Characterisation of a Hg(II) ion optrode based on Nafion®–1-(2-thiazolylazo)-2-naphthol composite thin films

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An optrode for Hg^{2+} ions based on a Nafion–1-(2-thiazolylazo)-2-naphtol (TAN) composite thin film has been developed. This optrode has a wide linear response range of 1–75 μM (pH 6) of Hg^{2+} ions with a limit of detection of 0.05 μM (10 μg l^{-1}), which is sufficiently adequate for many environmental monitoring applications. The reflectance signal response shows a close correlation with the theoretical model derived. The response time of the optrode thin film was within 5–7 min to reach 95% of the final signal, depending on the concentration of Hg^{2+} ions. The selectivity of optrode to Hg^{2+} ions in phosphate buffer is good, with Co^{2+} and Ni^{2+} ions as the main interferences.

Introduction

Optical fibre chemical sensors or optrodes based on organic polymeric thin and thick films have been investigated considerably in the last decade.[1–12] Organic indicator dyes and the immobilisation technique play important roles in the development and design of the optrode. Normally, the immobilisation of the indicator is achieved by absorption,[13] chemical bonding[14] or physical entrapment[6] into the support materials. However, after the immobilisation process the group which is responsible for the chemical interaction is often inaccessible to the analyte. Yet, the immobilised indicator often prohibits the reversible response, as a result of very strong binding of the analyte. Therefore, there is much interest in the immobilisation technique, which prevents leaching of indicators, and imparts a rapid response, reversibility and long lifetime to the sensors.

In the development of films for metal ion sensing, a different approach has recently been proposed using polymeric materials such as PVC[7], Nafion® [8] and sol–gel materials such as silicate or mixtures such as polyvinylformal–silica.[9] Optodes based on PVC membranes containing the ionophores methylene bis-(diisobutyldithiocarbamate),[10] 5-(4′-aminophenyl)-10,15,20-tris-(4′-sulfonatophenyl) porphyrin[11] and amphiphilic oxacarbocyanine dye[7] for heavy metal ion sensing have been published in the literature. In the case of Nafion®, the sensor is a thin film formed by entrapping the dye inside the film. They also have been used in conjunction with 1-(2-pyridylazo)-2-naphthol (PAN)[8] and 5,10,15,20-tetra(4-*N*-methylpyridyl)porphyrin[12] for heavy metal ion sensing. However, many of these sensor systems lack information on the optimisation parameters, such as pH, flow rate (if measurement is performed in the flow system), the lifetime, signal recovery process and the flow cell design.

Apart from chemical sensors, biosensors have also been demonstrated with high sensitivity for the detection of heavy metal ions. Novel microbial bioluminescent sensors using genetically engineered *E. coli*, have been used for detection of mercury with an exceptional sensitivity in the nanomolar concentration range.[15,16] However, generally, the biosensor has disadvantages in its complexity of the sensing set-up and frequently uses the complicated methods of biomolecular engineering. It is also less robust compared to a conventional chemical sensor in terms of stability for long periods of operation.

In this work, the simple reflectance technique has been applied in order to enhance sensitivity of the sensor and this technique is compared with the absorbance technique. Using the reflectance technique, rather than the absorbance one, the optrode will allow the design of the flow cell to be much simpler. This is due to geometrical reasons, where the sensing film can be attached to the bottom of the flow cell, perpendicular to the face of the bifurcated optical fibre, one arm of which serves for illumination of the film with the light source and the other arm to guide the reflected light back to the detector.

In terms of reagent dye, this work focussed on the use of 1-(2-thiazolylazo)-2-naphtol (TAN), particularly for Hg^{2+} ions, instead of PAN; TAN appears to be similar in character to PAN. However, as a result of the lower basicity of the thiazole nucleus, the substitution of the pyridine ring with a thiazole ring will lead to a shift of reaction conditions for the formation of complex towards the more acidic region. Therefore, TAN is more reactive with metal ions in acidic solution than PAN.[17] In this paper, TAN is also shown to be sensitive and selective to Hg^{2+} ions over the other metal ions particularly in a phosphate pH buffer solution.

