Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B
This journal is © The Royal Society of Chemistry 2013

Electronic Supplementary Information
for
Molecular Interactions between Charged Macromolecules: Colorimetric Detection and Quantification of Heparin with a Polydiacetylene Liposome

Yong-Suk Cho and Kyo Han Ahn*

Department of Chemistry and Center for Electro-Photo Behaviors in Advanced Molecular Systems, POSTECH, San 31 Hyoja-dong, Pohang 790-784 Republic of Korea

ahn@postech.ac.kr

CONTENT

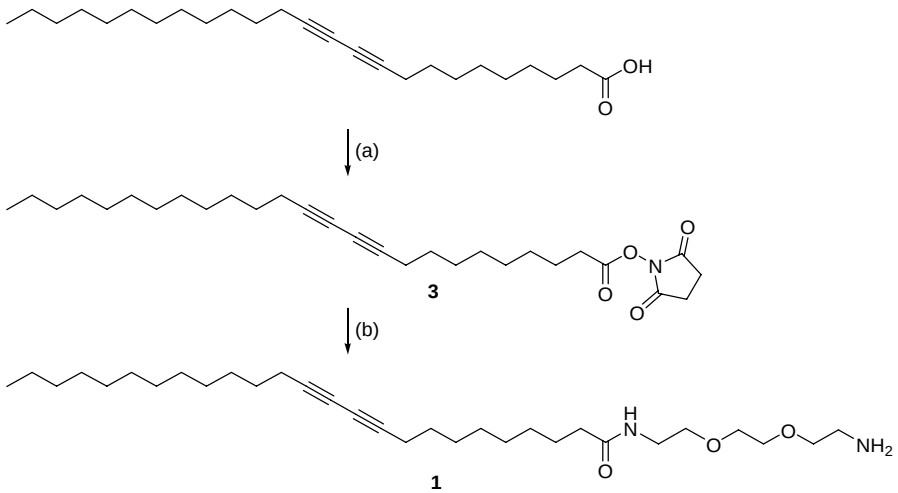
This journal is © The Royal Society of Chemistry 2013

General Information

All moisture or air sensitive experiments were performed under a positive pressure of argon in flame dried glassware equipped with a rubber septum inlet. Solvents and liquid reagents were transferred by an argon flushed syringe or cannula. Reaction mixtures were stirred with Teflon coated magnetic stirring bars. Commercial solvents and reagents were used without purification. Analytical thin layer chromatography was performed using Merk 60 F254 precoated silica gel plates. Subsequent to elution, ultraviolet illusion at 254 nm allowed for visualization of UV active materials. Column chromatography was carried out Merck silica gel 60 (230-400 mesh). Melting points were measured on a Thomas Hoover capillary melting point apparatus and were uncorrected. The nuclear magnetic resonance spectra were determined on an AM-300 Bruker [^{1}H NMR (300 MHz). ^{13}C NMR (75 MHz)] instrument unless otherwise noted. Mass spectral analysis was recorded on JEOL JMS-AX505WA and is reported in units of mass to charge (m/z). HRMS were performed by Korea Basic Science Center, Kyungpook National University. UV/Vis absorption spectra were taken on a Hewlett Packard Agilent 8453.

Synthetic Procedure

The synthesis of liposome components **1** and **3** was carried out according to the reported procedure.[1,2]



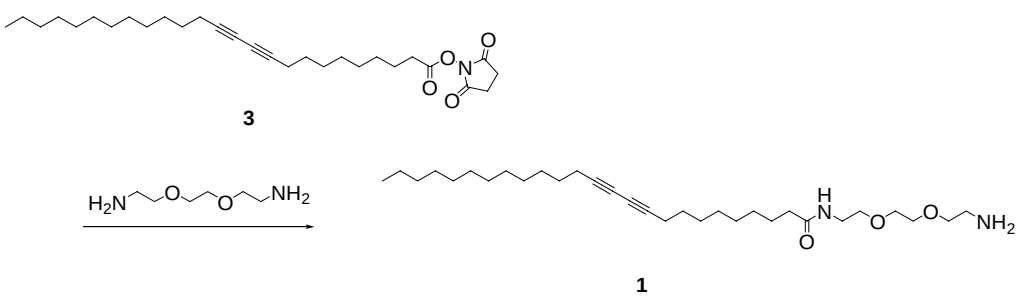
Scheme 1. (a) N-hydroxysuccinimide, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochlo ride, room temp., 2 h. (b) 2,2'-(ethylenedioxy)diethylamine, room temp., 3 h.

