Talanta 120 (2014) 470–474

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A novel method for the determination of dissolved methylmercury concentrations using diffusive gradients in thin films technique

Yue Gao*, Sam De Craemer, Willy Baeyens

Laboratory of Analytical and Environmental Chemistry (ANCH), Faculty of Sciences, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium

ARTICLE INFO

Article history:
Received 5 September 2013
Received in revised form
4 December 2013
Accepted 10 December 2013
Available online 25 December 2013

Keywords:
DGT
Agarose gel
3-Mercaptoproply functionalised silica resin gel
Acid extraction

ABSTRACT

A novel DGT probe and analysis protocol were developed for the determination of MeHg concentrations in aquatic system. The DGT probe consisted of an agarose (AG) gel as the diffusive hydrogel and a 3-mercaptoproply functionalised silica resin gel as the resin gel. The polyacrylamide (PA) hydrogel which is commonly used in DGT probes to assess trace metal concentrations in aquatic system appeared to be unsuitable for the determination of MeHg. The affinity of the PA hydrogel for MeHg is very high reducing its accumulation by the resin. In contrast, the AG hydrogel presents a by far lower affinity towards MeHg, which makes it suitable as diffusive layer in a DGT probe for MeHg determinations. Two extraction procedures to liberate MeHg from the resin were studied: one is involving thiourea as complexing agent, the other a simple acidic extraction. The extraction step was followed by an ethylation reaction of the liberated MeHg to determine low concentrations of MeHg species by Headspace-Gas Chromatography-Atomic Fluorescence (HS-GC-AFS). With the thiourea extraction method the recovery of the adsorbed MeHg compounds was extremely low while the recovery with the acid extraction method was 100%.

The reliability of the novel DGT probe and analysis protocol was studied. A linear dependency between the amount of MeHg accumulated on the resin gel and both the deployment time and the gel thickness were demonstrated. From those experiments a diffusion coefficient of MeHg in AG gel was determined: $5.1 \pm 0.20 \times 10^{-6}$ cm^2 s^{-1}. Additional experiments showed that the new DGT method can be used in most natural waters independent of the ionic strength and within a pH range of 3–8.

1. Introduction

Mercury is known as one of the most toxic trace metals in the environment. It is present in many chemical forms, but the most common ones are elemental mercury (Hg^0), inorganic (IHg or Hg^{2+}) and monomethylmercury (CH_3Hg^+, referred to as MeHg throughout this paper). Humans are exposed to Hg^0 mainly by inhalation and to IHg and MeHg mainly by ingestion of food. IHg and especially MeHg concentrations are usually very low in the environment, for example typical dissolved IHg and MeHg concentrations in the Belgian coastal zone are around 30 and 250 pg L^{-1}, respectively [1].

The determination of dissolved MeHg concentrations in the water column of rivers, estuaries or seas involves an ultra-clean sampling procedure followed by a preconcentration step, the separation of MeHg from other Hg species (mostly by GC) and atomic fluorescence spectrometry (AFS) or inductively coupled mass spectrometry (ICPMS) as detection step [2,3]. In the case of sediments, pore water has to be separated from the mineralogical part, generally by slicing the sediments and consequent centrifugation. The available volume of liquid is generally not large enough, even after a preconcentration step, for the determination of MeHg. The sampling and preconcentration procedures for the determination of dissolved MeHg either in the water column or in pore water are often the most crucial steps in the whole analysis chain. In that context, the Diffusive Gradients in Thin Films (DGT) technique has proven to be an excellent alternative since sampling and preconcentration steps are performed simultaneously, in situ and without any human intervention reducing strongly the range of uncertainty [4].

Several research groups have yet used the DGT technique to assess total mercury concentrations in natural waters and sediments [5–7]. The principle of this technique involves three key conditions: (1) the diffusive gel used in the technique should not bind with the interested solute(s); (2) the resin gel should be functional binding with the interested solute(s); (3) the elution procedure should be efficient and compatible with further steps of the analysis procedure such as the ethylation of the extracted MeHg ions.

