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Study on the Construction of Chemical Molecular Imaging Probes

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The chemical molecular probe belongs to chemical sensors, and they mainly use fluorescence to convey the change of chemical information produced by analytic target and sensitive molecules. The transfer of this information has the advantages of high sensitivity and high selectivity, and only needs a little samples. It is widely used in chemistry analysis, especially in biological analysis. The labeling reagents with strong fluorescent or the reagents generated by fluorescent can be used for the object to be tested to make it have fluorescent labeling characteristic, which will greatly improve the detection sensitivity and reduce the detection limit. The data show that the sensitivity detected by this method can reach 10-9, and the lowest has reached 10-12, which is the fluorescent probe technology. It has many advantages such as high selectivity, high sensitivity, simple operation, high accuracy and simple equipment. In recent years, many researchers have paid attention to it. In this paper, the small molecule fluorescent probe with thiophene [2, 3-b] thiophthene as fluorophore is designed, because thiophthene is a large conjugated system, the electron cloud density is relatively high, and it has hole transport ability and good optical properties. We chose benzothiazole as the main recognition perssad. In the study, we also use the chloroacetyl chloride reagent to adjust the distance between luminophor and the recognition objects, and to increase the number of atoms with coordination of probe molecules, and hope to strengthen complexing power of them with metal ion. The results show that when the recognition object is near the luminophor, the molecular probe has significant double recognition effect on Zn^{2+} ion and Hg^{2+} ion, and when they are far away from each other, it only has the recognition effect on the Hg^{2+} ion.

1. Introduction

In recent years, the detection technology of mercury ion and zinc ion is developing rapidly, and the reason is that they will have a great impact on life. As we all know, mercury is a highly toxic element (Cheng, et al., 2014), which will produce biological accumulation in the human body, thereby affecting the body's genes, immune system and nervous system, and endangers human health (Selvaraj et al., 2015; Busto et al., 2016; Syafiqah et al., 2017). Therefore, it is urgent to design a good mercury ion probe. On the other hand, zinc plays an indispensable role in the biological process, especially plays an important role in the immune function and brain function of the human body. If the static balance of the content of zinc in the body is broken (Lin et al., 2015), it will cause a series of diseases on the human body, such as anemia, cancer, diabetes, etc., which will seriously endanger human health (Pu et al., 2014).
Furan, thiophene and pyrrole these five membered heterocycle compounds all have aromaticity, because there are six π electrons in the ring, and they are adapted to the Hückel rules. In addition, the electron cloud density of thiophene is higher than that of the other two, which make the thiophene have better hole transport and optical properties. In this chapter, we choose thiophene [2, 3-b] and thiophthene as fluorophore (Kowada et al., 2015). Benzothiazole is a heterocyclic compound containing nitrogen, in addition to the introduction of sulfur atoms, which makes it appear more unique properties, such as: fluorescence characteristics, recognition properties, etc. In this study, the research of benzothiazole compounds started after 2-Chlorobenzophiazole was synthesized by Hoffman in 1879. Many scholars began to pay attention to benzothiazole compounds and to study them (Park, et al., 2015). By referring to many literatures, we find that benzothiazole not only has good

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fluorescence performance, but also has recognition performance, and the most characteristic is that it will participate in the reaction in some recognition process, that is, the reaction type fluorescent probe (Su, et al., 2017). An article published by MithunSantra et al in 2011 is about "reaction" proportion type fluorescence probe which has good detection effect on the mercury ion, the "reaction" proportion type fluorescence probe can detect mercury ion. The main reason is that mercury ions can promote the vinyl ether derivatives were hydrolyzed in buffer solution, and the reaction is quick, and the other metal ions had no such effect. When the mercury ion was added, the fluorescence emission peak of the vinyl ether compound appeared about 80nm red shift, which led to the color of compound changed from blue to cyan (Seddon and Workman, 2014; Miccio et al., 2017; Qiao, 2016; Zhao and Yue, 2016; Derian et al., 2016).
ArvindMisra et al. synthesize the compounds containing benzothiazole. In acetonitrile solvent, the compounds have better recognition ability on Cu^{2+} and Hg^{2+} ions, after the Cu^{2+} and Hg^{2+} ions are added, the UV absorption peak has obvious redshift, and the color of the solution was changed from yellow to blue and pink, which can an be seen with the naked eye and without using the instrument. For fluorescence recognition, Hg^{2+} ions can make fluorescence intensity increase, and Cu^{2+} ion has a strong quenching effect on the fluorescence emission. The naphthol OH in compounds, double bond N and thiophene N play an important role. The UV recognition effect of this compound is quite obvious, but the unicity of the fluorescent identification is not ideal.
Therefore, we design a small molecule fluorescent probe with thiophene [2, 3-b] thiophthene as fluorophore. Because thiophthene is a large conjugated system, the electron cloud density is relatively high, and it has hole transport ability and good optical properties. We chose benzothiazole as the main recognition perssad. In the study, we also use the chloroacetyl chloride reagent to adjust the distance between luminophor and the recognition objects, and to increase the number of atoms with coordination of probe molecules, and hope to strengthen complexing power of them with metal ion.

