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ORIGINAL PAPER

Synthesis and Characterization of New Light Emitter Symmetrical Phenoxazinium Salt and Its Potential Application as Sensor for Assessment of Hg^{2+}

M. S. Attia · A. O. Youssef · Abdel-Sattar S. H. Elgazwy · Samia A. El Abady · Samia M. Agami · Safaa I. Elewa

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Abstract The sensitization of the excited triplet state of a novel symmetrical Bis(dialkylamino)phenoxazinium salt was developed in the presence of Hg^{2+}. This effect was used to determine the concentration of Hg^{2+} in different water samples. The phenoxazinium salt sensor was characterized by different spectroscopic tools such as: UV, FTIR, NMR and fluorescence spectra. The sensor has an emission band at 347 nm in DMSO. Hg^{2+} in DMSO at pH 5.6 can remarkably quench the fluorescence intensity of the sensor at 347 nm and a new band was appeared at 436 nm due to the strong complex formation between Hg^{2+} and sensor. The quenching of the band intensity at 347 and the enhancement of the intensity of the new band at 436 were used to determine the Hg^{2+} in different waste water samples. The dynamic range found for the determination of Hg^{2+} concentration is 8.7×10^{-10} – 1.4×10^{-6} mol L^{-1} with a detection limit of 5.8×10^{-10} mol L^{-1} and quantification detection limit of 1.8×10^{-9} mol L^{-1}.

Keywords Hg^{2+} · Bis(dialkylamino)phenoxazinium salt · Sensor · Quenching · Fluorescence intensity

Introduction

Mercury is one of the most dangerous and ubiquitous of pollutants in the global environment, derives from both natural sources and human enterprise, and exists in a variety of forms (elemental, inorganic, and organic mercury). Mercuric ion (Hg^{2+}), much more common than mercurous ion (Hg^{+}), is a caustic and carcinogenic material with high cellular toxicity [1]. Methyl mercury, one primary form of organic mercury, can be formed naturally by bio-methylation of mercuric ion in the aquatic environment, is bio-accumulated in human body through biological food chains, and can cause brain damage and other chronic diseases [2, 3]. Therefore, it is important to monitor Hg^{2+}ions in many scientific fields, including medicine and environmental monitoring, etc.

In the past few years, many analytical methods such as atomic absorption spectrometry [4], inductively coupled plasma-mass spectrometry [5, 6], continuous flow cold vapor atomic fluorescence spectroscopy [7], inductively coupled plasma-atomic emission spectrometry [8], electrochemical methods [9–11], and UV–vis spectrometry [12] have been applied to detect the concentration of Hg^{2+}ions. Though these techniques are sensitive, selective, and accurate for Hg^{2+} assay, most of them are rather complicated, time-consuming, and costly as well as in- appropriate for on-line or field monitoring. Due to their advantages of simplicity, high sensitivity and low lost, a large number of fluorescent probes have been developed for the determination of mercuric ions so far. Hg^{2+} can cause fluorescence quenching of the fluorophores via the spin-orbit coupling effect [13], thus most of Hg^{2+} fluorescent probes were designed based on fluorescence quenching [14–19], which is very sensitive to the concentration of Hg^{2+} especially in the presence of the non-radiative environment or any source of quenching.

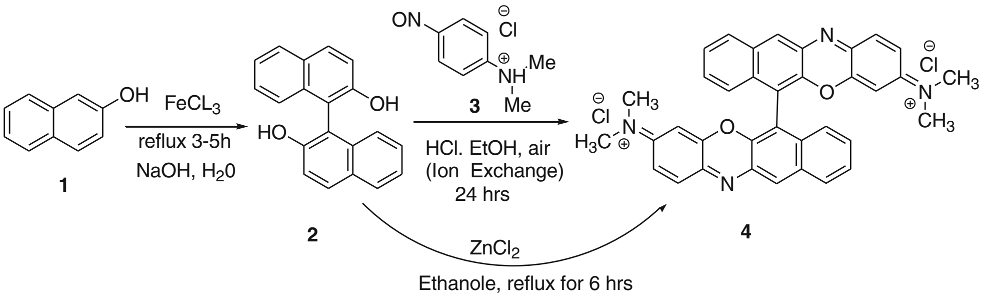
Because O, N, S binding sites might be a choice to be parts of a selective receptor for the selective recognition of

