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Molecularly imprinted polymeric film on semiconductor nanoparticles Analyte detection by quantum dot photoluminescence

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Abstract

Incorporation of semiconductor nanoparticles into molecularly imprinted polymer provides a sensor material which can be easily shaped and with better selectivity because the bound template would quench the photoluminescence (PL) emission of quantum dots significantly. In this work, artificial receptors of various templates were synthesized with functional monomers such as methacrylic acid (MAA), semiconductor like CdSe/ZnS core–shell derivatized with 4-vinylpyridine and ethylene glycol dimethacrylic acid as the cross-linker. The quenching of photoluminescence emissions is presumably due to the fluorescence resonance energy transfer between quantum dots and template molecules. The photoluminescence emission is unaffected upon incubation of analyte with the blank control polymer.

Keywords: Molecular imprinting; Quantum dot photoluminescence detection; Photoluminescence detection; Detection, LC

1. Introduction

Recognition of analyte molecules by utilizing the luminescent chromophoric nature of semiconductor nanoparticles, viz. quantum dots, is a well-established method in biological science [1,2]. In principle, this concept can be applied to the detection of specific analytes in competitive assay of analyte molecules in molecular imprinting technology (MIT) [3–5]. Optical detection is found to be a straightforward methodology especially in sensor applications for the detection of analytes. Organic fluorescent dyes have been used widely for analyte detection in molecular imprinting process [6]. But better results can be obtained from inorganic semiconductor nanoparticles. Due to its broad excitation spectra, which is effective to whole spectrum of colors, emission without red tailing and photodegradation stability of quantum dots make them more attractive as fluorescent labels. The hydrophobicity and the solvent-dependent quantum yields of dyes are the other issues that can be circumvented by replacing dyes with quantum dots as reporter molecules in a variety of bioassays [7,8]. Furthermore, quantum dots offer the potential for performing multiple targets on a single sample simultaneously.

There are reports on quenching of band-edge photoluminescence (PL) of CdSe as a tool to detect gas phase analytes like ammonia, trimethylamine, etc. by imprinting of polyacrylic acid on cadmium selenide crystal [9]. Solid analyte molecules can also be detected with a similar imprinting technique by making use of the photoluminescence behavior of semiconductor nanoparticles.

Molecular imprinting involves the synthesis of polymers in the presence of an imprint or template molecule to produce cavities in the polymer that are highly selective for the imprint. Drugs and foods can be tested for some special analyte by extracting it into solution and then ‘binding’ it selectively with molecularly imprinted polymer followed by quantification via various analytical techniques. Advantages of MIPs include simple preparation, high stability, high binding affinity and capacity and low cost. Earlier, we have reported the imprinting of caffeine molecule into the solid silica matrix using sol–gel process [10]. In this paper, we report the synthesis of polymeric host materials containing functionalized chromophores of CdSe coated with ZnS having an energy band gap of 2.12 eV based on molecular imprinting process. CdSe nanocrystals were functionalized with polymerizable organic moieties by simply exchanging

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the surfactant, trioctylphosphine, with 4-vinylpyridine in a repeated precipitation procedure. Small to medium sized analyte molecules were imprinted using aforementioned CdSe derived hosts. A variety of templates including caffeine were successfully imprinted and detected by quenching of photoluminescence emission, without the use of a transducer.

2. Experimental

2.1. Materials

Methanol (EL, Mallinckrodt) and toluene (HPLC, Mallinckrodt) distilled over magnesium/iodine and CaH_2, respectively, to remove trace amounts of water. Selenium (Riedel-de Haen), trioctylphosphine (TOP, 90% Aldirch), trioctylphosphine oxide (TOPO, Fluka), hexadecylamine (HDA, Fluka), stearic acid (Aldrich), cadmium acetate (>98%, Fluka), diethyl zinc (1 M solution in toluene, Aldrich), benzoyl peroxide (Fluka), 4-vinylpyridine (4-VP, Aldrich) and hexamethyldisilathiane [$(TMS)_2S$, Aldrich] were used as received. Caffeine (99 + % Lancaster), L-cysteine (Sigma), estriol (Sigma), uric acid (TCI-EP) and methacrylic acid (MAA) (TCI-EP), ethylene glycol dimethacrylate (EGDMA, Showa, Japan) were also used without further purification.

2.2. Synthesis of CdSe/ZnS

CdSe coated with ZnS was made by the reported methods from $Cd(OAc)_2$ and Se powder in a stearic acid/TOPO/TOP surfactant mixture at 300 °C [11,12]. It was subjected to a size selective precipitation and finally dispersed in anhydrous toluene. UV and PL spectra were recorded in toluene.

2.3. Functionalization of CdSe/ZnS with 4-vinylpyridine

CdSe/ZnS nanoparticles (0.795 mmol) were precipitated from toluene solution by the addition of anhydrous methanol. The nanocrystals were centrifuged and discarded the supernatant. Five milliliter of 4-vinylpyridine was added and stirred at room temperature for overnight. Nanocrystals were precipitated in anhydrous methanol and re-dispersed in 4-vinylpyridine in order to exchange the trioctylphosphine groups around the nanocrystals with 4-vinylpyridine moieties.

