

# **Design and Evaluation of a Mercury (II) Optode Based on Immobilization of 1-(2-Pyridylazo)-2-Naphthol on a Triacetylcellulose Membrane and Determination in Various Samples**

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## **Abstract**

The characterization of an optical sensor membrane is described for the determination of  $\text{Hg}^{2+}$  ions based on the immobilization of 1-(2-pyridylazo)-2-naphthol (PAN) on a triacetylcellulose membrane. The membrane responds to mercury ions by changing color reversibly from orange to red in universal buffer solution at pH 6. Under optimum conditions, the proposed membrane displayed a linear range of  $0.1\text{-}24\ \mu\text{g mL}^{-1}$  with a limit of detection of,  $0.02\ \mu\text{g mL}^{-1}$  at a wavelength of 558 nm. The response time of the optode was about 10-12 min, depending on the concentration of mercury (II) ions. The coefficients of variation (CV) of the sensor response for,  $1.0\ \mu\text{g mL}^{-1}$  of Hg (II) was, 1.8% and the CV between seven membranes was 2.3%. The sensor can readily be regenerated with the ethylene diamine solution. The optode is fully reversible and the selectivity of optode to  $\text{Hg}^{2+}$  ions in universal buffer is relatively good with  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  ions as interferences. The proposed optode was applied successfully for the determination of mercury (II) in various samples.

**Keywords:** Optode,  $\text{Hg}^{2+}$  ions, 1-(2-pyridylazo)-2-naphthol (PAN), Ethylene diamine, Triacetylcellulose.

## **1. Introduction**

Mercury is one of the most abundant heavy metals in the environment, and its toxic effects have been recognized for a long time. The mercury content of air, soil and water has been increasing in the past decades, because of the greater utilization of fossil fuels and for the expanded use in industry and agriculture. Due to its dangerous and harmful properties for the health of human being, the determination of mercury is very important for environmental protection [1].

A wide range of analytical methods have been used for the determination of mercury in real samples. These includes spectrophotometry [2], graphite – furnace atomic absorption

spectrometry (GFAAS) [3], inductively coupled plasma atomic emission spectrometry (ICP-AES) [4], high performance liquid chromatography inductively coupled plasma mass spectrometry (HPLC-ICP-MS) [5], Cloud point extraction [6], anodic stripping voltammetry [7], X-ray fluorescence spectrometry [8], inductively coupled plasma mass spectrometry (ICP-MS) [9], electrothermal atomic absorption spectrometry [10], atomic fluorescence spectrometry [11] and cold vapor atomic absorption spectrometry [12]. However these methods have good sensitivity, but they require expensive instruments, well-controlled experimental conditions, and profound sample – making. Optical sensors have drawn much attention in analytical chemistry because of their possible application in biology, biotechnology and ecology [13] and because of their advantages such as small size, feasibility of miniaturization, freedom from electrical interference, low cost, good sensitivity and selectivity [14]. Development of optochemical sensors (so called optodes) [15] has been mostly based on the immobilization of the reagent by either physical (adsorption, encapsulation, sol-gel, etc) or chemical (covalent bond) methods and incorporate this in the sensor design. The immobilization can be performed directly on the surface of optical fibers (intrinsic sensors), or on a suitable material which can then act as an interface between the sample and the fibre optic system (extrinsic sensors) [16]. The sensing phase consists of reagent dyes immobilized in organic or inorganic matrices. Reaction with the analyte changes the absorbance or fluorescence behavior of the sensitive layer. Organic dyes and metallochromic indicators, which are used in the spectrophotometric determination of various metals, play a main role in the design of optodes [17].

The azo-dye PAN (1-[2-pyridylazo]-2-naphthol) is a well-known metallochromic indicator for the quantitative and qualitative determination of variety of metal ions. PAN has many characteristics required an ionophore. This ionophore reacts highly colored complexes. The complexes can be reversed to form again the ionophore over a large number of repetitions [18].