M. S. Attia (✉) · A. O. Youssef
Analytical Chemistry Division, Faculty of Science, Ain Shams University, Cairo, Egypt
e-mail: saidattiasam78@gmail.com

A.-S. S. H. Elgazwy
Organic Chemistry Division, Faculty of Science, Ain Shams University, Cairo, Egypt

S. A. El Abady · S. M. Agami · S. I. Elewa
Organic Chemistry Division, Faculty of Women, Ain Shams University, Heliopolis, P.O.Box 11757, Cairo, Egypt

Scheme 1 Preparation of Bis(dialkylamino)phenoxazinium



Hg^{2+}ions [17, 20–25], an oxygen-based functional group was considered and introduced to chemosensor in the present work. In this work a highly selective, sensitive and rapid response chemosensor Bis(dialkylamino)phenoxazinium salt in DMSO at pH 5.6 is used to assessment the mercury in different waste water samples.

Experimental

Materials and Reagents

All chemicals used are analytical-reagents of higher grade. 2-Naphthalen-2-ol, $ZnCl_2$, 4-nitroso-N,N-dimethylaniline HCl, ethanol (99.9 %) and (acetone /petroleum ether 40–60) were purchased from Sigma-Aldrich Company. Highly pure salts; $HgCl_2$, $BaCl_2$, $SrCl_2$, $CaCl_2$, $CdCl_2$, $CuCl_2$, $ZnCl_2$, $CoCl_2$, $MnCl_2$, $PbCl_2$, $NiCl_2$, $FeCl_2$ and $CrCl_3$, were purchased from Sigma-Aldrich Company. Distilled water and pure grade solvents from Sigma-Aldrich are used for the preparation of all solutions and during the determinations. A stock solution of metal salts ($1x10^{-4}$ mol l^{-1}) is directly prepared and dissolved in distilled water. The working standard solutions of metal salts (1 $x10^{-4}$ mol L^{-1}) are freshly prepared by appropriate dilution with DMSO. A stock solution of chemosensor ($1x10^{-3}$ mol L^{-1}) is directly prepared and dissolved in DMSO. The working standard solution of Bis(dialkylamino)phenoxazinium salt (2 $x10^{-5}$ mol L^{-1}) is freshly prepared by appropriate dilution with DMSO.

Instruments

All fluorescence measurements are carried out on Shimadzu RF5301 Spectrofluorophotometer in the range (290 – 750 nm). The absorption spectra are recorded with a Unicam UV-Visible double-beam spectrophotometer from Helios Company. It employs a Tungsten filament light source and a Deuterium lamp, which have a continuous spectrum in the ultraviolet region. The spectrophotometer is equipped with a temperature-controller cell holder. Mercuric ion was determined by cold vapor atomic absorption spectrometry (AAS), using Perkin Elmer FIAS 400. All pH measurements are made with a pHs-JAN-WAY 3040 ion analyzer.

General Procedure

Preparation and Characterization of Chemosensor

Preparation of 3,3'-bis-(dimethyl [6,6']bi[benzo [b]phenoxazinyl]-3,3'-ylidene) ammonium; chlorides **4** by a two steps sequence from reaction of (racemic)- 1,1'-Binaphthalenyl-2,2'-diol (1.00 g, 1 mol) and *p*-nitroso-N,N'-dimethyl aniline **3** (0.65 g, 1 mol) in 25 mL ethanol was added to the reaction mixture and complete the reflux for about 72 hrs in the presence of acid, followed by auto-oxidation by air. Each compound was purified by column chromatography on silica gel and, if necessary, through an ion-exchange process to afford **4** (1.60 g, yield 76 %) as a dark violet, m.p >300 °C IR (KBr) cm^{-1}: 3483, 3400, 3055, 1598, 1512, 1435, 1379, 1321, 1274, 1174, 1145, 1066, 820, 778.6.; ^1H NMR (CDCl3) δ 3.01 (s, 6H), 3.11 (s, 6H), 6.91-6.93 (d, 2H, $^3J_{H,H}$=8.7Hz, Ar-H), 7.16-7.22 (m, 10H),