2.4. CdSe/ZnS-MIP synthesis: general protocol

CdSe/ZnS functionalized with 4-vinylpyridine (46 mmol) was transferred under an inert atmosphere to a mixture consisting of methacrylic acid (46 mmol), ethylene glycol dimethacrylate (139 mmol) and template (0.83 mmol). Polymerization was initiated by the addition of benzoyl peroxide (0.56 mmol) in toluene (5 ml) and at a bath temperature of 60–70 °C. The solid polymer obtained was ground to a fine powder and sieved. The powdered sample was subjected to a Soxhlet extraction process for removing the template molecules.

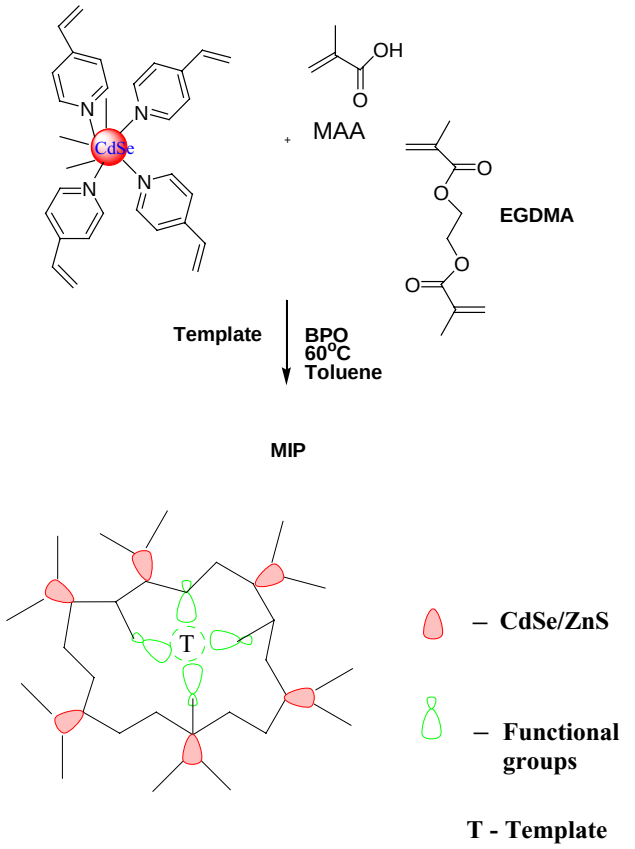

Fig. 1. Scheme for the preparation of CdSe/ZnS incorporated MIP.

A control polymer (NIP) was prepared similarly but without template.

2.5. Re-binding experiment

One hundred milligrams of MIP or NIP was incubated with an aqueous solution of analyte and its analogues molecule (200 ppm, 5 ml), respectively. The solution was centrifuged (5000 rpm, 20 min) and the separated polymer particles were dried thoroughly before the photoluminescence measurements. Concentrations of samples were the same for all experiments.

3. Results and discussion

Fig. 1 illustrates the general scheme for the preparation of imprinted polymers with CdSe/ZnS core–shell functionalized with 4-vinylpyridine as the quantum dots, methacrylic acid as the functional monomer and EGDMA as the cross-linker. Toluene was chosen as the porogen. The analyte detection was done by the incubation of MIP with the corresponding print molecule in an aqueous media. The

Table 1
Summary of photoluminescence results from various CdSe/ZnS/4-VP/EGDMA MIPs

Template/analyte	MIP composition	Emission intensity (a.u.) and emission maxima (nm)				
		CdSe/ZnS	MIP (with template)	MIP[a] (without template)	After re-binding[b]	Control polymer
Caffeine/caffeine	CdSe/ZnS/4-vinylpyridine/EGDMA	906 a.u. 556 nm	14.75 a.u. 594 nm	513 a.u. 589 nm	112 a.u. 587 nm	No response to analyte
Caffeine/theobromine	CdSe/ZnS/4-vinylpyridine/EGDMA	906 a.u. 556 nm	14.75 a.u. 594 nm	513 a.u. 589 nm	645 a.u. 584 nm	No response to analyte
Caffeine/theophylline	CdSe/ZnS/4-vinylpyridine/EGDMA	906 a.u. 556 nm	14.75 a.u. 594 nm	513 a.u. 589 nm	760 a.u. 587 nm	No response to analyte
Uric acid/uric acid	CdSe/ZnS/4-vinylpyridine/EGDMA	548 a.u. 562 nm	11.7 a.u. 585 nm	145/168 a.u. 581 nm	14.89 a.u. 586 nm	No response to analyte
L-Cysteine/L-cysteine	CdSe/ZnS/4-vinylpyridine/EGDMA	110 a.u. 584 nm	1.33 a.u. 589 nm	14.10 a.u. 587 nm	1.59 a.u. 586 nm	No response to analyte
Estriol/estriol	CdSe/ZnS/4-vinylpyridine/EGDMA	665 a.u. 594 nm	23.4 a.u. 566 nm	38.62 a.u. 603 nm	17.24 a.u. 535 nm	No response to analyte

[a] Template was washed away from MIP.
[b] MIP without template was re-bound with template.

