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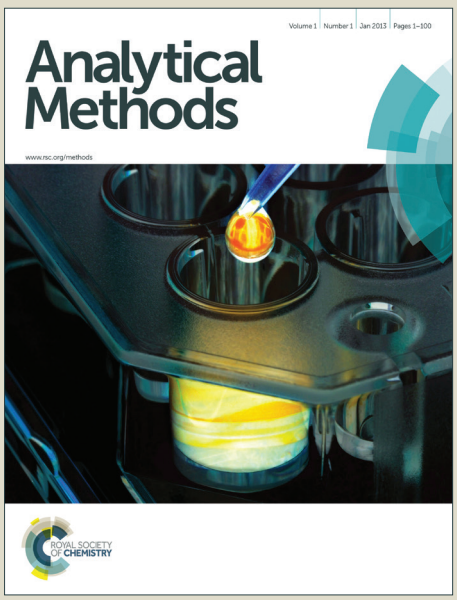

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Antifouling membrane integrated renewable gold microelectrode for *in situ* detection of As(III)

Romain Touilloux, Mary-Lou Tercier-Waeber*, Eric Bakker*

Department of Inorganic and Analytical Chemistry, University of Geneva, Quai Ernest-Ansermet 30, 1211 Geneva 4, Switzerland

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Abstract

Arsenic in the environment is of a global concern because of the widespread, chronic poisoning found in a number of countries and affecting large populations. Robust and sensitive analytical tools capable of direct, continuous *in situ* As(III) sensing is therefore of prime interest for As health risk assessment. For this purpose, we have developed here a microelectrode consisting of a gel integrated renewable Gold nanoparticles plated Iridium-based microelectrode (Au-GIME). The gel minimizes fouling problems by hindering diffusion of organic matters and inorganic colloids/macromolecules toward the sensor surface. Square Wave Anodic Stripping Voltammetry (SWASV) was used to characterize (i) the kinetics of As(III) diffusion in the agarose gel as a function of the gel thickness, (ii) the analytical performance of the sensor in synthetic and natural waters, and (iii) the influence of the temperature on the arsenic stripping peak current intensities. An evaluation of direct environmental application was performed in freshly collected lake water samples. The results reveal that the Au-GIME fulfills the requirements for direct measurements of (oxy)anions in freshwaters, i.e.: a reproducible mass transport of arsenite species in the gel; a gel equilibration time varying with the thickness of the gel in accordance with theory; a temperature effect on the SWASV As(III) signal intensities following a Arrhenius behavior

and thus readily corrected using a factor defined in laboratory; and a nanomolar detection limit at pH 8.

Introduction

In situ, real-time continuous measurements and speciation of trace metals in natural waters are strongly needed for quality monitoring purposes as well as to minimize artifacts such as contaminations, losses by adsorption, or speciation changes which often occurs during sampling, sample handling, and storage. Anodic Stripping Voltammetric (ASV) techniques coupled to micro-sized sensors are well designed for this purpose. They are highly sensitive and possess speciation capability based on metal complex lability and mobility or redox states.[1, 2] They are also readily automated with compact and moderate-cost instrumentation.[2] However, the reliability of electrochemical measurements in complex media, be it by ASV or other, is frequently compromised by the well-known fouling problem.[3-5] Fouling is related to the adsorption of polydispersed colloidal and dissolved organic matters (DOM), and inorganic colloids and particles. DOM are composed of a majority by fulvic and humic acids (FA/HA) as well as biopolymers such as polysaccharides, extracellular polymeric substances (EPS), peptides, proteins and cellular debris.[6, 7] They exhibit a large charge density owing to numerous dissociable functional groups as well as poly-functionality resulting from the presence of metal binding sites with different chemical nature.[8, 9] Inorganic colloids/particles include aluminosilicates (clays), calcium carbonates, silica, iron and manganese oxy-hydroxides.[6, 7] Inorganic colloids are usually considered as "compact" charged entities with strong affinity to solid surfaces. They are also sorbents of choice for trace metals.[10]

