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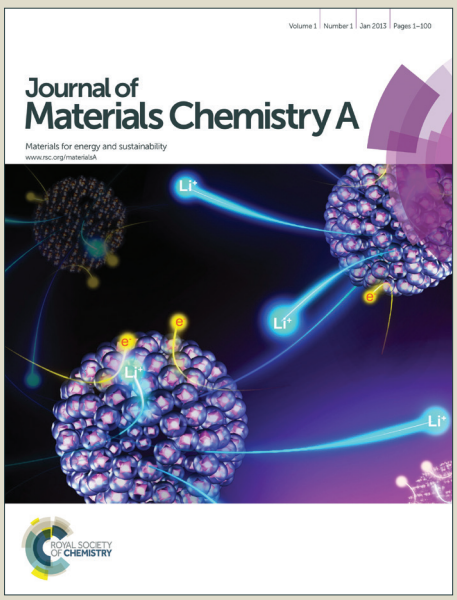

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PAPER

Polydiacetylene Liposome-Encapsulated Alginate Hydrogel Beads for Pb^{2+} Detection with Enhanced Sensitivity†

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The development of novel and simple method to trace lead ion (Pb^{2+}) has received great attention due to its high toxicity to human health and the environment. In this paper, we describe a new polydiacetylene (PDA)-based liposome sensor for colorimetric and fluorometric detection of Pb^{2+} in aqueous solution and in alginate hydrogel microbeads. In the sensor system, dopamine group was rationally introduced into diacetylene monomer to work as a strong binding site for Pb^{2+}. The dopamine-functionalized monomer and 10,12-pentacosadiynoic acid (PCDA) were then incorporated into PDA liposome in aqueous solution. After UV light-induced polymerization, deep blue colored liposome solutions were obtained. Upon the addition of various metal ions into the liposome solution, only Pb^{2+} could cause a distinct color change from blue to red and a dramatic fluorescence enhancement. To further improve its sensitivity and address its intrinsic aggregation, we then develop a liposome-immobilized detection system by encapsulating PDA-DA liposomes into alginate hydrogel beads through a microfluidic droplet-based method. The results showed that the PDA-DA liposome-contained hybrid hydrogel beads possessed excellent stability and high sensitivity. These interesting findings demonstrated that PDA liposome system developed in the current study may offer a new method for Pb^{2+} recognition in a more efficient manner.

Introduction

Inorganic contaminants such as heavy metals have caused large impact on the ecosystem in the past few centuries. Among the various heavy metal ions, lead ion (Pb^{2+}) in particular remains one of the most toxic metal ions and long-lasting danger to human health and the environment.[1] Even a very small amount of uptake of Pb^{2+} can cause severe poisoning, since it can damage the nervous system and brain function.[2] Currently, the most typical methods for Pb^{2+} detection are atomic absorption spectroscopy and inductively coupled plasma mass spectrometry.[3,4] These traditional methods allow to analyze the total content of Pb^{2+} with high sensitivity. However, the requirements of professional large-scale instrument and extensive pretreatment of samples limit their application for on-site and rapid detection.[5] To improve this situation, quite amount of fluorescent chemosensors based on DNAzyme,[6–8] peptides,[9] proteins,[10] nanoparticles[11–14] and small molecules[15–17] have been developed for Pb^{2+} detection over the past decades. Although some of the methods such as DNAzyme-, protein-, and peptide-based sensors displayed high sensitivity and selectivity in aqueous solutions, their costly fabrication and relative instability always prevent their practical applications.[18] In addition, some of the small molecule-based sensors often suffer from low solubility in water or low selectivity for detection.[19] Therefore, it is still highly desired and important to develop convenient and simple methods for Pb^{2+} detection in aqueous solution.

Polydiacetylene (PDA), a well-known conjugated polymer, has been extensively investigated and utilized as an attractive platform for sensing applications due to its unique optical properties.[20,21] The monomers of PDA can easily self-assemble into liposomal structures in aqueous medium. Upon UV light irradiation, the blue PDA can be easily obtained through rapid 1,4-addition polymerization of diacetylenes in the monomers. PDA can undergo a color shift to a red phase upon environmental stimulation, accompanied by fluorescent transition. The stimulus-induced blue-to-red transition and fluorescence enhancement of PDA have made the polymer an ideal material for the development of various chemosensors.[22] The dual signal generation is mainly ascribed to the interfacial perturbation of PDA caused by external stimuli, which can subsequently induce the conformational change of PDA conjugated backbone. To date, a variety of PDA-based sensors have been developed for chemical and biological analytes such as virus,[23] proteins,[24,25] enzymes,[26–28] metal ions[29,30] and organic solvents.[31,32] Although a few of the PDA-based sensors have been developed for Pb^{2+} detection,[33,34] the detection

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† Electronic Supplementary Information (ESI) available: Characterization and general methods, synthesis of PCDA-DA, NMR spectra of PCDA, optimization of PDA liposomes for Pb^{2+} detection, study on the size distribution of PDA-DA liposomes by DLS, study on the selectivity of PDA liposomes prepared from pure PCDA. See DOI: 10.1039/x0xx00000x

