

## Comparison of new optical sensor based on triazene ligand immobilized on PVC and triacetylcellulose membranes for Hg (II) ion

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### Abstract

For spectrophotometric analysis of Hg(II) ions, we have utilized and thought about two membranes preparation techniques utilizing different polymers; one is poly(vinyl chloride) (PVC) and the other is triacetylcellulose (TAC). In the case of TAC membrane, it was treated with a ligand solution ( $1.166 \times 10^{-3} \text{ mol L}^{-1}$ ) in ethylenediamine at ambient temperature for almost 2-5 min. However, in the case of PVC membrane, the ligand of triazene was incorporated into a plasticized membrane of PVC. The significant factors which had an impact on the determination of analytical performance from aqueous solutions are as follows: the limit of detections (LODs) by TAC membrane ( $64 \mu\text{g L}^{-1}$ ) and by PVC membrane ( $0.069 \text{ ng L}^{-1}$ ), linear dynamic range (LDR) by TAC membrane (7-90  $\text{mg L}^{-1}$ ) and by PVC membrane ( $0.125\text{-}10 \mu\text{g L}^{-1}$ ). In this study, LODs and linear dynamic range of PVC membrane were lower than that of TAC membrane by a factor of 10000. Nonetheless, TAC membrane was less demanding to work and not any more uncommon gadget required, but PVC membrane required much shorter time reaching a steady state. Both membranes were successfully applied in order to determine the level of Hg(II) in water samples.

**Keywords:** Sensor; spectroscopy; heavy metal; membranes; PVC; TAC.

### Introduction

For a long time, chemical and electrochemical sensors have been the subject of inquiry due to their extraordinary applications. Using such sensors is faster and economically more justifiable than monitoring environmental samples in order to determine heavy metal ions such as Hg, Cd, Cu and Zn which tend to be very hazardous elements [1-4].

Mercury metal is exceptionally poisonous, collective and constant in both the natural environment and living organisms. As a result, quantitative determination of mercury becomes increasingly important in the aspect of both environment and human health.

There is a wide range of techniques to determine the Hg(II) concentration in aqueous solutions. Indeed, atomic spectrometry [5-7], molecular spectrophotometry [8-12] and

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volatammetry methods [13-15] are among the techniques that can be expected to measure the trace of the amount of Hg(II) ions. Furthermore, there are various optical sensors in the literature that can be utilized to measure Hg(II) [16-23].

This strategy, i.e. the incorporation of chemical sensors based on an optical signal measurement has several advantages in analytical chemistry. The aforesaid elements can be miniaturized and fabricated at a low expense [24-26].

An ion-selective optode membrane can be set up by the incorporation of an ionophore into a hydrophobic membrane and utilized in contact with a sample solution containing a primary ion. Since ionophores are complex lipophilic agents, they possess the capability to control the reversibility of the ions and transport them crosswise over organic membranes by means of carrier translocation. With respect to the application of analytical chemistry for ionophores in the solvent polymeric membranes of bulk optodes or ion selective electrodes, some requirements such as adequate lipophilicity, sufficiently reversible exchange kinetics and high selectivity must concurrently be met [27-33].

Poly vinyl chloride (PVC) (high molecular weight) polymer is exceptionally well-known among all of the polymers that used to preparation of the ion-selective membranes. This polymer has certain advantages such as mechanistic aspects and its simplicity of membrane preparation. The fundamental objective is to create main membranes with better biocompatibility and adhesion properties [34].

In this study, we compared two membranes, one of which was TAC membrane and the other PVC membrane for fabrication of optodes.

Both membranes use the recently synthesized ligand, (E)-1-(2-ethoxyphenyl)-3-(4-ityrophenyl)triazene-1-ene (L), as the sensing reagent. This ligand can be utilized as an effective one for the spectrophotometric determination of Hg (II) ion in aqueous solutions. Triazenes constitute a different class of compounds which have thoroughly been studied for their anticancer potential. In triazene molecule, an amine was directly bonded to an azo group. The functional group is also known as the diazoamino group [35], and has a Tran's configuration in its basic mode [36]. Triazenes can bind to metals in both mono-dentate and bi-dentate manners. The products are stable and can resist both air and moisture. Moreover, they are easily prepared [37].