Fouling effects of DOM and inorganic colloids/particles on the ASV detection of various trace metals, including arsenic,[11] cadmium,[3, 12-14] copper,[3, 13, 15] manganese,[13] lead[3, 12-15] and zinc[13] have been studied using gold or Hg-plated microelectrodes. The results showed that fouling may (i) significantly increase the capacitive current;[16] (ii) impede the diffusion of the metal ions or the electron transfer of redox reactions, and thus decrease or supress the

voltammetric signals;[17] (iii) induce surface complexation of metals and thus modify ASV peak currents and potentials.[1]

An effective approach to minimize fouling problem involves a membrane with permselective properties that is placed between the sample and the electrode surface.[18] The permselective membrane imposes a separation step before voltammetric detection. This latter can be classified by the discriminative mechanism that confers permselective behaviour: charge, size and polarity discrimination. With an ideally behaving membrane, analyte transport to the electrode surface is not restricted while all fouling materials are excluded. Different types of thin permselective membranes have been developed[19-21] and applied as electrode antifouling coatings. They were mostly based on Nafion,[5, 15, 22] Nafion composites,[23-26] but also on polyaniline,[27] poly(ester sulfonic acid)[28] or poly(ethyl 3-thiopheneacetate).[29]

Nevertheless, due to their electrical charge, these membranes are not inert toward trace metals, nor effective enough to completely eliminate fouling by natural aquatic materials. Tercier et al. developed a reproducible and robust size exclusion permselective membrane placed directly on the microelectrode.[16] Microelectrodes were chosen because they exhibit a low *iR* drop and spherical diffusion that allows one to perform trace metals measurements by stripping techniques in quiescent solution[30]. The LGL agarose gel layer was chosen to be thicker than the diffusion layer thickness of the microelectrode. Consequently, voltammetric measurements are performed inside the gel after its equilibration with the sample. The LGL agarose gel was combined with Hg-plated Ir-based single and array microelectrodes.[16, 31] Systematic studies in synthetic solutions and field applications have demonstrated that these gel integrated microelectrodes (GIME) fulfill the key requirements for long-term, sensitive, and reliable simultaneous *in situ* Square Wave Anodic Stripping Voltammetric (SWASV) measurements of Cd(II), Pb(II), Cu(II) and Zn(II) as well as Mn(II) in fresh and marine

waters.[2, 16, 31] The membrane is chemically inert toward cationic metals and allows one to deposit and renew the Hg micro-layers through the gel. It displays anti-convective properties, ensuring purely diffusion-controlled mass transport of ions and small molecules within the gel, while aquatic natural fouling materials with radius typically higher than 35 nm are efficiently excluded from the gel.[32]

In the present study, the antifouling gel membrane described above[16, 31] is applied to a gold nanoparticles plated iridium-based microelectrode (Au-IrM). The Au-IrM was optimized earlier[33] for the detection of As(III) at trace level by SWASV in synthetic solutions at environmental pH. It shows reproducibility and robustness over 7 d with a limit of detection of 0.2 nM, and negligible interferences from 0.6 M of chloride and a 20-fold molar excess of copper(II). Here we study for the first time the diffusion characteristics of anions ($AuCl_4^-$ complex used for the electroplating of Au nanoparticles on the Ir-substrate) and oxyanions (As(III)) in the LGL agarose gel. The influence of temperature on the detection of As(III) with Au nanoparticles plated gel integrated microelectrode (Au-GIME) is investigated, along with the impact of DOM and inorganic colloids/particles electrodes with and without LGL agarose gel membrane. Finally, the reliability of the Au-GIME applied to the direct As(III) sensing in lakewater is evaluated by intercomparison with ICP-MS measurements of As(III) after separation on an ion-exchange resin.

Experimental

Chemicals and instrumentation

All solutions were prepared with Milli-Q water (18.2 MΩ.cm) from a Millipore system. The gold layer deposition was performed with tetrachloroauric acid ($HAuCl_4$, 99.99%) from Aldrich and the gold renewal with KSCN (Fluka, DE) and mercury acetate ($Hg(CH_3COO)_2$) (Acros Organics, USA). The phosphate buffer (di-potassium hydrogen

phosphate form), HNO_3, $NaNO_3$ and $NaOH.H_2O$ were of suprapur grade and purchased from Merck.