Experimental

Reagents

1-(2-thiazolylazo)-2-naphtol (TAN) (Sigma, UK) was used as purchased. Nafion® (Sigma, UK) was obtained as 5% m/m solution in a mixture of lower aliphatic alcohols. The pH buffer solutions (pH 3–10) were prepared by adjusting HCl–KCl, Na_2HPO_4–KH_2PO_4 and CH_3COOH–NaOH buffer systems; in all cases, the mixtures were 0.1 M and the ratios of the components were adjusted to the required pH. A stock solution of 5 mM Hg^{2+} ion was prepared by dissolving 0.1355 g of $HgCl_2$ (Aldrich, Poole, Dorset, UK) in 100 ml of buffer solution from which sample solutions were prepared by appropriate dilution. All reagents and inorganic salts were of analytical-reagent grade and made using deionised water (Elgastat Spectrum, UK).

Optrode film

The optrode film was prepared from a mixture containing 2% m/m TAN in Nafion. The mixture was cast from a solution made by dissolving 3 mg of TAN in 3 ml of 5% Nafion solution. The appropriate volume (200 µl) of the solution to give the desired film thickness (~5 µm) was placed on a silver reflective tape (50 mm^2) (3M, UK) and gently spread over the surface using the tip of a micropipette and followed by use of a spin-on device to enhance formation of a homogenous film. The film was dried by placing in a desiccator. The film thickness was estimated using a micrometer (RS, UK). Subsequently, the film size was cut to ~4 mm^2 and placed in the specially designed open flow cell. In the case of absorbance measurement, the film was prepared by casting Nafion-TAN onto a transparent plastic plate, and the procedure described above was then followed.

Apparatus

The apparatus used to measure reflectance and absorbance has been described elsewhere.[18] Basically, it consists of a quartz halogen lamp, an optical chopper, and a monochromator, a photomultiplier tube (PMT) and optical fibre as light guide. Furthermore, a current amplifier and lock-in amplifier was used to amplify the signal from the PMT, and the output relayed to a data acquisition system housed in a personal computer. In this system of measurement, TAN acts as the chemical transducer, converting chemical information from the analyte into optical signals, which are transmitted through the optical fibre to the detector. A peristaltic pump (Watson-Marlow 202 V, UK) was also used to pump sample and regenerating solution into the reagent compartment of the open flow cell.

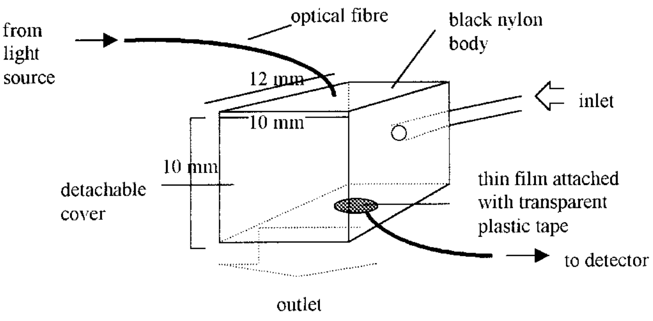

Fig. 1 The design of flow-cell for absorbance mode.

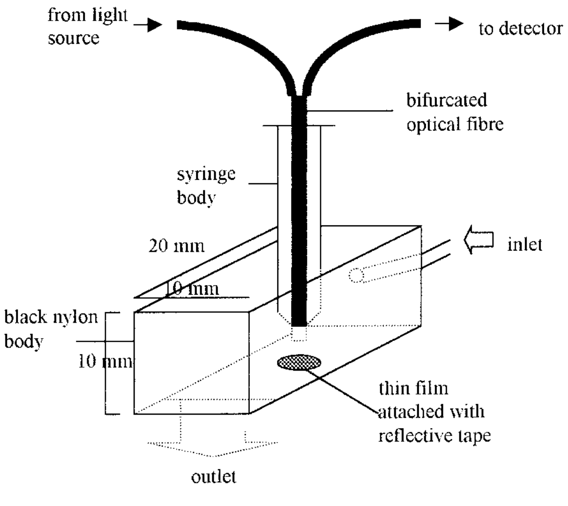

Fig. 2 The design of flow-cell for reflectance mode.

Flow cell design

Two types of open flow-cells were designed as shown in Figs. 1 and 2 for absorbance and reflectance modes respectively. These two types of flow cells allow measurements to be performed in a flow system free from backpressure effects, whereas in the conventional system such effects always occur due to the inlet and outlet having similar diameters. The effect of backpressure often creates noise in the signal response of the sensor, as a result of the sample solutions being unable to pass through the flow cell freely.

