Asian Journal of Chemistry Vol. 21, No. 3 (2009), 1747-1753

Development of Optical Test Strip for Rapid Determination of Trace Arsenic Using Immobilized Gallocyanine

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Irreversible test strip for the determination of arsenic has been developed. It has a rectangular sensing zone containing all the reagents necessary to produce a selective response to arsenic and formed by immobilized gallocyanine inside chitosan membrane. This method offer sensitivity and simplicity in detecting arsenic as no prior treatment or extraction is required. A linear response was attained in the arsenic concentration in the range of 10 to 30 ppm with calculated limit of detection of 0.04 ppm. This method also showed a reproducible result with relative standard deviation of 0.87 % and response time of *ca.* 5 min. Interference studies showed that Pb(II) and Ni(II) significantly interfered during the determination. The developed sensor has been validated against atomic absorption spectroscopy method and proven comparable.

Key Words: Arsenic detection, Optical test strip, Immobilized chitosan membrane.

INTRODUCTION

Arsenic occurs in the environment in organic and inorganic forms. Arsenic is generally found in the inorganic forms of arsenite and arsenate. Arsenic usually contaminates ground and surface water is toxic if swallowed. Concentration of arsenic in surface and ground waters generally range from 0.001 to 0.01 ppm, but elevated levels (1-50 ppm) have been reported in groundwaters in China, India and Bangladesh. Long term exposure to low concentration of arsenic has been linked to increased risk of cancer and can lead to death if ingested in large dose. Arsenic is usually exposed to human being through food and water. The maximum contaminant level of arsenic recommended for implementation in USA for drinking water is 0.01 ppm and maximum contaminant level in Malaysia is 0.003 ppm.

Numerous analytical techniques have been employed to detect arsenic including chromatography technique[1], atomic absorption spectrometry[2] and inductively coupled plasma mass spectrometry[3]. Although these methods are sensitive and accurate, most require a tedious sample pre-treatment, skilled operator and expensive equipment. Almost none of these equipments can be used for field screening or on-site

assay. Simple and fast field analytical test is indeed a necessity nowadays. Among these techniques, the dry reagent test strip method offered the simplest and the cheapest analytical method and has been widely used in clinical analysis. Test strips can be described as integral analytical elements typically in the form of thin pads or films which contain all the reagents required for an assay distributed in a dry form within a pad or film[4]. A drop or precise volume of a fluid problem is placed on one of the surfaces of the solid phase element by dipping or dropping from where it diffuses into the reaction zone. After the reaction optical or electrochemical property of the generated changes can be measured on the device itself.

Most analytical methods for arsenic determination are based on the arsenomolybdenum blue[5] formation, hydride generation[6-8] or arsine generation[9,10]. The arsenomolybdenum blue method is sensitive but suffers strong interferences from phosphates and silicate. As for arsine generation method, it can be dangerous since arsine is a very toxic substance.

There are different approaches in fabrication of test strip; (**a**) impregnation of solid particles[7-9]; (**b**) the use of porous carrier matrix impregnated with the selective reagent[10-14]; (**c**) the use of emulsions in both phases where hydrophilic polymers containing a buffer distributed in a hydrophobic film-forming polymer containing the reagents[15].

In this paper, the authors propose a new test strip to determine arsenic using a homogenous chitosan membrane impregnated with chromogenic reagent, gallocyanine that selectively and quantitatively respond to arsenic by modifying the colour.

EXPERIMENTAL

All chemical used were of analytical grade and deionized water was used throughout for solution preparation. Gallocyanine solution of 1.0×10^{-4} M was prepared by dissolving 0.006 g of gallocyanine in 200 mL of deionized water. A stock solution (1000 ppm) of As(III) was prepared by dissolving 0.100 g of As_2O_3 in 1000 mL of deionized water. Working standard solution of As(III) were prepared by appropriate dilution of the stock solution before used.

Procedure: Immobilization of gallocyanine inside the chitosan membrane is done by mixing 5 mL of 0.15 % (w/v) gallocyanine with 45 mL of 1 % chitosan solution. The mixture is stirred thoroughly for about 2 h and poured into a petri dish with diameter of 10 cm. The mixture was left to dry for around 14 d in room temperature to enable a smooth and even membrane to form. The membrane is then peeled off, soak in ionized water for about 2 h and left to dry for a few hours. The membrane is then cut into small square (0.5 cm × 0.5 cm) which weight about 43 mg and thickness of 20 mm. The membrane was then dipped into arsenic solution and the absorbance was measured using a double beam Varian (Model Cary 100) UV-Vis Optical Fibre Spectrophotometer.