Fulvic acids (FA) from the Suwanee river (1S101F) were purchased from the International Humic Substances Society (USA). A solution of about 400 ppm was prepared, filtered on 0.45 µm pore size nitrocellulose membrane and analysed by a Total Organic Carbon analyser (TOC-V CPH, Shimadzu, JPN). The final FA concentration was precisely determine as 392.6 ppm based on the carbon percentage in FA known as 52.44%.[34]

An As(III) stock solution of 13.3 mM was prepared by dissolving As_2O_3 (Fluka, DE) in 8.6 mM of NaOH. It was subsequently diluted in 8.6 mM NaOH to obtain a 0.1 mM As(III) solution. Both stock solutions were stored at 4°C before use. The 10 mM phosphate synthetic buffer was prepared in the presence of 10 mM $NaNO_3$ and the pH was adjusted to 8.0 with 0.1 M HNO_3.

Electrochemical experiments were performed using a PGSTAT101 workstation (Metrohm AG, Switzerland) controlled by NOVA software (v1.10/1.11) and a conventional three-electrode system. The working electrode was a Au-IrM or a Au-GIME described below. The reference and counter electrodes, purchased from Metrohm, were respectively a platinum wire and a Ag/AgCl (3 M KCl) electrode, protected by an additional bridge of 0.1 M $NaNO_3$ to avoid contamination.

All experiments were performed at room temperature (except for the temperature effect study) in a home-made Faraday cage. Synthetic phosphate buffer solutions were deoxygenated for 10 min using a nitrogen stream before measurements. A nitrogen atmosphere was maintained over these solutions during experiments.

Measurements made at controlled temperature were made using a thermostated glass cell (Metrohm) coupled to a Julabo F34 thermostated water bath. The precision on the temperature measured was on the order of 1°C.

A Lewatit MonoPlus M 500 (LM500), Lanxess (Leverkusen, Germany) resin was used for separation of As(III) and As(V).[35] The concentration of these inorganic species as well as the As total concentration were measured by ICP-MS (Agilent 7700x) made of a micromist nebulizer. ^{103}Rh and ^{185}Re were used as internal standards. The analytical accuracy and precision of the measurements were verified by the analysis of international natural reference certified materials (NRC-SLRS4 and NRC-SLRS5, Ottawa, Canada).

Numerical simulations were performed with Wolfram Mathematica 8.0 software installed on a PC.

Working Microelectrode

A Au-IrM or Au-GIME was used as working electrode. As described by Tercier et al.,[12] the IrM is made of an electroetched iridium wire sealed in a glass capillary. Cyclic Voltammetry in a deoxygenated 6 mM $K_3Fe(CN)_6$ + 1 M $NaNO_3$ solution[12] was used to determine the Ir microdisk radius as 2.1 µm. Gold deposition was achieved by chronoamperometric deposition in a solution of 1 mM Au(III), 0.1 M $NaNO_3$ at pH 2 (HNO_3) using a potential of -300 mV preceded by 10 consecutive pulses of +800 mV during 50 ms.[33] The renewal step was performed by Linear Sweep Voltammetry (LSV) with a solution of 5 mM $Hg(CH_3COO)_2$ and 1 M KSCN.[33] The potential was swept from +300 mV to +800 mV using a scan rate of 5 mV/s and a step potential of 2.5 mV.

1.5 % LGL-Agarose membrane preparation

A silicone tube was affixed to the extremity of the IrM electrode glass body. To define the gel thickness (typical range 350 to 650 μm), the silicone tube was made to extend over the edge of electrode tip (Fig. 1) as controlled by a brightfield microscope.