In this paper the fabrication of an optode for determination of low levels of mercury is described in which the sensing reagent is PAN immobilized on triacetylcellulose membrane. The reaction takes place within few minutes and a color change occurs from orange to red, which is spectroscopically detected in the absorbance mode. The sensor layer can be regenerated instantly and completely with the same carrier solution and the optode is fully reversible. Experimental results showed that, this optode could be used as an effective tool in analyzing the mercury content of various samples such as alloy for dental prosthesis, omega3 tablet, soil, water, fish and vegetables. In table 1 we compare the present optode with other reported optodes for determination of mercury.

**Table1.** Comparison of the proposed optode with other reported optodes for determination of mercury

Reagent immobilized on membrane	pH	$\lambda_{\text{Max}}$ / nm	Dynamic range/ $\mu\text{g mL}^{-1}$	D. L/ $\mu\text{g mL}^{-1}$	R.S.D %	Remarks	References
Dithizone on Triacetylcellulose membrane (optical sensor)	2	610	0.15-1.94	0.02	3.2	$\text{Cu}^{2+}$ interfere	[19]
1-(2-pyridylazo)-2-naphthol (PAN) on PVC membrane (optical fibre reflectance sensor)	5.4	560	2-200.59	0.11	3.5	$\text{Fe}^{3+}$ , $\text{Bi}^{3+}$ , $\text{Cr}^{3+}$ , $\text{Mn}^{2+}$ , $\text{Ni}^{2+}$ , $\text{Cd}^{2+}$ , $\text{Ag}^+$ , $\text{Co}^{2+}$ , $\text{Cu}^{2+}$ and $\text{Zn}^{2+}$ are interfere	[25]
Safranin – Iodid on naffion membrane (optical fibre reflectance sensor)	5	620	0-10	1.0	4	$\text{Ag}^+$ interfere	[26]
1-(2-pyridylazo)-2-naphthol on a triacetylcellulose membrane (optical sensor)	6	558	0.1-24	0.02	2.3	highly sensitive and selective	Proposed method

## 2. Experimental

### 2.1. Reagents

All the reagents such as PAN and ethylene diamine were supplied from Merck Company. The indicator 1-(2-pyridylazo)-2-naphthol (PAN) solution ( $1 \times 10^{-4} \text{M}$ ) was prepared by dissolving 0.0025 g of PAN in ethanol and diluting to 100 mL. A stock solution of  $1000 \mu\text{g mL}^{-1}$   $\text{Hg}^{2+}$  ion was prepared by dissolving 0.0680 gr of  $\text{HgCl}_2$  (Merck) in distilled water and diluted to the mark in a 50 mL volumetric flask.

Universal buffer solutions were prepared from boric acid / acetic acid / phosphoric acid (0.04 M each). The final pH was adjusted by the addition of 0.2M sodium hydroxide.

Stock solutions of  $6000 \mu\text{g mL}^{-1}$  of interfering ions were prepared by dissolving appropriate amounts of suitable salts in double distilled water.

### 2.2. Apparatuses

A Shimadzu 1601 PC UV-Vis spectrophotometer with a 1cm cell was used for recording all spectra and absorbance measurements. A Jenway 3510 pH-meter which calibrated against two standard buffer solutions at pH 4.0 and 10.0 was used to measure the pH of the solutions.

A Hamilton syringe (10  $\mu$ l) was used to deliver small volumes of reagent into the cell. The reference cell was contained a membrane without any indicator. All measurements were made in the absorbance mode.

### **2.3. Preparation of the sensor membrane**

The immobilized indicator on triacetylcellulose was prepared according to the following procedure. The transparent triacetylcellulose membranes were produced from waste photographic film tapes that were previously treated with commercial sodium hypochlorite for several seconds to remove colored gelatinous layers.

The films were treated with a clear solution of PAN (20 mg) in 20mL ethylene diamine for 5-7 min at ambient temperature. Then they were washed with water for removing ethylene diamine and loosely trapped indicator. The membranes were finally washed with detergent solutions and water. Prepared membranes were kept under water when not in use [19].

### **2.4. Samples preparation**

#### **2.4.1. Determination of mercury in amalgam and omega3 tablet samples**

The commercial form of the amalgam takes form of little balls, each weighing 1.0 g. One of these exactly weighing balls was dissolved in 1:1 HNO<sub>3</sub> and the mixture was carefully boiled until complete dissolution of the amalgam was achieved. The pH was adjusted to 6 and diluted by appropriate dilution of the mother solution to 100 mL.

