

## 基于铈配合物的荧光分子印迹的制备及其 选择性检测痕量 2,4,6-三氯苯酚

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**摘要:** 设计并制备了用于检测 2,4,6-三氯苯酚的荧光分子印迹。由于铈配合物对模板分子的荧光淬灭作用, 可用于荧光检测工具。荧光分子印迹在模板分子浓度范围 0~70 nmol·L<sup>-1</sup> 内具有良好的荧光线性响应, 相关系数为 0.996 28。荧光分子印迹展现出了良好的灵敏度, 其最低检出限为 3.12 nmol·L<sup>-1</sup>。荧光分子印迹具有较好的热稳定性, 同时选择性检测实验证明荧光分子印迹对 2,4,6-三氯苯酚具有良好的特异选择性。实验结果说明荧光分子印迹可用于选择性荧光检测低浓度氯酚类物质。

**关键词:** 铈配合物; 荧光检测; 分子印迹聚合物; 2,4,6-三氯苯酚

中图分类号: O614.33·8

文献标识码: A

文章编号: 1001-4861(2018)08-1531-07

DOI: 10.11862/CJIC.2018.169

## Synthesis of Fluorescent Molecularly Imprinted Polymers Based on Europium(III) Complex for Selective Determination of Trace 2,4,6-Trichlorophenol

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**Abstract:** Fluorescent molecularly imprinted polymers (FMIPs) for selective determination 2,4,6-trichlorophenol were devised and fabricated. Here, europium(III) complexes were used as fluorescence detection tool via the fluorescence quenching with templates. The FMIPs have a linear fluorescent response in 0~70 nmol·L<sup>-1</sup> concentration range, and the correlation coefficient was 0.996 28. The FMIPs exhibit excellent sensitivity with the limit of detection of 3.12 nmol·L<sup>-1</sup>. In addition, FMIPs show good thermal stability and appreciable selectivity over several analogues. The experimental results showed that FMIPs could be used for selective fluorescence detection of low concentrations of chlorophenols.

**Keywords:** europium complex; fluorescence detection; molecularly imprinted polymer; 2,4,6-trichlorophenol

收稿日期: 2018-03-05。收修改稿日期: 2018-04-18。

国家自然科学基金(No.21576112), 吉林省自然科学基金(No.20180101181JC), 吉林省科技创新建设项目(No.20180623042TC), 吉林省科技发展计划项目(20150623024TC-19), 吉林省人力资源和社会保障厅项目(No.2017956)和中国博士后基金(No.2017M611732)资助。

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## 0 Introduction

Chlorophenols (CPs) are a kind of synthetic organic compounds which are proved to have adverse effects on humans. CPs are included as priority pollutants by the US Environmental Protection Agency in 2007<sup>[1]</sup>. CPs are considered as endocrine disruptors; CPs and their derivatives would lead carcinogenicity, genotoxicity and mutagenicity to human body<sup>[2]</sup>. Through mother to child transmission, CPs can cause prenatal CPs exposures in utero<sup>[3-4]</sup>. CPs can be used in synthesis insecticides, wood preservatives, bactericides, fungicides, herbicides and process intermediates. They are distributed throughout the environment and have been detected in sludge products, surface waters, wastewater, groundwater and various types of human biological samples<sup>[5-6]</sup>. CPs include mono-, di-, tri-, tetra- and penta-chlorinated phenols, and 2,4,6-trichlorophenol (2,4,6-TCP) is a representative characteristic of CPs and have been studied extensively as a target substance for catalysis<sup>[7]</sup>, detection<sup>[8]</sup> and adsorption<sup>[9]</sup>. Existing detection technology for 2,4,6-TCP have some shortage such as complex operations, lacking of detection accuracy or anti-interference. Therefore, it's necessary to find an affordable, highly sensitive and selective technique to detect the trace target in complex environment.