Milli-Q water was heated in a boiling water bath. 30 mg of agarose was weighted and placed in a second test tube before adding 2 mL of hot Milli-Q water. The test tube was immersed into the boiling water bath and the agarose was found to dissolve after ~10 min, yielding a transparent solution. The heating was stopped and the test tube kept in the water bath until air bubbles in the gel disappeared. A drop of hot agarose was deposited with a Pasteur pipette into the silicone tube fixed at the tip of the IrM. Any excess was immediately removed to give a geometrically flat surface. After curing, the gel was observed through a microscope to confirm that no air bubbles were trapped. The gel coated Ir microelectrode tip was then immersed in Milli-Q water for 1 h and subsequently stored in 0.1 M $NaNO_3$ for at least 8 h before use.[16]

Stripping voltammetric detection of As(III)

SWASV was used for the quantification of As(III). The SWASV protocol consisted of the following steps performed in an uninterrupted sequence. (I) Conditioning of the electrode: $E_{conditioning}$ = +500 mV, $t_{conditioning}$ = 30 s ; (II) Preconcentration step: E_{prec} = -1.0 V, t_{prec} = 3 min (As(III) ≥ 10 nM) or 36 min (As(III) ≤ 10 nM); (III) Equilibration step, as step (II) but for a period of 10 s; (IV) Stripping step: initial potential (E_i) = -1.0 V, final potential (E_f) = +0.3 V, frequency (f) = 200 Hz, potential pulse amplitude (E_{sw}) = 25 mV, potential step height (E_s) = 8 mV. Subsequently a background scan was recorded with the same parameters but without preconcentration and conditioning steps. The SWASV voltammograms in this work are presented after the subtraction of the background scan.[33]

As(III) diffusion through the gel

The protocol used to measure diffusion of As(III) from the solution to the sensor surface and conversely was as follows: after the electroplating of the Ir microdisk substrate with the Au nanoparticles through the gels, three replicates were measured in a phosphate buffer electrolyte. This blank solution was then spiked with 50 nM of As(III), and SWASV measurements were run every 2 min until a plateau is reached (t_{prec} was decreased to 110 s instead of 3 min). The solution was then quickly replaced by the blank phosphate buffer solution, and SWASV measurements repeated as before.

Inorganic arsenic speciation by ICP-MS after separation on resin

Previous work[35] demonstrated that the LM500 resin, a strong base anion exchange resin, is well suited for separation of As(III) and As(V). The design of the column as well as the flow rate (~1.4 $mL.min^{-1}$), controlled by an Ismatec pump (Switzerland), used here were as reported.[35] The only difference was that, to further improve the inorganic As species separation, the pH of the samples was adjusted with HNO_3 solution (pH 1) to pH 5 instead of 7. Efficiency and reliability of the As(III) and As(V) separation were evaluated using an Arve river (Geneva, Switzerland) matrix spiked with As(III) and As(V) at various ratios prior to its elution through the resin (Fig. S1). As(III), predominantly present in neutral form at $5 \leq pH \leq 8$, was detected in the effluent. A 0.1 M NaOH solution was used to release the bonded negatively charged As(V) species and regenerate the resin. The various collected fractions were immediately acidified to pH 1 with HNO_3 and analysed by ICP-MS. A good recovery was obtained for all As(III)/As(V) ratios tested (Fig. S1).

Sample collection

For optimization, samples were collected from the Arve river (Geneva, Switzerland), and stored in a polyethylene container. Analyses were performed within 30 min after

sampling. As part of a field campaign on Lake Greifen (Zurich, Switzerland) samples were collected at various depths from a moored platform (EAWAG, Switzerland). The polyethylene containers were immediately stored in a cold box. SWASV analyses of As(III) in unfiltered collected samples were performed at the EAWAG lab (Dübendorf, Switzerland) within maximum 22 h after sampling. The pH of the freshwater samples was adjusted to 8.0 by a gas mix of nitrogen and 5% CO_2 in nitrogen (N_2/CO_2).[16] In parallel to SWASV measurements, As(III) separation on a LM500 resin were performed. The collected effluent samples were acidified and stored at 4°C prior ICP-MS analysis. The polyethylene sampling bottles were pre-cleaned for 24 h in 0.1 M HNO_3, 2 times 24 h in 10^{-2} M HNO_3, followed by immersing in Milli-Q water for 12 h after each acid washing step. They were then dried in a laminar flow hood and stored in double polyethylene bags prior to use.