For preparation of the omega3 tablet sample, 20 tablets (from Zahravi Manufactory) were accurately weighed and powdered in a mortar. A mass corresponding to a tablet was dissolved in 0.1 M HCl in 100 mL calibrated flask. After 30 min of mechanically shaking, the solution was filtrated in a 100 mL calibrated flask through Whatman no: 40 filter papers. The pH was adjusted to 6 and then was diluted 1:500 with water. The mercury content of the each samples solution was determined by the proposed optical sensor.

#### **2.4.2. Determination of mercury in soil and water samples**

Soil sample (from W.30381 location of 404 well, Ahvaz , Iran) was homogenized and dried at 105°C. After that, 10 gr of soil was taken and 3 mL of HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> mixture (2/1) were added to the soil. This mixture was slowly shaken and dried on a hot plate. After cooling, 2 mL of 0.75 M nitric acid were added to the remainder and centrifuged. After adjustment of pH to 6 and dilution of the mother solution, the mercury content of the sample solution was determined by the proposed optical sensor.

River water samples (from different locations of kor river, Marvdasht, Iran) did not need previous treatment and was analyzed for mercury (II) ion concentration using the proposed optode. pH of river water was adjusted by the addition of 2 mL of universal buffer (pH6) to 8 mL of river water.

#### **2.4.3. Determination of mercury in fish and vegetable samples**

2.0 g of the fish was dried and ashed at 700 °C. After that the ash was dissolved with 10 mL of concentrated HCl and diluted to 50 mL with distilled water and 1.0 M NaOH, so that the pH 6 was reached for the analysis 0.5 mL of the solution was transferred into a 25 mL calibrated flask.

For the analysis of vegetables, all samples were washed and then dried at 100 °C. Approximately, 0.5 gr of each dried sample put into 250 mL of pyrex beaker about 0.5 mL of conc. H<sub>2</sub>SO<sub>4</sub> was added to it. Beaker containing sample was placed into ashing furnace at 480 °C for 4-5 hour. Then, 3.0 mL of conc. HNO<sub>3</sub>/ H<sub>2</sub>O<sub>2</sub> mixture (2/1) was added to the ashed sample and dried on a hot plate. After residue was dissolved by using 2.0 mL of 1.0 M HNO<sub>3</sub> and, if necessary diluted to suitable volume and pH adjusted to 6.

#### **2.5. Absorbance measurements**

The prepared membranes were put in a buffer solution of pH 6 for 30 min to reach equilibrium. Then membrane was placed vertically inside in sample cuvette containing 3 mL buffer solution of pH6, and a blank membrane (without indicator) was put in the reference cuvette containing the buffer solution. The sample cell was finally titrated with mercury (II) ions solution and the absorbance value of system was measured over the wavelength range of 400-800 nm after 12 min ( equilibrium time).

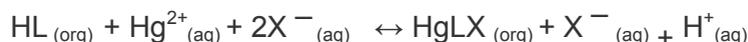
Absorbance of membrane before and after the sample solution was added, at 558 nm and 473 nm was measured.

### **3. Results and discussion**

#### **3.1. Properties of PAN as a chromoionophore**

The complexation reactions of PAN as a chromoionophore with metal ions are well known to be strongly dependent on the pH [20]. The extraction of Hg (II) from the aqueous sample solution into the membrane phase and its complexation by the indicator (PAN) involved the loss of one proton from the hydroxyl group of one PAN molecule. This ion-exchange process depends on the electroneutrality conditions in the organic membrane phase. The

overall equilibrium between the aqueous sample solution (aq) and the organic membrane phase (org) is:



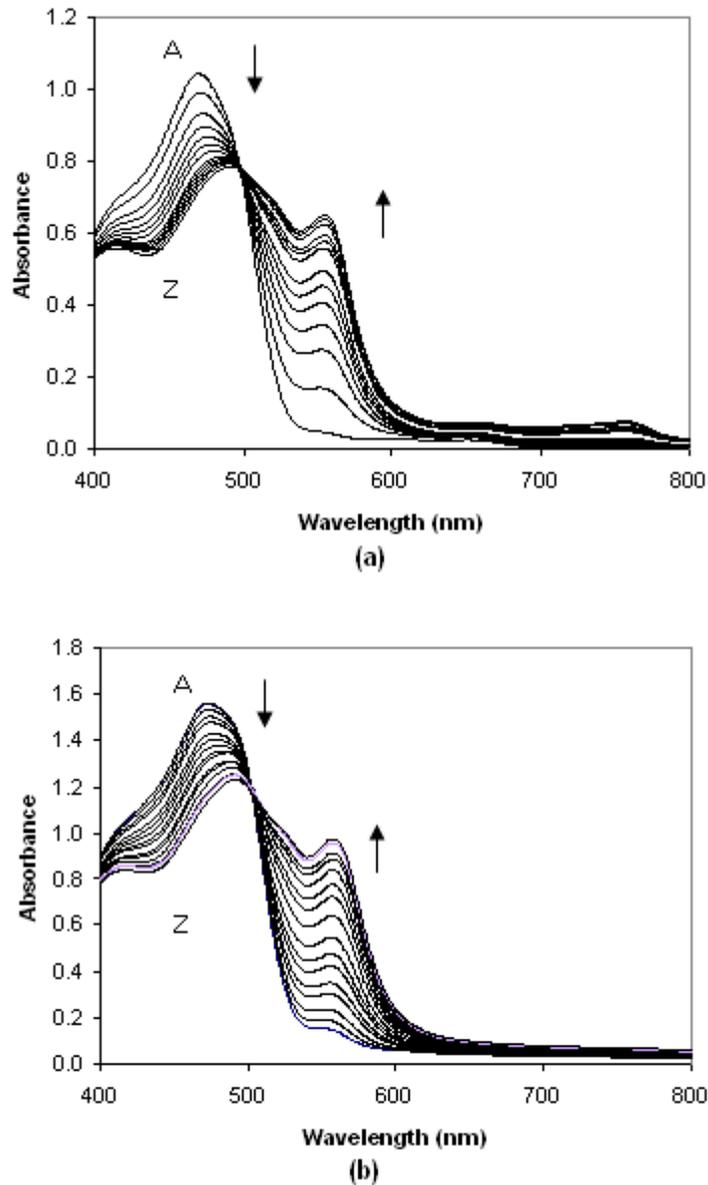
where HL is PAN. It may be assumed that a 1:1 Hg (II)-PAN complex is formed in the optode membrane since this was the stoichiometry found in aqueous solution for PAN [21].

### 3.2. Spectral characteristics

Fig.1 shows the absorption spectra of free and immobilized PAN, which were obtained after being equilibrated in buffer solution (pH6) containing different concentrations of mercury. The spectral change (increase in absorption band at 558 nm and decrease in absorption band at 473 nm) is result of increase of mercury ions concentration in the membrane, which is due to the extraction of mercury ion into the membrane and complex formation. The absorbance maxima of the immobilized PAN are located at 558 nm and those of free dye at 550 nm. It is important to note that the absorption spectra of immobilized dye is red shift in comparison to those of their soluble form (558 instead 550 nm). This suggests that the structural conformation of the immobilized dye is more planar than that of its solution analogue [22]. The wavelength of 558 nm was selected for further studies because of higher selectivity and sensitivity at this wavelength.

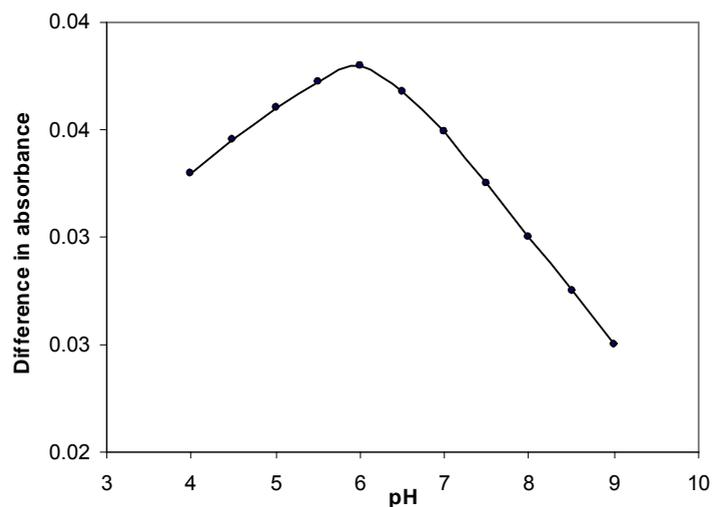
### 3.3. Effect of pH on response of the optode