Procedure

All measurements were carried out in the flow system. The sample solutions were pumped through the flow cell at a constant flow rate. The spectral responses of the sensors in absorbance and reflectance mode were expressed as the measured intensity (mV). The measurements of the sensor were expressed as a reflectance signal (R), which is defined as the intensity signal (mV) of the Hg^{2+}-TAN complex divided by the intensity signal (mV) of the immobilised TAN alone in reflectance mode. Thus, the reflectance values will be between 1 and 0, which are the reciprocal values of the absorbance values. The maximum reflectance signal (R_{max}) and the minimum reflectance signal (R_{min}) were determined with the optrode film in contact respectively with 0.1 M phosphate buffer at the optimum pH value and with a solution containing high amounts of Hg^{2+} ions (~500 µM) to make the optrode film fully saturated.

Results and discussion

Optrode film composition

Nafion® is a polyfluorinated hydrocarbon based polymer with sulfonic acid sites built into its structure.[19] It is supplied as a mixture solution with aliphatic alcohols, which also makes it ideal for casting films by evaporation of the alcohols. Yet, the presence of sulfonic acid groups can serve to preserve electroneutrality in the film in the presence of a cationic metal ion complex, such as Hg^{2+} ions, without the need for additional reagents. Therefore, it might be a better alternative for use as a polymeric support in optrode films rather than other materials, such as cellulose acetate, PVC or silicate. In addition, the film developed in this work was orange in colour, homogenous, transparent and mechanically strong, and no leaching of the ligand from the film was observed in water, acid buffer or even in slightly alkali solution.

In this application of the optrode film in the flow system, the response to the analyte involves the extraction process of the analyte into the film. This process is also accompanied by a chemical reaction in the film, producing a colour change, which can be monitored spectrophotometrically. Thus, these processes can also be classed as the ion-exchange systems. These processes are relatively slow compared to a potentiometric sensor system, where in the latter case, the interfacial exchange processes occur only at the surface of a potentiometric film. Therefore, the application of the optrode film requires careful consideration as to the way in which the film functions, where the overall response depends on the contact time with analyte, the film thickness and the concentration of analyte.

Reaction with Hg^{2+} ions

As the optrode film belongs to the class of ion-exchange systems, the extraction of Hg^{2+} ion from the aqueous solution into the film phase and its complexation by indicator TAN proceeds with the loss of proton from the hydroxy group of each of the two indicator molecules.[17] This ion-exchange process is determined by the electroneutrality in the polymeric

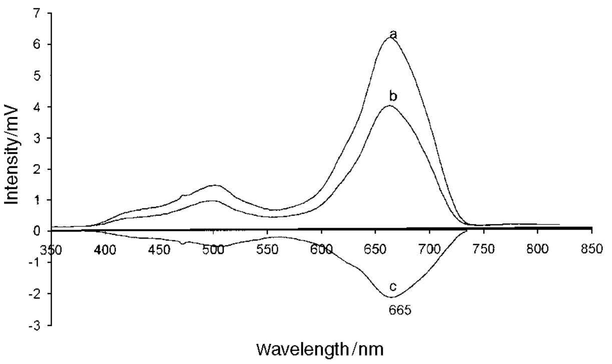

Fig. 3 The spectra of the optrode film in the absorbance mode in the absence (a) and the presence (b) of 125 μM Hg^{2+} at pH 6, (c) the difference between (b) and (a).

film phase. Figs. 3 and 4 show the spectra of the optrode film in the absence and the presence of Hg^{2+} ions recorded using absorbance and reflectance modes, respectively (phosphate buffer, pH 6). In these systems, the reflectance mode shows higher intensity change than the absorbance mode. This is due to the fact that in the former, the light passes through the film twice, due to the presence of the shiny silver reflective tape. Thus, the reflectance mode was selected as a spectrometric technique in these measurements.