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systems are mainly working in PDA liposome solution,[18] which has a few of limitations such as intrinsic aggregation for long-term storage and low sensitivity due to homogeneous dilution of liposome and targets in solutions.[35] An improved strategy used in current studies is to immobilize PDA liposomes on a solid matrix such as thin films,[36] electrospun fibers[31,37] and microbeads.[35,38,39] However, the detection of Pb^{2+} by using PDA-immobilized system is rather limited.[40,41]

With the aiming to develop a more efficient PDA-based sensor for Pb^{2+} recognition, in this paper we first designed and synthesized a dopamine-functionalized diacetylene monomer PCDA-DA since the catechol moiety in dopamine possesses high complexing power toward Pb^{2+} and could form lead catecholate (Scheme 1) in the presence of Pb^{2+}.[42,43] By co-assembly of PCDA-DA and 10,12-pentacosadiynoic acid (PCDA), we then provided a new PDA liposome-based chemosensor for colorimetric and fluorometric detection of Pb^{2+}. Upon the addition of various metal ions into the liposome solution, only Pb^{2+} could cause a distinct color change from blue to red and a dramatic fluorescence enhancement. To further improve its sensitivity and address its intrinsic aggregation, we then develop a liposome-immobilized detection system by encapsulating the PDA-DA liposomes into alginate hydrogel beads through a microfluidic droplet-based method. In the sensor system, PDA liposomes could be easily separated from solutions and immobilized within hydrogel beads at high concentrations, which endowed the PDA liposomes with enhanced stability and sensitivity for Pb^{2+} detection. Additionally, the PDA liposome-encapsulated hydrogel beads could realize a non-intrusive detection since the hydrogel beads could be easily removed from sample solutions. The current work may offer a new method for Pb^{2+} recognition in a more efficient manner.

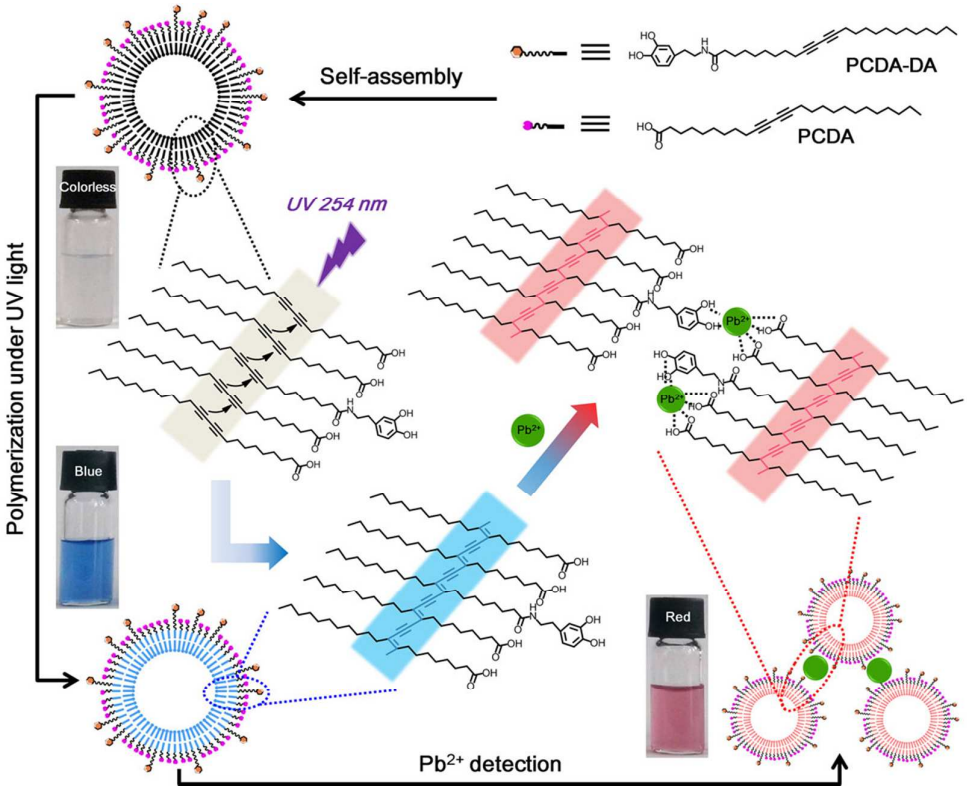
Experimental

Materials and apparatus

10,12-Pentacosadiynoic acid (PCDA) was purchased from Alfa Aesar (Lancaster, England). The PCDA powder was dissolved in chloroform and filtered through a 0.45-μm nylon filter to remove oligomers prior to use. Sodium alginate, M8410 mineral oil and sorbitan monooleate (SPAN 80) were purchased from Sigma-Aldrich (MO, USA). Dopamine hydrochloride was obtained from Aladdin (Shanghai, China). Dopamine modified diacetylene monomer (PCDA-DA) was synthesized in our laboratory. The detailed experimental procedures and characterization can be found in Supporting Information. Dry solvents used in the synthesis were purified by standard procedures. All other reagents and solvents were of analytical grade and supplied by local commercial suppliers. Ultrapurified water in all experiments was supplied by a Milli-Q system (Millipore).

Preparation of PDA liposomes

The PDA liposomes used in the current study were prepared following the probe sonication method.[28] Briefly, a mixture of



Scheme 1 Self-assembly of PCDA-DA and PCDA to form PDA lipsomes and schematic illustration of PDA liposomes for the colorimetric detection of Pb^{2+}.