## Experimental

### Materials and reagents

All of the chemicals were in the analytical grade and purchased from Merck (Darmstadt, Germany). Doubly distilled water was used throughout the experiments. A 1000 mg L<sup>-1</sup> Hg (II) stock solution was prepared by dissolving 0.1708 g Hg(NO<sub>3</sub>)<sub>2</sub>•H<sub>2</sub>O in doubly distilled water and 0.5 mL of 1 mol L<sup>-1</sup>HNO<sub>3</sub> and diluted to 100 mL in a volumetric flask. Subsequently, sample solutions were prepared by appropriate dilution. Buffer solutions were prepared from boric acid/acetic acid/phosphoric acid (0.04 mol L<sup>-1</sup> each), and the pH adjustments were made by the addition of 1 mol L<sup>-1</sup>NaOH or 1 molL<sup>-1</sup>HCl solutions. A Shimadzu 1601 (Kyoto, Japan) PC UV-Vis spectrophotometer with 1 cm cell quartz was used in order to record all spectra and absorbance measurements. A Metrohm 827 (Switzerland) pH-meter with a combined glass electrode was used after calibration against

standard Merck buffer for pH adjustment.

### Synthesis of (E)-1-(2-ethoxyphenyl)-3-(4-nitrophenyl) triazene-1-ene

The preparation of 1-(2-ethoxyphenyl)-3-(4-nitrophenyl)triazene-1-ene (L) was performed as described in the previous experiment and its structures have been approved [38]. The main compound,  $C_{14}H_{14}N_4O_3$ , was prepared by the following method: A 100 mL flask was charged with 10 g of ice and 15 mL of water and then cooled to 0 °C in an ice-bath. To this, 2 mmol (0.344 g) of 4-nitroaniline and 2 mmol of hydrochloric acid (36.5%) and 2 mL of

water was added. Then, a solution containing  $NaNO_2$  (2 mmol, 0.16 g) was added to 2 mL of water during a 15-minute period. After mixing for 15 minutes, the obtained solution was added to a solution of 2 mmol (0.261 mL) of o-phenetidin and 2 mL of methanol and 2 mL of water. After that, a solution containing 36 mmol (2.95 g) of sodium acetate in 10 mL of water was added. After mixing for 24 hours, the orange product was filtered off and dissolved in dimethyl sulfoxide (DMSO). Recrystallization from DMSO turned the product into an orange crystalline material.

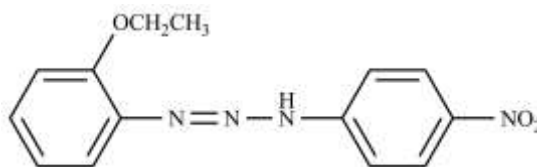


Figure 1. The general structure of a triazene

### Preparation of the triacetylcellulose membrane

To immobilize the triazene ligand (L), TAC membrane was treated with a ( $1.166 \times 10^3 \text{ mol L}^{-1}$ ) ligand solution, in ethylenediamine, and for 2-5 minutes at ambient temperature. The resulting orange color membranes were thoroughly washed in a detergent solution and water. Prepared membranes were kept under water when not in use. Each time six membranes were made, and they can still be used after 1 month when kept under water. A 1 cm  $\times$  3 cm bit of the fabricated membrane sensor was cut and placed inside the quartz cell of the spectrophotometer. All the measurements on the TAC membranes were performed in the aqueous medium. For the purpose of analysis, about 2.5 mL of the sample was

transferred to a 1 cm quartz cell equipped with the membrane sensor. Subsequently, the absorbance was measured at 405 nm and subtracted from an absorbance reading for a buffer solution at the same wavelength. After that, the Hg(II) concentration was obtained using an ordinary calibration curve method.

### Membrane preparation of PVC

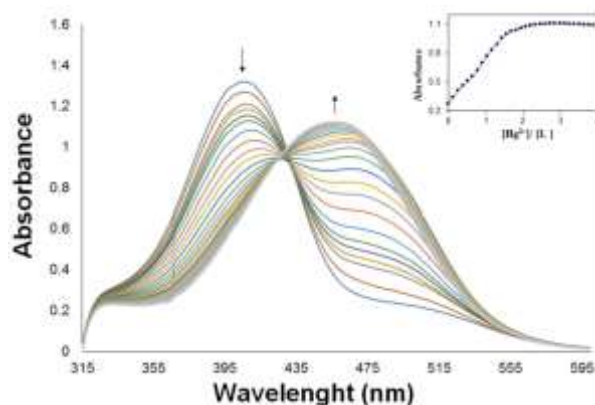
Microscope slides cut into 9 mm  $\times$  50 mm dimensions will prepare glass slides to fit easily into a standard spectrophotometer glass cell (10 mm  $\times$  10 mm  $\times$  50 mm). Membrane solutions were prepared by dissolving 32 mg PVC, 64  $\mu\text{L}$  dibutyl phthalate (DBP) and 8 mg ligand in 2.0 mL tetrahydrofuran (THF). The solution was stirred with a magnetic stirrer to obtain a homogeneous mixture. To improve the adhesion of the membrane,