The overall equilibrium between the polymeric film phase(s) and the aqueous sample phase (aq) is described as follows:

$$\mathrm{Hg(Ind)_{2(s)}} + 2\mathrm{H^+_{(aq)}} \overset{K_e}{\rightleftharpoons} \mathrm{Hg^{2+}_{(aq)}} + 2\mathrm{HInd_{(s)}} \qquad (1)$$

Where the equilibrium constant, K_e, for the reaction can therefore be written as

$$K_e = [\mathrm{Hg^{2+}}][\mathrm{HInd}]^2/[\mathrm{Hg(Ind)_2}][\mathrm{H^+}]^2 \qquad (2)$$

Therefore, the equilibrium concentration of the analyte (Hg^{2+}), the ligand (HInd) and the concentration of the complex ($Hg(Ind)_2$) depend on K_e. In this case, the concentration of the complex is correlated to the complex formation constant, whereas the concentration of Hg^{2+} and Hind is also correlated to the distribution coefficients of the Hg^{2+} and H^+ ions between the aqueous phase and the polymeric film phase. It is assumed that the formation of a coloured complex in the optrode film occurs with a Hg^{2+} to ligand ratio of 1 : 2, as this stoichiometry has been found in aqueous solution for TAN.[17] By introducing α, as the concentration of the protonated ligand in the polymeric film phase and by substituting their concentration with the activities of the species in the film phase,[20,21] the response of the optrode film can be given as

$$a_{\mathrm{Hg}}{}^{2+} = (a_{\mathrm{H}}{}^{+})^2 K_e (1-\alpha)/(4\alpha^2) \qquad (3)$$

where $a_{\mathrm{Hg}}{}^{2+}$ and $a_{\mathrm{H}}{}^{+}$ denote the activities of the Hg^{2+} and the H^+ ions, respectively. The activity coefficient of Hg^{2+} can be assumed to be constant, as the high concentration of phosphate buffer (0.1M) in the sample solution provides nearly constant ionic strength. It also can be assumed that in this ion-exchange system, not all metal ions are being complexed by the ligand. Therefore, the relative amount of free Hg^{2+} ion should be considered as well, which is approximately proportional to its total concentration, $[Hg^{2+}]_{tot}$. In order to determine the Hg^{2+} ion concentration simply, lets assumed that K_e are constants in these systems. Hence, the $[Hg^{2+}]_{tot}$ can be derived from eqn. (3).

$$[\mathrm{Hg^{2+}}]_{\mathrm{tot}} = k(1-\alpha)/\alpha^2) \qquad (4)$$

where k is the value correlated to K_e and all other constants. If the optrode films obey the Kubelka–Munk function, [21] which is related to the Beer–Lambert law, and if the measurements were performed in the absorbance mode.[22] The measured reflectance can be directly related to α, as follows:

$$\alpha = (\Delta R_{\mathrm{min}} - \Delta R_{\mathrm{exp}})/(\Delta R_{\mathrm{min}} - \Delta R_{\mathrm{max}}) \qquad (5)$$

where ΔR_{max} and ΔR_{min} are the limiting reflectance values for $\alpha = 1$ (fully protonated ligand) and $\alpha = 0$ (fully complexed ligand), respectively. By definition ΔR_{min} is the difference in reflectance signals between protonated ligand (at certain pH of phosphate buffer) and complexed ligand or fully saturated optrode film. ΔR_{exp} is the difference in reflectance signals between that of the base line and that obtained after the optrode film exposure to Hg^{2+} ion samples (95% of the final signal). ΔR_{max} is the difference in reflectance signals between the protonated ligand immersed in phosphate buffer of certain pH value and that obtained after the steady state signal is reached.

Theoretically, if the optrode film did not lose any significant amounts of the indicator, the optrode would produce a linear signal for similar pH values, as the ligand is still under protonated conditions. This also means that ΔR_{max} would be very low (~ 0) compared to ΔR_{min}, which would be close to 1. Therefore, for simplification of calculation of α, the eqn. (5) can be written as shown below.

$$\alpha = (\Delta R_{\mathrm{min}} - \Delta R_{\mathrm{exp}})/(\Delta R_{\mathrm{min}}) \qquad (6)$$