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PCDA-DA and PCDA with various mole ratios (0/10, 1/9, 2/8, 3/7, 4/6, 5/5, 10/0) was dissolved in 1 mL of methylene chloride. Then, the organic solvent was removed by a stream of nitrogen gas and an appropriate amount of ultrapurified water was subsequently added to yield a total lipid concentration of 1 mM. The resulting solution was sonicated for 20 min at 80 °C to afford a clear or translucent solution. The formed liposome solution was stored at 4 °C overnight and then warmed to ambient temperatures. Photo-induced polymerization was carried out at 254 nm lamp irradiation for 15 min to obtain a deep-blue solution. The obtained PDA liposome solutions can be stored at 4 °C for one week without the formation of precipitate.

Characterization of PDA liposomes

The morphology of PDA liposomes prepared from PCDA-DA and PCDA (mole ratio, 1/9) in the absence or presence of Pb^{2+} were characterized by using a transmission electron microscopy (TEM, HT7700, Hitachi, Japan). For typical experiments, one drop of fresh prepared samples was dropped onto a carbon-supported copper grid and dried gradually at room temperature before observation. The morphology of the PDA-DA liposome-encapsulated hydrogel microbeads was characterized by a field emission scanning electron microscope (FE-SEM, S-4800, Hitachi, Tokyo, Japan). Samples were freshly prepared and dropped on small silicon wafers. After drying at room temperature, samples were coated with gold nanoparticles for 10 min and then observed by FE-SEM. The size distribution of PDA-DA liposomes in the presence of different concentrations of Pb^{2+} (0, 50 and 100 μM) was determined by using a Zetasizer (Malvern Instruments Co, UK) equipped with a He-Ne laser at room temperature. The particle size distribution was related to the scattered light intensity.[44]

Detection of Pb^{2+} using PDA liposomes

For a typical experiment of Pb^{2+} detection, 300 μL of PDA liposome solution (1 mM) was placed in a test tube and diluted with 2700 μL of HEPES buffer (10 mM, pH = 7.4) to obtain 100 μM of the stock solution. The stock solution was titrated with Pb^{2+} solution (0-100 μM) and the resulting solutions were allowed to equilibrate for 15 min at room temperature. The color changes were captured by a digital camera. The UV-Vis absorption and fluorescence emission spectra were respectively recorded on a Shimadzu UV1800 spectrometer (Shimadzu, Kyoto, Japan) and a Shimadzu RF-5301PC fluorescence spectrometer (Shimadzu, Kyoto, Japan). The fluorescence excitation wavelength was at 520 nm and both the excitation and emission slit widths were 5 nm.

To quantify the color change of the PDA liposomes, the colorimetric response value (CR, %) was employed to determine the extent of color transition.[23,28] The formula was defined as follows:

$$CR = [PB_0 - PB_1]/PB_0 \times 100\%$$

where $PB = A_{blue}/[A_{blue} + A_{red}]$. A_{blue} and A_{red} repressent the absorbance at 637 nm ("blue" phase) and 537 nm ("red" phase), respectively. PB_0 is the ratio of the absorbance at 637 nm to that at 537 nm in the absence of Pb^{2+}, while PB_1 is the ratio of the absorbance at 637 nm to that at 537 nm when different concentrations of Pb^{2+} were added.

Preparation of PDA liposome-encapsulated hydrogel microbeads

The PDA liposome-encapsulated hydrogel microbeads were prepared by using a droplet microfluidic device. The microfluidic device used for droplet formation was fabricated according our previous reported methods.[45] The alginate solution containing PDA liposomes was prepared by mixing 1 wt% sodium alginate and 1 mM PDA liposome with a volume ratio of 1:5. Mineral oil containing 5 wt% SPAN as a stabilizer was used as the immiscible phase. To generate droplets, mineral oil and alginate solution containing PDA liposomes were introduced into the microfluidic channels using a syringe pump (LSP01-1A, Baoding Longer Precision Pump Co., Ltd., Hebei, China). The flow rate of oil phase and aqueous phase kept constant at 6 μL/min and 3μL/min, respectively. The formed droplets were externally collected and gelled in 5 wt% $CaCl_2$ in water-ethanol (1:1, *v/v*). After gelation, the hydrogel beads were washed thrice with ultrapurified water. Polymerization of the PDA liposome-encapsulated hydrogel beads was performed under 254-nm UV light irradiation for 30 min. The obtained blue colored hydrogel beads were stored at 4 °C before use.

Detection of Pb^{2+} using PDA liposome-encapsulated hydrogel microbeads

PDA liposome-capsulated hydrogel microbeads (approximately 200 microbeads) were incubated with different concentrations

