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Janus-Compartmental Alginate Microbeads Having Polydiacetylene Liposomes and Magnetic Nanoparticles for Visual Lead(II) Detection

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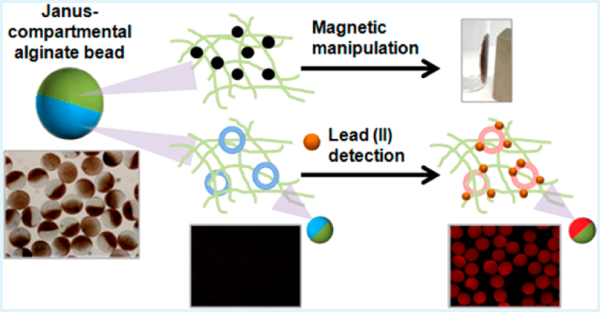
Supporting Information

ABSTRACT: Janus-compartmental alginate microbeads having two divided phases of sensory polydiacetylene (PDA) liposomes and magnetic nanoparticles were fabricated for facile sensory applications. The sensory liposomes are composed of PDA for label-free signal generation and 1,2-dipalmitoyl-*sn*-glycero-3-galloyl (DPGG) lipids whose galloyl headgroup has specific interactions with lead(II). The second phase having magnetic nanoparticles is designed for convenient handling of the microbeads, such as washing, solvent exchange, stirring, and detection, by applying magnetic field. Selective and convenient colorimetric detection of lead(II) and efficient removal of lead(II) by alginate matrix at the same time are demonstrated.



KEYWORDS: *Janus microbead, alginate, polydiacetylene liposome, magnetic nanoparticle, lead(II) detection*

INTRODUCTION

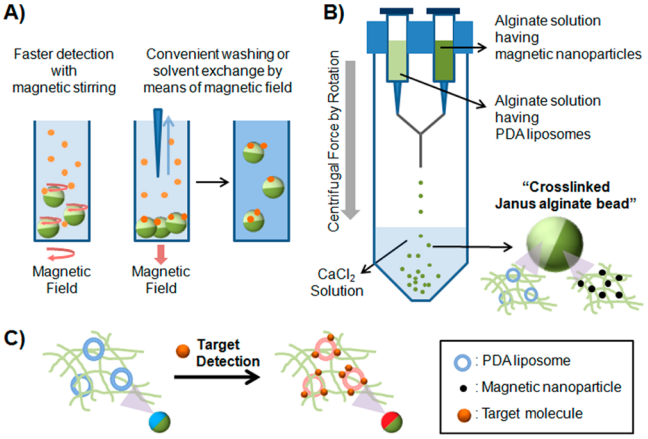
Polydiacetylenes (PDA) have been uniquely utilized in numerous colorimetric sensory systems due to their convenient spontaneous color change and fluorescent emission development under various external stimuli. When environmental stimuli, such as heat,[1,2] mechanical stress,[3] certain metal ions,[4−11] chemicals,[12−16] biomolecules,[17,18] and bacteria,[19−21] are applied or present, PDA changes its color from blue to red and emits red fluorescence, providing desirable self-signaling and dual-signaling properties. The signal generation mechanism of PDA is believed to stem from distortion of the conjugated yne-ene main chain of PDA by mechanical stress caused by external stimuli. PDA-based sensors are commonly prepared by self-assembly of rationally designed PDA monomers into liposome. During the self-assembly process, amphiphilic PDA monomers are closely and parallel packed as such subsequently UV irradiation efficiently photopolymerizes PDA monomers into blue-colored PDA. In order to give selectivity to PDA liposome, PDA monomers or lipids are rationally designed to have a particular receptor, which has specific interactions with an aimed target, or a functional group for attaching such a receptor at the surface of PDA liposomes.

Though the liposome based sensing platforms of PDA can be readily used in homogeneous solution detection schemes, there are a few limitations: (i) long-term storage of liposomes in solutions is troublesome because of intrinsic aggregation,[17] (ii) buffer exchange or washing steps (e.g., dialysis, centrifuge) for removal of unbound receptor units or nonspecific targets is challenging, and (iii) sensitivity is rather low due to homogeneous dilution of liposome and targets in solutions. Therefore, immobilization of PDA liposomes on a solid substrate or in a microparticles and fibers have been actively investigated. Some examples include microarrays,[5,6,14,22] thin films,[16,21,23] microfibers,[11,24,25] and microbeads.[26−28] In this regard, our group previously reported a multiphasic PDA microbead system, which has embedded sensory PDA liposomes in calcium-medicated cross-linked alginate hydrogel microbeads.[26] The developed PDA microbead system showed enhanced sensitivity, selective multitarget detection, and long-term storage stability.