Fluorescence analysis is a useful detection technique, it can be used to detect inorganic matter, organic matter, ion and biomolecules based on fluorescence spectroscopy or fluorescence colorimetry<sup>[10-13]</sup>. In recent years, various of materials have been used as fluorescence sensing such as quantum dots, organic dyes and lanthanides<sup>[14-16]</sup>. Due to their unique 4f electrons, lanthanides relative to other fluorescent materials have high luminescence quantum yield, narrow bandwidth, long-lived emission and large Stokes shifts. As they have an excellent signal-to-noise ratio which improved detection sensitivity, low toxicity, high extinction and variety colors, lanthanides can be widely used in biological fluorescence related applications<sup>[17-18]</sup>. Furthermore, exclusive near infrared radiation leads to less photodamage of the biological

samples and more efficiently penetrate in tissues than those in the visible range<sup>[19]</sup>. Based on these features, lanthanides have been designed as coordination compounds, chelate compounds and metal-organic frameworks for probe, energy storage, catalysis, magnetic, luminescent materials<sup>[18,20-21]</sup>. Though lanthanides showed excellent luminescent properties for detection, their thermal and chemical stability still need to improve for adapting complex sample environment.

Molecular imprinted polymers (MIPs) are highly selective polymeric materials with good thermal, chemistry and mechanical stability that have been widely applied to various fields<sup>[22]</sup>. According to the different forms such as nanoparticles, nanocapsules, nanorods, nanotubes and membranes, MIPs have been employed in a variety of applications including food analysis, contamination detection, diagnosis and targeted treatment, membrane separation and proteomic analysis<sup>[23-24]</sup>. Traditional MIPs have low detection sensitivity, and the recognition process of target need simplification, so fluorescence analysis has been introduced to improve the performance of MIPs<sup>[25-26]</sup>.

Fluorescence quenching between fluorophores and target molecule can realize the transformation of chemical signals to optical signals. Fluorescent molecularly imprinted polymers (FMIPs) are a kind of optical sensors with high detection sensitivity and rapid detection capability. In recent years, FMIPs are widely used in sample and biomarkers detection<sup>[27-29]</sup>.

In the present study, FMIPs had been synthesized through precipitation polymerization with the specific recognition sites for 2,4,6-TCP molecules. The fluorescence was provided by highly luminescent Eu(MAA)<sub>3</sub>phen coordination compounds. The obtained composite materials were characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron microscope (SEM), photoluminescence (PL) and thermogravimetric analysis (TGA). The FMIPs were applied as sensors to detect 2,4,6-TCP based on their luminescence, and the results showed that this type of sensor is suitable for detecting 2,4,6-TCP; the emission intensity of Eu<sup>3+</sup> decreased with the increasing concentration of 2,4,6-TCP in the system, making the

chlorophenols residues be easily tracked, identified and monitored by the change of luminescence.

## 1 Experimental

### 1.1 Reagents and chemicals

Acetonitrile ( $\geq 99.0\%$ ), ethyl alcohol ( $\geq 99.7\%$ ) hydrochloric acid (HCl), and  $\text{Eu}_2\text{O}_3$  ( $\geq 99.99\%$ ) were purchased from Sinopharm Chemical Reagent Co (Shang Hai, China). Ethylene glycol dimethacrylate (EGDMA), 2,2'-azobis (2-methylpropionitrile) (AIBN), methacrylic acid (MAA) were all obtained from Aladdin Chemistry Co. Ltd (Shanghai, China). Double distilled water was prepared and used for cleaning processes. All other chemicals used were analytical grade and obtained commercially.

### 1.2 Instruments

Infrared spectra ( $4\ 000\sim 400\text{ cm}^{-1}$ ) were obtained on a Nicolet iS50 FTIR apparatus (Thermo Scientific). The morphologies and sizes of FMIPs and FNIPs were measured using a SEM (JEOL, JSM-7800F). Fluorescence intensity of the samples was observed by F-4600 FL-SPECTROPHOTOMET (HITACHI). TGA was obtained on a STA 449 F3 (NETZSCH).

### 1.3 Synthesis

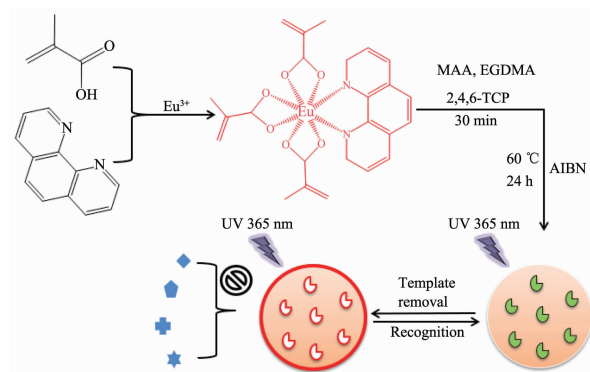
#### 1.3.1 Preparation of $\text{Eu}(\text{MAA})_3\text{phen}$