Reference

1. Kim, K. M.; Oh, D. J.; Ahn, K. H. *Chem. Asian. J.* **2011**, *6*, 122–127.

2. Kim, J.-M.; Ji, E.-K.; Woo, S. M.; Lee, H.; Ahn, D. J. *Adv. Mater.* **2003**, *15*, 1118–1121.

This journal is © The Royal Society of Chemistry 2013



N-(2-(2-(2-Aminoethoxy)ethoxy)ethyl)pentacosa-10,12-diynamide (1).

To a solution of **3** (2.06 g, 4.36 mmol) in dichloromethane (20 mL) was slowly added 2,2'-(ethylenedioxy)diethylamine (4.25 g, 28.66 mmol). The solution was stirred for 3 h at room temperature. The solution was concentrated in vacuum, and the residue was dissolved in ethyl acetate. The organic solution was washed with water, and the organic layer was separated and concentrated to give a residue. The residue was purified by silica gel column chromatography (EtOAc/Hex= 3/7) to afford N-(2-(2-(2-aminoethoxy)ethoxy)ethyl)pentacosa-10,12-diynamide **1** as a white solid (900 mg, 40 %). mp 60–61 °C: ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.57 (s, 1H), 3.49-3.57 (m, 6H), 3.37 (s, 4H), 2.84 (m, 2H), 2.17 (m, 6H), 1.19–1.55 (m, 32H), 0.80 (t, 3H).

Quantification of heparin in heparin spiked samples.

Two heparin-spiked samples (A and B) were prepared, which contain a therapeutic dose level of heparin (sample A, 9 μM; sample B, 33 μM). Each sample was divided into ten separate volumes, which were then treated with liposome **1** incrementally from 10 to 100 μM (for sample A) or 110 to 200 μM (for sample B). As the liposome concentration increased, the sample solutions show a progressive color change from red to blue

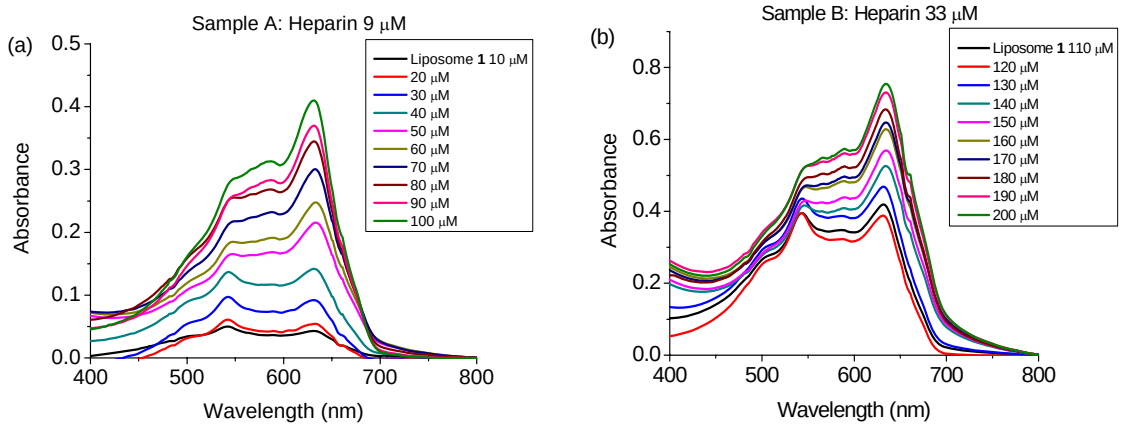


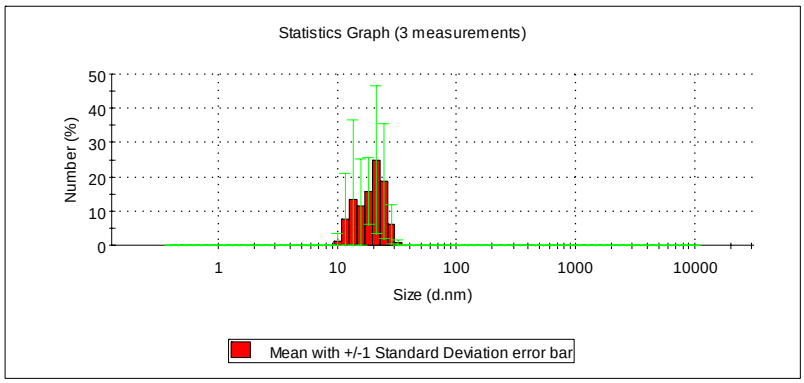
Figure S1. UV/Vis titrations of heparin-spiked samples with liposome **1**: (a) A: 9 μM heparin-spiked; (b) B: 33 μM heparin-spiked in a buffer solution (10 mM HEPES, pH 7.4).