In this contribution, we developed Janus-compartmental microbeads composed of two divided phases having sensory PDA liposomes and magnetic nanoparticles, respectively, for convenient sampling, handling, and detection. As illustrated in Scheme 1A, magnetic field can be conveniently used to stir the microbeads in the solution, which helps the sensory microbeads

Received: April 16, 2014
Accepted: June 4, 2014
Published: June 13, 2014

Scheme 1. (A) Janus Magnetic Microbeads Provide Fast and Convenient Handling and Detection of Target Molecules in Homogeneous Solutions. (B) Schematic Illustration of the Fabrication Procedure of the Janus-Compartmental Microbeads Having Sensory PDA Liposomes and Magnetic Nanoparticles. (C) Colorimetric Detection of Target Lead(II) Ions by the Janus Magnetic Microbeads



sample larger number of target molecules in a faster time frame thereby enhances sensitivity and reduces detection time. In addition, selective collection of the magnetic microbeads by means of magnetic field enables convenient washing off unbound and/or nonspecifically bound molecules and buffer/solvent exchange. The Janus microbeads were fabricated by cross-linking alginate solutions having sensory PDA liposomes and magnetic nanoparticles, respectively, with calcium ions as shown in Scheme 1B.[26,29,30]

Water and land contamination by lead is a serious problem, because lead is a poisonous heavy metal, which accumulate in animal and human bodies and damages nervous system, kidney, bone, and/or other tissues.[31,32] Various methods for lead(II) detection have been developed based on gold nanoparticles,[33−35] photonic crystals,[36] fluorophores,[37] and polymers.[8,9,38] In parallel to the development of detection schemes, elimination of lead(II) and other heavy metals has been an active research and development focus. Absorbents such as alginate,[39−44] polyphenolic compounds,[39,45] and chitosan[39,46] have been utilized for that purpose. Especially, alginate has been widely used as a low cost and nontoxic absorbent because alginate has abundant carboxylic groups, which have inherent high affinity to heavy metal ions.[39−44]

Unique system integration of sensory PDA and efficient absorbents alginate into the magnetic Janus microbeads can realize sensitive detect and convenient removal of lead(II) ions simultaneously.[42,47] If the sensory PDAs embedded in the alginate absorbent can provide quantitative detection of lead(II) ions, the sensory signal would be a nice self-indicator visualizing the degree of lead(II) absorption. For selective lead(II) detection, we designed a novel PDA liposomes (PDA-DPGG liposome) composed of PDA monomers (10,12-pentacosadiynoic acid) and 1,2-dipalmitoyl-*sn*-glycero-3-galloyl (DPGG, a lipid). The galloyl groups of DPGG are known to bind with lead(II) ions with high affinity, forming phenolic metal complexes.[33−35,48] As designed, our PDA-DPGG liposome in the Janus microbeads showed sharp color change and red fluorescence upon exposure to lead(II) ions, demonstrating convenient lead(II) detection and removal.

MATERIALS AND METHODS

Materials. Chemicals such as a PDA monomer, 10,12-pentacosadiynoic acid (PCDA), magnetic nanoparticles (Fe_3O_4, 50−100 nm), lead(II) ion (chloride salt), other heavy metal ions (chloride salt), sodium alginate, calcium chloride, solvents, and buffers were purchased from Sigma-Aldrich Chemicals. A lipid for lead(II) detection, 1,2-dipalmitoyl-*sn*-glycero-3-galloyl (DPGG) was ordered from Avanti Polar Lipids.

Assembly of PDA-DPGG Liposome. PDA liposomes having DPGG lipids (PDA-DPGG liposome) were self-assembled by the following injection method. PCDA and DPGG were dissolved in 100 μL tetrahydrofuran and the mixture solution was injected into 10 mL of 5 mM HEPES buffer at pH 7.4. The total concentration of PCDA and DPGG (4:1 molar ratio) was 1 mM. The solution was sonic treated by means of 120 W probe sonicator for 10 min and filtrated through a 0.8 μm cellulose acetate syringe filter. The liposome solution was stored at 5 °C overnight before use.