$\text{Eu}(\text{MAA})_3\text{phen}$  were synthesized by the co-precipitation method<sup>[30]</sup>. Solution of europium chloride ( $0.25\text{ mol}\cdot\text{L}^{-1}$ ) was prepared from high purity  $\text{Eu}_2\text{O}_3$  by dissolving in concentrated hydrochloric acid ( $6\text{ mol}\cdot\text{L}^{-1}$ ). MAA in anhydrous ethanol (15 mL) was added dropwise into the stirred solution of europium chloride in anhydrous ethanol (30 mL), and then the pH value of the mixture was adjusted to 8 using ammonium hydroxide. The mixture was stirred for 30 minutes, and then the solution of 1,10-phenanthroline monohydrate (phen) in anhydrous ethanol ( $0.1\text{ mol}\cdot\text{L}^{-1}$ , 15 mL) was added dropwise. The resulting solution was stirred at room temperature for 3.0 h and standing overnight. The precipitate was filtered and washed with anhydrous ethanol by 3 times, and finally dried at  $80\text{ }^\circ\text{C}$  to obtain  $\text{Eu}(\text{MAA})_3\text{phen}$ .

#### 1.3.2 Preparation of FMIPs and FNIPs

FMIPs and FNIPs were both prepared via

precipitation polymerization according to the following procedure.  $\text{Eu}(\text{MAA})_3\text{phen}$  (50mg) were added to a mixture of MAA (40  $\mu\text{L}$ ), EGDMA (100  $\mu\text{L}$ ), a certain amount of 2,4,6-TCP and acetonitrile (60 mL). The mixture was ultrasonically degassed for 5 min. Self-assembly was performed in a water bath orbital shaker at  $40\text{ }^\circ\text{C}$  for 3.0 h under nitrogen protection. The temperature subsequently rose to  $60\text{ }^\circ\text{C}$ , and 30 mg of AIBN was added to initiate polymerization for 24 h. After polymerization, the mixture was centrifuged at  $3\ 700\text{ r}\cdot\text{min}^{-1}$  for 5 min, and the suspension was removed. The solid particles were then washed with ethanol and water three times. Then the polymers were eluted with methanol/acetonitrile (4:1, V/V) to remove the templates, and then dried in a vacuum chamber. FNIPs were used as a control to evaluate the molecular recognition properties of imprinted materials. The FNIPs were synthesized in the same manner as FMIPs but without the addition of 2,4,6-TCP. The synthesis routes of FMIPs were shown in Scheme 1.



Scheme 1 Schematic Illustration of the fabrication of FMIPs

#### 1.3.3 Fluorescence detection of 2,4,6-TCP

The fluorescence of FMIPs and FNIPs was detected with the excitation wavelength of 335 nm. In brief, 50 mg FMIPs were dispersed in 100 mL volumetric flask with ethyl alcohol solution, then the solution was preserved in the dark. Afterwards, 5.0 mL FMIPs solution was mixed with 5.0 mL of 2,4,6-TCP solutions with different concentrations, respectively. In order to adequately adsorb on target molecule, the mixed solution was dispersed with

ultrasonic concussion for 20 min. Then, the fluorescence intensity of mixture was measured. Moreover, the fluorescence intensities of FNIPs with 2,4,6-TCP solutions with different concentrations were obtained in the similar procedure.

## 2 Results and discussion

### 2.1 Characterization of FMIPs and FNIPs

Fig.1 (a,b) compares SEM images of FMIPs and FNIPs, whose size, morphology and dispersibility are

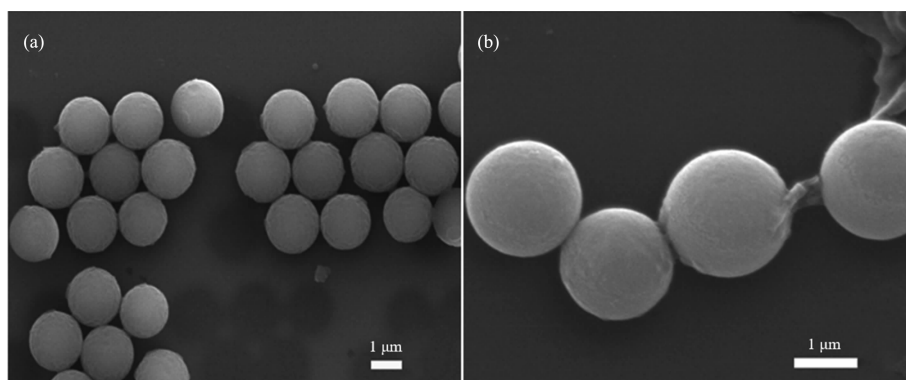


Fig.1 SEM images of FMIPs (a) and FNIPs (b)