The diffusive gels that are reported in the literature for the analysis of trace metals are agarose (AG) and polyacrylamide (PA). Earlier studies reported that PA binds mercury, possibly with its

* Corresponding author. Tel.: +32 2 629 3261; fax: +32 2 629 3274.
E-mail address: yuegao@vub.ac.be (Y. Gao).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.12.023

amide groups, causing it to compete with the resin gel instead of just providing a diffusive gradient, and making it impossible to get reliable results [5]. Therefore, DGT Probes including an AG diffusive gel have been proposed for the determination of total mercury [5–7]. Regarding the determination of MeHg ions in the water column and sediments, Clarisse and Hintelmann [8,9] used a DGT probe with a PA hydrogel. However, they did not take into account the possible affinity of MeHg for that hydrogel.

Common resin gels included in the DGT to bind IHg and MeHg ions are all sulfhydryl (for example 3-mercaptopropyl) functionalized silica gels. For the release of IHg species from solid materials such as sediments, hair, resins etc., several extraction solutions have been used: an acidic solution [2], a basic solution [2] and a thiourea solution [7]. For the release of MeHg ions from the resin only a thiourea solution was reported [8,9].

In this study, the binding affinity of AG and PA hydrogels towards MeHg was tested. In addition, a protocol to extract efficiently MeHg from the resin gels and to allow afterwards ethylation of the extracted MeHg ions was developed. Next, the diffusion coefficient of MeHg in the AG hydrogel was assessed as well as the influence of pH and ionic strength variability on the DGT efficiency regarding MeHg accumulation.

2. Method and materials

2.1. DGT assembly

2.1.1. Reagents and materials

Acrylamide solution (40%, pa, Merck), agarose (certified molecular biology grade, bio-rad), ammonium peroxydisulfate (APS, >98%, Merck), DGT gel crosslinker (DGT research Ltd), 3-mercaptopropyl functionalized silica gel (200–400 mesh, Aldrich), MilliQ water (Millipore, >18 MΩ cm), tetramethylethylenediamine (TEMED, >99%, Merck), sodium nitrate (suprapur, Merck). DGT pistons (DGT research Ltd).

2.1.2. Diffusive and resin gel preparation

Polyacrylamide (PA), agarose (AG) and resin gels were prepared similar to the methods reported by Gao et al. [7,10]. Briefly, for the resin gel preparation 2.5 g of 3-mercaptopropyl functionalized silica resin was added to 10 mL gel solution (15% acrylamide, 0.3% DGT cross-linker) and this solution was mixed well. Then 50 μL 10% ammoniumpersulfate solution and 15 μL *N,N,N,N*-tetraethylenediamine (TEMED) were added. The solution was mixed and cast between two glass plates with a spacer separating the plates. The glass assembly was placed in an oven at 45 °C for 1 h, and afterwards the resin gel was peeled off and hydrated in MilliQ water for at least one day until use.

2.1.3. Assembling DGT units for solution deployment

Resin gels were cut into 2.5 cm circles with a plexi-glass gel cutter. The resin gel was mounted on the DGT piston base with the resin side face up. Then the diffusive gel was placed on top of the resin gel and covered by a Millipore Durapore membrane filter (HVLP). The cap was then placed on the piston and pressed down to the bottom of the base.

2.1.4. MeHg accumulation test on diffusive gels

Twelve PA gel discs and 12 AG gel discs were separately deployed in 24 vessels with 15 mL of 40 ng L^{-1} MeHg solution. In addition, 8 control vessels containing 15 mL of 40 ng L^{-1} MeHg solution were also prepared. All those vessels were continuously shaken. After 4, 8, 24 and 48 h, 3 PA discs and 3 AG discs were each time retrieved from their deployment solution. From those 6 vessels 10 mL solution were sampled as well as from two control vessels. MeHg concentrations were assessed in all those solutions.

2.1.5. DGT performance test experiment

Performance tests of the DGT assembly were carried out in a 2 L MeHg solution of 50 μg L^{-1} containing 0.03 M $NaNO_3$. During the experiment, the MeHg concentration in the solution was monitored and used for comparison with the DGT derived MeHg concentration. The pH of the solution was around 5. Eight DGT pistons were plugged into the holes of a square housing rack. After 2, 4, 8 and 24 h, 2 of the DGT pistons were removed from the solution, the resin gel was transferred to a 20 mL FEP bottle and MeHg was extracted.