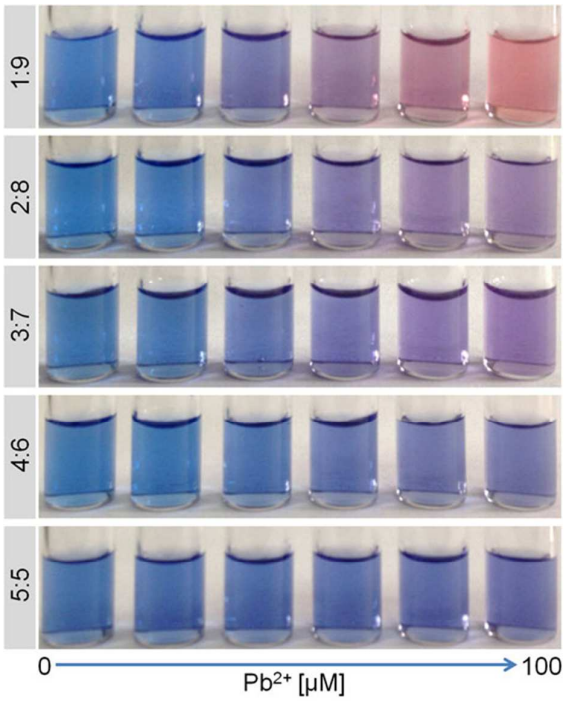

Fig. 1 Color changes of PDA liposomes (100 μM) prepared from different mole ratio of PCDA-DA and PCDA (1:9, 2:8, 3:7, 4:6, 5:5) in the presence of different concentrations of Pb^{2+} (0, 10, 30, 50, 70 and 100 μM).

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of Pb^{2+} (0, 1, 2, 5, 10 μM) for 1 h at room temperature. The optical and fluorescence images of the post-reaction solutions were recorded simultaneously using an inverted microscope (Olympus, CKX41) with a charge-coupled device camera (Olympus, DP72) and a mercury lamp (Olympus, U-RFLT50). The fluorescence intensity of the images was analyzed using Software Image-Pro Plus 6.0 (Media Cyternetics).

Results and discussion

Optimization of PDA liposome components

To obtain an efficient PDA liposome sensor for Pb^{2+} detection, it is desirable to investigate the optimal PDA liposome components since the monomer compositions in the liposome can notably affect its recognition and sensing behaviors.[44] We first prepared series of PDA liposome solutions by using different mole ratios of PCDA-DA and PCDA. After photoinduced polymerization under 254nm UV light, blue colored suspensions were obtained. The colorimetric responses of each kind of PDA liposomes in the presence of different concentrations of Pb^{2+} were then examined as shown in Fig. 1. It can be seen that PDA liposomes prepared from PCDA-DA and PCDA with different mole ratios displayed different color responses to Pb^{2+}. Among them, PDA liposomes prepared by 1:9 mole ratio of PCDA-DA and PCDA showed the most distinct color changes from blue to red upon the addition of increasing amounts of Pb^{2+}. A discernible color change from blue to purple was directly observed when 10 μM of Pb^{2+} was introduced, revealing the potential application of PDA liposomes for naked-eye recognition of Pb^{2+}. The colorimetric response value (CR, %) calculated by the UV-Vis absorbance spectroscopic data was about 50% when 100 μM Pb^{2+} was added (Fig. 2A). With the component increase of monomer PCDA-DA in the liposomes, the color responses of PDA liposomes to Pb^{2+} gradually decreased. When the mole ratio of PCDA-DA to PCDA reached to 1:1, the PDA liposomes exhibited little color change, even 100 μM of Pb^{2+} was added (CR, ~11%). These results indicate that the mole ratio of carboxyl acid group to dopamine group on the surface of PDA liposomes plays a key role in the recognition of Pb^{2+}. To further substantiate this issue, PDA liposomes derived from pure PCDA and pure PCDA-DA were respectively investigated (Fig. S3†), showing that the two kinds of PDA liposomes prepared from pure PCDA and pure PCDA-DA all displayed slight color changes upon the addition of 100 μM Pb^{2+}. Such observation demonstrates that both the carboxylic acid groups and dopamine groups were all essential for colorimetric detection of Pb^{2+} and it is desirable to note that a suitable local "micro-environment" produced by the two groups is important for the detection of Pb^{2+}.[44] In view of the color change results, PDA liposomes prepared from 1:9 mole ratio of PCDA-DA to PCDA (denoted as PDA-DA) was chosen as the optimal detection system for further investigation on Pb^{2+} detection.

Size distribution and morphology studies

To gain inside into the detection mechanism of PDA-DA liposomes for Pb^{2+}, the size changes of PDA-DA liposomes after the addition of different concentrations of Pb^{2+} (0, 10, 30, 50, and 100 μM) were investigated by using the dynamic light scattering (DLS) method.[44] The fresh prepared PDA-DA liposomes exhibited an average size of 102 nm before UV light irradiation. The size of the liposome decreased to 86 nm after UV light treatment because the polymerization of the diacetylene monomers could make the lipid molecules compact (Fig. S4†). Upon the addition of different concentrations of Pb^{2+}, the size of PDA-DA liposomes gradually increased (Fig. 2B). The addition of 100 μM Pb^{2+} to PDA-DA liposomes led to an extreme increase of 1106 nm in size (Fig. S5†). Morphological studies by using transmission electron microscopy (TEM) also showed that PDA-DA liposomes were almost spherical and well separated in the absence of Pb^{2+} (Fig. 2C). However, the addition of Pb^{2+} (100 μM) caused extensive aggregation of the liposomes (Fig. 2D). These phenomena confirmed the strong complexation of Pb^{2+} with both carboxyl and dopamine groups, which further resulted in the aggregation of PDA-DA liposomes, as depicted in Scheme 1. These intra- and inter-molecular interactions were believed to produce the interfacial perturbations of PDA and subsequently caused the conformational change of PDA conjugated backbone. As a result, the color change and fluorescence transition were generated, which could be used for naked-eye detection of Pb^{2+}.