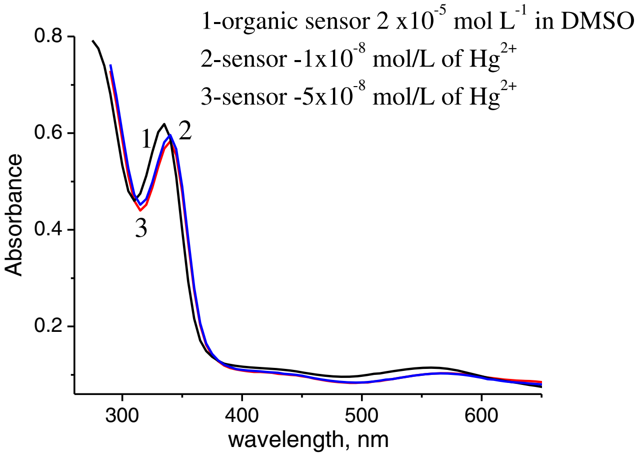


Fig. 1 Absorption spectra of 2×10^{-5} mol L^{-1} of Bis(dialkylamino)phenoxazinium in the presence of different concentrations of Hg^{2+} in DMSO

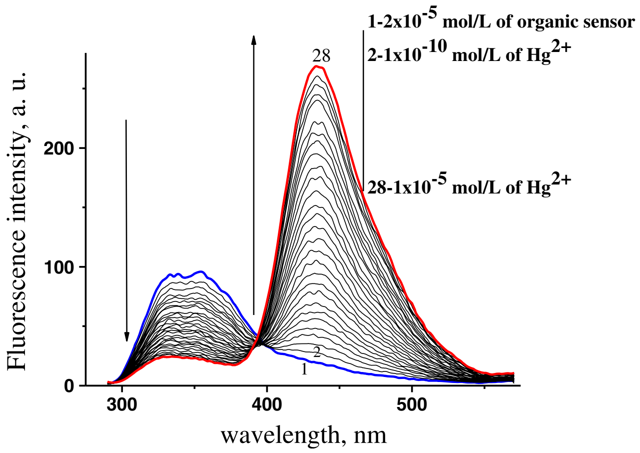


Fig. 2 Fluorescence emission spectra of 2×10^{-5} mol L^{-1} of Bis(dialkylamino)phenoxazinium in the presence of different concentrations of Hg^{2+} in DMSO at λ_{ex}=280 nm and pH=5.6

Table 1 Sensitivity and regression parameters for chemosensor

Parameter	Method
$\lambda_{\varepsilon m}$, nm	436
Linear range, mol L^{-1}	1.4×10^{-6}- 8.7×10^{-10}
Limit of detection (LOD), mol L^{-1}	5.8×10^{-10}
Limit of quantification (LOQ), mol L^{-1}	1.8×10^{-9}
Intercept (a)	25.0
Slope (b)	17.28×10^{8}
Standard deviation	0.30
Variance (Sa^2)	0.09
Regression coefficient (r)	0.99

7.31-7.33 (d, 2H, $^3J_{H,H}$=6.3 Hz, Ar-H), 7.83 –7-94 (d, 2H, $^3J_{H,H}$=5.4 Hz, Ar-H) . ^{13}C[^{1}H NMR] (75mHz, DMSO-d6), δ=619 m/e (34 %), 594(39 %), 567 (43 %), 431 (39 %), 330 (90 %), 105(100 %) Scheme 1.

General Procedure

To 10 mL measuring flasks, solutions are added in the following order: 2.0 ml (1.0×10^{-4} mol L^{-1}) of 3,3'-bis-(dimethyl [6,6']bi[benzo[b]phenoxazinyl]-3,3'-ylidene) ammonium; chlorides sensor solution and different additions of (1×10^{-4} mol L^{-1}) $HgCl_2$ solution. The pH of solutions was adjusted by using appropriate volume of HCL/NH_4OH, and then the mixture is diluted to the mark with DMSO at room temperature. The above method is used for the subsequent measurements of absorption, emission spectra, effect of solvent, effect of pH as well as the effect of time. The fluorescence intensity was measured at λ_{max}=280 nm.