the glass slides were cleaned with THF, sulfuric acid and sodium hydroxide solutions, thoroughly rinsed with distilled water, and finally dried in an oven at 110 °C. The membranes were cast by setting 25  $\mu$ L of the homogenized membrane solution on the glass slide and spread quickly using a spin-on device (1650 rpm rotation frequency). The ready membranes were dried in ambient temperature for 15 minutes and then kept in a clean and dark place for 12 hours to completely evaporate the solvent. Blank (reference) membranes were prepared in a similar way excluding ligand from the membrane solution. All measurements were carried out at room temperature.

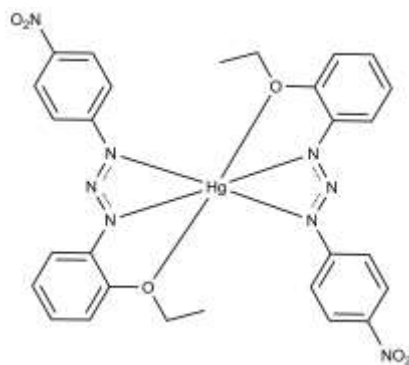
### A complex study

In order to determine the stoichiometry and stability of the resulting metal-ligand complexes in a typical procedure, 2.0 mL of ( $5.0 \times 10^{-5}$  mol L<sup>-1</sup>) ligand solution in dimethyl formamide (DMF) were placed in the spectrophotometer cell and the absorbance of the solution in the range of 315-600 nm was measured. Thereupon, a known amount of the

concentrated solution of Hg(II) in ( $1.30 \times 10^{-3}$  mol L<sup>-1</sup>) DMF was added in a stepwise manner using a 10  $\mu$ L Hamilton syringe. The absorbance spectrum of the solution was recorded after each addition. The absorbance curves are shown in Figure 2. Through addition of Hg(II), a decrease in absorbance was observed at 405 nm and a new peak at about 465 nm was formed which corresponded to the formation of a Hg-L complex. The resulting plots of the absorbance at 465 nm against metal ion/ligand mole ratios are shown in Figure 2. From the sharp inflection point observed for Hg(II) at a mole ratio of 2, it can be concluded that 1:2 complex of [Hg-L<sub>2</sub>] is formed in DMF solution. The formation constant of the resulting complex between Hg(II) and L ligand was evaluated and found to be equal to 7.81 from the absorbance versus [Hg(II)]/ [L] mole ratio data using known equations and utilizing a non-linear least-squares curve fitting program, KINFIT [39]. The molecular structure of synthesized triazene ligand with Hg(II) ion has been confirmed in Figure 3 [38].



**Figure 2.** Absorbance spectra of ligand solution ( $5.0 \times 10^{-5}$  mol L<sup>-1</sup>) in DMF and increasing concentration of Hg(II) ion solution ( $1.30 \times 10^{-3}$  mol L<sup>-1</sup>). The arrows show the direction of absorbance by increasing the ion concentration.



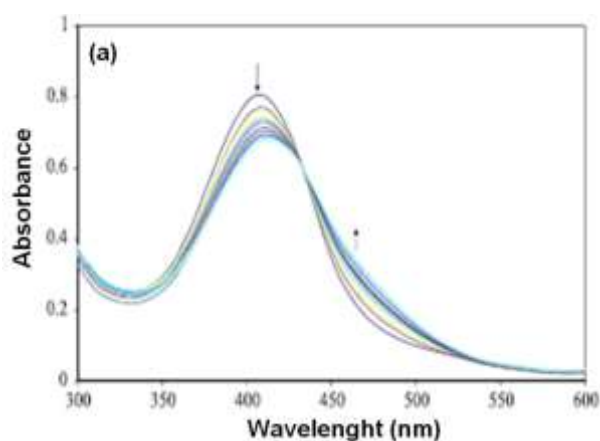
**Figure 3.** The molecular structure of  $[\text{Hg}(\text{C}_{14}\text{H}_{13}\text{N}_4\text{O}_3)_2]$