Surface Treatment of Magnetic Nanoparticles. Before mixing with alginate solution, Fe_3O_4 magnetic nanoparticles were passivated with citrate ions to prevent interactions with carboxylate of alginate.[49,50] 0.2 g Fe_3O_4 magnetic nanoparticles in 10 mL DI water containing 0.2 g citric acid were heated to 95 °C for 2 h and subsequently precipitated in acetone at 25 °C.

Fabrication of Janus Microbeads Having PDA Liposomes and Magnetic Nanoparticles. This procedure was based on our previously developed calcium-mediated cross-linking of alginate hydrogel.[26] As shown in Scheme 1B, our homemade microbead fabrication device consists of two syringes having 100 μL alginate solutions having embedded PDA liposomes and magnetic nanoparticles, respectively. The alginate solution for PDA phase was made by mixing of 4 wt % alginate solution and 1 mM PDA-DPGG liposome solution (1:2 volume ratio) while the alginate solution for magnetic phase was a mixture of 4 wt % alginate solution and the surface-modified magnetic nanoparticle solution (1:2 volume ratio). The two syringe needles (25 Gauge) were combined parallel to each other in such a way that when the alginate solutions in the syringes are injected dropwise into $CaCl_2$ solution (2.5 wt %) by 100*g* centrifugal force for 5 min Janus particles can be formed. The fabricated Janus beads were further hardened in the $CaCl_2$ solution for additional 20 min and washed three times with DI water and then stored at 5 °C before use.

Lead(II) and Heavy Metal Ions Detection Using PDA-DPGG Liposomes. For confirming the colorimetric response of PDA-DPGG liposome to lead(II) and heavy metal ions in solution, 0.66 mM PDA-DPGG liposome solution was polymerized by 254 nm UV irradiation (1 mW/cm^2) for 5 min and a 0.26 mM heavy metal ion solution (Ca^{2+}, Cu^{2+}, Hg^{2+}, Fe^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, or Pb^{2+}) was introduced into the PDA liposome solution. After 1 h incubation, optical microscope images were obtained and UV−vis adsorption spectra were taken from PerkinElmer Lambda 45 UV−vis spectrometer. Fluorescence spectra were also recorded on PTI Quantamaster spectrofluorometer.

Lead(II) Detection Using Janus Microbeads. For detection tests, 8 mg of wet Janus microbeads (approximately 100 microbeads) were incubated with various concentrations of lead(II) solutions for 1 h with or without magnetic stirring (1000 rpm). Optical and fluorescence microscopic images were obtained on Olympus BX 71 microscope or Nikon eclipse Ti microscope.

Lead(II) Removal Using Janus Microbeads. To confirm the lead(II) removal by alginate matrix of Janus microbeads, 8 mg of the Janus microbeads were incubated with 1 mM lead(II) solution with 1000 rpm stirring. The supernatant solutions were collected at various time intervals, and the lead(II) concentrations of the collected supernatnat were measured by Shimadzu ICPS-7500 ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectrometer). The exact number of microbeads used was counted after the removal experiment and the adsorbed lead(II) ions (nmol) per hundred beads were calculated.