2. Experimental section

2.1 Experimental reagents and instruments

Table 1 shows the experimental reagents and table 2 shows the experimental instruments.

Table 1: Experimental reagents

Experimental drug	Drug purity	Manufacturer
Tetrahydrofuran	AR	Guangdong Xilong Chemical Co., Ltd.
Lithium aluminium hydride	AR	Shanghai Han Xiang Biotechnology Co., Ltd.
2- mercaptobenzothiazole	AR	Shanghai Leji Biochemical Technology Co., Ltd.
Anhydrous potassium carbonate	AR	Tianjin Yongda Chemical Reagent Development Center
Potassium iodide	AR	China Medicine and Health Products Co.,Ltd. (Beijing branch)
Acetone	AR	Hengyang Kaixin Chemical Reagent Co. Ltd.
Three ethylamine	AR	Yabang chemical (Shanghai) Co., Ltd.
4- dimethyl pyridine	AR	Ningbo Sailun Chemical Co. Ltd.
Chloroacetyl chloride	AR	Shanghai Leji Biochemical Technology Co., Ltd.
Dichloromethane	AR	Tianjin Fuyu Fine Chemical Co., Ltd.
Sodium bicarbonate	AR	Shanghai Puzhen Biological Technology Co., Ltd.

2.2 Preparation of molecular probes

(1) Preparation of 3, 4-dimethyl -2, 5- demethyl carbinol thiophene [2, 3-b] thethiophthene:
250mL tetrahydrofuran and 5g (131mmol) $LiAlH_4$ were added to 1000mL three flasks, then 7.8g (25mol) 3, 4-dimethyl -2, 5- dioctyl phthalate diethyl ester thiophene [2, 3-b] thethiophthene were dissolved in tetrahydrofuran, and added dropwise into the first solution, dropping for 1 hour, stirring at room temperature for 24h. After cooling with ice bath, 5mL deionized water and 5mL 15% NaOH solution were added to them, finally again 20mL deionized water was added, vacuum filtration, and the filtrate was taken out, and the rotary evaporator was used to do reduced pressure distillation to get the product.
(2) Preparation of 3, 4-dimethyl -2, 5- dichlorne thiophene [2, 3-b] thethiophthene:
1.14g (5mmol) 3, 4-dimethyl -2, 5- demethyl carbinol thiophene [2, 3-b] thethiophthene was dissolved in dry CH_2C_{l2}, then 1.0mL SOC_{l2} (13.6mol) heavy steam was dropwise added in -5 to -10 °C, stirring at room temperature above 5h, and the brown solid will be obtained after spinning dry. The petroleum ether with low boiling was used for recrystal, and the supernatant was taken for cold storage one night, and then the solid will be obtained.
(3) Preparation of chemical molecular probe:

0.29g (0.76mol) compounds (2), 0.34g (2mol) 2- mercaptobenzothiazole, 0.279g (2mol) K_2CO_3 and 0.48g (2.9mmol) catalyzer KI were placed in 50mL round bottom flask. 20mL acetone was added into it after three times of replacement of nitrogen, and the reactant will be refluxing overnight under the protection of nitrogen. The mixture was poured into a large amount of ice water, and then filtration. The filter cake was dried in vacuum after washed three times, and then the products will be obtained.