PL emissions from CdSe/ZnS core–shell nanoparticles were detected with bound template as well as in free form. The PL emission intensities of MIP with template were drastically reduced from the values of which the templates were removed from MIP (Table 1). The photoluminescence emission from CdSe/ZnS core–shell nanoparticles was quenched upon analyte binding to the receptor site indicated a successful recognition phenomenon. The re-binding experiment of quantum dot-MIPs with the print molecule showed a four- to five-fold reduction in emission intensity when caffeine was used as the print molecule (Fig. 2). Similar trends in the emissive behavior of CdSe/ZnS core–shell nanoparticles were observed with uric acid, L-cystein and estriolm, etc. as the templates (Table 1). The photoluminescences were measured in several solvents such as methanol, tetrahydrofuran, chloroform and water as well. The largest response was obtained when water was used as the solvent. This is ideal for the detection of biological samples. Selectivity of the recognition sites was demonstrated for caffeine-imprinted polymers. The as made MIPs exhibited no response to caffeine analogues such as theophylline and theobromine, in terms of PL emission quenching. The emission intensity of quantum dot-MIP is unaffected with these molecules. All the imprinted polymers exhibited much higher template recognition toward the corresponding print molecule than its analogous structures, while a control polymer (NIP)

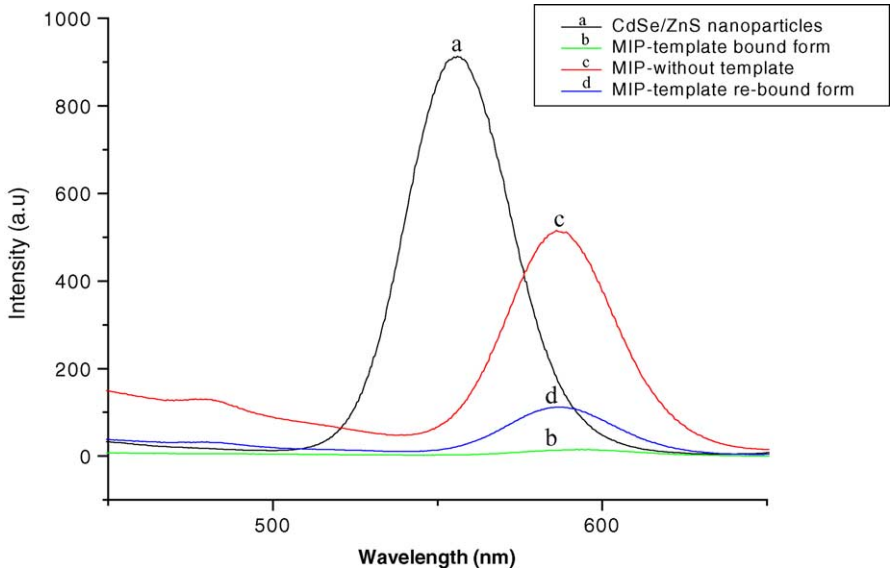

Fig. 2. Comparison of PL emission spectrum of the CdSe/ZnS with those of caffeine imprinted MIP in the template bound, free and re-bound forms.

did not show any change in photoluminescence emission derived from the quantum dots.

Re-binding experiment was also conducted for a solution containing caffeine, theophyllline and theobromine. When the caffeine-imprinted MIP was incubated with a mixture with 66 ppm each of the above three compounds, the quenching of photoluminescence was diminished to only a limited extent. This is presumably due to the blocking of the receptor sites by the analogous structures and hence hindering caffeine molecules to enter the actual recognition sites. Sensor responses in terms of quenching of photoluminescence emission from CdSe/ZnS for several different molecules such as uric acid, L-cysteine, estriol upon binding with the corresponding MIPs are also tabulated in Table 1. Higher responses for uric acid, L-cysteine and estriol can be explained due to their higher hydrophilicities as compared with caffine and its analogous, which help them to enter the imprinted hydrophilic recognition sites.

The optimized results led us to conclude that the template binding to receptor sites of the host polymer significantly affects the intensity of photoluminescence emission from quantum dots due to the fluorescence resonance energy transfer (FRET), which involves radiation-less energy transfer that occurs between CdSe/ZnS and bound guest molecule. This phenomenon can be exploited in enzyme-linked immunosorbent assay (ELISA) type assay applications. These observations of quenching of fluorescence using the core–shell layered solid structure of MIPs are qualitatively similar to the homogeneous soluble cases of biomolecules explored elsewhere [13].

4. Conclusion

In conclusion, this work demonstrates that the analyte-dependent emissive properties of quantum dots coupled with selective recognition capacity of molecularly imprinted polymers opens a straightforward methodology for making optical chemical sensors.

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