The infrared spectra of  $\text{Eu}(\text{MAA})_3\text{phen}$ , FMIPs and FNIPs are shown in Fig.2. The feature peaks at  $1\,568$  and  $1\,428\text{ cm}^{-1}$  from  $\text{Eu}(\text{MAA})_3\text{phen}$  could be attributed to  $-\text{COO}-$  stretching vibration, which suggests that the  $-\text{COOH}$  from MAA is involved in the coordination, and the feature peaks at  $1\,646\text{ cm}^{-1}$  conforms to the  $\text{C}=\text{C}$  stretching mode of MAA vinyl groups. Meanwhile, FMIPs and FNIPs have the same peaks at  $1\,729$ ,  $1\,254$  and  $1\,155\text{ cm}^{-1}$ , which indicated  $\text{C}=\text{O}$

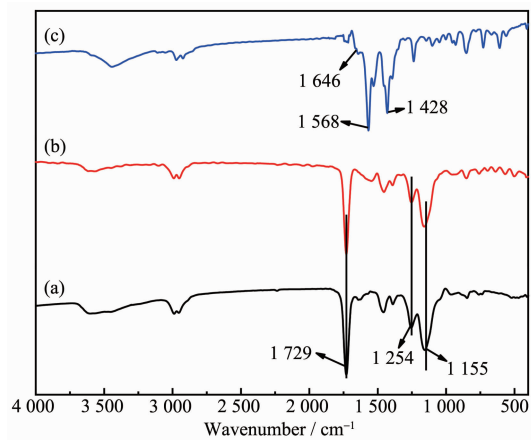


Fig.2 FTIR spectra of FMIPs (a), FNIPs (b) and  $\text{Eu}(\text{MAA})_3\text{phen}$  (c)

clearly observed. As exhibited in Fig.1(a), the size of FMIPs is  $1.5\sim 2\text{ }\mu\text{m}$  and the monodispersity is excellent with a regular appearance. Moreover, the FNIPs in Fig.1(b) have shown that the range of size is  $1.5\sim 2\text{ }\mu\text{m}$ . Both of the two species have a smooth surface. In brief, it demonstrated that microspheres of FMIPs and FNIPs have been successfully prepared and the molecularly imprinted microspheres were better than FNIPs in term of size and monodispersity.

stretching vibration of carboxyl,  $\text{C}-\text{O}$  asymmetric and symmetric stretching vibration of ester from EGDMA, respectively. All the results confirm that precipitation polymerization reaction was successfully initiated.

Thermal stability of  $\text{Eu}(\text{MAA})_3\text{phen}$  (black), FMIPs (blue) and FNIPs (red) were evaluated by TGA under  $\text{N}_2$  atmosphere (Fig.3). As shown in Fig.3, the prepared nanospheres demonstrate excellent thermal stability at temperatures lower than  $200\text{ }^\circ\text{C}$ . The TGA curve of FMIPs shows a high rate of weight loss about 90.53% at  $600\text{ }^\circ\text{C}$ , as compared to 95.38% of FNIPs. The tiny

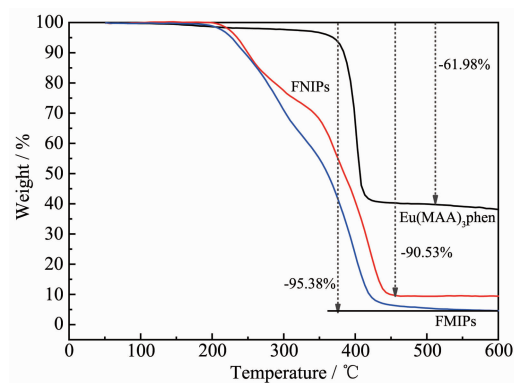


Fig.3 TGA curves of  $\text{Eu}(\text{MAA})_3\text{phen}$ , FMIPs and FNIPs

weight loss differences of FMIPs and FNIPs are due to that the templates have combined more europium complexes. The TGA results suggest that FMIPs have excellent thermal stability in nature environment.