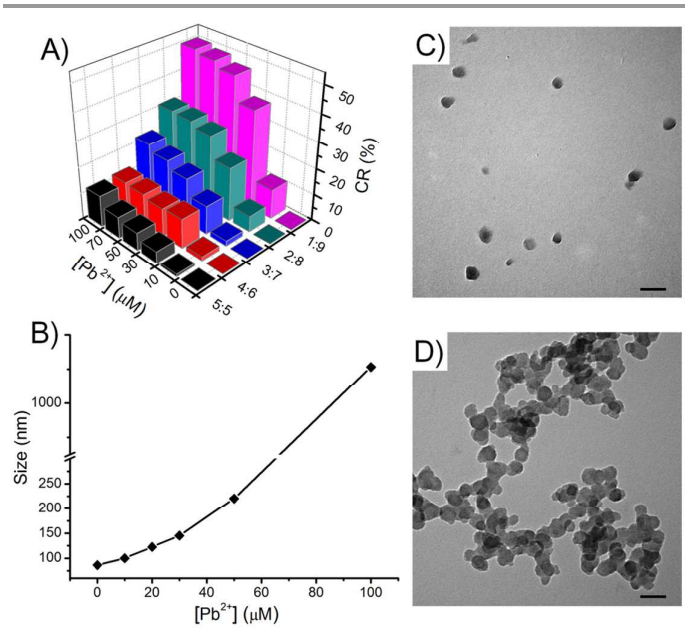

Fig. 2 (A) CR (%) values of PDA liposomes (100 μM) prepared from different mole ratios of PCDA-DA to PCDA (1:9, 2:8, 3:7, 4:6, and 5:5) in the presence of different concentrations of Pb^{2+} (0, 10, 30, 50, 70 and 100 μM). (B) Particle sizes of PDA-DA liposomes in the presence of different concentrations of Pb^{2+} (0, 10, 20, 30, 50 and 100 μM). TEM images of PDA-DA liposomes (C) before and (D) after the addition of 100 μM Pb^{2+}. Scale bar : 100 nm.

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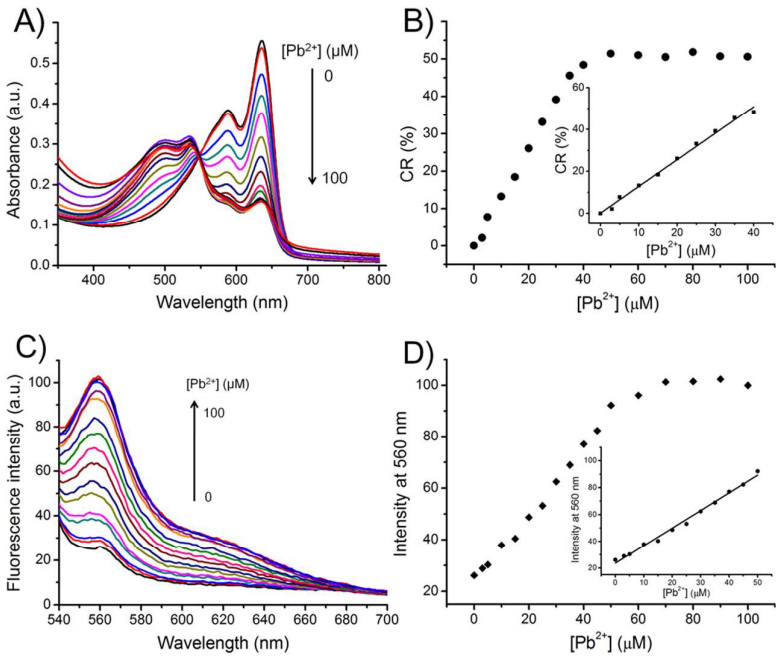

Fig. 3 (A) UV-vis absorption spectra and (B) related CR (%) values of PDA-DA liposomes (100 μM) after adding different concentrations of Pb^{2+} (0-100 μM) in HEPES buffer (10 mM, pH 7.4) at room temperature. Inset: the linear relationship of PDA-DA liposomes between the CR (%) value and Pb^{2+} concentration. (C) Fluorescence emission spectra and (D) related fluorescence intensity changes at 560 nm of PDA-DA liposomes (100 μM) upon the addition of different concentrations of Pb^{2+} (0-100 μM) in HEPES buffer (10 mM, pH 7.4) at room temperature. Inset: the linear relationship of PDA-DA liposomes between the fluorescence intensity and Pb^{2+} concentration.

An *in situ* NMR spectral test was performed to further verify the intense complexation of Pb^{2+} with carboxyl and dopamine groups. As shown in Fig. S6†, before the addition of Pb^{2+}, it was hardly to observe the resonances of the hydroxyl and carboxylic protons, probably due to the formation of strong hydrogen bonds among the two molecules. However, the peaks of these two kinds of protons were sharply appeared after the addition of Pb^{2+}, and the peaks were getting slightly stronger with the time increasing. Such observation reveals that Pb^{2+} could form cathecolate-Pb^{2+} complex with carboxyl and dopamine groups, which may break the hydrogen bond interactions among the two molecules.