An additional experiment was carried out to study the effect of the diffusion layer thickness on the MeHg accumulation by the resin gel. DGT pistons with AG gel thicknesses from 0.04 to 0.12 cm were prepared in duplicate and deployed in a 50 μg L^{-1} MeHg solution containing 0.03 M $NaNO_3$ for 2 h. The resin gel was then treated in the same way as mentioned above.

2.1.6. pH and Ionic strength test experiments

The DGT pistons were exposed to MeHg solutions (50 μg L^{-1}) at different pH values (2 to 12) for 24 h. The pH value was adjusted using diluted HCl and NaOH. The effect of ionic strength was studied by adjusting the ionic strength of a MeHg solution (50 μg L^{-1}) with $NaNO_3$ in the range of 100 nM to 1 M. The MeHg concentration measured by DGT (C_{dgt}) was compared with that of the deployment solution (C_s).

2.2. MeHg determination

2.2.1. Reagents and standards

Acetic acid (100%, Merck), copper sulphate ($CuSO_4.5H_2O$, Merck, pa), dichloromethane (CH_2Cl_2, >99.8%, Suprasolv), hydrochloric acid (suprapur, Merck), MeHg stock solution (1000 mg L^{-1}, Chemlabpotassium Bromide (Merck, pa), potassium hydroxide (KOH pellets, Vel), sodium acetate (Merck, pa), sodium tetraethylborate ($NaBEt_4$, min 98%, Strem Chemicals), sulphuric acid (95–97%, Merck, pa), tetramethylammonium hydroxide (25% in methanol, Acros organics).

A $CuSO_4$ solution (1 M), an 18% (w/v) KBr solution and a 5% (v/v) H_2SO_4 solution were prepared from the purchased reagents in Milli-Q water. A 100 ppm MMHg stock solution is prepared from a 1000 ppm MeHg stock solution (1000 ppm, Alfa) in Milli-Q water and stored in a brown glass bottle at 4 °C. Working standard solutions of 5, 10, 20, 40 ng L^{-1} are prepared daily. One gram of sodium tetraethylborate ($NaBEt_4$, Strem Chemicals) is dissolved in a 100 mL, 2% KOH solution, which was cooled for 2 h in the deep freezer. This 1% $NaBEt_4$ solution is further diluted ten times and these solutions are stored in 20 mL FEP bottles in the deep freezer. The 0.1% $NaBEt_4$ deep frozen reagent is stable for several weeks, but once in use its lifetime is limited to one day. Acetate buffer solution is prepared in a FEP bottle by dissolving 272 g of sodium acetate and 118 mL of glacial acetic acid in 1 L Milli-Q water.

2.2.2. Extraction of MeHg from the resin gels

Twenty 3- mercaptopropyl functionalized silica resin gels were deployed separately in 10 mL of 40 ng L^{-1} MeHg solution for 48 h. Afterwards two extraction methods were applied: (1) 10 resin gels were extracted by the protocol reported by Clarisse and Hintelmann [8]. Two mL of thiourea solution (a concentration range of 0.5 to 50 mM was used) at pH 1 (0.1 M HCl) were added to each resin gel and the solution was shaken for 24 h; (2) the other 10 resin gels were extracted by a protocol that is similar to the one reported by Gao et al. [11]. Five mL of a 5% H_2SO_4 and 18% KBr mixture and 1 mL $CuSO_4$ (1 M) were added to each resin gel in a 20 mL FEP bottle and

shaken for 40 min. After adding 10 mL of CH_2Cl_2, the mixture was shaken again for 1.5 h, followed by centrifugation for 15 min at 3000 rpm. The water layer was removed and MeHg species in the CH_2Cl_2 layer were back-extracted in Milli-Q water by solvent evaporation at 60 °C under a constant N_2 flow.