Table 2: Experimental instruments

Instrument name and type	Manufacturer
DF-101B thermal-arrest test constant temperature magnetic stirrer	Gongyi Ying Yu Hua Instrument Factory
RE-85A rotary evaporator	Gongyi Ying Yu Hua Instrument Factory
JJ-1Precision timing electric stirrer	Jintan ronghua Instrument Manufacturing Co., Ltd.
SHB-B type circulating water multipurpose vacuum pump	Zhengzhou Changcheng Instrument Factory
SHZ-D (III) type circulating water vacuum pump	Gongyi Ying Yu Hua Instrument Factory
AB204-N electronic analytical balance	Mettler-Toledo Instrument Co., Ltd.
Frame-pan balance	Zhejiang Dong'ou Instrument Factory
Three ultraviolet analyzer	Shanghai Gu village electro optical instrument factory
CL-2 type constant temperature heating magnetic stirrer	Gongyi Ying Yu Hua Instrument Factory
Nuclear magnetic resonance (400MHz)	Bruker AVANCE company
Agilent 7890A GC / 5975C MSD(EI) GC-MS	Agilent company
Bruker auto flex smart beam MALDIⅢ-TOF MS	Bruker company
Vario EL III elemental analyzer	Vario company
Lambda 25 Ultraviolet visible spectrophotometer	Pekin-Elmer company
LS 55 fluorescence spectrophotometer	Pekin-Elmer company

3. Performance test and result analysis

Through the figure1 we can more directly observe that the absorption intensity of the fluorescent probe has greatly changes before and after adding Hg^{2+} ion, so the fluorescent probes have excellent specific recognition ability for Hg^{2+} ion.

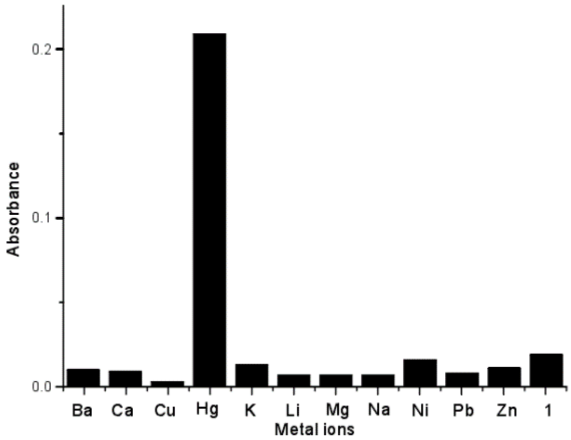

Figure 1: UV absorption histogram of chemistry molecular probe (2x105) at 546nm after adding different metal ions (50 times the amount of metal ions) in acetonitrile solvent

In order to make better use of the fluorescent probe, we need to research the environment and the impact of other metal ions, so we carry out competitive experimental study of chemical molecular probe (as shown in figure 2). The concentration of the chemical probe is 2×10-5M, and the concentration of the target metal ions and other competing metal ions are about the 50 times the amount, and the testing environment is the pure acetonitrile solvent. It can be seen from the picture that other metal ions have little effect on the recognition of Hg2+ ions, which shows that this kind of probe may have good anti-interference ability.

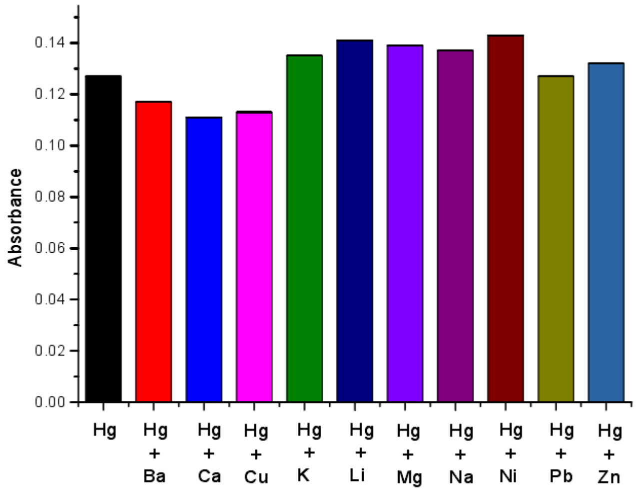

Figure 2: UV absorption histogram of chemistry molecular probe (2×10-5M) after adding different metal ions (50 times the amount of Hg2+,50 times the amount of the other metal ions) in acetonitrile solvent

We mix the Zn^{2+} ions with many other metal ions such as Ba^{2+}, Ca^{2+}, Cu^{2+}, Hg^{2+}, K^{+}, Li^{+}, Mg^{2+}, Na+, Ni2+, Pb^{2+}, respectively, and then added them to the acetonitrile solution of chemical molecular probe to determine their fluorescence emission spectra. The purpose of that is to study that if the metal ions have interfere to the recognition of chemical molecular probe on Zn^{2+} (competitive experiment). As shown in figure 3, it can be observed that although Ca^{2+} and Hg^{2+} ions have a certain influence on the fluorescence emission of the chemical probe, it will not have fluorescence quenching. To sum up, the fluorescent probe has good selectivity and good anti-interference ability.