Fig. 2 shows the effect of pH values on the response of the optode membrane. The absorbance measurements were made for  $1.5 \mu\text{g mL}^{-1}$  mercury ion in the pH range of 4 – 9 at 558 nm. The absorbance measurements were expressed as absorbance difference, which was defined as the difference between the absorbance of the immobilized PAN alone and the absorbance of the Hg - PAN complex at 558 nm. As can be seen in Fig.2, the pH increases from 4 to 6.0 as the value of the difference in absorbance increases. At pH values more than 6, the response decreases. This phenomenon might be due to the fact that at lower pH values (pH<6), complexation is weak. At pH values higher than 6,  $\text{Hg}^{2+}$  forms different hydroxide species which make it unable to form complex with PAN [23]. Thus, pH6 was selected for further studies.



**Fig.1. (a)** Absorption spectra for a  $1 \times 10^{-4}$  M PAN solution in the presence of  $(0-28 \mu\text{g mL}^{-1}) Hg^{2+}$  at pH6 (A-Z show increase in concentration of  $Hg^{2+}$  by addition of  $1.7 \mu\text{g mL}^{-1} Hg^{2+}$  in each interval)

**(b)** Absorption spectra of optode film in the presence of  $(0-28 \mu\text{g mL}^{-1}) Hg^{2+}$  at pH6. (A-Z show increase in concentration of  $Hg^{2+}$  by addition of  $2.3 \mu\text{g mL}^{-1} Hg^{2+}$  in each



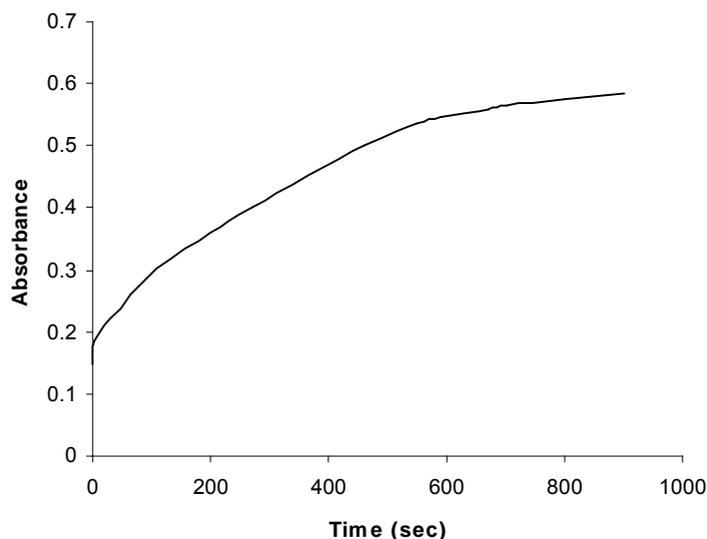
**Fig.2.** Effect of pH on the response of membrane in the presence of  $1.5 \mu\text{g ml}^{-1}$  mercury at 558 nm

### 3.4. Response time

The response time of the optode is controlled by the time required for the analyte to diffuse from the bulk of the solution to the membrane interface and to associate with the indicator [24].

The response time of the present optode was tested by recording the absorbance change at 558 nm from a pure buffer (pH 6) to a buffered mercury solution of  $12 \mu\text{g mL}^{-1}$ .

The membrane was found to reach 95% of the final signal at 10-12 min depending on the concentration of the  $\text{Hg}^{2+}$  (Fig. 3). In general, the response time is lower in concentrated solutions than dilute solutions.



**Fig.3.** Typical response curve of the film optode at 558 nm as a function of time when film was exposed to  $12 \mu\text{g mL}^{-1}$   $\text{Hg}^{2+}$  ion

### 3.5. Dynamic range

Fig. 4 shows the absorbance signals of the optode film to various concentrations of  $\text{Hg}^{2+}$  ions in the range of 0-28  $\mu\text{g mL}^{-1}$  that exhibits a linear range from 0.1 to 24  $\mu\text{g mL}^{-1}$  and 28  $\mu\text{g mL}^{-1}$  was found as the concentration of  $\text{Hg}^{2+}$  ion that saturates the film.