Determination of Hg^{2+} in Waste Water Samples

Five milliliter of waste water sample was diluted to 250 mL measuring flask by distilled water, then an appropriate volume from each sample was analyzed by atomic absorption spectroscopy.

Assay Principle

2.0 mL of 1.0×10^{-4} mol L^{-1} Bis(dialkylamino)phenoxazinium salt sensor was added to 10 μL of diluted waste water samples at pH 5.6 and complete to mark by DMSO. The fluorescence intensity of the sensor was measured without and with the waste water sample and ΔF was obtained.

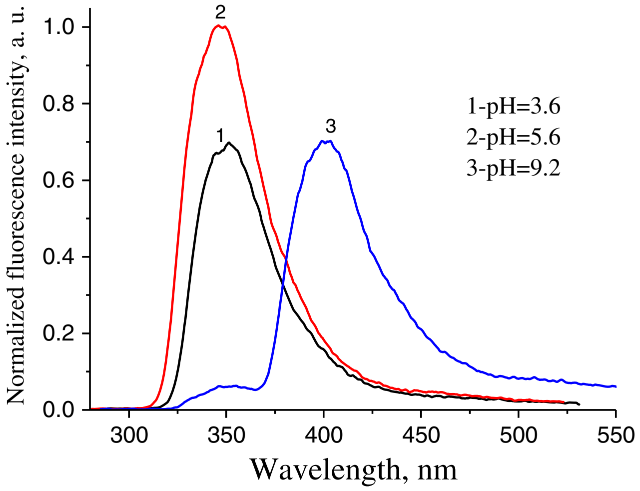


Fig. 3 Fluorescence emission spectra of 2×10^{-5} mol L^{-1} of Bis(dialkylamino)phenoxazinium in different pH

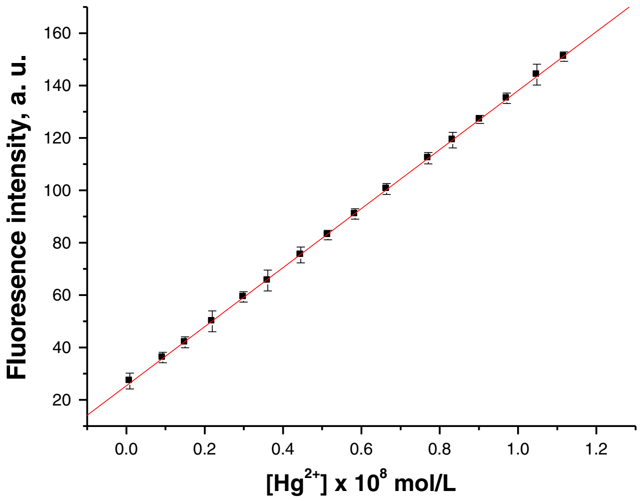


Fig. 4 Linear relationship between fluorescence intensity of chemosensor at 437 nm and [Hg^{2+}]

Results and Discussion

Spectral Characteristics

The absorption spectrum of 2×10^{-5} mol L^{-1} of Bis(dialkylamino)phenoxazinium salt sensor in DMSO shows a band in UV region at 335 due to $\pi\rightarrow\pi^*$ transition and two weak and broad bands in Vis region at 430 and 555 nm due to $n\rightarrow\pi^*$. Upon addition different concentrations of Hg^{2+} in DMSO and at pH 5.6 a red shift was observed in all three bands by 5, 10 and 20 nm, respectively, as shown in Fig. 1.

The emission spectrum of Bis(dialkylamino)phenoxazinium salt in DMSO and pH 5.6 shows a band at 347 nm at $\lambda_{ex}=280$. Upon addition of different concentrations of Hg^{2+} this band diminishes and a new band appear at 436 nm due to the complex formation between the sensor and Hg^{2+} ions in DMSO Fig. 2.

The ion titration revealed that the number of quencher (Hg^{2+}) attached by Bis(dialkylamino)phenoxazinium salt chemosensor equal 0.5, the formation constant of the complex between Hg^{2+} and the chemosensor $K=1.23\times10^7$ mol/L and M: L ratio (1: 2), which indicates that the ligand may coordinate to the metal from more than one different coordination sites.