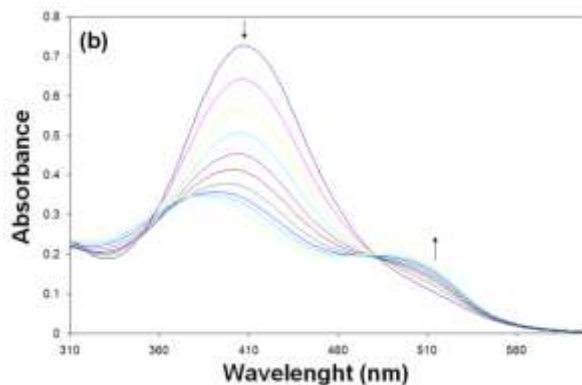
### Results and discussion

#### Absorbance measurement

The prepared membranes (TAC and PVC) were placed into a buffer solution at pH 3.0 for 2 minutes to reach equilibrium. Then, the membranes were placed in a quartz cell containing 2.0 mL of universal buffer (pH 3.0) in the sample path of the spectrophotometer. The quartz cell along the reference path of the spectrophotometer consisted of a blank film. The baseline was corrected in the intended area. Then, the sample solution containing an appropriate amount of Hg(II) ions was injected into the cell with a micro syringe. After 10 minutes, the absorbance was measured

at 405 nm for both the membranes. The spectral change (increase in the absorption band at 476 nm and decrease in the absorption band at 405 nm) is the result of the increase of Hg(II) ion concentration in the membrane and complex formation (Figure 4). This illustrates that the optode membranes can be used in order to assay Hg(II) in aqueous sample solutions. The maximum absorbance of the immobilized ligand (L) is located at 405 nm. The wave length of 405 nm was selected for further studies due to its higher selectivity and sensitivity at this point.



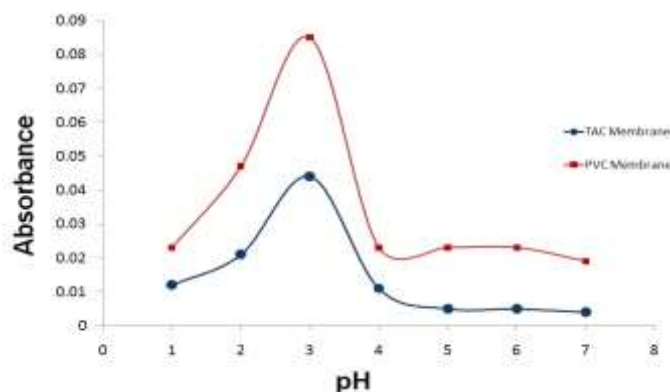


**Figure 4.** Absorption spectra of optode film: (a) TAC membrane in the presence of (0.0-5.0 mg L<sup>-1</sup>) Hg(II) at pH 3.0, by addition of (0.5 mg L<sup>-1</sup>) Hg(II) at each interval, (b) PVC membrane in the presence of (0.0-5.0 μg L<sup>-1</sup>) Hg (II) at pH 3.0, by addition of (0.5 μg L<sup>-1</sup>) Hg(II) at each interval.

#### Effect of test solution pH on the sensor response

pH could obviously affect the response of the proposed Hg (II) ion-selective optical sensor as illustrated in Figure 5. The response characteristic of the membrane sensor was deduced as highly dependent on pH. The absorbance measurements were made in the pH range of 1-7 at 405 nm for (10 mg L<sup>-1</sup>) and (10.0 μg L<sup>-1</sup>) concentrations of Hg (II) ion for TAC and PVC membranes, respectively. As it is obvious from Figure 5, the

absorbance increased rapidly by changing the pH from 2 to 3, while it decreased at pH values higher than 4. This phenomenon might be a result of the fact that at lower pH values (pH < 3), complexation is weak as pH can change the conditional stability constant of the resulting metal complex. On the other hand, the reduced optical response of the proposed sensor at pH > 3 could be due to a possible hydroxide formation of Hg (II) ions. Thus, in subsequent experiments, pH 3 was used for further investigations.

