonate (2 mmol), 1,2-hexadecanediol (10 mmol), oleic acid (6 mmol), oleyl-amine (6 mmol), and benzyl ether (20 mL) were mixed in a three-neck round flask under an N₂ atmosphere. Next, the mixture was heated to 200°C for 2 h and further heated to 300°C for 1 h under reflux. After the mixture was cooled to room temperature, excess ethanol was used to wash the reactant. Nanoparticles were collected by centrifugation. When phenyl ether (20 mL) was used instead of benzyl ether, and the mixture was heated to 200°C for 30 min and further heated to 265°C for 30 min under reflux, nanoparticles 4 nm in diameter were obtained. Nanoparticles 8 nm and 11 nm in diameter were produced using a seed-mediated growth method using 6 nm nanoparticles .

ESI 1. Confirmation of synthesis of polymers

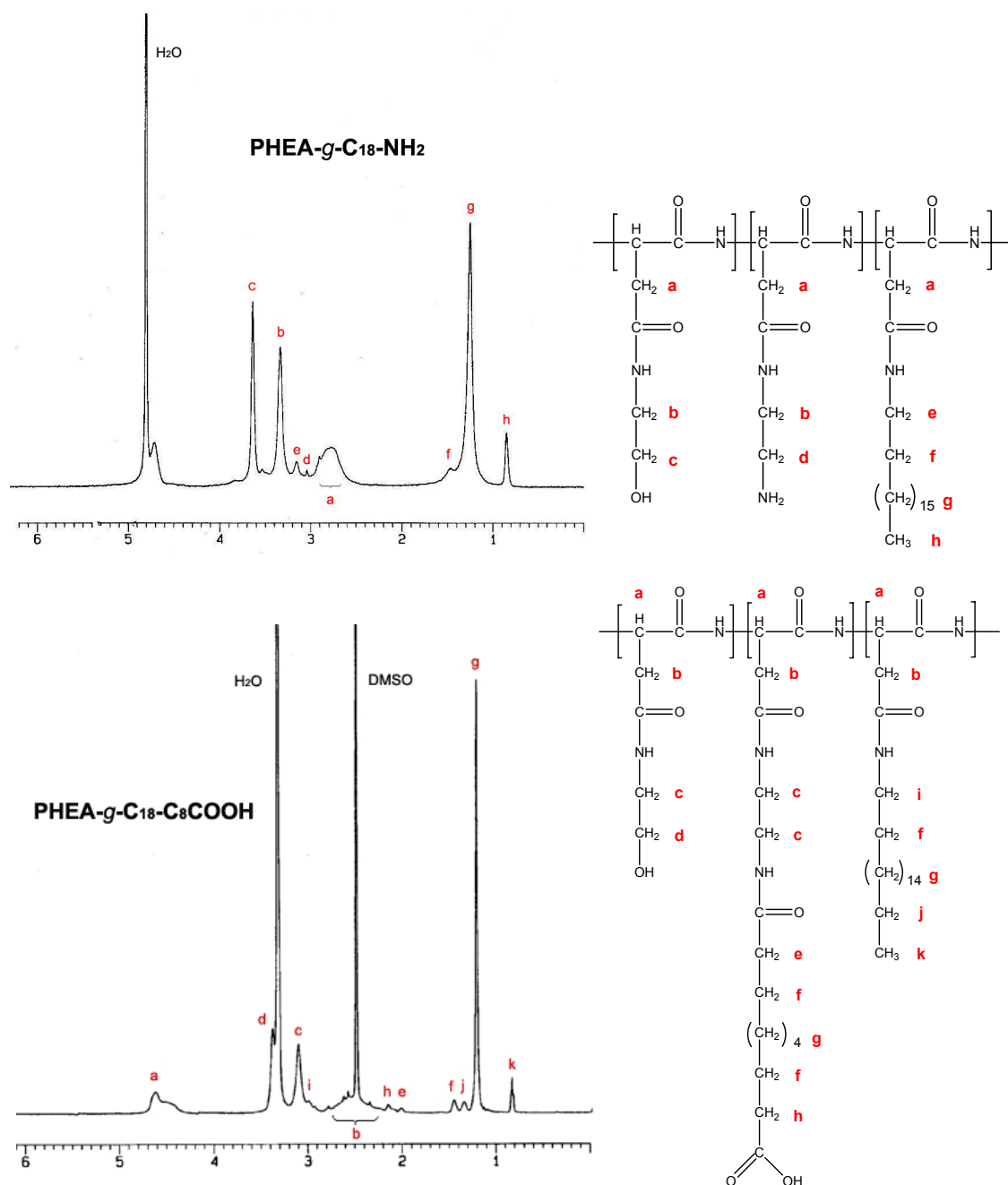


Fig. 1S. ¹H NMR spectra of PHEA-g-C₁₈-NH₂ in D₂O and PHEA-g-C₁₈-C₈COOH in DMSO-d₆