This journal is © The Royal Society of Chemistry 2013

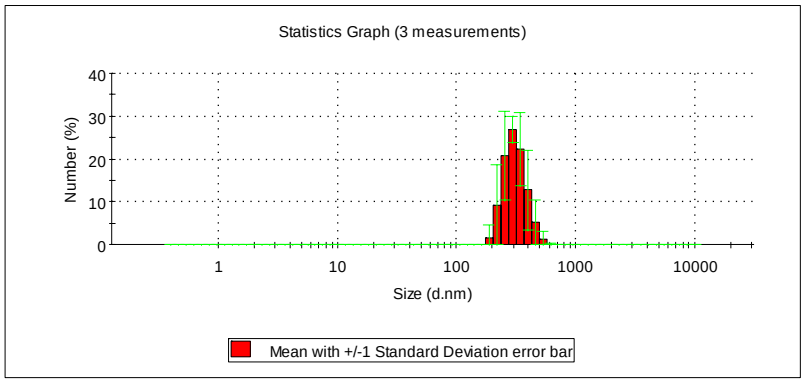
Dynamic Light Scattering (DLS) Analysis.

Each solution of liposomes **1** (10–100 μM) in a buffer solution (10 mM HEPES, pH 7.4) after treatment with the given amount of heparin (0–32 μM) was analyzed after 10 min.

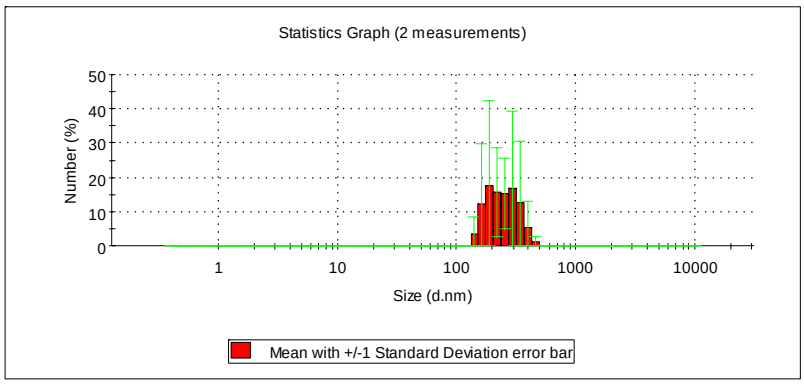
1. Liposome **1**



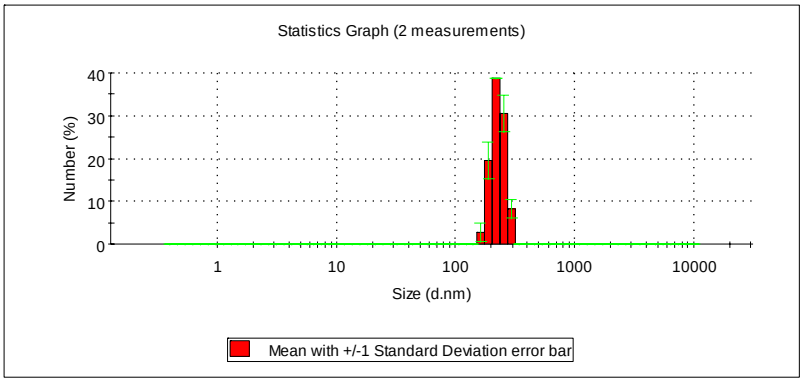
2. Liposome **1** treated with heparin (10 μM)



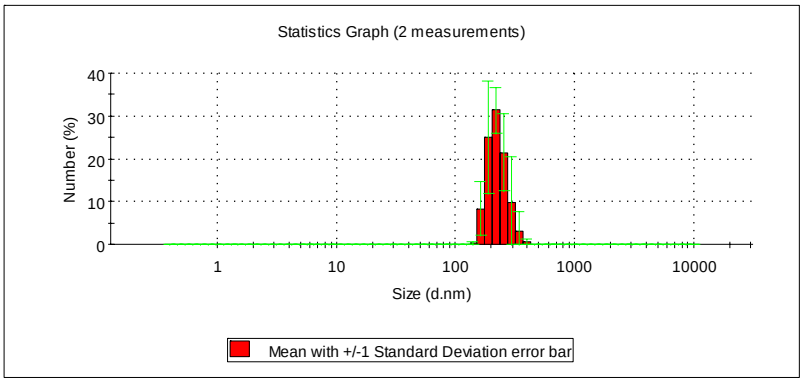
3. Liposome **1** treated with heparin (14 μM)