## 2.2 Fluorescence detection of 2,4,6-TCP@FMIPs

In this study, our aim is to demonstrate the recognition ability of FMIPs for 2,4,6-TCP and we are able to intuitively observe the detection effect. Because 2,4,6-TCP molecular added into the FMIPs solvent could give rise to decline of fluorescence intensity, we concluded FMIPs could detect 2,4,6-TCP molecule. As shown in Fig.4, the fluorescence intensities of both FMIPs and FNIPs are gradually quenched with the increasing concentration of 2,4,6-TCP. Moreover, it was observed that fluorescence intensity of FMIPs declines more obviously than that of FNIPs, which indicates that FMIPs have a more excellent detection capacity with 2,4,6-TCP compared with FNIPs.

To obtain precisely the detection range of synthetic material, the quenching efficiency of micro-

spheres was evaluated by the Stern-Volmer equation:

$$I_0/I = 1 + K_{sv}C \quad (1)$$

$I_0$  is the initial fluorescence intensity without analyte.  $I$  is the fluorescence intensity in the presence of analyte with the concentration of  $C$  ( $0 \sim 100 \text{ nmol} \cdot \text{L}^{-1}$ ), and  $K_{sv}$  ( $\text{L} \cdot \text{mol}^{-1}$ ) is the quenching constant with 2,4,6-TCP.

To avoid the contingency of linear relation, the uniform experiment processes were repeated five times and acquired Fig.5 by their average. As manifestation in Fig.5(a), the excellent linear relationship of Stern-Volmer equation appears in the range of  $0 \sim 70 \text{ nmol} \cdot \text{L}^{-1}$ . The linear equation of FMIPs is  $I_0/I - 1 = 0.00889C + 0.01277$  and the corresponding correlation coefficient was  $R^2 = 0.99628$ . Hence, the limit of detection (LOD) was found to be  $3.12 \text{ nmol} \cdot \text{L}^{-1}$ , which was calculated with the equation:  $\text{LOD} = 3\sigma/S$  ( $\sigma$  was the standard deviation of the blank signal and  $S$  was the slope of the linear calibration plot). In addition, the linear relationship of FNIPs were shown in Fig.5(b) and

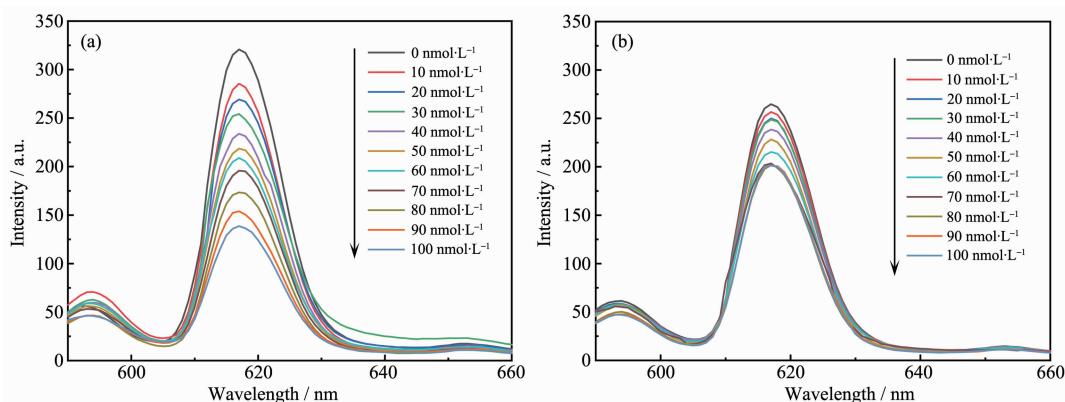


Fig.4 Response of (a) FMIPs and (b) FNIPs to 2,4,6-TCP in the concentration range from 0 to  $100 \text{ nmol} \cdot \text{L}^{-1}$