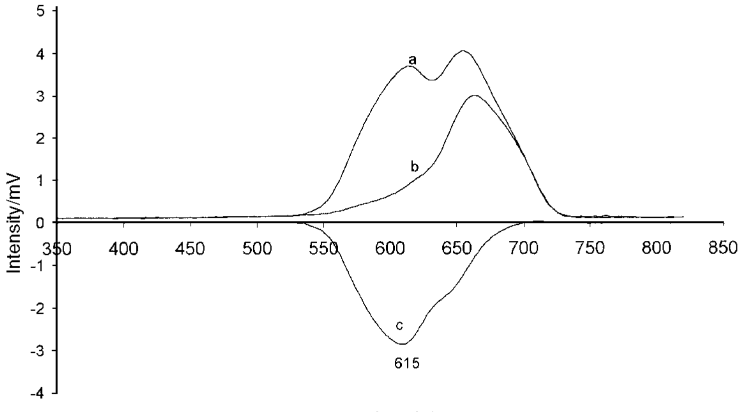

Fig. 4 The spectra of the optrode film in the reflectance mode in the absence (a) and the presence (b) of 125 μM Hg^{2+} at pH 6, (c) the difference between (b) and (a).

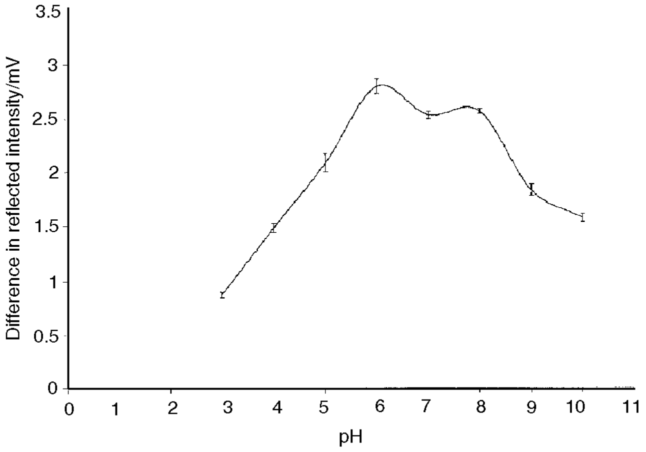

Fig. 5 Effect of pH on the optrode film response between pH 3 and 11.

Optimisation of experimental parameters

In this work, optimisation of the experimental parameters were carried out including pH and flow rate, as the measurements being performed in the flow system and measured at room temperature (~25 °C). Fig. 5 shows the effect of pH (between 3 to 10 in phosphate buffer) on the optrode film response. In this case, pH 6 was found to be the optimum pH for the optrode response, in all subsequent measurements this pH was used.

Fig. 6 shows the effect of flow rate (between 0.25–1.50 ml min^{-1}) on the optrode film response and Fig. 7 indicates the time response recorded at different flow rates. It is apparent that there is no leaching problem observable at this range of flow rates. The flow rate of 1 ml min^{-1} was selected as the optimum flow rate in this flow system, due to the fact that this flow rate can produce stable signals with a reasonable response time and sample consumption and is also considered as a compromise between the response time, sensitivity, repeatability of response for Hg^{2+} ions and the possible loss of appreciable amounts of the indicator in the film, which are affected by high flow rate. Thus, all measurements were carried out at the flow rate of 1 ml min^{-1}.

Response behaviour

Fig. 8 shows the relative reflectance signals of the optrode film to the various concentrations of Hg^{2+} ions in the range 0–500 μM. In this case, 250 μM was found to be the concentration of Hg^{2+} ions that makes the polymeric film become saturated. This is due to the fact that at this concentration the relative reflectance signal of the optrode film is close to the value of R_{min}, where the film is fully saturated ($[Hg^{2+}]$ = 500 μM). In order to determine the value of k from Fig. 8, it is important to select the concentration of Hg^{2+} ions that is within the linear portion of the optrode film response. In this case, the concentrations of Hg^{2+} ions in the range of 1–75 μM with 95% of final signals were found to be the linear range. Fig. 9 shows the reflectance signal of the optrode film *versus* the concentration of Hg^{2+} ions. The calibration plot is quite reproducible. Thus, this reveals the justification of the assumptions made in the derivation of eqn. (4).