Detection of Pb^{2+} using PDA-DA liposomes in aqueous solution

Having obtained the optimal detection system, we then evaluated the optical spectral changes of PDA-DA liposomes at a given concentration (100 μM) in the presence of different concentrations of Pb^{2+} (0-100 μM). As shown in Fig. 3A, pristine blue-phase PDA-DA liposomes showed a maximum absorption at 637 nm. The addition of increasing amount of Pb^{2+} caused a gradual decrease of this absorption intensity, accompanied by a remarkable increase of a new absorption band at 537 nm. It should be noted that when the concentration of Pb^{2+} was greater than 50 μM, the color transition of PDA-DA liposomes became slightly and only negligible change in absorbance was observed, suggesting that the binding sites on the surface of PDA-DA liposomes were saturated through the formation of Pb^{2+} complexes. In addition, the color response was quite fast, which could reach the maximum within 15 min (Fig. S7†). It was found that the CR values showed a linear increase for the concentration of Pb^{2+} in the range of 3 μM to 40 μM with R^2 = 0.993 (Fig. 3B, inset). The maximal CR value (~50%) was gained after adding 50 μM of Pb^{2+}, and the CR values almost remained constant when the concentration of Pb^{2+} was over 50 μM (Fig. 3B). This result is consistent with the observation of color and spectral changes mentioned above, which further indicated the saturation of PDA-DA liposomes by Pb^{2+}. The detection of Pb^{2+} using the PDA-DA liposomes was also evaluated by fluorescence spectroscopy because the color change of PDA is often accompanied by fluorescence enhancement. As expected, gradual fluorescence enhancement appeared as the concentration of Pb^{2+} increased (Fig. 3C). Also, a linear correlation (R^2 = 0.992) was obtained with the concentration of Pb^{2+} in the range of 3 μM to 50 μM (Fig 3D, inset).

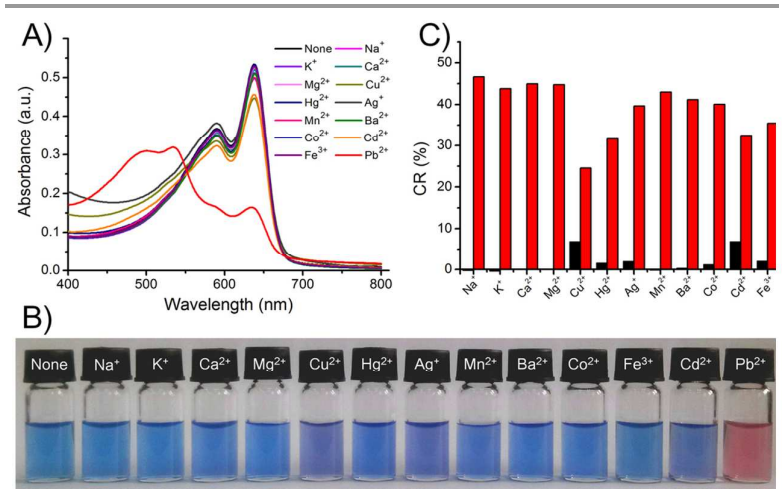

Fig. 4 (A) UV-vis absorption spectra and (B) the color changes of PDA-DA liposomes (100 μM) upon the addition of different metal ions in HEPES buffer (10 mM, pH 7.4) at room temperature. (C) Related CR (%) values of PDA-DA liposomes in the presence of different metal ions. Black bars represent the CR (%) values of the addition of the given metal ions (100 μM). Red bars represent the CR (%) values of the addition of Pb^{2+} ions (100 μM) to the respective solution.

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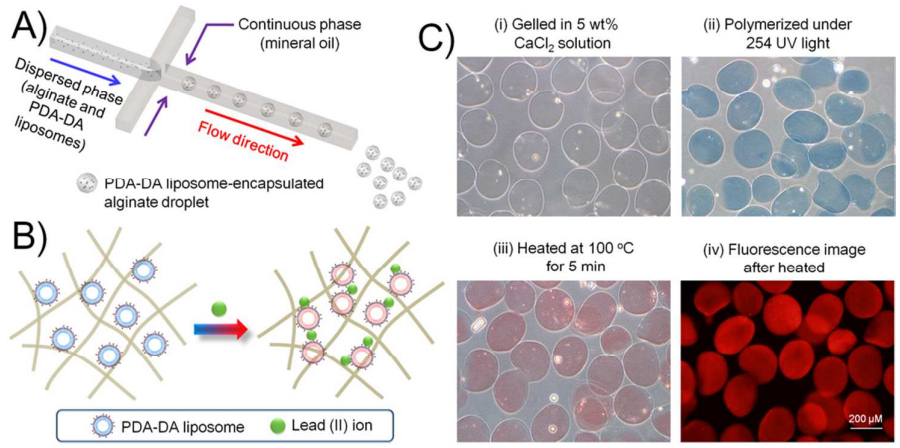

Fig. 5. (A) Schematic illustration of the droplet-based preparation of PDA-DA liposome-encapsulated hydrogel microbeads. (B) Colorimetric detection of Pb^{2+} by using the PDA-DA liposome-encapsulated hydrogel microbeads. (C) Optical and fluorescence images of the PDA-DA liposome-encapsulated hydrogel microbeads: (i) gelation in 5 wt% Ca_2Cl solution to form hydrogel microbeads; (ii) polymerization under 254 UV light to form blue-colored microbeads; (iii) heating at 100 °C for 5 min to form red-colored microbeads; (iv) fluorescence image of the microbeads after heated.