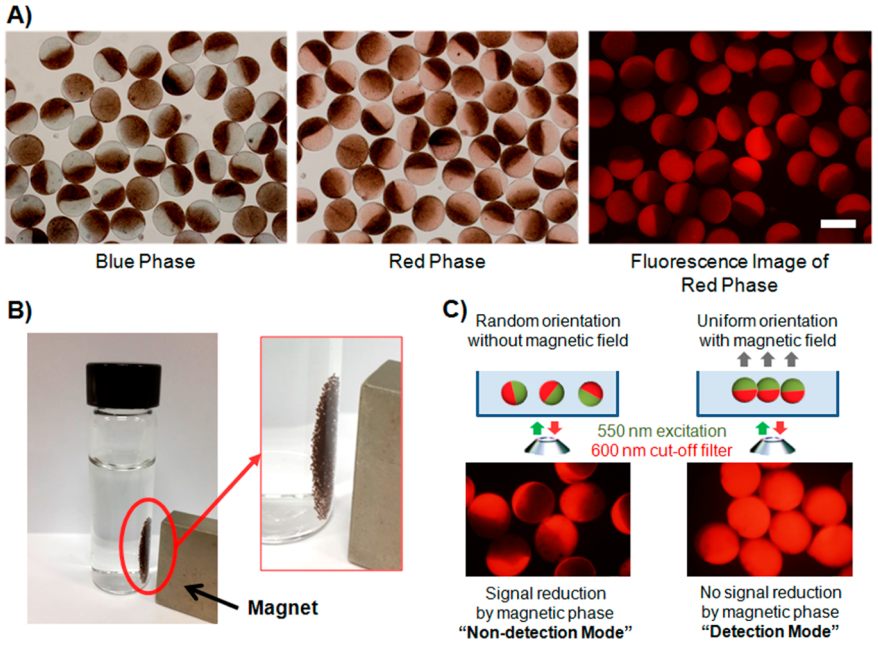
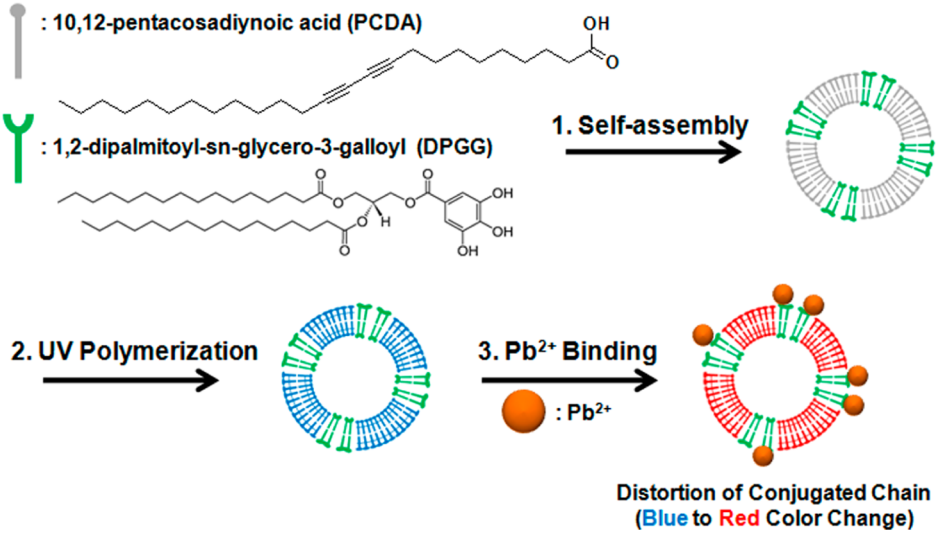


Figure 1. (A) Optical and fluorescent microscope images of Janus microbeads having either blue or red phase PDA liposomes (scale bar: 500 μm). (B) Manipulation of Janus microbeads in an aqueous solution by magnetic field. (C) Uniform orientation of Janus microbeads by magnetic field in a detection mode.

Scheme 2. Co-assembly of PCDA with DPGG to Form PDA-DPGG Liposomes and Schematic Illustration of the Colorimetric Lead(II) Detection



RESULTS AND DISCUSSION

Sensory Behaviors of Janus Microbeads. The fabricated Janus microbeads showed two distinct compartments of PDA liposomes and magnetic nanoparticles (Figure 1A). The size of the microbeads was about 500 μm and uniform. In the alginate hydrogel matrix of the Janus microbeads, the PDA liposomes produced blue color by photopolymerization and maintained their colorimetric property, showing the blue-to-red transition upon heating (70 °C, 5 min). The red phase PDA liposomes also generated red fluorescence. In addition, the PDA liposomes in the microbeads retained the blue color over 30 days of storage in 5 °C, implying enhanced stability of PDA liposome in the microbead environment due to the suppressed aggregation among PDA liposomes.