RESULTS AND DISCUSSION

As shown in Fig. 1, gallocyanine showed a maximum absorbance at λ = 610 nm, whereas the complex showed a maximum absorbance at λ = 585 nm. This different in the maximum values for gallocyanine and its complex is due to a sharp colour change of the gallocyanine and arsenic-gallocyanine complex from navy blue to violet, respectively. Gallocyanine is a green crystal, practically insoluble in cold water, slightly soluble in hot water and is soluble in alcohol and glacial acetic acid. It is soluble in alkali carbonate to give a red solution and is soluble in concentrated hydrochloric acid to give a blue solution which becomes red when diluted with water. As_2O_3, when dissolved in water, attracts H^+ and OH^- ions and may rearrange their structures. In the case of As_2O_3, the reaction involved is as shown below:

$$As_2O_3 + 3H_2O \longrightarrow 2H_3AsO_3.$$

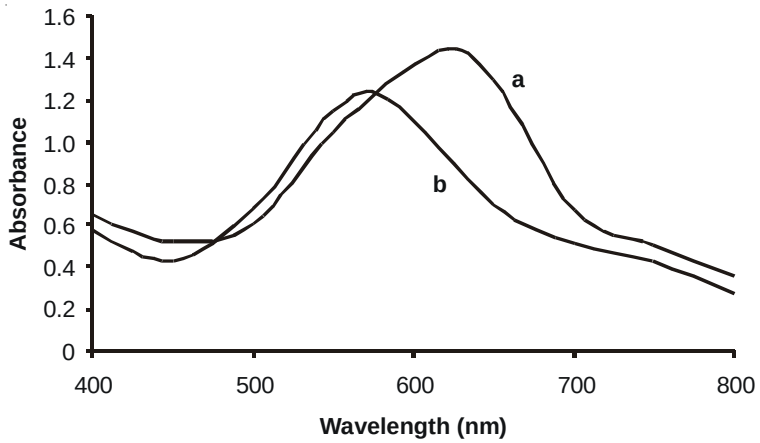

Fig. 1. Absorption spectra of Gallocyanine (a) and As_2O_3-Gallocyanine (b) complex

The molecule formed is arsenious acid whose salts are the arsenites[16]. The possible interaction between gallocyanine and arsenic is shown in Fig. 2.

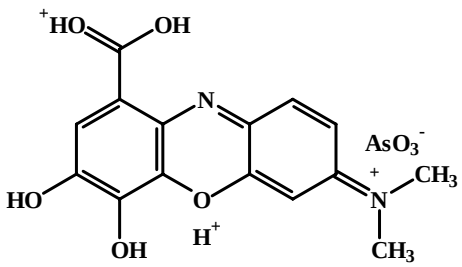

Fig. 2. Interaction between AsO_3 and diprotonated gallocyanine

pH is an important factor that influences arsenic compound. Thus, the solubility of arsenic depends on its oxidation state and pH of the solution. For example, the solubility of arsenic of As(V) decreases with decrease in pH, whereas the solubility of As(III) decreases as the pH increases[17]. In order to determine the optimum pH of immobilized gallocyanine and 200 ppm As_2O_3 solution in forming a stable complex, the absorbance of complex at various pH values was studied. The highest absorbance was obtained at pH 2. In acidic medium (pH < 4), gallocyanine exist as diprotonated ligand and appear in red-violet colour whereas in basic medium (pH 5.5 to 8.0), gallocyanine exist as monoprotonated ligand and appear in blue colour. For pH > 8, gallocyanine exist as nonprotonated ligand and appear in red-violet colour. The maximum interaction occur in pH lower than 4 because of the diprotonated condition is the most suitable condition for negatively charged molecule (arsenite ion) to form complex with gallocyanine. The graph of absorbance at different pH (pH 1 to pH 10) is shown in Fig. 3.

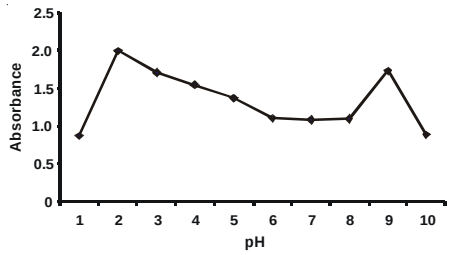

Fig. 3. Absorbance of the complex (As_2O_3-Gallocyanine) at different pH

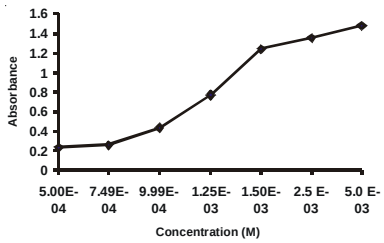

Fig. 4. Effect of the concentration of immobilized gallocyanine complex

The effect of the reagent concentration on the sensor response was studied by using different initial concentrations of the reagent (5.0×10^{-4} to 5.0×10^{-3} M) during its immobilization. The higher the reagent concentration used for immobilization, the higher the reflectance signal obtained for the same concentration of arsenic. A higher initial concentrations of reagent allowed more of it to be immobilized, resulting in more reaction between arsenic and gallocyanine producing a higher absorbance (Fig. 4). At the concentration of 4.996×10^{-3} M, the reflectance signal is leveled off. Therefore this initial reagent's concentration was used for further experimental purposes.

A study on the photo stability of the sensor was carried out to monitor the possibility of photo leaching or photodecomposition of the reagent phase when it was continuously exposed to a light source for a long period of time. For a continuous monitoring period of 7 h, the result shows that the reagent phase is stable and no leaching occur.