Selectivity of PDA-DA liposomes

To investigate the selectivity of PDA-DA liposomes for Pb^{2+}, other metal ions including Na^+, K^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Hg^{2+}, Ag^+, Mn^{2+}, Ba^{2+}, Co^{2+}, Fe^{3+}, Cd^{2+} were chosen to introduce into the PDA-DA liposomes. The UV-Vis absorption spectra and corresponding color changes in the presence of various metal ions (100 μM) were vividly shown in Fig. 4A and 4B. It can be seen that only Pb^{2+} could cause a significant spectral change accompanied by a distant color change. Other metal ions such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Hg^{2+}, Ag^+, Mn^{2+}, Ba^{2+}, Co^{2+}, Fe^{3+} almost had no color and spectral changes to PDA-DA liposomes. It is worth noting that PDA-DA liposomes showed a slightly color and spectral changes in the presence of Cu^{2+} and Cd^{2+}. However, compared with Pb^{2+}, the color responses of Cu^{2+} and Cd^{2+} were quite mild. As a control, we also investigated the color change of PDA liposome prepared from pure PCDA to these metal ions. However, no obvious color changes were observed in the presence of these metal ions (Fig. S8†). Such observation indicates that the outstanding selectivity of PDA-DA liposomes for Pb^{2+} may be plausibly ascribed to the strong interactions of Pb^{2+} with dopamine and carboxyl groups.[19] To further verify the selectivity of PDA-DA liposomes for Pb^{2+}, an interference experiment was also performed by adding Pb^{2+} into these metal ion-contained solutions and their CR values were calculated (Fig. 4C). They only had relative low CR values (0-7%) of PDA-DA liposomes in the presence of other metal ions. However, the CR values of the mixed suspensions were enhanced after the addition of Pb^{2+} with a clear color change from blue to red. These results thus indicate that the presented PDA-DA liposome system could act as sensitive and selective colorimetric sensor to detect Pb^{2+} with no or little interference from other competitive metal ions.

Detection of Pb^{2+} using PDA-DA liposome-encapsulated alginate hydrogel microbeads

The promising results above demonstrated that PDA-DA liposome could be served as a chemosensor for naked-eye detection of Pb^{2+} in a simple and cost-effective manner. However, the inherent limitations (e.g. low sensitivity and intrinsic aggregation) of PDA liposomes in aqueous solution would notably affect the sensing performance for Pb^{2+} recognition.[41] To realize more efficient sensing, we then developed a liposome-immobilized detection system which can detect Pb^{2+} with enhanced sensitivity. In this system, alginate sodium was employed as matrix material due to its facile and rapid gelation in aqueous solution in the presence of Ca^{2+}. PDA-DA liposomes were directly mixed with a solution of alginate sodium, and the mixture could be conveniently converted into alginate hydrogel microbeads through a microfluidic droplet system reported by our group.[45] As illustrated in Fig. 5A, mineral oil and sodium alginate containing PDA-DA liposomes were respectively introduced into the microdevice through the oil and hydrogel channels. By controlling the flow rate of the oil and aqueous phase, uniform droplets were formed through the system. After collected and gelled in 5 wt% $CaCl_2$ solution, spherical hydrogel microbeads with the diameter of ~200 μM were obtained (Fig. 5C, i). Under 254 UV light irradiation, blue colored microbeads produced duo to photopolymerization of PDA-DA liposomes (Fig. 5C, ii). The blue colored microbeads can be stored at 4 °C for two months without any color changes, suggesting that the capsulation of PDA-DA liposome in hydrogel microbeads can

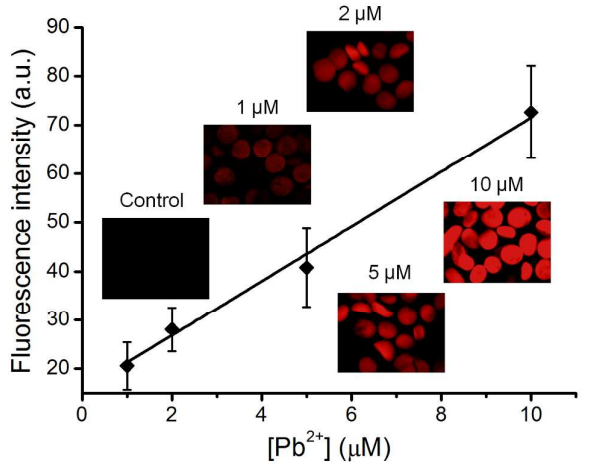

Fig. 6 Plot of the relative fluorescence intensity as a function of Pb^{2+} concentration. Inset: typical fluorescence images of the PDA-DA liposome-encapsulated hydrogel microbeads upon incubation with different concentrations of Pb^{2+} for 1 h.

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enhanced its stability. The increased stability could be ascribed to the framework of the alginate hydrogel, which can prevent the nonspecific aggregation of PDA-DA liposomes. The morphologies of the obtained PDA-DA liposome-encapsulated hydrogel beads were further characterized by FE-SEM. As seen from Fig. S9†, the hydrogel beads were almost spherical with the diameter about 150 μM, which were smaller than that observed by optical microscope. The smaller size of the hydrogel beads observed by SEM was probably due to the shrinkage during the drying process. In addition, the internal morphologies of the hydrogel beads were also observed by SEM with a high magnification (Fig. S9†). Many smaller nanoparticles (~100 nm) existed in the internal microbeads, indicating the successful encapsulation of PDA-DA liposomes.