The Janus microbeads showed actuating responses to applied magnetic field due to the embedded magnetic nanoparticles as designed. The microbeads could be conveniently collected by applying static magnetic field (Figure 1B) or efficiently stirred on a magnetic stirrer (Supporting Information video). In many biosensor or assay experiments, magnetic actuations are routinely used because the effective manipulation by using magnetic field enables easy washing off unbound molecules, efficient exchange of buffer or reactants, and faster detection by stirring.[51−53] Through examination we could also confirm that while magnetic field effectively actuates the Janus microbeads, it had no effect on the colorimetric property and stability of the microbeads. As shown in Figure 1C, a possible concern about masking colorimetric signal of the PDA half sphere phase by the rest of magnetic phase can be solved by applying magnetic

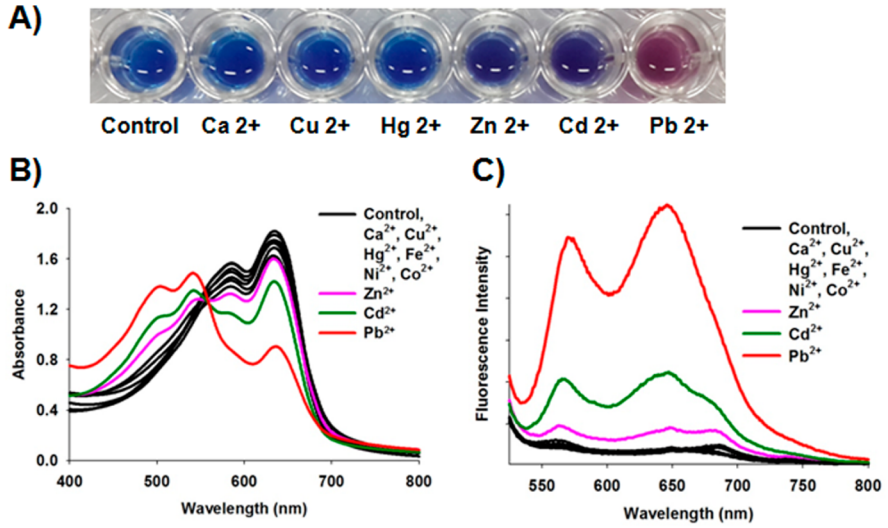


Figure 2. (A) Optical images of PDA-DPGG liposomes (0.66 mM) after 1 h incubation with lead(II) and other heavy metal ions (the final concentration of all metal ions is fixed at 0.26 mM), and corresponding (B) UV−vis spectra and (C) PL spectra.

field in "detection mode". Therefore, the developed Janus microbeads system equipped with the sensory PDA liposomes can be usefully applied to various bioassay systems and biosensors. In the following detection experiments, all the fluorescence images of Janus microbeads were obtained in the detection mode.

Lead(II) Detection Using PDA-DPGG Liposomes in Solution. The chemical structure of PCDA and the DPGG lipid, coassembly of PCDA with DPGG to form PDA-DPGG liposome, and schematic illustration of the colorimetric lead(II) detection are shown in Scheme 2. The galloyl headgroup of DPGG lipid can form phenolic metal complexes with heavy metal ions and therefore have been used in heavy metal removal applications.[45,54,55] This galloyl group has a high affinity particularly to lead(II). Because lead(II) ions can make strong complex with multiple galloyl groups, we envisioned that immobilized galloyl groups at the surface of PDA-DPGG liposomes would form multiple inter- and intraliposomal interactions and produce strong colorimetric signal.[13,33−35] The colorimetric and fluorometric response of the PDA-DPGG liposomes after 1 h incubation with various metal ions were investigated via UV−vis absorption spectra and PL spectra as shown in Figure 2. As we anticipated, the PDA-DPGG liposomes exposed to lead(II) showed the sharpest color change from blue to red and the strongest red fluorescence development. In contrast, only slight color change from blue to violet and weak red fluorescence development were observed in case of zinc(II) and cadmium(II). In case of calcium(II), copper(II), mercury(II), iron(II), nickel(II), and cobalt(II), we could not observe any noticeable color change. Moreover, even a cocktail solution of calcium(II), copper(II), mercury(II), iron(II), nickel(II), and cobalt(II) did not prevent the specific response of PDA-DPGG liposomes to the lead(II), demonstrating selective detection of lead(II) by PDA-DPGG liposomes. (Supporting Information Figure S1).