The reproducibility refers to the successive runs made by using an immobilized gallocyanine, as reagent to estimate discrepancies in its response. In order to test the accuracy of the test strips, 10 replicates following measurements was carried

out with standard solution of 200 ppm As_2O_3. The standard deviation (SD) and relative standard deviation (RSD) were found to be 0.0119 and 0.8715 %, respectively. Relative standard deviation (RSD) is just the standard deviation expressed as a fraction of the mean; usually it is given as the percentage of the mean (% RSD), which is often called the coefficient of variation[18].

The degree of interference measured for some foreign ions at a 1:1 mole ratio of arsenic:foreign ion is summarized in Table-1. The interference of foreign ions in the present system was studied with the determination of 10 ppm arsenic. Negative interference can result from the reaction of the interference with the analyte being determined, leading to an incomplete reaction. The most common type of such interference is the complexing of the analyte by interfering ion. Many cations will form complexes in solution with a variety of substances that have a pair of unshared electrons (*e.g.*, on N, O, S atoms in the molecule) capable of satisfying the coordination number of the metal. At pH 2, the gallocyanine exist in deprotonated form, which means possible interference from cations are low. Positive interference normally occur when the interfering ion react with the reagent along with the analyte and produce more intense coloured species resulting a higher reading in absorbance. Possible interference from cations and anions were studied and it was observed that ammonia and citrate has the highest degree of interference (Table-1). However the degree of interference is less than 10 % for 1:1 mole ratio and considered insignificant

TABLE-1
PER CENT INTERFERENCE OF FOREIGN IONS

Anions/cations	% Interference	Anions/cations	% Interference
Mn(II)	1.33	NH_3	7.84
Cd(II)	1.79	Citrate	7.16
Co(II)	1.84	Phosphate	2.71
Pb(II)	1.46		

Typical analytical curve (Fig. 5) of signal response as the function of arsenic concentration. It shows that the method developed produced a linear response when the As(III) concentration is within the range of 1.0 to 7.0 ppm. The limit of detection (LOD) of As(III), defined here as the concentration equivalent to a signal blank plus 3 times the standard deviation of the blank, was calculated to be 0.04 ppm. Hashemi and Modasser[6] who carried out detection of arsenic based on hydride generation and bleaching of permanganate has achieved LOD as low as 3.0 ppb, whereas Afkhami *et al.*[19] who utilized inhibitory effect of arsenic on the redox reaction between bromate and hydrochloric acid have been able to detect arsenic at sub ppb level. Kundu *et al.*[20] proposed a simpler method for arsenic detection based on colour bleaching of methylene blue in micellar medium with LOD of 0.03 ppm. Even though better LOD have been reported by these researchers, the usage of multiple steps reaction (hydride generation, inhibitory effect and arsine release for

colour bleaching) will usually have some effect on the response time. The use of multiple reagents also limits the possibilities of miniaturization. Compared to these researches, the current reported work offered single step detection with short response time and fairly good LOD.

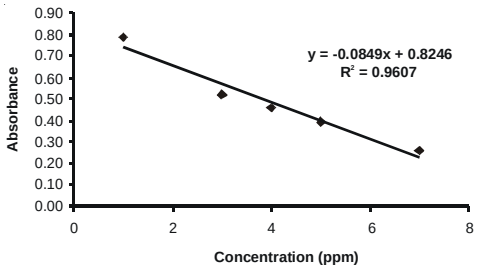

Fig. 5. Response of different concentration of arsenic

In order to asses the reliability of the test strips for the determination of arsenic, it was applied to real samples of waters of various provinces (mineral and tap). Recovery studies were made using 1 mL of water sample spiked with 1 ppm arsenic mixed with 9 mL of buffer solution. Table-2 shows the result obtained using the test strip proposed here compared with atomic absorption spectrometry (AAS) as the reference method. A statistical analysis (t-test) has been carried out. The t test is carried out to compare the mean from 2 sets of analysis. The result shows that the mean from both methods were not significantly different indicating that the method developed is within good agreement with the establish method.

TABLE-2
APPLICATION ON REAL SAMPLE

Method	Mineral water (ppm)	Tap water (ppm)	Recovery (%)	
			Mineral	Tap
AAS	0.97	1.01	97	101
Test strip	0.89	0.97	89	97

Three replicate samples

Conclusion

Optical test strip based on immobilized gallocyanine in chitosan membrane was found suitable to be used as the sensing element for determination of trace arsenic. This study has shown that upon reaction with arsenic, the optical test strip produces a reproducible response, with a good limit of detection (RSD = 0.87 %, LOD = 0.04 ppm) and exhibits fairly short response times (*ca.* 5 min). The developed test strip offered a simple and direct detection of arsenic and can be operated in mild condition (pH 2) at room temperature.

ACKNOWLEDGEMENT

The authors would like to acknowledge Ministry of Environmental and Science Department of Malaysia for funding this research through research grant IRPA 09-02-02-0028.

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(*Received*: 21 January 2008; *Accepted*: 31 October 2008) AJC-6982