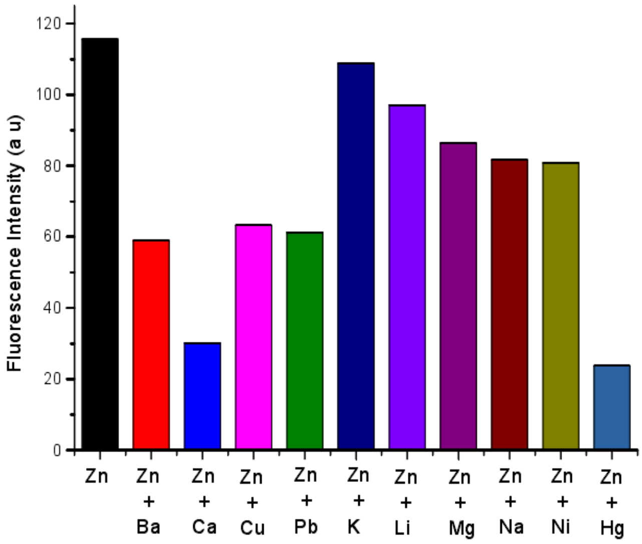

Figure 3: Fluorescence emission of of chemistry molecular probe (2×10-6M) after adding different metal ions (50 times the amount of Hg2+,50 times the amount of the other metal ions) in acetonitrile solvent

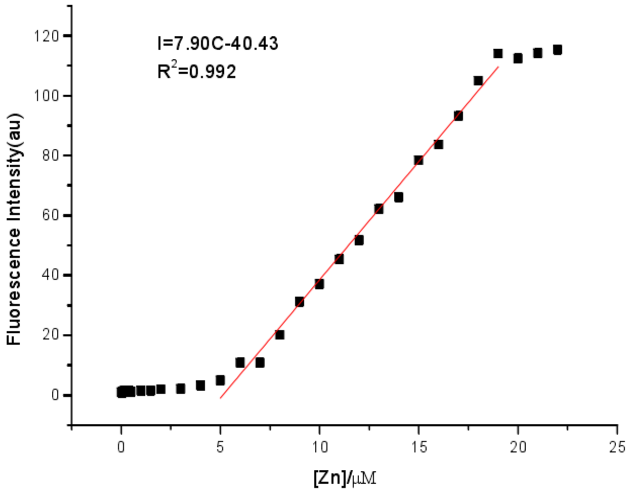

Figure 4: Variation of the molecular probe (2×10-6M) with the amount of Zn2+ ions in pure acetonitrile solvent

Figure 4 is the variation of the chemical molecular probe in the pure acetonitrile solvent with the amount of Zn^{2+} ions and, and it was calculated by the linear regression equation. The linear regression equation is: I=7.9c-40.43, R2=0.992. The results show that when the content of Zn^{2+}ion is 5-20 μm, the fluorescence emission of the compound is linear with the concentration of Zn^{2+} ion. We can conclude that the complexation of molecular probe which is taken as a fluorescent probe and Zn2+ ion is strong and stable.

4. Conclusion

In this paper, the small molecule fluorescent probe with thiophene [2, 3 -b] thiophthene as fluorophore is designed, because thiophthene is a large conjugated system, the electron cloud density is relatively high, and it has hole transport ability and good optical properties. We chose benzothiazole as the main recognition perssad. In the study, we also use the chloroacetyl chloride reagent to adjust the distance between luminophor and the recognition objects, and to increase the number of atoms with coordination of probe molecules, and hope to strengthen complexing power of them with metal ion. The results show that when the recognition object is near the luminophor, the molecular probe has significant double recognition effect on Zn^{2+} ion and Hg^{2+} ion, and when they are far away from each other, it only has the recognition effect on the Hg^{2+} ion.

Although the fluorescent probe as new detection technology has attracted much attention recently, because the current preparation of the fluorescence probe has bad reproducibility, low yield, high cost and other problems, causing its practicability is not very well, and the single identification ability for the object is poor. It is easy to be effected by the external environmental, and the selectivity is not high enough. Therefore, through the cross-over study of the subjects of analytic chemistry, supramolecular chemistry, organic chemistry, life sciences and organic chemistry, the design of probe molecule materials with high selectivity and high sensitivity according to the analysis of specific objects is one of the effective means to solve these problems. At the same time, the compound technology of the base material and the fluorescent substances should be optimized, which can promote the development of fluorescent probes towards miniaturization, fast and easy operation. We believe that fluorescent probe technology will certainly play a huge role in antibody immunity, environmental testing, medicine and food analysis and biological measurement field in the future.

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