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Development of a visual optode sensor for onsite determination of Hg(II)

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ABSTRACT

A color changeable cellulose triacetate (CTA) membrane-based optode has been developed for the highly selective and sensitive determination of Hg(II) ions in aqueous solutions. The sensing layer of the optode consists of phenylthiosemicarbazide derivative of rhodamine 6G immobilized in a plasticized CTA membrane. The colorless optode film on reaction with Hg(II) turned pink, in color, and the intensity of the color was found to be directly proportional to the amount of Hg(II). The sensing mechanism is based on the mercury-induced spirolactum ring opening followed by its cyclization to form highly conjugated system which leads to the development of pink color. Various experimental parameters such as pH, optode composition and response time have been optimized. Under these conditions, the sensor showed a wide linear dynamic range of 10–5000 ng mL^{-1} Hg(II), with a detection limit of 1.3 ng mL^{-1}. In addition to its stability and reproducibility, optode showed a high selectivity toward Hg(II) ions as compared to other coexisting ions in real water samples. The developed optode is suitable for rapid and onsite detection of Hg(II) ions in a variety of aqueous samples. The sensor was successfully applied for the determination of Hg(II) ions in groundwater samples.

1. Introduction

The adverse health effects caused by mercury poisoning are well known since Minamata disease of 1950s [1]. Symptoms of mercury poisoning can be numerous and may occur either rapidly or over a long period of time. Human beings are exposed to different forms of mercury under different circumstances. Among these, inorganic mercury and methylmercury are the most toxic. The inorganic salts of mercury are corrosive to the skin, eyes and gastrointestinal tract and may induce kidney toxicity if ingested, whereas exposure to methylmercury can produce harmful effects on the nervous, digestive and immune systems of living beings [2–4]. These severe health problems caused by mercury even when present at an ultra-trace level have led to the development of methods with improved detection limits and a dynamic range of quantification.

The common methods for quantification of mercury in the different matrices are cold-vapor atomic absorption spectrometry (CV-AAS), spectrophotometry, anodic stripping voltammetry, energy dispersive X-ray fluorescence (EDXRF) and high-performance liquid chromatography hyphenated with inductively coupled mass spectrometry (HPLC-ICPMS) [5–13]. Although these methods offer a wide working range and low detection limit, these suffer from one or more drawbacks such as high cost of instrumentation, tedious sample preparation and long analysis time. In addition to these, they are not suitable for onsite monitoring of mercury. To overcome these limitations, over the past few years, efforts are continuously being made to develop various optical sensors for detection of mercury [14–19]. In this regard, the development of different colorimetric or fluorescence-based optical sensors for mercury ion detection plays an important role [20–25]. Most of the reported colorimetric sensors are based on using organic molecules that usually work in organic media and, therefore, do not meet the selectivity and sensitivity requirements for the detection of mercury in aqueous solutions [26,27]. The strong hydration of mercury ions and the lack of solubility of synthesized organic derivatives lead to a poor solid phase extraction of mercury from aqueous solutions. This poses a challenge for the development of

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optical sensors for the detection of mercury ions in the aqueous medium.

Development of optode sensors, also known as optochemical sensors, for mercury detection has recently gained interest among chemists [28–32]. The commonly used optodes consist of a plasticized polymer matrix containing a molecular sensor which is responsible for targeting the desired analyte and generating optical signal proportional to the concentration of the analyte. The sensor molecule is immobilized by either physical or chemical methods. On interaction of the sensor with the analyte, a distinct change in the absorbance or fluorescence of the sensing layer is obtained which is responsible for the optical signal. The choice of molecular sensor responsible for generating the optical signal plays an important role in the design of optodes [33].

Recently, the rhodamine framework has attracted wide attention in the field of constructing fluorescent chemosensors, owing to its excellent photophysical properties such as higher absorption and emission wavelengths, high absorption coefficient and high fluorescence quantum yield [34]. The rhodamine derivatives usually display a color change and strong fluorescence in acidic solution due to a carbonyl group in its spirolactone or spirolactam moiety. Similarly, the spirolactam ring can cause color as well as fluorescence changes on the addition of metal ions, depending on the solvent system [35,36]. The rhodamine derivatives are being explored for the colorimetric determination of various metals (e.g. antimony, iron, zinc, silver and osmium) in the biological samples [37–40]. Basically, these methods are based on the on/off switch of the spirocyclic moiety, mediated by the metal ion [40].