2.2.3. Headspace vial reactions

Ethylation parameters (concentration of ethylating agent, reaction time), headspace parameters (thermostatic heating time, temperature, pressurization time, injection time, sample volume), and GC parameters (column temperature, gas flow rate) were based on the values reported by Gao et al. [11]. Ten mL of working standard solution of 5, 10, 20, 40 ng L^{-1} or 0.1–5 mL aqueous sample extract were transferred to the headspace vials and diluted to 10 mL. Next, 60 μL acetate buffer to obtain a pH of about 4.9 and 100 μL $NaBEt_4$ to obtain a concentration of 10 μg L^{-1} in the vial, were then added. Finally, the vials were sealed with Teflon-coated butyl rubber septa and Al crimp caps and allowed to react for 1 h before analysis. Every 4 samples, a 20 ng L^{-1} standard solution was inserted as a control sample.

2.2.4. HS–GC–AFS analysis

MeHg samples (standard solutions and DGT extracts) were analyzed with a Perkin Elmer Turbo Matrix 40 Trap headspace sampler coupled to a Perkin Elmer Clarus 500 gas chromatograph through a heated fused silica transfer line. Ar (Oxhydrique 5.0) is used as the carrier gas. The outlet of the GC is coupled to an atomic fluorescence detector (TEKRAN 2500) via a pyrolytic column. During this study we used the Perkin Elmer Turbo Matrix 40 headspace sampler without the trap. The 22-mL Pyrex glass headspace vials are closed with Teflon-coated butyl rubber septa and Al caps.

2.2.5. Precision and detection limit (LOD)

Ten replicate analyses of a 20 ng L^{-1} MeHg standard solution showed a RSD of 5.5%. The detection limit (LOD) calculated as three times the standard deviation of the noise was 0.08 ng-Hg L^{-1}.

3. Results

3.1. Extraction efficiency

The extraction protocol of Clarisse and Hintelmann [8] was thoroughly tested by using various concentrations of thiourea for the elution of MeHg from the resin gels. However, all results of the ethylated MeHg compound, observed with our HS-GC-AFS system, lay below the detection limit (0.08 ng L^{-1}). Thiourea is a very strong complexing agent for MeHg which makes it an excellent candidate to liberate the MeHg ions adsorbed on the resin gel but at the same time it is a very bad reagent for their consequent ethylation by tetraethylborate.

In contrast, the extraction protocol of Gao et al. [11] allowed to determine quantitatively the amount of MeHg that was adsorbed on the resin gel. An average recovery factor of 1.01 ± 0.08 was obtained and was further used in all experiments.

3.2. MeHg accumulation on diffusive hydrogels

Polyacrylamide (PA) and Agarose (AG) hydrogels were tested regarding their affinity for MeHg ions. The control solution, which is a standard solution of 40 ng L^{-1} MeHg free of gel, remained stable during the whole period of the experiment (48 h). This was also the case when AG gels were immersed in a similar solution (Fig. 1). However, when testing the PA gels, an obvious decrease of the MeHg concentration in the vessels was seen after 8 h and this

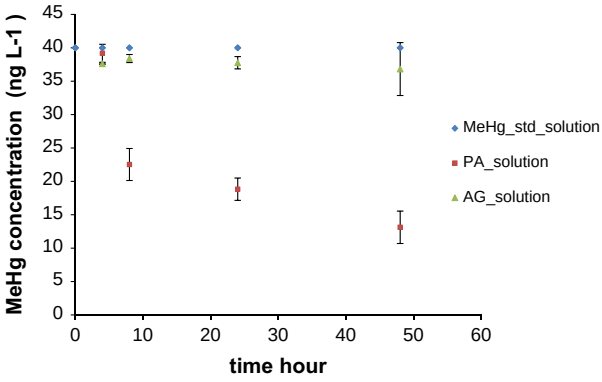

Fig. 1. MeHg accumulation on the PA and AG diffusive gels.