Effect of pH

The pH of the medium has a great effect on chemical structure and the fluorescence intensity at 347 nm of the Bis(dialkylamino)phenoxazinium salt sensor. Where in strong acid medium all hetero atoms were protonated and the conjugation was diminishes and the emission intensity decreases at 347 nm. By elevation of pH the emission intensity increases till pH 5.6 then decreases and a red shift was observed in alkaline medium and a new band was appeared due to the conjugation and the rigidity of the Bis(dialkylamino)phenoxazinium salt increases. The more preferred pH at which the complex formation between the sensor and Hg^{2+} ions and a perfect quenching process was occurred at pH=5.6, Fig. 3.

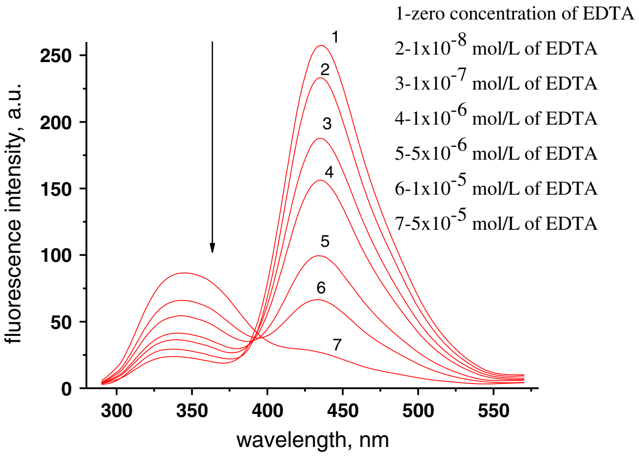


Fig. 6 Effect of EDTA concentrations on the fluorescence intensity of complex Hg^{2+} with chemosensor

Effect of the Addition Order of Reagents

Addition of the reagents in different orders has great influence on the fluorescence intensity. The experimental results indicate that the optimum fluorescence intensity is obtained when solutions are added in the following order: (Bis(dialkylamino)phenoxazinium salt , Hg^{2+},

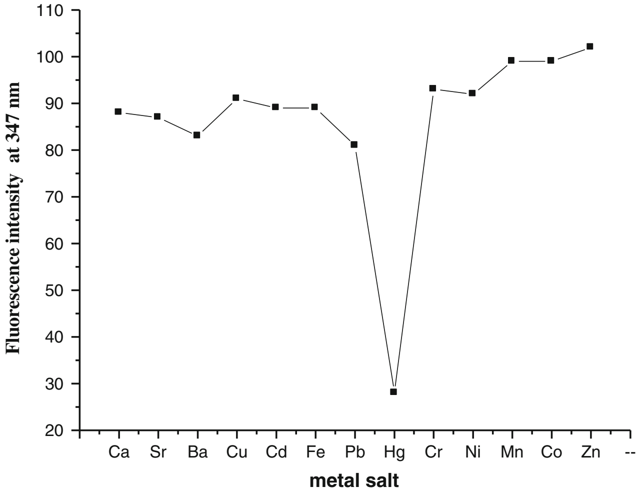


Fig. 5 Relationship between fluorescence intensity of sensor at 347 nm and different metal salts

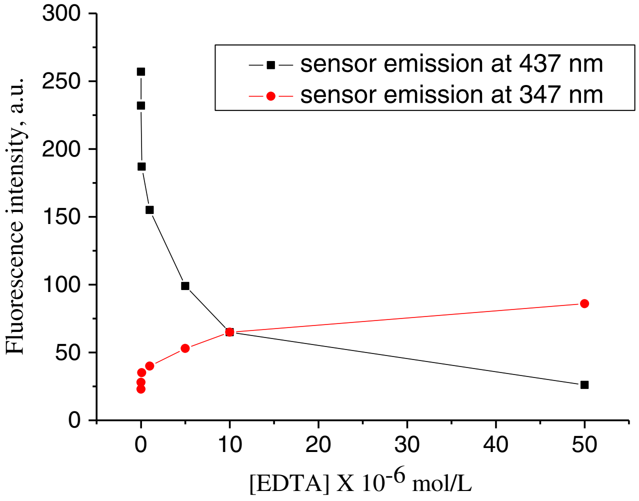


Fig. 7 Relationship between EDTA concentrations and fluorescence intensity of the chemosensor in presence of Hg^{2+}