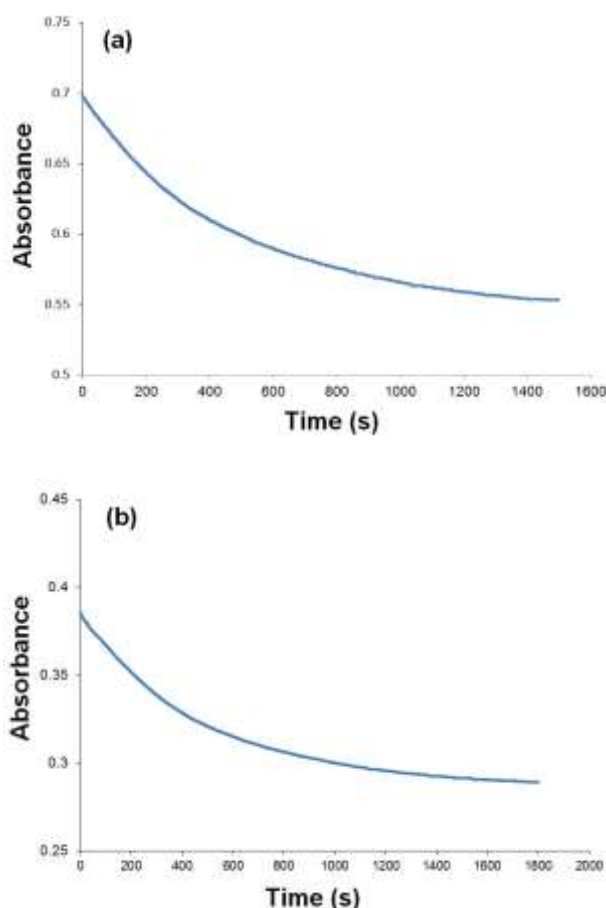


**Figure 5.** Effect of pH on the response of: (a) TAC and (b) PVC membranes in the presence of (10.0 mg L<sup>-1</sup>) and (10.0 μg L<sup>-1</sup>) of Hg(II) ions, respectively

### Sensor response time

As we know, the time required to response of the sensor towards a certain concentration of the measuring ion, the response time value ( $t_{95\%}$ ) of the sensor when reaches to 95% of its final value (steady state) [40], is one of the main parameters that must be determined experimentally. Figure 6 demonstrates the time-dependent response characteristics of the optical films,

immersed into a buffer solution at pH 3 containing  $10 \text{ mg L}^{-1}$  and  $10 \mu\text{g L}^{-1}$  Hg(II) ions in TAC and PVC films, respectively. This Figure indicates that the shortest response time of the TAC and PVC films are about 15 minutes and 8 minutes respectively, in which the sensors are able to monitor the reversibility and reproducibly of the Hg(II) ion.



**Figure 6.** Typical response curve of the optode film: (a) TAC and (b) PVC membrane at 405 nm as a function of time when the film was exposed to  $10 \text{ mg L}^{-1}$  and  $10 \mu\text{g L}^{-1}$  Hg(II), respectively

### Regeneration of the optode membranes

The optical layers ought to be recovered utilizing an appropriate stripping reagent after it contacts with the Hg (II) solution and prepares for the

accompanying measurements. A few reagents, including HCl, NaOH,  $\text{H}_2\text{SO}_4$ ,  $\text{HNO}_3$ , ethylenediamine and EDTA were considered as recovering reagents. It was perceived that the best outcome was acquired by applying EDTA which

produced short membrane recovery times (40-50s). After this recovery and for the next Hg(II) concentration measurement, the optode must be put in buffer pH 3 for 10 minutes.

#### Linear dynamic range

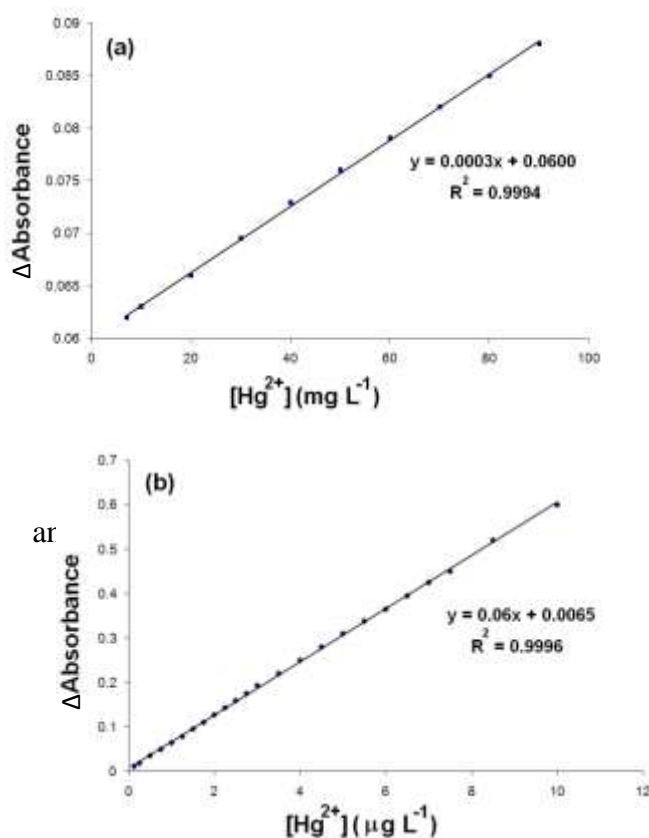
Figure 7a shows the absorbance signals of the TAC optode film to various concentrations of Hg(II) ions, in the range of (7.0-90.0 mg L<sup>-1</sup>) at pH 3 and (90.0 mg L<sup>-1</sup>) found as the concentration of Hg(II) ion saturating the films. The limit of detection (LOD) which was calculated as the