Results and Discussion

Electrodeposition and renewal of gold film through the gel layer

The gold layer deposition and removal on the Au-GIME was performed through the gel layer under the same conditions as reported earlier[33] without gel coating. A fixed time of 86 s was used, giving a reduction charge (Q_{red}) that was determined for 9 different gold layers. The average gold layer thickness was found as 0.943 ± 0.069 µm (Table S1). This negligible difference comparing to the deposition performed without gel (1.059 ± 0.030 µm)[33] allows us to keep the same previous parameters for the deposition.

As(III) diffusion properties in the gel

As(III) diffusion through LGL agarose gels of various thicknesses were measured from the solution toward the sensor surface and conversely. These operations were repeated several times. Typical examples of the monitored diffusion profiles are shown in Fig. 2a.

The time required for equilibration of the 1.5% LGL agarose gel with the As(III) (Table 1) was evaluated from the values t_{95}, i.e. the time needed for As(III) concentration at the microelectrode surface to be 95% of the concentration in bulk solution (i/i_{max} = 95%). The average values of the diffusion coefficients of As(III) in the 1.5% LGL agarose gel, reported in Table 1, were determined with eqn (1),[36] based on calculation of free diffusion of a substances through a membrane of thickness l under the limiting condition (0.2 C_{sol} + 0.8 Cm) ≤ C ≤ (0.9 C_{sol} + 0.1 C_m)[16, 36]:

$$\ln\left[\frac{C_{sol} - C}{C_{sol} - C_m}\right] = 0.2306 - 2.452\,\frac{D.t_{eq}}{l^2} \qquad (1)$$

where C_{sol} is the solution concentration (constant), C_m the initial uniform concentration in the gel, C the concentration at time $t = 0$, D the diffusion coefficient, t_{eq} the equilibration time and l the thickness of the gel.

Diffusion coefficients were found to be independent of the gel thickness (Table 1). This observation, coupled with the absence of As(III) retention in the gel demonstrated by the diffusion profiles reported in Fig. 2, confirms that the LGL agarose gel is chemically inert to As(III). Chemical inertness is also supported by the linear relationship observed between the equilibration time, t_{95}, and the square toot of the gel thickness (Fig. 2b) as expected from the theoretical eqn (1).

The average diffusion coefficient for As(III) in the agarose gel, calculated from the different diffusion profiles (Table 1), was found to be 6.6 ± 0.3 x 10^{-6} $cm^2.s^{-1}$. This value corresponds to 61% of the value of 10.8 x 10^{-6} $cm^2.s^{-1}$ determined by Tanaka et al.[37] in solution at pH 8.

A finite difference numerical simulation was used to further confirm the experimental data. For this purpose, the mass transport in a homogeneous phase for a one-dimensional diffusion problem was simulated with Fick's second law:

$$c_n(t+\Delta t) = c_n(t) + \{c_{n-1}(t) - 2c_n(t) + c_{n+1}(t)\}\frac{D.\Delta t}{\delta^2} \quad (2)$$

where c is the concentration, D the As(III) diffusion coefficient in the gel, t the time, Δt the time increment, δ the thickness of each element and n the position. Values for Δt and δ were chosen as 0.01 s and 25 μm, respectively, with a t_{max} of 1200 s (20 min). The diffusion coefficient was adjusted until a good correspondence between simulated and observed behaviour was obtained (Fig. 2a). An As(III) diffusion coefficient in the gel of 5.4 x 10^{-6} $cm^2.s^{-1}$ was then calculated, allowing for an uncertainty in the gel layer thickness of 25 μm.

When this value is compared to the one determined by Tanaka et al.[37] as 10.8 x 10^{-6} $cm^2.s^{-1}$, the ratio $D_{gel}/D_{free\ sol}$ is 0.50. The numerical simulation confirmed the experimental values with only a difference of ~10% which is reasonable. Tercier et al.[16] demonstrated than the diffusion coefficient in 1.5% LGL agarose decreased by about half for cations analysis due the physical properties of the gel (mesh size, fiber diameter, macroreticualte networks), which influence ion mobility.[38] We may now confirm that this statement is also true for oxyanions, and the gel is behaving the same as for the detection of cations.