Table 2 Determination of (Hg^{2+}) in waste water using the Bis(dialkylamino)phenoxazinium salt sensor

Proposed method

Sample studied	Mercury added, × 10^{-7} mol/L	Mercury added, × 10^{-9} mol/L	Average mercury found, × 10^{-7} mol/L	Average mercury found, × 10^{-9} mol/L	Mercury recovered (percent±RSD*)10^{-7} mol/L	Mercury recovered (percent±RSD*)10^{-9} mol/L
Ismailia Canal (Abou-Zabaal)	1.0	6.0	1.10	6.20	110.0±2.27	103.0±1.27
	4.0	8.0	4.15	8.25	103.7±1.78	103.1±1.22
	8.0	10.0	7.91	10.11	98.87±1.22	101.3±1.55
Karun Lake (Alfyuom)	1.0	6.0	1.05	5.95	105.0±2.90	99.1±1.88
	4.0	8.0	4.08	7.98	102.0±2.10	99.75±1.22
	8.0	10.0	8.11	10.11	101.40±1.67	101.10±1.80
Tap water	1.0	6.0	1.05	5.98	105.0±2.88	99.6±1.99
	4.0	8.0	4.06	8.06	101.5±1.27	100.75±1.33
	8.0	10.0	7.95	9.93	99.37±1.65	99.30±1.77

DMSO and adjusting the pH 5.6. So this order was chosen in the all experiments.

Effect of the Concentration of Hg^{2+}

The influence of the concentration of Hg^{2+} ions on the fluorescence intensities of the solutions containing 2.0×10^{-5} mol L^{-1} of Bis(dialkylamino)phenoxazinium salt was studied under the same experimental conditions shown above. The quenching of the fluorescence intensity at 347 nm in the same time the enhancement of the intensity of the band at 436 nm were increased with the increasing amount of Hg^{2+} ions up to 1×10^{-5} mol L^{-1}.

Effect of Solvent

The influence of the solvent on the fluorescence intensities of the solutions containing 2.0×10^{-5} mol L^{-1} of Bis(dialkylamino)phenoxazinium salt was studied under the conditions established above. The high intensity of the fluorescence of Bis(dialkylamino)phenoxazinium salt was observed in aprotic solvents like DMSO and DMF. This can be attributed to the formation of anhydrous solvates of Bis(dialkylamino)phenoxazinium salt introducing solvent molecules into sphere of the studied sensor leading to the enhancement of fluorescence intensity [26–30].

Analytical Parameters

A linear correlation was found between fluorescence intensity of Bis(dialkylamino)phenoxazinium salt sensor at λ_{em}=436 nm and concentration of Hg^{2+} ions in the ranges given in Table 1.

The sixteen-points calibration curve was obtained by plotting the peak intensity of sensor at λ_{em}=436 nm versus the concentration of Hg^{2+} shown in Fig. 4 and the graph was described by the regression equation:

$$Y = a + bX$$

Where

Y	fluorescence intensity of Bis(dialkylamino)phenoxazinium-Hg complex at λ_{em}=436 nm
a	intercept
b	slope
X	(concentration) in mol L^{-1}

Regression analysis of fluorescence intensity data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) and the values were presented in Table 1. The limit of detection (LOD) and quantization (LOQ) calculated according to IUPAC [31] using the

Table 3 Comparison of the proposed method with different methods for the determination of (Hg^{2+})

Method	Linear range (mol/L)	Detection limit (mol/L)	References
Electrochemical method	1.25×10^{-1} to 1.0×10^{-5}	8.9×10^{-6}	[32]
Electrochemical method	1×10^{-1} -10^{-6}	2×10^{-6}	[33]
Optical sensor	3.2×10^{-3}- 2.9×10^{-8}	1×10^{-8}	[34, 35]
Fluorimetric method	$(0–2)\times 10^{-6}$	6.0×10^{-8}	[36]
Chemosensor method	1.4×10^{-6}- 8.7×10^{-10}	5.8×10^{-10}	Present work

formulae: LOD=3.3 S/b and LOQ=10 S/b, (where S is the standard deviation of blank fluorescence intensity values, and b is the slope of the calibration plot).