There has been significant development in the synthesis of various rhodamine derivatives for mercury ion detection. Kim and co-workers have [41] reported rhodamine-based tren(triethylenetetramine) and diethylenetriamine derivatives with tosyl groups, in which the Hg(II) was entrapped by diethylenetriamine groups. The addition of Hg(II) ions to the CH_3CN solutions produced a color change, with enhanced fluorescence intensity. Duan and co-workers [42] reported a Hg(II) probe based on rhodamine containing a carbohydrazone unit in DMF solution. Yoon et al. reported rhodamine-urea-based derivatives in acetonitrile solution. A multisignaling optical–electrochemical sensor based on a rhodamine-ferrocenyl derivative and an 8-hydroxyquinoline moiety for sensing Hg(II) in ethanol–HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer solution was synthesized by Huang and co-workers [43]. Shi and Ma [44] and Xu and co-workers [45] reported a rhodamine-B thiolactone derivative as a sensor for Hg(II). Most of the reported derivatives as stated above are applicable for Hg(II) detection in the non-aqueous media. Therefore, the current research is focussed on developing chemical sensor for the detection of mercury ions in the aqueous medium. Recently, Yang et al. [46] have developed a rhodamine-6G phenylthiosemicarbazide derivative, which works as a highly selective and sensitive chemodosimeter for Hg(II) in aqueous solution. In the present work, we have selected this derivative to be incorporated in an optically transparent solid matrix to form a Hg(II) selective optode. Although a large number of molecular probes are reported for Hg(II) detection in solution phase, only a few examples of membrane/optode probes are found in the literature. The major reason for this lies in the problems encountered during the synthesis of any solid phase reagent immobilized sensors. Agglomeration and leaching of the incorporated indicator reagent from the matrix are some of the common problems. The covalent linkage of the incorporated reagent to a suitable matrix appears to be an efficient method to overcome the leaching but most of the time it is accompanied by the decreased activity of the sensing sites.

In the present work, the main objective was to synthesize a polymeric film-based optode sensor for highly selective and sensitive onsite detection of Hg(II) present in the natural waters. Rhodamine-6G phenylthiosemicarbazide derivative (Rh6G-P) was immobilized in a plasticized polymer matrix as it binds with Hg(II) ions. The binding of Hg(II) was followed by ring opening and cyclization reactions in the rhodamine derivative, leading to the development of pink color. Based on the preliminary experiments, the cellulose triacetate (CTA) matrix was selected due to its optical quality and the ease with which signal generating molecule (rhodamine-6G phenylthiosemicarbazide) can be immobilized in it. The conditions for the synthesis of the optode were optimized to obtain maximum analytical signal for Hg(II) ions. The tolerance of the synthesized optode sensor toward co-existing ions in groundwater has been studied. The optode showed a high reproducibility and selectivity toward Hg(II) ions. Therefore, it was explored for a detection of mercury in the groundwater samples consisting of a high concentration of interfering ions.

2. Experimental study

2.1. Reagents and instrumentation

CTA (molecular weight 72,000–74,000, acetyl value = 43.2%), rhodamine 6G, tri-(2-ethylhexyl) phosphate (TEHP), hydrazine monohydrate and phenyl isothiocyanate were obtained from Sigma-Aldrich (Steinheim, Germany) and used as received. Polystyrene ($M_w \approx 250{,}000$) was procured from Acros Organics. Dichloromethane and elemental mercury standard ($1000\,\mathrm{mg\,L^{-1}}$) were procured from E. Merck, Germany. The working standards of Hg(II) were prepared by a serial dilution of the stock standard solution. Nano pure water ($18.2\,\mathrm{M\Omega\,cm^{-1}}$) collected from Elix, Millipore Merck ultra pure water system was used throughout the experiments. Hydrochloric acid and sodium hydroxide used for adjusting the pH of solutions were of supra pure grade procured from E. Merck, Germany. Aqueous stock solutions of $1000\,\mathrm{mg\,L^{-1}}$ K^+, Na^+, Ca^{2+}, Mg^{2+}, Cd^{2+},Cr^{3+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Fe^{2+}, Fe^{3+}, Al^{3+} and Zn^{2+} were prepared from their respective salts.

The surface morphology of the synthesized optode film was studied by atomic force microscopic (AFM) studies. The tapping mode AFM measurements were performed at ambient temperature conditions using a Nanosurf easy Scan 2 AFM (Nanosurf, Switzerland), with 10 μm scanner head. The cantilevers used were NCLR-10 (Nano World), with a resonance frequency of 190 kHz and a force constant of 48 N/m. The AFM data were analyzed using Nanosurf Report 4.1 software.

All absorbance measurements were carried out using USB 4000 Spectrophotometer (Ocean Optics, Germany). Absorbance spectra of the optode were recorded in the reflectance mode. Steady-state fluorescence spectra of the optode films were recorded using Hitachi F-4010 spectrofluorometer (Tokyo, Japan). All the pH measurements were made with a Metrohm 780 pH meter.

2.2. Synthesis of rhodamine derivative

Phenylthiosemicarbazide rhodamine 6G derivative (Rh6G-P) was synthesized following a detailed two-step procedure described by Yang et al. [46]. In the first step, rhodamine 6G chloride (300 mg) was dissolved in 20 mL methanol, and to this hydrazine monohydrate (0.2 mL) was added. The reaction mixture was refluxed for 6 h until the pink color disappeared. The resultant solution was diluted with 30 mL ethyl acetate and then washed with water and 1 N NaOH (10 mL). This organic phase was dried over magnesium sulfate, concentrated and purified using silica-gel column with a mixture of hexane: CH_2Cl_2: MeOH = 10:2:1 as an eluent. The yield of rhodamine 6G hydrazide obtained was 250 mg (94%). In the second step, rhodamine 6G hydrazide (200 mg) was added