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Agarose Cleaning Optimization for DGT Devices Specific for Cesium

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Abstract. Agarose gels commonly used in electrophoresis are regularly checked by its manufacturers for the absence of contaminating nucleases or residual sulfates. It is also tested for several metals and trace elements: Pb, Cd, Co, Cu, Zn, K, Ca, Cr Mg, Mn, Fe, Ni, Na, and Cl. However, no data are available for Cs-impurity measurements. A Diffusive Gradients in Thin films (DGT) device specific for cesium (Cs) is intended to be developed. Therefore, it is important to assess the impurity degree of agarose with Cs. Direct dissolution of agarose in 4 mol·L^{-1} HNO$_3$ gives a concentration of 2.1×10^{-8} mol·L^{-1} of Cs, which is higher than that is found in natural environments (e.g. [Cs]$<1 \times 10^{-8}$ mol·L^{-1} in water). Two methods of Cs impurity removal have been tested, involving acidic cleaning with HCl and extraction by exchanger resin (KCuFC). Agarose powder was mixed with HCl in different concentrations, rinsed and dried. This method shows that the HCl-cleaning method is not quite efficient in removing the Cs impurity compared to untreated agarose gel. The second method, KCuFC resin was mixed with agarose powder in ultra-pure water, heated afterwards, and filtered. This method was able to reduce the amount of Cs by 99% compared to the untreated agarose gel. It appears that acceptable Cs levels (i.e. with respect to ambient Cs concentration in natural waters) are achievable by the removal of preexisting Cs in the agarose powder.

Keywords: Diffusion Gradient in Thin Film, Cesium, Potassium Copper Ferrocyanide, Agarose

1. Introduction

Diffusive gradient in thin film (DGT) is a method used for in-situ preconcentration of trace and ultra-trace labile compounds. It allows estimation of the concentration and the labile fraction of an element. This method does not disturb the dynamics of the particle dissolution, colloid formation or aggregation, and solutes' concentrations in natural water [1]. It has been widely used to probe heavy metals and, more recently, the persistent organic pollutants or radionuclides.
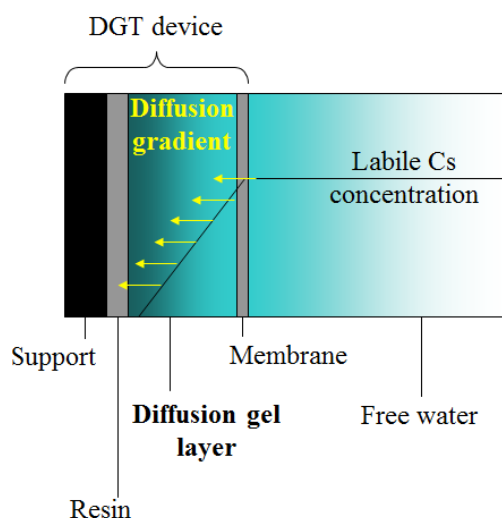
Figure 1. Schematic view of the functioning of a DGT device and concentration evolution during steady state diffusion in water.

Most of the DGT devices include a polyacrylamide as the diffusion gel layer. Polyacrylamide gel may be toxic to the local environment, because monomeric acrylamide is toxic, and polymerization is not complete Polyacrylamide gel for DGT purposes consists of 15% by volume acrylamide monomer, 0.3% by volume agarose based crosslinker, for 10 ml of gel solution 70 µl of ammonium persulphate 10% (APS) initiator and 20 µl TEMED catalyst. In some cases, the crosslinker can be substituted with bis-acrylamide 2% [2].

Cesium (Cs) is a chemical element that displays a high environmental mobility. It travels long distances when suspended in the atmosphere onto particles or dissolved in natural waters. Once deposited on the soil, its mobility vastly depends on local environmental factors including the soil nature and ongoing pedological processes [3]. Clayey soil presents high affinity with Cs, in particular with minerals presenting specific sorption sites such as illite, montmorillonite [3,4].

Cs is a natural chemical element but it can also be anthropogenically produced in the nuclear sector and the military industry. Actually, amounts of radioactive Cs have been released into the atmosphere during nearly all nuclear weapons tests and several nuclear accidents, notably the Chernobyl and Fukushima disaster. In fact, ^{137}Cs was among these isotopes widely distributed into the local and global environment. It thus constituted on of the greatest risk to human health [5]. All these aspects make Cs a useful element for probing the transfer mechanisms and, as a consequence, to monitor the dissemination of radionuclide into pristine and anthropized areas caused by a nuclear accident or the use of nuclear weapons.

In spite of its extreme ecotoxicity, the level of ^{137}Cs may be extremely low. Reportedly, radioactive Cs in the water samples from Fukushima area is less than 0.01 Bq·L^{-1}. Comparatively, germanium semiconductor detectors show a limit of detection (LOD) of 0.3 Bq·L^{-1}, for 12 hour measurement. Besides, stable Cs is found in free and pore waters at ultra-trace levels (0.004 to 2 µg·l^{-1} with an average of 0.05 µg·l^{-1} [3]). Direct determination is therefore not always possible [6,7].