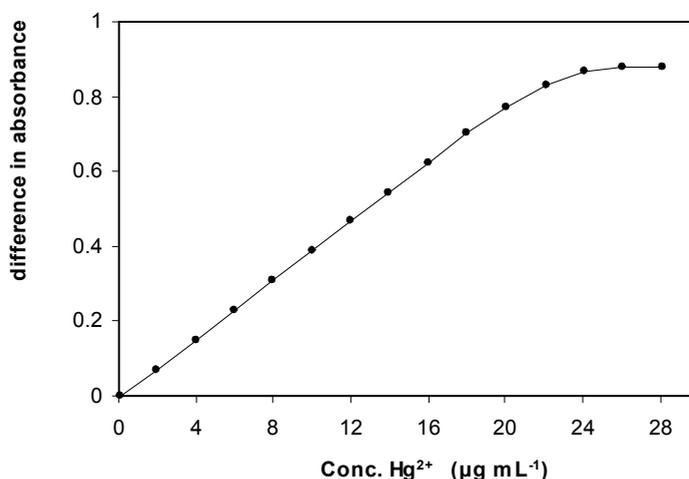


Fig.4. Calibration curve of the membrane at 558 nm in the range of 0-28  $\mu\text{g mL}^{-1}$

The regression equation was:  $\Delta A = 0.0068 C + 0.0783$  with a correlation coefficient of 0.9991, where  $\Delta A$  is the increase in absorbance of the film at 558 nm for a fixed time of 12 min, the C is the concentration of  $\text{Hg}^{2+}$  in  $\mu\text{g mL}^{-1}$ .

The detection limit which was estimated as the concentration of analyte producing an analytical signal equal to three times the standard deviation of the blank signal was found to be 0.02  $\mu\text{g mL}^{-1}$ .

### 3.6. Regeneration of the optode membrane

Some reagent including HCl, NaOH, EDTA,  $\text{H}_2\text{SO}_4$ ,  $\text{HNO}_3$ , SCN, ethylene diamine and uric acid were studied as regenerating reagents. It was found that the best result was obtained by applying ethylene diamine which gave short membrane regeneration times (10-30 S). After this regeneration and for the next mercury concentration measurement, the optode should be placed in buffer (pH 6) for 10-15 min.

### 3.7. Selectivity

To determine of selectivity of the optode membrane, the membrane was tested for the determination of 0.2  $\mu\text{g mL}^{-1}$  of  $\text{Hg}^{2+}$  ions in the presence of some metal ions . including,  $\text{Cd}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ti}^+$ ,  $\text{Bi}^{3+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Sn}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cr}^{3+}$ .

The tolerance limit was taken as the concentration causing an error of  $\pm 5\%$  in the analytical signal for determination of  $\text{Hg}^{2+}$  [25]. At the applied pH value, no interference was observed from even 1000-fold excess of the above metal ions except  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$ . Ions such as  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Bi}^{3+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cr}^{3+}$  are precipitated at universal buffer (pH 6) which can be eliminated by filtering. The results showed that the  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  ions interfered at concentrations higher than  $10 \mu\text{g mL}^{-1}$  and  $23.5 \mu\text{g mL}^{-1}$ , respectively which can be masked by addition of murexide (0.01 M).

### **3.8. Reproducibility and Repeatability**

These parameters of the sensing phase membrane in the determination of mercury was evaluated by repeatedly exposing the sensing phase membrane to a  $1.0 \mu\text{g mL}^{-1}$  mercury solution and a  $1 \times 10^{-4}$  M ethylen diamine solution. The repeatability was evaluated by performing seven determinations with the same standard solution of  $\text{Hg}^{2+}$ . The relative standard deviation (R.S.D) for the response of one membrane towards a  $1 \mu\text{g mL}^{-1}$  of mercury solution was 1.8% ( $n=7$ ).