^1H NMR spectra were measured using a Bruker AMX500 spectrometer. Samples were dissolved in DMSO-d_6 or D_2O for NMR analysis. Figure 1S shows the ^1H NMR spectrum of PHEA- g - $\text{C}_{18}\text{-NH}_2$, revealing the characteristic proton peaks of the octadecyl chain and ethylenediamine grafted to a polymer backbone. The ^1H NMR spectrum of PHEA- g - $\text{C}_{18}\text{-C}_8\text{COOH}$ is also shown in Figure 1S. After conjugation of sebacic acid onto the PHEA graft copolymer using the EDC-NHS coupling method, the proton peak intensity of the alkyl chain increased, compared with the ^1H NMR spectrum of PHEA- g - $\text{C}_{18}\text{-NH}_2$.

ESI 2. Crystalline property of Fe_3O_4 nanoparticles

X-ray diffraction was performed to explore magnetic nanoparticles crystal structure using a Rigaku D/max-RB apparatus and a $\text{Cu K}\alpha$ source ($\lambda = 0.154 \text{ nm}$); powder samples were analyzed.

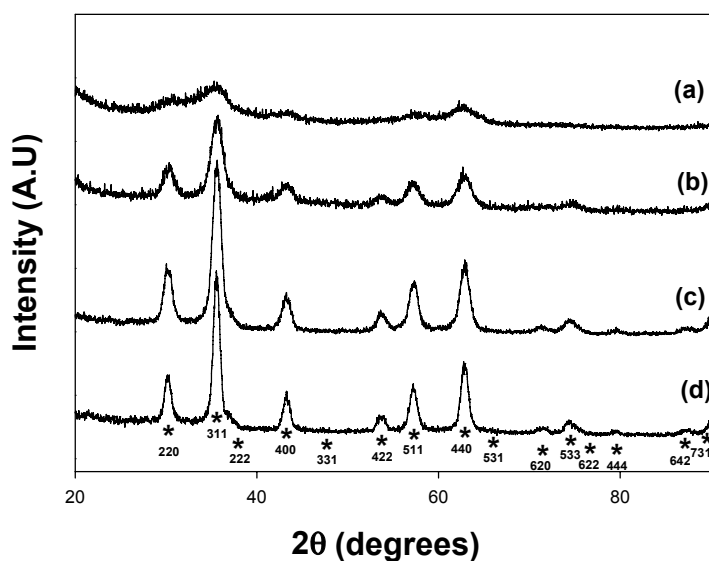


Fig. 2S. X-ray diffraction patterns of (a) 4 nm, (b) 6 nm, (c) 8 nm, (d) 11 nm as-synthesized Fe_3O_4 nanoparticles.

ESI 3. Iron oxide nanoparticles content in PAION determined by Thermogravimetric analysis (TGA).

The TGA was carried out on a setsys 16/18 (Setaram, France). We analyzed the iron oxide nanoparticles and PAIONs at a temperature range of 100-500°C and 100-1000°C, respectively. The heating rate is 10°C/min. At the end of TGA experiment, the detected amount of residuum reflects the iron oxide nanoparticles content.