Thermochromatism is the inherent characteristic of PDA.[21] To explore the sensing behaviours of PDA-DA liposomes encapsulated in the hydrogel microbeads, thermochromatism of PDA was also investigated in the current study. The result (Fig. 5C, iii) showed that the blue colored microbeads could change their color to red after being heated at 100 ^{o}C for 5 min. Moreover, the red colored microbeads exhibited strong red fluorescence emission (Fig. 5C, iv). The red colored microbeads couldn't return to the original blue during the process of cooling to room temperature. Combining with the increased stability, these phenomena indicated that the PDA-DA liposome-encapsulated hydrogel microbeads could be used for sensor applications. Thus, the detection of Pb^{2+} by using the PDA-DA liposome-encapsulated hydrogel microbeads was then performed. The detection principle of the PDA-DA liposome-encapsulated hydrogel microbeads for Pb^{2+} is given in Fig. 5B. Pb^{2+} can directly penetrate into the hydrogel beads and form complexes with the carboxyl acid and dopamine groups on the surface of PDA-DA liposomes when the hydrogel beads are immersed in the sample solution. The strong intro-molecular interactions thus could lead to the perturbation of the PDA conjugated backbone and produce the colorimetric change and "turn on" fluorescence signal. As shown in Fig. 6 and Fig. S10†, the exposure of the blue-colored microbeads to Pb^{2+} caused clear color change from blue to red and a gradual red fluorescence enhancement at room temperature (Fig. 6, inset). The fluorescence intensity exhibited a good linear relationship with Pb^{2+} concentrations. The detection limit is as low as 200 ppb, while this detection limit is imposed by microscope we used and can be further improved if more sensitive equipment was used. The color changes of the hydrogel microbeads can be quantified through a sample image analysis of the RGB values.[46] The relative changes of the red component denoted as red chromaticity level (r) was directly calculated by using the RGB values obtained from each image shown in Fig. S10a†. The red chromaticity level showed a linear increase with the concentration of Pb^{2+} increasing. These results indicated that the PDA-DA liposome-encapsulated hydrogel microbeads could be used for Pb^{2+} detection in a more efficient manner. Alginate has strong affinity toward heavy metal ions (especially for Pb^{2+}) and has been widely used as a nontoxic absorbent for toxic metal ions.[47] In the current detection system, alginate hydrogel microbeads provided not only a three dimensional environment but also a preconcentration of Pb^{2+}. These effects could enhance the interactions between PDA-DA liposomes and Pb^{2+}, and thus increased the sensitivity for Pb^{2+} detection. In addition, the immobilization of PDA-DA liposomes in alginate hydrogel microbeads can realize a non-intrusive detection. The microbeads can be easily removed from the solution when the detection completed.

Conclusions

In summary, we have designed and synthesized a new diacetylene monomer PCDA-DA, in which dopamine groups was rationally modified as a strong binding site for Pb^{2+}. After incorporated this new monomer and PCDA into liposomes, we first provide a novel polydiacetylene (PDA)-based liposome sensor for colorimetric and fluorometric detection of Pb^{2+} in aqueous solution. Upon the addition of various metal ions, only Pb^{2+} could cause a significant color change from blue to red accompanied by a remarkable fluorescence enhancement. A clear color change could be directly observed by naked-eye in the presence of 10 μM Pb^{2+}. To improve the storage stability and detection sensitivity, we then developed a PDA-DA liposome-immobilized detection system by facilely embedded the liposomes into alginate hydrogel microbeads through microfluidic droplet technique. Compared with PDA-DA liposomes, the hydrogel microbead-based detection system possessed high stability and sensitivity for Pb^{2+}. The detection limit is as low as 200 ppb for Pb^{2+}. Moreover, the microbead-based detection system could achieve a non-intrusive detection and could be utilized to develop other PDA-based sensors for different applications.

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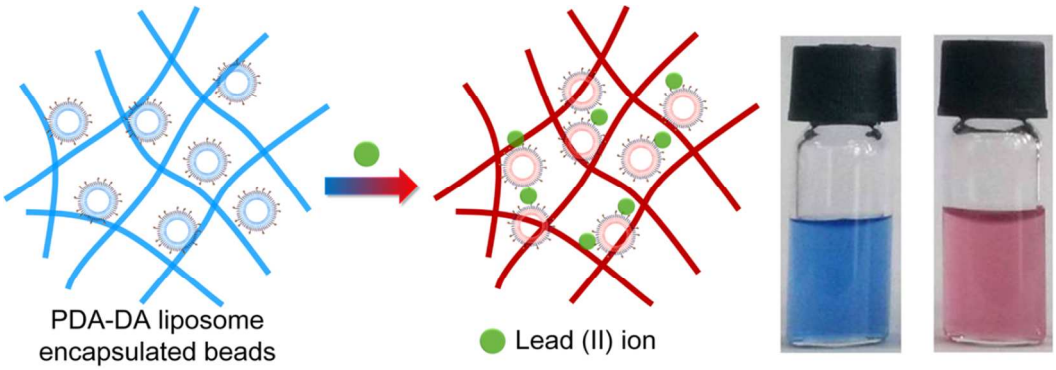
Graphic Abstract:



A new polydiacetylene liposome-based sensor was successfully constructed for colorimetric and fluorometric detection of Pb^{2+}.

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