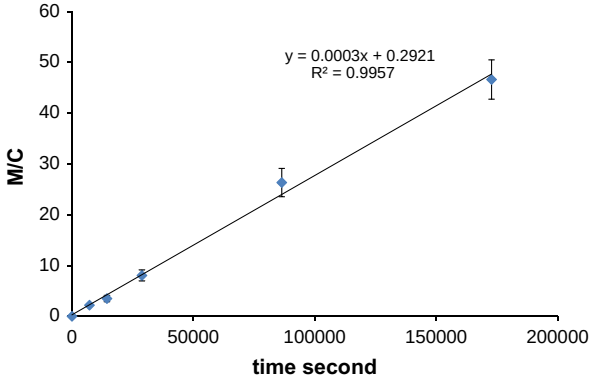

Fig. 2. MeHg accumulation on 3-mercaptopropyl functionalized silica gels.

decrease continued until the end of the experiment (after 48 h, the initial MeHg concentration decreased by a factor between 3 and 4). This result shows that the PA gel has a much higher affinity for MeHg than the AG gel. In an earlier study, Docekalova et al., [5] showed that the PA gel also has a high affinity for inorganic mercury. For the speciation of Hg in aquatic systems by DGT probes, it is thus recommended to using AG as the hydrogel.

3.3. DGT performance test

Eight DGT pistons, consisting of a filter, AG diffusive gel and 3-mercaptoproply functionalised silica resin gel, were deployed in a 50 μg L^{-1} MeHg solution containing 0.03 M $NaNO_3$. The DGT pistons were inserted into a housing rack facing into the deployment solution, which was continuously stirred. After 2, 4, 8, 24 and 48 h, 2 DGT pistons were taken out, along with 1 mL of the solution. The pH of the solution was around 5. Sub-samples of the deployment solution were analysed immediately after collection while the resin gels were transferred to the FEP bottles for MeHg extraction. The experiment was done in duplicate.

The average concentration in the deployment solution, which was around 46 μg L^{-1}, was used to calculate the diffusion coefficient. The 3-mercaptoproply functionalised silica resin gel shows a linear accumulation of MeHg with time (Fig. 2). The diffusion coefficient of MeHg in AG gel was then calculated with the following formula:

$$M/C = (DAt)/\Delta g \quad (1)$$

with M is the accumulated mass of MeHg, C is the MeHg concentration in the bulk solution, D is the diffusion coefficient

of MeHg in the AG gel, A is the exposure area of the gel to the bulk solution, t is the deployment time and Δg is the thickness of the diffusive layer. When the accumulated mass of MeHg (M) in the resin gel divided by the concentration (C) in the deployment solution was plotted against the deployment time (t), the angular coefficient of the curve (k) equals:

$$k = (DA)/\Delta g \quad (2)$$

The diffusion coefficient can then be calculated as:

$$D = (k\Delta g)/A \quad (3)$$

The MeHg diffusion coefficient in AG gel at 20 °C observed in this study equals $5.1 \pm 0.20 \times 10^{-6}$ cm^2 s^{-1}. The diffusion coefficient of MeHg in seawater at 20 °C is 5.0×10^{-6} cm^2 s^{-1} and the diffusion coefficient in PA gel reported by Clarisse and Hintelmann is $5.1 \pm 0.3 \times 10^{-6}$ cm^2 s^{-1} [8].

In an additional study, DGT pistons with different thicknesses of diffusive gel (from 0.04 to 0.12 cm) were prepared and deployed in a 50 μg L^{-1} MeHg solution during 2 h. Fig. 3 shows that the measured MeHg mass (M) accumulated on the resin gel was inversely proportional to the diffusion hydrogel layer thickness (Δg) in agreement with Eq. (1). Plotting the accumulated mass of MeHg against different diffusive gel thicknesses also allows, in a different and independent way however, to determine the MeHg diffusion coefficient in an AG gel according to Eq. (1). A diffusion coefficient of $5.1 \pm 0.40 \times 10^{-6}$ cm^2 s^{-1} was now obtained, which is identical to the diffusion coefficient previously determined.

3.4. pH and ionic strength test

In order to test the influence of the pH on the MeHg uptake by the resin gel, DGT probes were deployed in a 40 ng L^{-1} MeHg solution for 24 h at a pH between 2 and 12. MeHg was extracted from the resin gels with the acid extraction protocol and this extraction solution as well as the deployment solution were then analysed with HS-GC-AFS after ethylation of the MeHg ions. At different pH, the ratio of DGT MeHg concentrations (C_{dgt}) to the MeHg solution concentrations (C_s) presents the uptake efficiency of this type of DGT probe for MeHg.