Calibration slopes determined for the same solution are different for electrodes with and without gel coating (Fig. S2). As discussed above, this is explained with a decrease of the As(III) diffusion coefficient in agarose. The value of the resulting ratio of the calibration slopes determined for electrode with and without gel was typically 0.69. This value is similar to the resulting ratio $Dgel/D_{free\ sol}$ value determined as 0.61, confirming the statement above that chemical interactions with the gel appear to be insignificant, as they would influence the mass transfer rates in the gel.

Gel efficiency against fouling

The influence of FA (which represents typically 40 to 60% of DOM in freshwater waters[39]) and suspended inorganic matters (SPM) was studied by intercomparison of SWASV As(III) peak current intensities monitored using Au-IrM and Au-GIME. For the former, SWASV measurements were performed in 10 mM phosphate buffer at pH 8.0 in the presence of 5 nM As(III). Aliquots of Fulvic Acids (FA) were added stepwise up to 10 and 20 ppm, which is 10 to 20 times more than the FA average concentration level in surface waters.[40] To allow more readily the intercomparison of a potential fouling effect on the Au-IrM and Au-GIME, the peak currents in the presence of gel were normalized by dividing them by the ratio

$D_{gel}/D_{free\ sol}$ = 0.69 determined above. Fig. 3 reveals the harmful effect of FA on As(III) detection in the absence of gel layer, with a loss of 30% on the As(III) current after the addition on 10 ppm FA. This behaviour can be explained by FA adsorption on the gold electrode surface or by the possible formation of a poorly reversible complex of As-FA, as already observed by Salaun et al.[11] In contrast, peak currents for electrodes with a gel layer did not change in the presence of FA up to a concentration of 20 ppm (Fig. 3). SPM potential fouling effect on SWASV As(III) measurements was studied by intercomparison of the calibration slopes obtained in unfiltered Arve sample containing 107 ppm of SPM and in the phosphate electrolyte using Au-IrM coated or not with 1.5% LGL agarose gel. Fig. 4 shows that for the Au-GIME, the resulting calibration slope obtained in the unfiltered Arve river sample was found to be similar to the one obtained in synthetic media, respectively 0.143 ± 0.008 and 0.148 ± 0.005 $nA.nM^{-1}$. In contrast, for the Au-IrM the calibration slope obtained in the non-filtered Arve river water (0.107 ± 0.004 $nA.nM^{-1}$) was 45 % lower than the one obtained in phosphate electrolyte (0.195 ± 0.005 $nA.nM^{-1}$). The same Arve river water was then filtered on 0.2 µm pore size nitrocellulose membrane and a new calibration was performed. The slope recovery increased to 94% (0.184 ± 0.009 $nA.nM^{-1}$ for filtered Arve river water). This demonstrates that the loss of sensitivity observed for measurements in the unfiltered sample originates from a fouling effect by the SPM, which was successfully eliminated in the presence of the gel membrane (Fig. 5).

These overall results confirm the efficiency of the 1.5% LGL agarose gel to protect the Au-IrM sensor surface against fouling by DOM and SPM. Note that no deformation of the SWASV voltammograms, with the exception of the decrease in the peak current intensities, was observed for the measurements performed on Au-IrM in the presence of FA and SPM. Therefore, an application of such a sensor for environmental monitoring without careful characterisation may have resulted in a significant underestimation of As(III) concentrations.