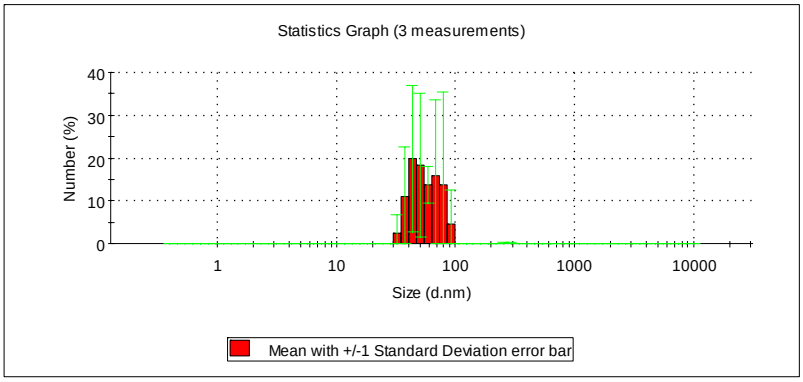
4. Liposome **1** treated with heparin (16 μM)



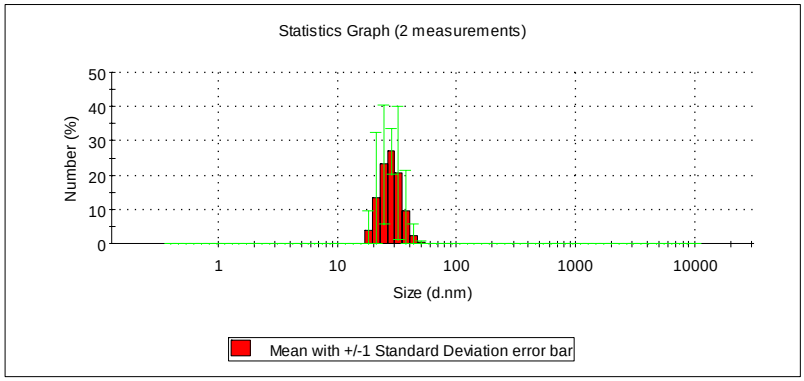
5. Liposome **1** treated with heparin (24 μM)



6. Liposome **1** treated with heparin (26 μM)



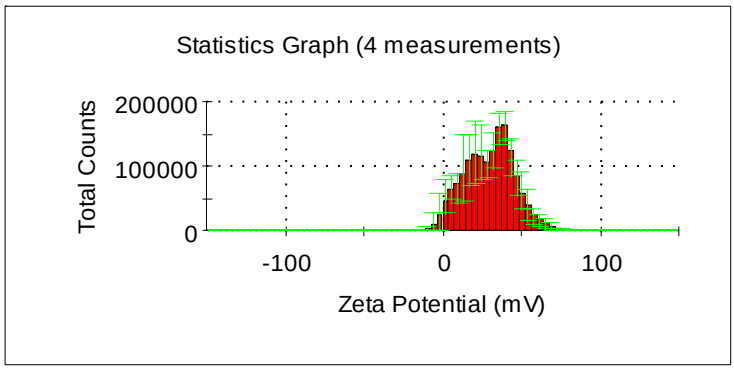
7. Liposome **1** treated with heparin (30 μM)



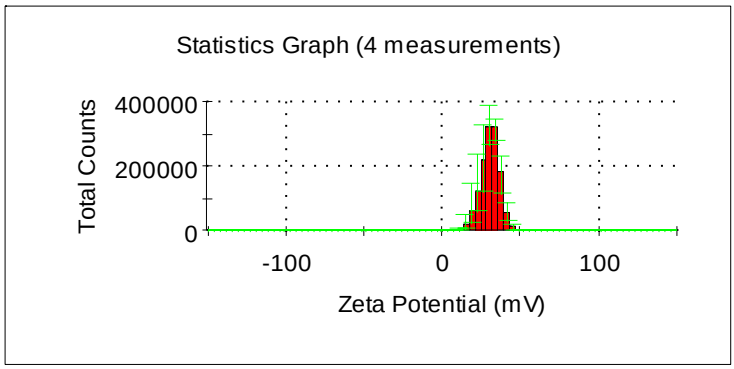
Zeta Potential Measurement Data.