Lead(II) Detection Using Janus Microbeads Embedding PDA-DPGG Liposomes. Based on the above results in the solution, we incorporated the lead(II)-sensitive PDA-DPGG liposome into the Janus microbead system for more convenient and practical lead(II) detection and removal. When the Janus microbeads containing PDA-DPGG liposomes were exposed to lead(II), they showed similar UV−vis adsorption spectra and fluorescence spectra to the PDA-DPGG liposome solution (Supporting Information Figure S2). Figure 3

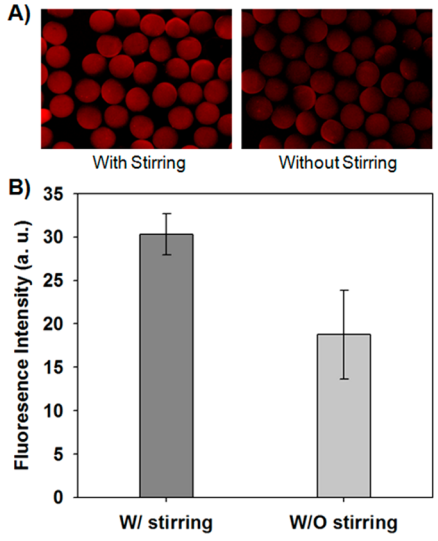


Figure 3. (A) Fluorescence microscope images of Janus microbeads having embedded PDA-DPGG liposomes after 1 h incubation in 1 mM lead(II) solution with or without stirring, and (B) corresponding relative fluorescence intensity comparison.

demonstrates the effects of stirring on the sensory properties of the Janus microbeads. On 1-h incubation with 1 mM lead(II) solution, the fluorescence intensity of the stirred Janus microbeads (approximately 100 microbeads) was about 1.5 times stronger than that of the microbeads without stirring. The stirred Janus microbeads also showed more uniform fluorescence intensity confirmed by the smaller standard deviation. It can be explained that magnetic stirring helps the microbeads to sample more lead(II) ions in a given volume and a given time thereby enhances sensitivity.[52] We further conducted a detection limit study by incubating approximately 100 microbeads in lead solutions at various concentrations. The fluorescence intensity of the Janus microbeads became stronger as the lead(II) concentration increased (Figure 4A). We could

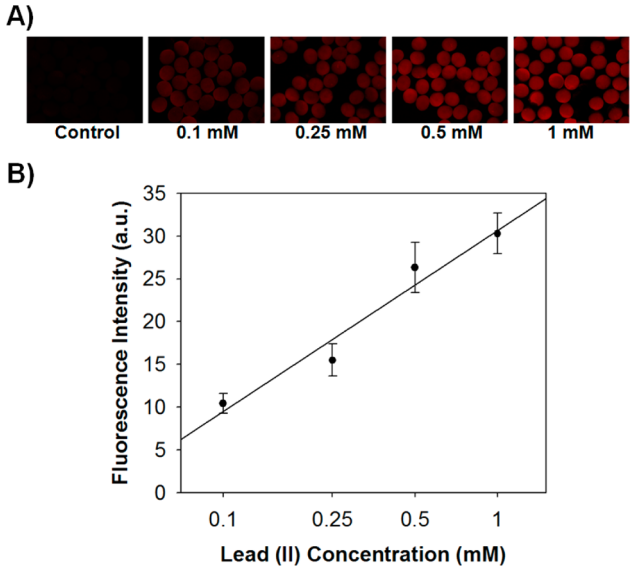


Figure 4. (A) Fluorescence microscope images of magnetic microbeads having PDA-DPGG liposomes after 1 h incubation in various concentrations of lead(II) solution with stirring, and (B) corresponding relative fluorescence intensity.

develop a good correlation between the fluorescence signal intensity and the lead(II) concentration as plotted in Figure 4B, and the detection limit of the system was 0.1 mM (~20.7 ppm).