Influence of temperature

Temperature is well known to influence the rate of diffusion.[41] In surface waters, temperatures may vary between 25 to 5°C as a function of the depth, necessitating a temperature correction of the voltammetric data. As a first approximation, for an electrode size of a few micrometers, Belmont-Hebert et al.[31] demonstrated that the SWASV peak current can be considered directly proportional to the diffusion current. According to Arrhenius' law (eqn (3)), the diffusion coefficient D is ideally expected to be proportional to $\exp(E_a/RT)$. Since the peak current intensity at microelectrodes is directly proportional to D, a linear relationship may be expected between ln(i) and 1/T (eqn (4)/eqn (5)).

$$D = D_0 . e^{\frac{-E_a}{RT}} \quad (3)$$

$$\rightarrow i \propto D \rightarrow i = i_0 . e^{\frac{-\Delta G}{RT}} \quad (4)\ (5)$$

where D is the diffusion coefficient, D_0 the maximum diffusion coefficient (at infinite temperature), E_a the activation energy, ΔG the free enthalpy, T the temperature and R the gas constant. ΔG is similar to E_a for reversible systems. For quasi-reversible and irreversible systems, charge transfer kinetics becomes the limiting factor, and ΔG is significantly higher than E_a.

Eqn (5) suggests that the slope $\Delta G/R$, which is the temperature effect correction factor, can be experimentally determined and later on applied to correct the influence of T on the current monitored *in situ* using eqn (6):

$$i_{T_{room}} = i_{T_{in\text{-}situ}} . \exp\left(\frac{\Delta G}{R}\left(\frac{1}{T_{room}} - \frac{1}{T_{in\text{-}situ}}\right)\right) \quad (6)$$

where $T_{in-situ}$ is the temperature of the sample measured *in situ*, T_{room} is the temperature in the lab (room temperature), $i_{T_{in-situ}}$ is the current measured *in situ* at $T_{in-situ}$ and $i_{T_{room}}$ is the current which should be measured at T_{room}.

The experiment was performed with a 50 nM As(III) solution without a gel layer, assuming that the correction factor should be close to the one obtained with gel coating.[31] A linear ramp was applied from 25°C to 5°C and 5°C to 25°C with a step of 5°C and repeated three times. Fig. 6 shows that a linear relationship was indeed observed between ln(i) and 1/T. The temperature effect correction factor, ΔG/R, determined from the slope was found to be -5552 ± 111 (2 %) K. This value can be compared to the theoretical value for a reversible system (E_a/R = 2563 K), given by the Stokes-Einstein equation,[31] where the linear relationship is determined between ln D and 1/T. The slope for As is higher than predicted for diffusion controlled systems and it may be concluded that the peak current behaves as for a not entirely reversible system under the fast SWASV scan rate conditions used (1.6 V/s).

Validation for environmental applications

Evaluation of the Au-GIME for direct environmental monitoring of As(III) at natural pH, via intercomparison with ICP-MS measurements of As(III) after separation on the LM500 resin, was performed during a field campaign on Lake Greifen (Zurich, CH). This lake was chosen based on the work of Kuhn et al.,[42] which demonstrated the presence of As(III) in oxic water and its possible link to biological activity.

Measurements from two depth profiles sampling at the same time, 9:00 am, at an interval of 3 d are presented in Fig. 7 and Fig. S7. Two replicates were measured for each depth. Even if the As(III) concentrations were close to the limit of detection, the comparison between the data observed with the Au-GIME and the resin/ICP-MS measurements is satisfactory. For the profile of the 19th of August, when the oxygen level and the *chlorophyll a*

decrease to near-zero, no more As(III) was detected, suggesting a link between As(III) concentration and biological activities (Fig. 7). This could support the hypothesis made by Kuhn et al.[42], namely the reduction of As(V) is related to phytoplankton activities. As(V) is chemically similar to o-phosphate and can be taken up by phytoplankton. This bio-uptake creates a cellular stress which induces a detoxification process via the intracellular reduction of the As(V) into As(III), the exudation of the As(III) resulting. Of course more studies are required to confirm this hypothesis. For this purpose, in situ Au-GIME profiling at high resolution over appropriate time scale may be of particular interest.