Zeta potential measurement was carried out for each solution of liposomes **1** (100 μM) in a buffer solution (10 mM HEPES, pH 7.4) after treatment with the given amount of heparin (0–50 μM).

1. Liposome **1**

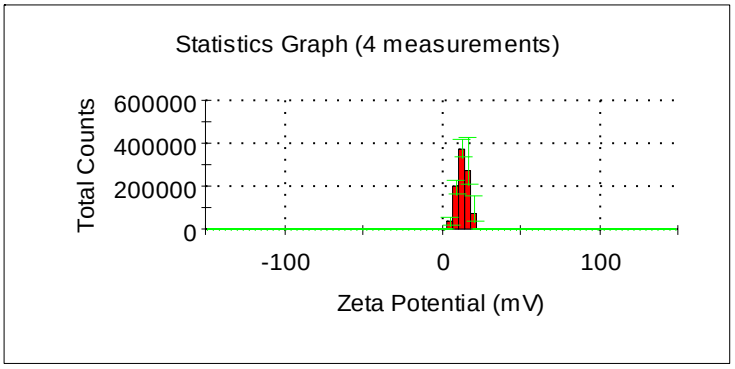


2. Liposome **1** treated with heparin (10 μM)

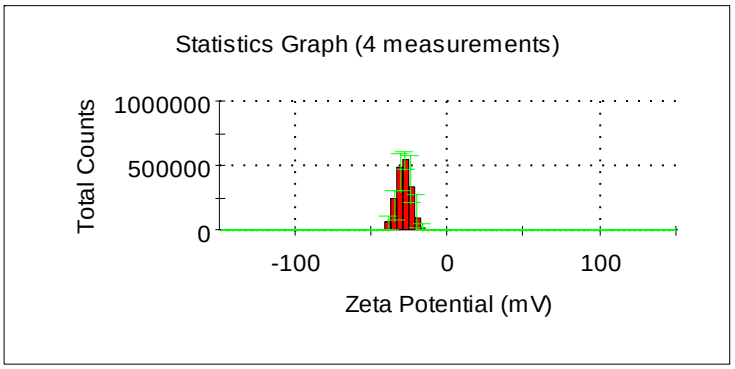


This journal is © The Royal Society of Chemistry 2013

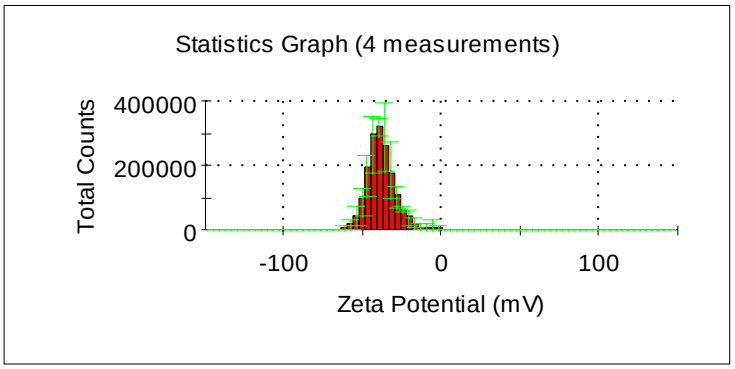
3. Liposome **1** treated with heparin (20 μM)



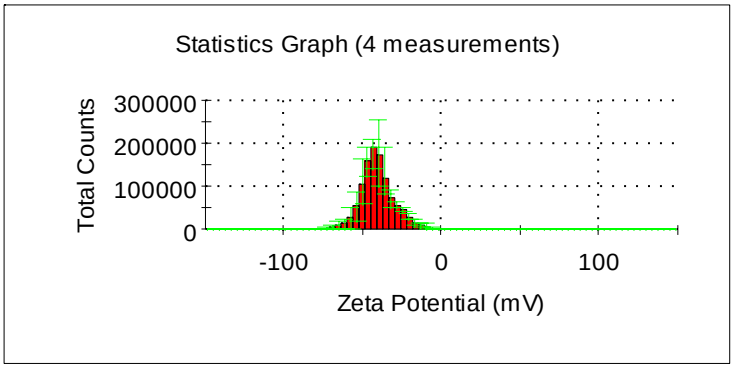
4. Liposome **1** treated with heparin (24 μM)



5. Liposome **1** treated with heparin (30 μM)



6. Liposome **1** treated with heparin (50 μM)



^{1}H NMR spectra of component **1**