The best results are obtained in a pH range of 4–6 (Fig. 4), but in a pH range of 3–8 the uptake efficiency is always above 70%. The pH of natural fresh waters normally falls into this range. However, care should be taken with marine water samples of higher pH, or very acidic waters, since the uptake efficiency diminishes outside the pH range of 3–8. The DGT MeHg concentration decrease at higher pH is due to the formation of stable MeHg compounds. For example at pH = 9 the ratio of non-dissociated MeHgOH molecules to dissociated ones is $10^{4.37}$ (equilibrium constant from Westöö [12]). These results are in agreement with the results reported by Clarisse and Hintelmann [8].

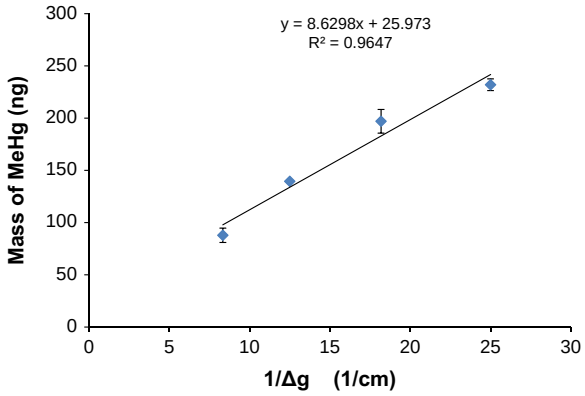

Fig. 3. Measured mass of MeHg in the resin gels with different gel layer thickness.

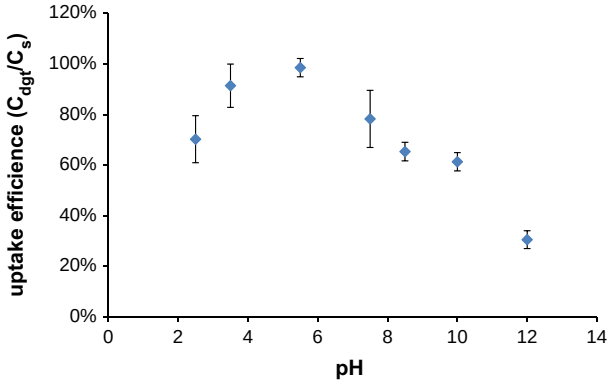

Fig. 4. MeHg uptake efficiency of the resin gel at different pH values of the MeHg solution.

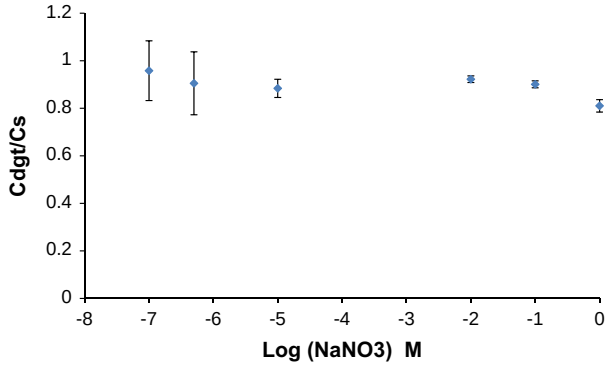

Fig. 5. Effect of ionic strength on DGT measurements assessed by the ratio of MeHg concentrations measure by DGT (C_{dgt}) to the concentrations obtained by direct measurement of the MeHg solution (C_s).

Next to temperature and pH, the ionic strength of an aquatic system is another important parameter that may influence the efficiency of the DGT technique, but no information about MeHg is available in the literature. The effect of this parameter was studied by adjusting the ionic strength of a MeHg solution (50 μg L^{-1}) via the addition of $NaNO_3$. The ionic strengths of the solutions were in the range of 100 nM to 1 M (100 nM, 10 μM, 10 mM, 100 mM, 500 mM and 1 M), corresponding to a range of 5.8 μg to 58 g of $NaNO_3$. Most natural waters fall into this range. To visualize the effect of the ionic strength on the MeHg accumulation by the resin gel, the DGT MeHg concentration (C_{dgt}) divided by that of the deployment solution (C_s) is plotted versus the log value of the ionic strength. At all ionic strengths, a good agreement between the MeHg concentration measured by DGT and the MeHg concentration directly measured in the solution is observed (Fig. 5).