In Fig. 10, the relative reflectance values, α, are given as a function of $[Hg^{2+}]_{tot}$ for pH 6. The curve fitting for experimental data points were calculated from eqn. (4) with k evaluated to be $1.78 \pm 0.61 \times 10^{-5}$ ($\log k = -4.75$). This value is greater than the formation constant obtained ($\log k = -6.08$) for

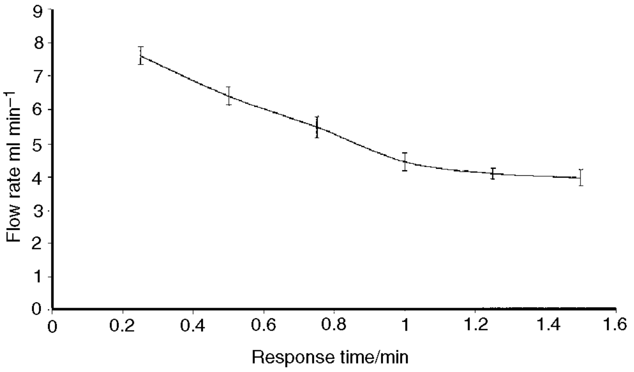

Fig. 6 Effect of flow rate on the response time of the optrode film.

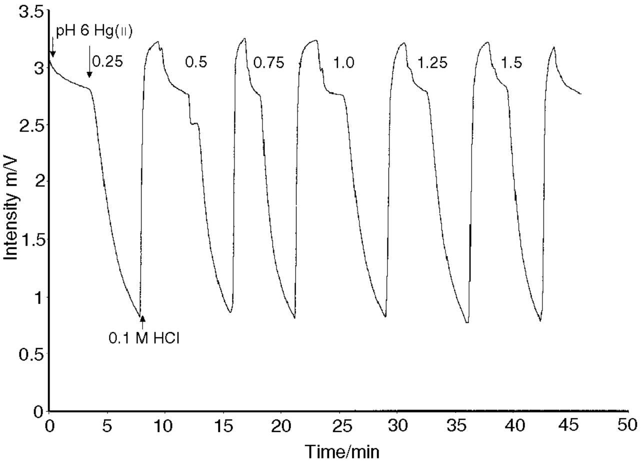

Fig. 7 Effect of flow-rate on the response time of the optrode film.

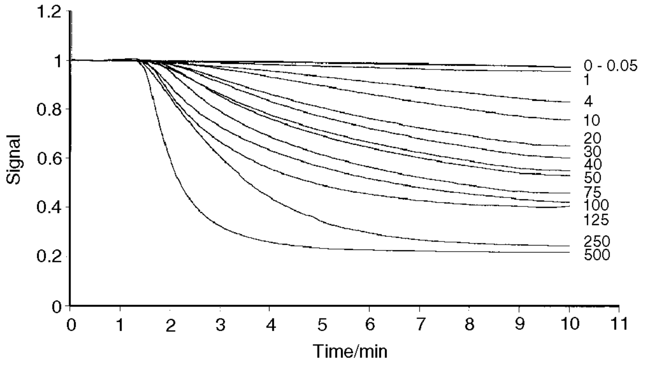

Fig. 8 The optrode film response to Hg^{2+} ions in the range 0–500 μM at pH 6.

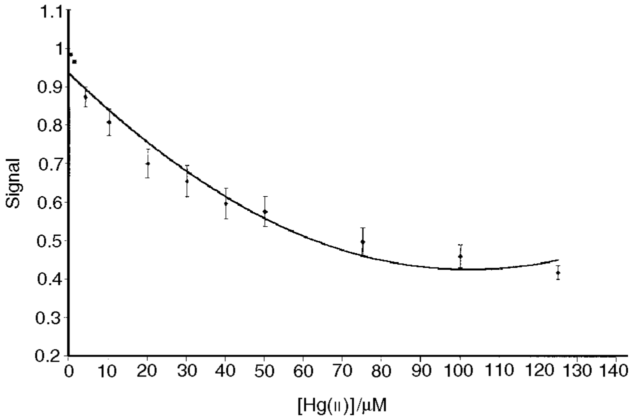

Fig. 9 The optrode film response *vs.* Hg(II) ion concentration. The solid state line is the polynomial fit.

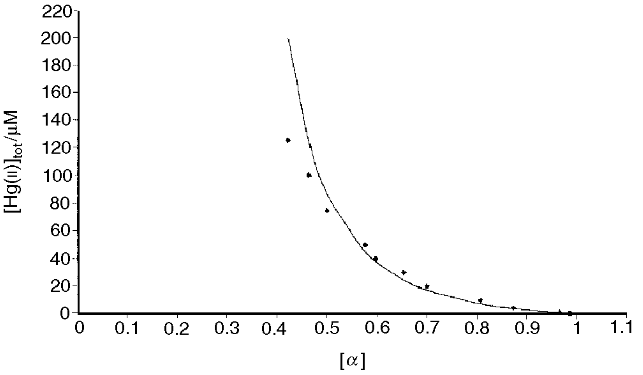

Fig. 10 The optrode film response to Hg^{2+}. The solid state line is calculated from eqn. (4).