The reproducibility of the response of different membranes was also studied. Seven different membranes were prepared from the same batch and they were evaluated by performing the determination of  $1 \mu\text{g mL}^{-1}$  mercury. The relative standard deviation for the response of between membranes was 2.3%. The results show that the reproducibility is satisfactory and the membrane could be regenerated easily by using ethylene diamine solution.

### **3.9. Life time and stability**

The lifetime of membrane was determined by adding a buffer solution (pH 6) in a cuvette including the film. The signal was recorded at wavelength of 558 nm over a period of about 10 h. No significant loss of the indicator and no drift in signal occur during this time and the sensing phase was stable over the experiment with no leaching of the indicator. It should be noted at pH 6, PAN exists its neutral form (yellow). Additionally the stability of response of the film was investigated over six weeks under water, which indicated that the film was stable over this period.

### **3.10. Accuracy and Analytical applications**

The proposed optical sensor was found to work well under laboratory conditions. To test the practical application of the present sensor, the omega3 tablet, vegetable and fish digest samples spiked with different amounts of mercury ions were measured by the proposed optode (Table 2). The mercury content of water and soil digest were analyzed by standard addition

method and then determined by the proposed optode (table 3). From the data given in Table 2, 3 and part 3.10.1, of this paper is readily seen that the present optical sensor is useful for the determination of mercury in real samples.

**Table2.** Results of mercury (II) ion determination in spiked samples (N=3)

Sample <sup>a</sup>	Mercury (II) Added	Mercury (II)found <sup>b</sup>	RSD%	Recovery (%)
Omega3 (1) <sup>c</sup>	0	N.D <sup>d</sup>	---	---
	1	0.96	1.8	96.0
Omega 3 (2)	0	N.D	---	---
	6	5.7	1.9	95.0
Omega3 (3)	0	N.D	---	---
	20	19.6	1.9	98.0
Vegetable 1	0	N.D.	---	---
	2.0	1.85	1.8	92.5
Vegetable 2	0	N.D.	---	---
	12.0	11.81	1.9	98.5
Vegetable 3	0	N.D.	---	---
	22.0	21.5	1.9	97.7
Fish 1	0	0.13	2.0	---
	3	3.06	1.9	102.0
Fish 2	0	0.13	2.0	---
	8.0	8.00	1.8	100.0
Fish 3	0	0.14	2.0	---
	14.0	14.10	1.9	100.7

a) All values are  $\mu\text{g g}^{-1}$

b) Average of three determinations found by the proposed method

c) Number of samples

d) No detected

**Table3.** Determination of  $\text{Hg}^{2+}$  in soil and water samples

Sample	$\text{Hg}^{2+}$ added	$\text{Hg}^{2+}$ found	RSD%
Soil <sup>a</sup>	0	2.21	2.1
	5	6.92	1.9
	10	11.68	1.8
	15	16.73	1.8
	20	21.96	1.9
Water <sup>b</sup>	0	0.58	1.7
	5	5.24	1.9
	10	9.91	1.9
	15	15.10	1.9
	20	19.96	1.8

a) All values are  $\mu\text{g g}^{-1}$

b) All values are  $\mu\text{g mL}^{-1}$

### 3.10.1. Determination of mercury (II) in an alloy

The certified composition of the alloy was Ag 34.38% , Zn 16.05% , Cu 6.88% and Hg 42.69%. The average value of five determination by the proposed optode was 41.52% mercury (n=5, standard deviation =  $\pm 0.83$ ). The recovery of the experimental mean obtained in relation to the known certified value was 97.3%.

## 4. Conclusion

The optode described in this work is easily prepared and provides a simple and inexpensive means for the determination of  $\text{Hg}^{2+}$  ions. The membrane responds to mercury ions by changing color reversibly from orange to red. The sensor can be regenerated readily with ethylene diamine solution and has a long life time. The response of the optode was reproducible and the optode presented a good selectivity for  $\text{Hg}^{2+}$  over other metal ions except for  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$ . Since the sensor does not require solvent extraction, it can compete with standard optical fibers. The sensor can be applied for the analysis of various samples. Also by comparison of this method with some of the other sensors for determination of  $\text{Hg}^{2+}$  ions, has been recognized that the proposed method has more sensitive and selective. It has wide dynamic range and even more easy fabrication and low cost.

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