Lead(II) Removal Using Janus Microbeads Embedding PDA-DPGG Liposomes. Alginate has the affinity to divalent metal ions (including heavy metal ions) as following order: $Pb^{2+} > Cu^{2+} > Cd^{2+} > Ba^{2+} > Sr^{2+} > Ca^{2+} > Co^{2+}$, Ni^{2+}, $Zn^{2+} > Mn^{2+}$.[56] We conducted an experiment to quantify the amount of lead(II) by the Janus alginate microbeads and understand the absorption kinetics. As shown in Figure 5, initial absorption of

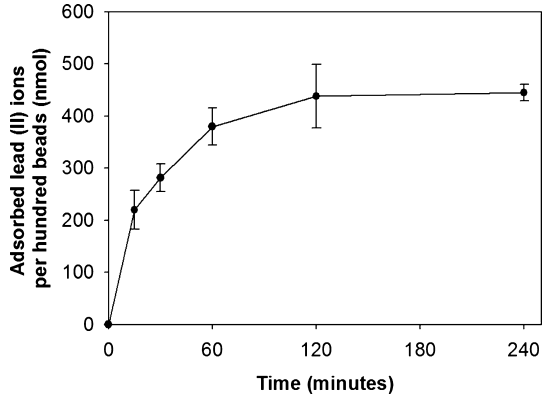


Figure 5. Amount of lead(II) ion absorbed by Janus alginate microbeads in 1 mM lead(II) solution versus incubation time.

lead(II) by the microbeads is fast but the absorption slows down as time goes and saturates at about 4 h of stirring. About 450 nmol of lead(II) ions were absorbed by hundred Janus microbeads for 4 h. Our calculation shows that hundred Janus microbeads have 1.10 μmol of alginate monomeric units (each alginate monomeric unit has one carboxylic groups), 50.5 nmol of citrate coated on the magnetic nanoparticles (though a citrate has three carboxylic groups, most of its carboxylic groups are bound to the surface of the nanoparticle, blocking the interactions between the nanoparticles and alginate), and 5.34 nmol lipids (PDA and DPGG). Based on this calculation, we believe that lead(II) absorption by Janus microbeads is mainly by alginate matrix. Even though many carboxylic acid groups of alginate should be used during the cross-linking with calcium ions, it is reasonable to expect that lead(II) ions replaced calcium ions due to much stronger affinity of carboxylic acid to lead(II).

CONCLUSIONS

Janus-compartmental alginate microbeads having two divided phases of sensory PDA liposomes and magnetic nanoparticles were fabricated for facile sensory applications. We designed and prepared PDA liposomes coassembled with 1,2-dipalmitoyl-*sn*-glycero-3-galloyl lipids (DPGG), whose galloyl headgroup has specific interactions with lead(II). Recognition of lead(II) at the PDA liposome surface by DPGG induced distortion of conjugated yne-ene main chain of PDA and caused ensuing color change from blue to red and red fluorescence development. The Janus microbeads having the sensory PDA liposomes and magnetic nanoparticles showed the same selective sensory property toward lead(II) with a few additional advantageous features such as easy manipulation and convenient collection by applying magnetic field for washing and solvent exchange, and stirring for enhanced sensitivity and fast detection. The alginate matrix of the Janus microbeads provides additional feature of lead(II). Therefore, unique system integration of sensory PDA and efficient absorbents alginate into the magnetic Janus microbeads renders sensitive detect and convenient removal of lead(II) ions simultaneously. The presented magnetic sensory microbead system can be readily adapted to many other sensor development and environmental applications.

ASSOCIATED CONTENT

Supporting Information

Video of Janus alginate microbeads being stirred on a magnetic stirrer; UV−vis and PL spectra of PDA liposome to the lead(II) in coexisting of other metal ions, UV−vis and PL spectra of PDA-DPGG liposome embedded in alginate microbeads to lead(II). This material is available free of charge via the Internet at http://pubs.acs.org/.

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Author Contributions

[▽]D.H.K. and H.-S.J. equally contributed to this work.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

K. Kim acknowledges financial support from the National Research Foundation (NRF) of Korea funded by the Ministry of Science, ICT & Future Planning (2012M3A9B4028738). D.H. Kang has been supported by Global Ph.D. Fellowship Program through the NRF of Korea funded by the Ministry of Education (2011-0007317). H. Jung thanks financial support from the Ministry of Oceans and Fisheries, Korea (20110171) and Basic Science Research Program through the NRF of Korea funded by the ministry of Education (2013055323). J. Kim

acknowledges the financial support from Animal, Plant and Fisheries Quarantine and Inspection Agency of Korea (I-AD14-2011-13-11) and the Converging Research Center Program funded by the Ministry of Science, ICT and Future Planning (Project No. 2013K000314).

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