There is thus no appreciable dependency of the accumulation of MeHg by the DGT on the ionic strength of the deployment solution. This is consistent to what was reported in the literature [13] for other types of DGT pistons (PA diffusive gel and chelex resin gel) used to determine other trace metals. The independency from the ionic strength is a considerable advantage when carrying out field work, as there is no need for multiple calibrations when sampling is done in aquatic environments of different ionic strengths.

4. Conclusion

In this study, a reliable DGT technique was developed for methylmercury determination in aquatic system. Our method differs strongly from the one reported in the literature because we found two major drawbacks in applying that method in our laboratory. First, the extraction of MeHg, accumulated on a resin gel, with thiourea prevented a further ethylation of the MeHg ion with tetraethylborate. Therefore, we applied an acid extraction protocol which we used before for the analysis of MeHg in sediment and hair [2,11]. This extraction method allows quantitative ethylation afterwards showing a recovery of 1.01 ± 0.08. In addition, this extraction method is simple and fast. Second, we found that Polyacrylamide (PA) gel accumulates MeHg ions, which makes it less suitable for MeHg assessment by DGT. Contrary to PA, Agarose gel (AG) accumulates by far less MeHg.

Therefore, the DGT probe consisting of an AG diffusive gel and a 3-mercaptoproply functionalised silica resin gel was characterized and validated by using the new analytical protocol described in this paper. The theoretical principles of DGT such as linear dependency (1) between the amount of MeHg accumulated on the resin gel and the deployment time as well as (2) between the amount of MeHg accumulated on the resin gel and the thickness of the diffusive gels, are demonstrated. A diffusion coefficient of MeHg in AG gel of $5.1 \pm 0.2 \times 10^{-6}$ cm^2 s^{-1} was obtained at 20 °C, which is comparable to the one in natural water. The pH dependency of MeHg uptake on the resin gel was similar to results reported in the literature, being most efficient in the pH range of 3–8. Finally, there exists no appreciable dependency of the accumulation of MeHg on the ionic strength of the deployment solution.

Acknowledgements

The authors thank two anonymous reviewers for their constructive comments on the manuscript. Dr Yue Gao thanks to FWO for a postdoc fellowship.

References

[1] M. Leermakers, S. Galletti, S. De Galan, N. Brion, W. Baeyens, Mar. Chem. 75 (2001) 229–248.
[2] M. Leermakers, H.L. Nguyen, S. Kurunczi, B. Vanneste, S. Galletti, W. Baeyens, Anal. Bioanal.Chem. 377 (2003) 327–333.
[3] M. Leermakers, W. Baeyens, P. Quevauviller, M. Horvat, TrAC 24 (2005) 383–393.
[4] W. Davison, H. Zhang, Nature 367 (1994) 546–548.
[5] H. Docekalova, P. Divis, Talanta 65 (2005) 1174–1178.
[6] P. Divis, M. Leermakers, H. Docekalova, Y. Gao, Anal. Bioanal. Chem. 382 (2005) 1715–1719.
[7] Y. Gao, E. De Canck, M. Leermakers, W. Baeyens, P. Van Der Voort, Talanta 87 (2011) 262–267.
[8] O. Clarisse, H Hintelmann, J. Environ. Monit. 8 (2006) 1242–1247.
[9] O. Clarisse, D. Foucher, H. Hintelmann, Environ. Pollut. 157 (2009) 987–993.
[10] Y. Gao, M. leermakers, C. Gabelle, M. Wartel, G. Billon, B. Ouddane, J.C. Fischer, W. Baeyens, Sci. Total Environ. 362 (2006) 266–277.
[11] Y. Gao, S. De Galan, A. De Brauwere, W. Baeyens, M. Leermakers, Talanta 82 (2010) 1919–1923.
[12] S. Westöö, Acta Chem. Scand. 21 (1967) 1790–1800.
[13] H. Zhang, W. Davison, Anal. Chem. 67 (1995) 3391–3400.