**A highly selective chromogenic probe**

**for Hg2+**

Hossein Tavallali

Department of Chemistry

Payame Noor University, PNU

19395-4697 Tehran, Iran

tavallali@yahoo.com

Shiva Rajaei Dastghaib

Department of Chemistry

Payame Noor University, PNU

19395-4697 Tehran, Iran

shivarajaei@yahoo.com

**Abstract**

In this paper, indigo carmine (IC) was proposed as a highly accurate and sensitive colorimetric chemosensor for the determination and detection of mercury (II) (Hg2+), which is a very toxic and dangerous pollutant. The chromogenic probe (IC) in the aqueous medium shows a color change from indigo to light blue upon interaction with Hg2+. The range of linear and the limit of detection of Hg2+ were 0.15-20.2 μM and 0.06 μM, respectively. Also, the relative standard deviation (RSD) based on ten replicates calculated for two different concentrations of 7.4 μM and 15.8 μM of Hg2+ was 2.1% and 1.4%, respectively. The proposed method has advantages such as: simplicity, low cost, high accuracy, wide linear concentration range, low limit of detection, high sensitivity and selectivity, no separation and preconcentration steps, and can be used in neutral water media. The concentration of Hg2+ in tap water, mineral water and plasma samples can be easily detected and determined using this method. It should be noted that this ideal colorimetric chemosensor for Hg2+ has not been reported before.

Keywords: chromogenic probe, colorimetric chemosensor, mercury (II) detection, indigo carmine (IC).

1. **Introduction**

 The development of selective and sensitive chemosensors is an important assignment for detecting transition metal ions, which play important roles in chemical, biological, medicinal, and environmental processes [1, 2]. Among them, the most toxic and ubiquitous heavy-metal ions of pollutants, Mercury ion (Hg2+) arising from a variety of natural and anthropogenic sources, has attracted a particular attention [3, 4]. Water-soluble divalent mercuric ion (Hg2+) can cause several serious diseases such as the brain damage, gastrointestinal system diseases, nephropathy and neurological diseases even at very low concentrations since they can easily enter the body through the skin or the esophagus [5, 6]. The United States Environmental Protection Agency (EPA) has set a maximum Hg2+ contaminant level in food and drinking water at 0.002 ppm (0.01 µM) [7].

 Common methods for detecting Hg2+ include atomic absorption spectrometry (AAS) [8], inductively coupled plasma optical emission spectrometry (ICP-AES) [9], inductively coupled plasma mass spectrometry (ICP-MS) [10] and High-Performance Liquid chromatography (HPLC) [11] have been explored. These practices do not offer a cost-effective, rapid and real time-monitoring system for Mercury ions [12]. In this regard the colorimetric chemosensors are a powerful tool for the sensing of Mercury ions [13]. Therefore, development of simple colorimetric chemosensor that can selectively sense Hg2+ in aqueous media is significant.

 Indigo carmine (3,3'-dioxo-2,2'-bis-indolyden-5,5'-disulfonic acid, IC) (scheme 1) is an available commercial dye with promising features that make it suitable for use as a chemosensor. Previously we have reported some spectroscopic applications of Indigo Carmine in the determination of some cations [14-16]. IC as a fluorescent and colorimetric chemosensor was used for the detection of Cu2+ and HPO42- in H2O/DMSO media [17].



**Scheme 1. Structure of IC.**

1. **Experimental**
2. **Chemical substances and reagents**

 All chemicals with analytical grade were purchased from Merck and used as soon as received. Indigo Carmine dye, di sodium salt, was used for preparing the solutions. The solution of Hg2+ and all cations, including Na+, K+, Mg2+, Ca2+, Sr2+, Ba2+, Fe2+, Fe3+, Co2+, Ni2+, Cu2+, Zn2+, Ag+, Cd2+, Pb2+ was prepared with its nitrate salt in deionized water.

1. **Apparatus**

 Shimadzu 1601 PC UV-Vis spectrophotometer with 10.0 mm diameter quartz cuvettes was used to record and measure all absorption spectra. Deionized water was used in the reference cell. A Jenway 3510 digital pH meter calibrated with two standard buffer solutions was used to measure pHs. Using a 50 μL Hamilton syringe, the desired amount of analyte solution was introduced into the cuvette.

1. **Measurement methods**

 Stock solutions of IC and Hg(NO3)2 were prepared with concentrations of 1.0 × 10-3 M and 1.0 × 10-2 M in deionized water, respectively. Then, 3-540.0 µL of Hg(NO3)2 solution with a concentration of 0.1 mM was added to 2.0 mL of IC with a concentration of 25.0 µM. They were mixed and then the UV-vis spectra were taken at room temperature.

1. **Results and discussion**

Different metal ions were added to the solution of IC, and the UV-Vis absorption spectrum was investigated. Among various metal species such as Na+, K+, Mg2+, Ca2+, Sr2+, Ba2+, Al3+, Cd2+, Sn2+, Pb2+, Bi3+, Ti2+, Cr3+, Mn2+, Fe2+, Fe3+, Co2+,Ni2+, Cu2+, Zn2+, Ag+, and Ce3+, only Hg2+ showed a color change from indigo to light blue accompanied by a distinct spectral shift at 610.0 nm. Other species did not show changes in the spectrum and color of IC solution (Fig. 1).