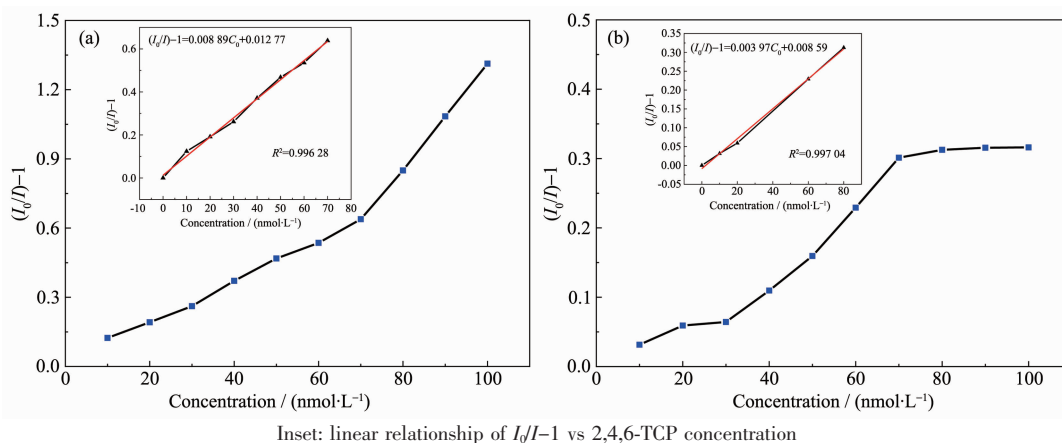


Fig.5 Dependence of fluorescence quenching efficiency on 2,4,6-TCP concentration



linear equation was  $I_0/I-1=0.003\ 97C-0.008\ 59$ , with the concentration range of  $0\sim80\ \text{nmol}\cdot\text{L}^{-1}$  and  $R^2=0.997\ 04$ .

### 2.3 Selectivity determination of FMIPs and FNIPs

Several analogs with the similar structure including 2,4-DCP, 2,5-DCP, 2,6-DCP were chosen as chaff interferent to investigate the selectivity determination of FMIPs further. In brief,  $0.5\ \text{g}\cdot\text{L}^{-1}$  of FMIPs (5 mL) were dispersed in  $20\ \text{nmol}\cdot\text{L}^{-1}$  of 2,4-DCP, 2,5-DCP, 2,6-DCP and 2,4,6-TCP (5 mL), respectively, which were vibrated with ultrasonic for 20 min.

In Fig.6, it is obviously observed that the quenching efficiency of FMIPs for 2,4,6-TCP is superior to that of FNIPs. Moreover, the quenching efficiency for 2,4,6-TCP is also much better than those for other chlorophenols. To sum up, a good selectivity determination of FMIPs for 2,4,6-TCP was well illustrated without interference of other chlorophenols.

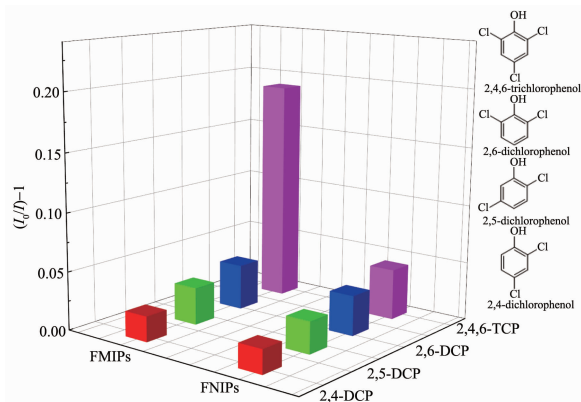


Fig.6 Quenching amount of FMIPs and FNIPs by different kind of chlorophenols

### 3 Conclusions

In summary, we presented a new application for the use of europium complexes as fluorescent functional monomer in the synthesis of FMIPs. The newly prepared FMIPs are an efficient and selective detection method for trace level 2,4,6-TCP, which was successfully discriminated from other chlorophenols. The FMIPs have a linear fluorescent response in  $0\sim70\ \text{nmol}\cdot\text{L}^{-1}$  concentration range, and the correlation coefficient is 0.996 28. The FMIPs show excellent sensitivity and the limit of detection is  $3.12\ \text{nmol}\cdot\text{L}^{-1}$ .

In addition, FMIPs show good thermal stability and appreciable selectivity over several analogs. Further studies are expected to design a multifunctional sensor based on europium complexes. In addition, our study have provided a new way to fabricate FMIPs with potential application in the recognition and sensitive sensing of analytes. In principle, this technique offers a general approach to fluorescence detection of chlorophenols which quench fluorescence in complex systems.

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