Hg^{2+} ions with *o*-(2-thiazolyl azo)-4-methoxyphenol (TAM) in solution.[23] The difference between the values shown here, suggests that Hg^{2+} ions would preferentially form a stronger complex with the TAN in the immobilised phase rather than TAM in solution phase. The measured value of the *k* was re-substituted into eqn. (4) to give predicted values of Hg^{2+} concentrations (Fig. 10). The experimentally determined values were observed to follow predicted values except at high concentrations (>75 μM). This deviation may be due to the optrode film becoming saturated with Hg^{2+} ions. This means that the assumption made in deriving eqn. (4) may not be valid at high Hg^{2+} concentrations. The approximation used to derive eqns. (4) and (6) would be valid only for the lower concentrations of metal ions, where an approximately linear relationship may be discerned. The 1 : 2 stoichiometry of the reaction between Hg^{2+} ions and the TAN in solution may also still exist in the immobilised phase.

The detection limit (LOD) of the sensor film, which is defined as the concentration of sample yielding a signal equal to the blank signal plus three times of its standard deviation was found to be 0.05 μM (or 10 μg l^{-1}). This is sufficiently adequate for many monitoring applications in environmental analysis.[24]

Response and reversal times

In this work the optrode film was found to reach 95% of the final signal (t_{95}) at between 5 and 7 min depending on the concentration of Hg^{2+} ions (see Fig. 8). At high concentrations of Hg^{2+} ions a rapid response was achieved, which resulted in a large change in gradient of the response. At low concentrations of Hg^{2+} ions a longer response time has been produced by the optrode film. This is due to the response time of the optrode film and is mainly governed by three processes: (a) diffusion process in the film, (b) the rate of complex formation between metal ion and ligand, and (c) the rate of complex dissociation. Therefore, these three factors might play an importance role in such optrode response. Thus, is due to the nature of the film response to various Hg^{2+} ions, the kinetic method can be applied to this system easily, whereby a fixed time response function can be used for the optrode film.

In terms of reversal time, the optrode film produces a rapid reversal time within 1 min when contacted with 0.1 M HCl (Fig. 11). These processes occur rapidly due to the fact that acid re-protonates TAN, which causes the destruction of Hg^{2+} complex, and the metal returns to the acidic phase. These processes are also quite repeatable without the loss of indicator in the polymeric film.

Reproducibility

By using the kinetic method of analysis, the reproducibility of the optrode film was determined with 50 μM of Hg^{2+} at a fixed time of 5 min (Fig. 9). The mean of the relative reflectance signals ($n = 13$) and their standard deviations were found to be reproducible (0.271 ± 0.011).

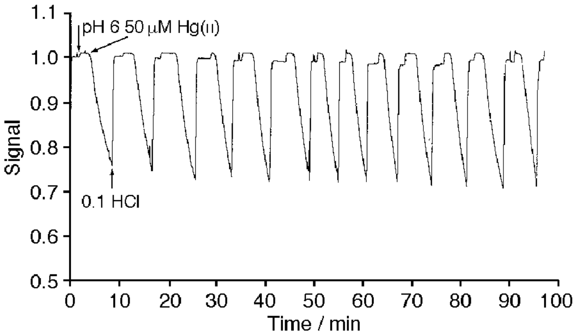

Fig. 11 Reproducibility of the optrode film to 50 μM Hg^{2+} at pH 6, 0.1 M used as regenerating solution.

Life time

The lifetime of the optrode film was determined by pumping 0.1M phosphate buffer solution (pH 6) over a period of time ranging from 100 min to about 17 h (Fig. 10). From Fig. 10, it can be found that no significant loss of the indicator occurs during time. However, in the early stages of the film response studies, a slight change in its response due to the conditioning of the film is noted; under the wet conditions the film gives stable responses. Therefore, in order to make the film stable, it would be necessary to immerse in buffer solution or pump the buffer solution through the film for conditioning, before it can be used for the measurements. Additionally, the stability of response of the film has been investigated over 6 months under ambient conditions, which indicated that the film was stable over this period (Fig. 12).