producing an analytical signal equal to 3σ of the blank signal, was 64.0 μg L<sup>-1</sup>.

The absorbance signals of the PVC optode film to various concentrations of Hg(II) ions in the range of (0.125-10.0 μg L<sup>-1</sup>) at pH 3 are shown in the Figure 7b. 10.0 μg L<sup>-1</sup> was found as the concentration of Hg(II) ion which saturated the film. The limit of detection (LOD) was 0.069 μg L<sup>-1</sup>.

In Table 1, comparison of the significant factors affecting the determination of Hg(II) ion in both of the membranes has been shown.

concentration of ar



**Figure 7.** Calibration curve of the (a) TAC and (b) PVC membrane at 405 nm in the range of (7-90 mg L<sup>-1</sup>) and (0.125-10 μg L<sup>-1</sup>) Hg(II), respectively



### Determination of Hg(II) ion in water samples

To assess the applicability of the proposed sensors for determination of Hg(II) in real samples, the sensors were kept in a water sample in order to determine the Hg(II) ion. For the purposes of analysis, tap water and ground water were collected from Islamic Azad University of Lamerd (Iran) and filtered. As no distinct signal of Hg(II) was observed, the standard

addition method was used, and the samples were spiked with different amounts of Hg(II). Each sample with the proposed sensors was analyzed for 3 replicates for TAC and 5 replicate for PVC membranes. The results are given in Tables 2 and 3. The outcomes demonstrate that the sensors are appropriate for determination of Hg(II) concentrations in such samples with great precision and accuracy.

**Table 1.** Comparison of the significant factors affecting the determination of Hg(II) ions

Parameter	Membtane Type	
	TAC	PVC
pH	3	3
Response time	15 min	8 min
Detection Limit (LOD)	64 $\mu\text{g L}^{-1}$	0.069 $\mu\text{g L}^{-1}$
Linear Dynamic Range (LDR)	7- 90 $\text{mg L}^{-1}$	0.125-10 $\mu\text{g L}^{-1}$
Line equation	$y = 0.0003x + 0.06$	$y = 0.06x + 0.0065$
R <sup>2</sup>	0.9994	0.9996

**Table 2.** Determination of the Hg(II) ions in different water samples for TAC film

Water sample	Hg(II) ( $\text{mg L}^{-1}$ )		RSD% (n=3)	RR* %
	added	found		
Ground water	10	9.52	0.097	95.2
	30	29.53	0.014	98.4
Tap water	30	29.2	0.086	97.3
	50	47.4	0.011	94.8

\*Relative recovery

**Table 3.** Determination of the Hg (II) ions in environmental water samples for PVC film

Water Sample	Hg(II) ( $\mu\text{g L}^{-1}$ )		RSD% (n=5)	RR %
	added	found		
1	1.25	1.91	7.05	104.8
2	2	2	4.23	95.5
3	5	5.06	1.61	101.2
4	7	6.98	1.03	99.71

\*Relative recovery

## Conclusion

In this study, PVC membrane optode was compared with TAC membrane in terms of affecting factors, analytical performance and application to real samples. It was demonstrated that both TAC and PVC membranes showed similar analytical performance for Hg(II) ion, however, PVC is suggested to have fast response time, outstanding wide linear dynamic range, very low detection limit, high selectivity, and good reproducibility. By comparison, TAC membrane has easy preparation (was easier to operate) and demanded no extra device. Due to the selectivity of newly synthesized ligand, both of the two membranes could be applied directly to the determination of Hg(II) ion in real samples. From the two membranes, PVC membrane suffers from difficult preparation.

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