Selectivity

The fluorescence responses of chemosensor to various cations and its selectivity for Hg^{2+} ions were illustrated in Fig. 6. The experiments were carried out by fixing the concentration of Hg^{2+} at 1.0×10^{-5} mol L^{-1}, salts of cations were added as chlorides. As can be seen from Fig. 5.

The fluorescence almost did not change in the 1x 10^{-4} mol L^{-} of Cr^{3+} solution. Furthermore, the fluorescence interferences were negligible in the $1x10^{-4}$ mol L^{-1} Zn^{2+}, Cd^{2+}, Co^{2+}, Ca^{2++} and Cu^{2+} solutions. The fluorescence was affected to some extent in the 8 $x10^{-5}$ mol L^{-1} Pb^{2+} ,Sr^{2+} and Ba^{2+} solution. However, below the mentioned concentrations of Zn^{2+}, Cd^{2+}, Co^{2+}, and Al^{3+} solutions, these ions did not induce any obvious fluorescence interferences, which were probably due to several combined influences cooperating to achieve the unique selectivity for the Hg^{2+} ion.

Reversibility and Response Time

The reversibility is an important matter to obtain an excellent chemical sensor. Thus, the EDTA-adding experiments were conducted to examine the reversibility of chmosensor. It is shown clearly that the fluorescence intensity at 347 nm increases and the intensity of the band at 436 nm diminishes with increasing EDTA concentration. Besides, the color also gradually changed from faint blue to dark violet color. When Hg^{2+} was added to the system again, the fluorescence could be reproduced and the dark violet color solution turned to faint blue. These findings indicated that chemosensor reversibly coordinated with Hg^{2+}ions, The above results also further elicit that the spectral response of chemosensor to Hg^{2+}ion is likely due to the equilibrium between the free form of the sensor and the complexed form with Hg^{2+} rather than other possible reactions, Figs. 6 and 7.

We also investigated the time course of the response of chemosensor to 1.0×10^{-5} mol L^{-1} Hg^{2+} in pH 5.6. The results indicate the recognition interaction was completed immediately after addition of Hg^{2+} without any detectable time-delay. Thus, this system might be used for the real-time monitoring of Hg^{2+} ion in practical analysis

Preliminary Analytical Applications

In order to examine the applicability of the proposed method in practical sample analysis, the chemosenor was applied in the determination of Hg^{2+} in tap, lake and canal water samples. The lake and canal water samples were obtained from Ismailia Canal (Abou-zabaal) and Karun Lake (Alfyuom) then simply filtered. No Hg^{2+} was found in these samples. All these water samples were spiked with standard Hg^{2+} solutions and then analyzed with proposed chemosensor. The recovery study of spiked Hg^{2+} was calculated and it gives satisfactory results (Table 2). Thus, the present probe seems useful for the determination of Hg^{2+} in real samples.

By comparison with some existing methods, as shown in Table 3, the present method has the advantages in terms of high sensitivity, good selectivity, rapid response and a wide linear range of applications. It avoids potential background fluorescent emission interferences from water sample solution background. So this method may provide a new kind of sensor for the determination of Hg^{2+} in water samples.

Conclusion

In conclusion, a fluorescent chemosensor for mercuric ions based on a symmetrical Bis(dialkylamino)phenoxazinium salt sensor has been developed, in which the signal was transduced through the enhancement of the band at 436 nm upon Hg^{2+}ion binding. chemosensor exhibits the feature of a reversible dual-responsive colorimetric and fluorescent response to Hg^{2+}ions, and shows a high sensitivity and selectivity for Hg^{2+} sensing in comparison to other cations in DMSO and pH 5.6.

The proposed probe can be applied to the quantification of Hg^{2+} with a linear range covering from 8.7×10^{-10} to 1.4×10^{-6} mol L^{-1} and the detection limit is 5.7×10^{-10} mol L^{-1}. The proposed probe has been used for the determination of Hg^{2+} in different waste water samples and shows satisfactory results.

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