(a)


(b)



**Fig. 1. (a) Absorption spectrum, (b) The color changes of IC (25.0 µM) with the different metal ions (1.0 equiv.).**

 The interaction of IC with Hg2+ was investigated by UV–vis titration (Fig. 2). When Hg2+ was added to IC, the absorption band at 610.0 nm gradually decreased and a new absorption band appeared at 666.5 nm. In such a curve, an obvious isosbestic point at 640.5 nm was observed, indicating the interaction between IC and Hg2+. The molar extinction coefficient of the peak at 610.0 nm is 3.58 × 104 M-1cm-1 and the new peak at 666.5 nm is 4.36 × 103 M-1cm-1, indicating a mercury-based d-d transition and can be considered as the metal-to-ligand charge transfer mechanism. (MLCT) mechanism.



**Fig. 2. UV–vis IC spectrum (25.0 μM) with stepwise addition of Hg2+ (0.15–21.26 μM) and**

**the color change of IC.**

 Benesi-Hildebrand analysis [18] was used to determine the stoichiometry of IC binding to Hg2+. Absorption titration data (Fig. 3) show that the complex formed between IC and Hg2+ has a stoichiometric ratio of 1:1 (Eq. 1) and the binding constant of Hg2+ is 2.77×103 M-1.

Eq. 1 $\frac{1}{(A-A\_{0})}=\frac{1}{(A\_{max}-A\_{0})}\left[\frac{1}{K\_{ass}\left[Hg^{2+}\right]^{n}}+1\right]$

**Fig. 3. Benesi-Hildebrand plot of IC (1/(A0-A) change at 610.0 nm versus 1/[Hg2+] function) based on 1:1 binding stoichiometry with Hg2+.**

 In order to apply to the environmental systems, the pH dependence of IC in the absence and presence of Hg2+ was performed at different pH (2.5-12.6) (Fig. 4). In the pH range (2.5-7.0), IC showed no change without the presence of the Hg2+ ions. The increase of in absorption caused by the addition of Hg2+ ion was observed at pH 7.0. This result guarantees its use in environmental conditions (pH 5.2-9.0) without any change in Hg2+ detection. Therefore, pH 7.0 was chosen for all colorimetric measurements.

 To investigate the application of the proposed method in environmental systems, the effect of pH was studied. It was performed in the absence and presence of Hg2+ at different pH (2.5-12.6) (Fig. 4). The IC did not change in the pH range (2.5-7.0) without the presence of Hg2+ ions, but in its presence an increase in the absorbance was observed at pH 7.0. Therefore, pH 7.0 was selected for the proposed method.

**Fig. 4. The effect pH on the proposal method.**

 The interaction time of the IC sensor with Hg2+ was investigated (Figure 5). The results showed that the reaction occurs in the first few seconds and remains constant for the next 40 minutes at the absorption wavelength of 610.0 nm.

**Fig. 5. The effect of time on the proposal method.**

 To verify the linear range of the proposed method, a calibration curve was plotted (Fig. 6). The linear range was 0.15-17.36 μM, and the regression equation for the data was A610.0 = 0.8766–0.025CHg (μM) with R2 = 0.9901. The limit of detection and limit of quantification for the measurement of Hg2+ were calculated to be 0.06 and 0.22 μM, respectively. The relative standard deviation for two concentrations of Hg2+, 7.4 and 15.8 μM and 10 replicate measurements, was 2.1% and 1.4%, respectively.

**Fig. 6. Calibration curve.**

 Different metal cations were used to determine the selectivity for Hg2+ (Fig. 7). The results showed that the proposed method has good selectivity and the IC can be used as a potential chemosensor for the detection of Hg2+.



**Fig. 7. Effect of coexisting substances on (a) Absorption spectrum,**

**(b) absorbance of IC in the presence of Hg2+.**

 To determine Hg2+, the proposed method was used in real samples, but Hg2+ could not be detected in the samples. Therefore, the spiking method was carried out. The measurement of Hg2+ in the samples was repeated three times, and the recovery values between 96.00 and 103.90% showed good accuracy of the proposed method (Table 1). The results show that this method can be used to measure Hg2+ in real samples.

**Table. 1. Determination of Hg2+ in real samples by spiking method.**

|  |  |  |  |
| --- | --- | --- | --- |
| Sample(n = 3) | Hg2+ (μM) | Average recovery (%) | Relative error (%) |
| Amount added | Amount found |
| Tap water | 0.00 | - | - | - |
| 4.00 | 3.98 ± 0.02 | 99.50  | -0.50 |
| 8.00 | 8.31 ± 0.05 | 103.90 | 3.00 |
| 10.00 | 10.15 ± 0.03 | 101.50 | 3.88 |
| Mineral water | 0.00 | - | - | - |
| 4.00 | 3.95 ± 0.03 | 98.75  | -1.25 |
| 8.00 | 8.08 ± 0.02 | 101.00 | 1.00 |
| 10.00 | 9.76 ± 0.04 | 96.00  | -2.40 |
| plasma | 0.00 | - | - | - |
| 4.00 | 4.01 ± 0.04 | 100.25 | 0.25 |
| 8.00 | 8.06 ± 0.05 | 100.75 | 0.75 |
| 10.00 | 10.10 ± 0.06 | 101.00 | 0.06 |

1. **Conclusion**

 A method for the determination of the toxic substance Hg2+ was proposed, which has advantages over other methods, such as simple, fast, no separation and preconcentration, low cost, high accuracy, wide linear concentration range, low detection limit, high sensitivity, compatibility, and the selectivity is excellent. Therefore, these results show that IC as a chemosensor can be used to detect Hg2+ by a colorimetric method in an aqueous solution at room temperature.

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