Conclusions

The important second step of the development of an Au-IrM was presented here with the implementation of an antifouling 1.5% LGL agarose membrane. The effect of the gel coating on the As(III) diffusion coefficient was studied experimentally and by numerical simulation. The ratio between D_{gel} and $D_{free\ sol}$ was found to be relatively similar between these two calculation methods and demonstrated that mass transfer is not influenced by chemical interactions with the gel. As in earlier work for cationic metals,[16] it was confirmed that the gel is chemically inert toward (oxy)anions as arsenite compounds and anionic gold complex ($AuCl_4^-$). The temperature correction factor was determined for a range between 5 and 25 °C in view of future *in situ* measurements. We confirmed as well that this gel acts as an efficient antifouling membrane against FA and SPM. Finally, reliable direct As(III) measurements were obtained in freshwater at concentration close to the limit of detection.

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Table

Table 1. Experimental diffusion coefficients and gel equilibration time of As(III) in 1.5% LGL agarose determined by SWASV

Gel thickness / µm (error ± 25 µm)	Gel eq. time / min ($t_{i/imax}$ = 95%)	D x 10^6 / $cm^2.s^{-1}$	
0	0	10.8	
375	4.7 ± 0.4	6.6 ± 0.9 [a]	6.5 ± 0.9 [b]
500	9.1 ± 0.2	6.3 ± 0.6 [a]	6.5 ± 0.6 [b]
625	13.1 ± 0.4	6.5 ± 0.5 [a]	7.2 ± 0.6 [b]

[a] mass transfer from solution to the sensor surface
[b] mass transfer from sensor surface to the solution

Figure Captions

Fig. 1. Scheme of the gel integrated Gold nanoparticles-plated Iridium-based microelectrode (Au-GIME).

Fig. 2. (a) Experimental (symbols) and theoretical (in grey) diffusion profiles of As(III) toward (open) or from (solid) the microelectrode surface, in 375 ± 25 µm (●), 500 ± 25 µm (■) and 625 ± 25 µm (▲) thick 1.5% LGL agarose membrane (b) Gel equilibration time is plotted in function of (gel thickness)^2 from Table 1. Sample: 50 nM As(III) in 10 mM phosphate, 0.01 M $NaNO_3$, pH 8.0. SWASV conditions: $E_{precleaning}$ = +500 mV (10s); E_{prec} = -1.0 V; t_{prec} = 110 sec; E_i = -1.0 V; E_f = +0.3 V; f = 200 Hz; E_{SW} = 25 mV; E_s = 8 mV.

Fig. 3. As(III) stripping peak currents measured in presence of FA on the Au-GIME (▲) and on the Au-IrM (□, ○). Currents obtained by Au-GIME are normalized by $D_{gel}/D_{free\ sol}$ = 0.69. SWASV conditions: $E_{precleaning}$ = +0.5 V (30s); E_{prec} = -1.0 V; t_{prec} = 36 min; E_i = -1.0 V; E_f = +0.3 V; f = 200 Hz; E_{SW} = 25 mV; E_s = 8 mV.

Fig. 4. As(III) calibration curves (n = 3) obtained from Au-GIME SWASV measurements in (□) unfiltered Arve river water and (○) 10 mM phosphate at pH 8 spiked with increasing concentrations of As(III) in a range of 1 to 7 nM. SWASV conditions as in Fig. 3.

Fig. 5. As(III) calibration curves (n = 3) obtained from Au-IrM SWASV measurements in (□) unfiltered Arve river water , (○) 10 mM phosphate at pH 8 and in (Δ) filtered Arve river water spiked with increasing concentrations of As(III) in a range of typically 1 to 10 nM . SWASV conditions as in Fig. 3.

Fig. 6. Influence of the temperature on the As(III) stripping peak current intensities. Temperature ramp varies from 25°C to 5°C (solid) and 5°C to 25°C (open), repeated three times. Sample as in Fig. 2. SWASV conditions as in Fig. 3, except t_{prec} = 3 min.

Fig. 7. (a) As(III) concentration profiles in Lake Greifen (19th August 2015) determined from Au-GIME SWASV measurements and ICP-MS after separation on LM500 resin. Also shown are profiles of total dissolved As measured in pH 1 acidified samples by ICP-MS, (b) dissolved oxygen and *chlorophyll a* measured in situ using an Idronaut OS316-multiparameter probe.