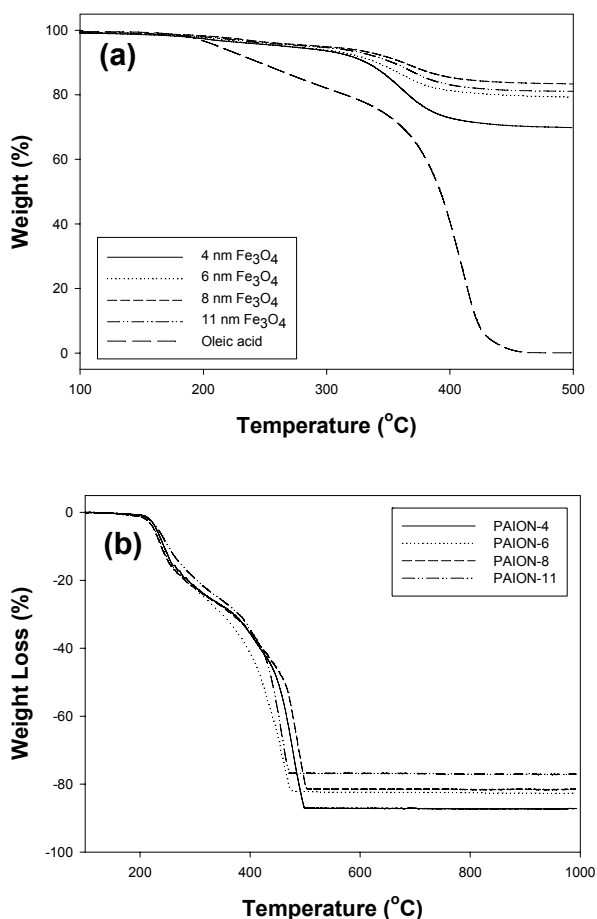


Fig. 3S. TGA analysis of (a) Fe_3O_4 nanoparticles and (b) PAIONs after coating Fe_3O_4 nanoparticles with PHEA-*g*- C_{18} - C_8COOH

ESI 4. Colloidal stability and Influence of pH on the zeta potential of PAION-11

PAION colloidal stability was determined using dynamic light scattering to measure PAION-11 size change over wide ranges of NaCl concentration and pH. A PAION suspension (100 μ L of a 20 mg/mL preparation) was added to 3 mL amounts of NaCl solutions of varying concentration, and of different pH values (0-5.0 M sodium chloride and pH 4-10), at room temperature.² After 5 days, PAION size was measured using a ZetaPlus instrument (He-Ne laser, Brookhaven Instruments Co., Holtsville, NY) operating in the “particle size” mode. Zeta potentials of PAION-11 in buffer solution standards at pH 4, pH 7, and pH 10 (DC Chemical Co., Ltd.) were also measured using the ZetaPlus equipment.

Table S1. Zeta potential of the PAION in various pH.

pH	4	7	10
Zeta potential (mv)	-4.20 \pm 2.26	-13.50 \pm 4.03	-21.71 \pm 4.92

ESI 5. The magnetic behaviour of Fe₃O₄ nanoparticles and PAIONs measured by a SQUID magnetometer.

A superconducting quantum interference device (SQUID) magnetometer, operating at fields intensities up to 5 T, was used to measure saturation magnetization of poly(amino acid)-coated iron oxide nanoparticles.

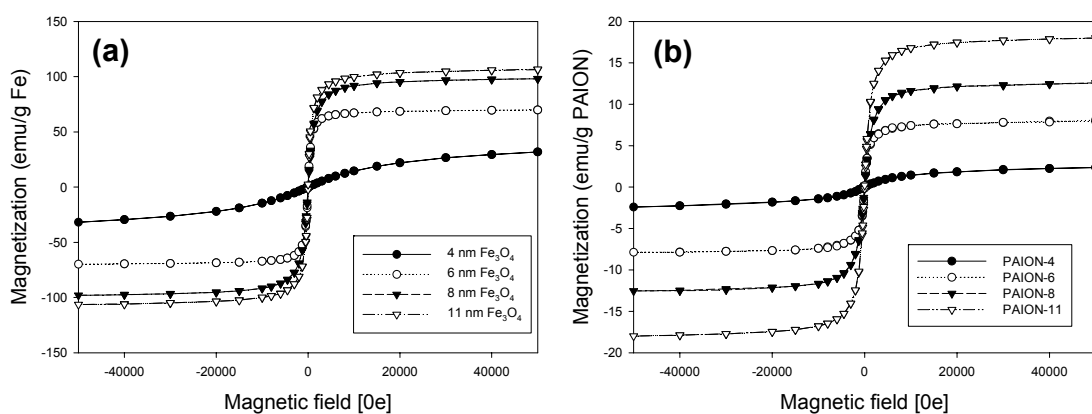


Fig. 4S. Magnetic hysteresis loops of (a) as-synthesized Fe₃O₄ nanoparticles and (b) Poly(amino acid)s coated iron oxide nanoparticles(PAION) at room temperature.

Reference

1. S. H. Sun, H. Zeng, D. B. Robinson, S. Raoux, P. M. Rice, S. X. Wang and G. X. Li, *J. Am. Chem. Soc.*, 2004, **126**, 273-279.
2. J. Yang, T.-I. Lee, J. Lee, E.-K. Lim, W. Hyun, C.-H. Lee, Y. J. Song, J.-S. Suh, H.-G. Yoon, Y.-M. Huh and S. Haam, *Chem. Mater.*, 2007, **19**, 3870-3876.