Selectivity

To determine the selectivity of the optrode film, the film was tested for the determination of 5 μM of Hg^{2+} ions in the presence of other metal ions as summarised in Table 1. The criterion for interference has been estimated as an error greater than twice the standard deviation obtained in the absence of

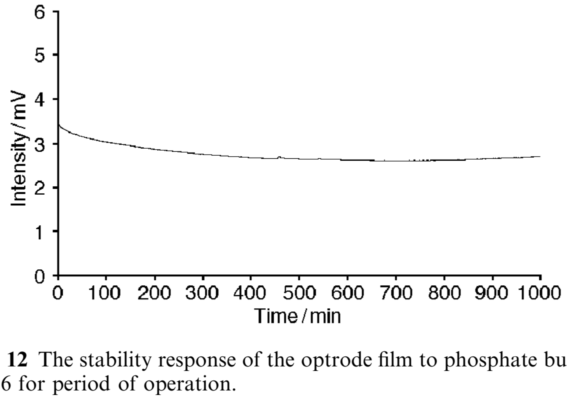

Fig. 12 The stability response of the optrode film to phosphate buffer, pH 6 for period of operation.

Table 1 Effect of foreign ions on the determination of 5 μM Hg^{2+}

Ion	Tolerance limit/μM
Pb^{2+}, Mg^{2+}, Li^{2+}, Pd^{2+}, CH_3COO^-, Ga^{3+}, Bi^{3+}, SO_4^-, PO_4^-	1000[a]
Zn^{2+}, Cd^{2+}, NO_3^-, Cl^-, ClO_3^-	100
Ag^+, Cu^{2+}, Fe^{3+}	10
Ni^{2+}, Co^{2+}	5

[a] Large amount tested.

foreign ions.[25] The main interferences in this case were Ni^{2+} and Co^{2+}. In the case of Ni^{2+}, the rate of complex dissociation was slightly slow, while the dissociation of Co^{2+} complex was almost irreversible, with the colour change from yellow to dark green. This is due to the high binding constant of Co^{2+} toward ligand. Therefore, it can be seen that appreciable discrimination of the optrode is obtained for these two metals, where in the first case, the pH is low for effective complexation. In the latter case, the colour change is noticeably different to that of Hg^{2+}, so some wavelength discrimination can be achieved, in order to differentiate between these metal ions. In addition, due to the strong binding of Co^{2+} and Ni^{2+} with the ligand compared to Hg^{2+}, a rapid reversal of sensor action can be achieved for these metal ions by passing high concentrations of acid solution (~1 M), particularly for Co^{2+}. However, at high concentrations of acid solution (1 M), it has been observed that the acid can also attack TAN which, in turn, causes loss of appreciable amounts of TAN film. Thus, a 0.1 M acid solution was found to be sufficient for the regeneration process without the loss of reagent from the sensing film layer.

Actually as an indicator, the TAN acts as a non-selective chelating agent towards many metal ions, particularly for the transition metal ions. However, as the system is similar to extraction systems, the interference from foreign metal ions can be reduced by prior extraction or by the use of masking agents. Furthermore, it is also apparent from this study that the film can be useful for determination of other transition metal ions or discrimination between different metal ions can be effected using chemometrics, by controlling several parameters, *viz*. the wavelength, the pH and the reaction kinetics of metals with TAN.

Conclusions

This sensor system uses simple procedures and is regenerable for Hg(II) ion sensing. The film has response functions that are in reasonable agreement with theoretical models and has a wide linear range 1–75 μM (pH 6) with limit of detection 0.05 μM ($10\ \mu g\ l^{-1}$) for Hg(II) ions. Thus, it allows the development of the optrode film suitable for environmental monitoring of Hg(II) ions. Furthermore, the response time of the optrode was within 5–7 min to reach 95% of the final signal, with good selectivity for Hg^{2+} over other metal ions except for Co^{2+} and Ni^{2+}, which are the main interferences.

Further development of film optrode studies involving film thickness, ligand concentration and contact time between the film and analyte are currently under investigation. These parameters are related to the film function, which represents the rate of complex formation and dissociation, as these might play an important role in this sensor system.

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