Accordingly, a novel Cs-specific DGT device was developed in this work that uses agarose as the diffusion gel layer. Agarose is made from agar by removing the agaropectin which has a lower gelling ability (Figure 2). It is a thermoreversible and ion independent gelling agent. Some types of agarose are sufficiently devoid of charged residues so it can be considered neutral. This makes agarose suitable for electrophoresis as well as for investigating the most charge-sensitive environmental processes [8] (e.g. such as ionic Cs-Illite interactions).
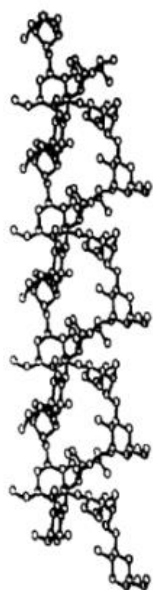
Figure 2. Agarose double helix viewed perpendicular to the helix axis [9].

Ultimately, because one of the most common use of agarose gels is electrophoresis, the impurities that the manufacturers check are the absence of contaminating nucleases or residual sulfates. It is also tested for several metals and trace elements: Pb, Cd, Co, Cu, Zn, K, Ca, Cr Mg, Mn, Fe, Ni, Na, and Cl. However, to our knowledge, there is no available information about the Cs content. Here we ask the question if commercial agarose can be sufficiently cleaned to allow the assessment of natural and radioactive Cs levels in the environment by means of agarose gel DGT devices. To answer this question, it is important to first assess the impurity degree of agarose with Cs and propose a cleaning procedure that gives access to the determination of environmental Cs concentrations.

The information available in the bibliography is given first. Then, the methodology and materials used for agarose gel cleaning are presented. The principal results are then displayed and discussed with respect to Cs concentration in collected water samples.

2. Overview on previous researches on Cs specific DGT devices

There exist several studies about Cs-specific DGT development. In most of these studies, authors use polyacrylamide as a diffusive gel according to the procedure from Zhang and Davison [2]. The difference in all the studies are the type of resin types of resin are tested in order to bind ionic Cs.

For instance, in the research by Chang et al. (1998), the binding agent consisted of AG50WX8 cation exchange resin. The resin is dispersed into an acrylamide solution [10]. Authors observed that the resin is readily saturated due to the continuous uptake of major cations present in the solution. Utilizing AG50WX8, they proved their hypothesis where for Cs and Sr, there was no evidence for the presence of inert complexes which would dissociate too slowly to be accumulated in DGT. There could be situations, however, where colloidal forms are significant and, in these cases, DGT would discriminate by measuring only labile solution species. Furthermore, it can easily be deployed in situ so that the selective binding of labile species would be assessed [10]. Chang et al. concluded that DGT with a general cation exchanger will be suitable for applications that measure multiple cations at low concentrations [10].

Later, in the research of Murdock et al. (2001) [11], they used Ammonium Molybdophosphate (AMP) as binding agent. They found that using known concentrations of ^{134}Cs, measurements with DGT devices with AMP binding agent closely reproduced the actual concentration, with a good degree of accuracy and precision for sampling periods up to 1 day. The DGT deployment period was limited to <1 month because of the significant progressive reduction in performance over longer periods [11]. The hypothesis was to consider that this was due to the growth of biofilms of algae/bacteria, which were seen to color the filter surface after deployment. Another explanation lies

in the progressive degradation of the AMP resin. Hence, authors recommended another Cs specific binding agents: namely, potassium cobalt ferrocyanide (KCuFC) that may extend deployment periods [11]. They found a nonlinear effect on the diffusion coefficient of variations in water temperature during the DGT sampling period. With unchanging solution concentration, the error will depend on the accuracy of the temperature record obtained and can become negligible with the use of a temperature logger. When there is variation in both temperature and solution concentration during the sampling period, the error is unquantifiable, although within definable limits. The worst case would occur if all the activity was discharged as a spike when the temperature was either at a maximum or minimum [11].

Finally, in the work of Li et al. (2009) [12], the authors used Copper Ferrocyanide (CuFCN) as the binding agent is immobilized on Chelex 100 resin gel (Chelex-CuFCN) and on polyacrylic acid gel (PAA-CuFCN). They found that both binding phases are suitable to be used as binding phase for Cs accumulated in DGT. Both are capable to selectively bind Cs ions in a high alkali and alkaline earth metal ions environment. But the labile concentration in PAA-CuFCN is closer to the dissolved Cs concentration compared to those in Chelex-CuFCN. The difference is caused by the different binding kinetics of Cs ions on the gel. DGT coupled with a gamma detector can be used for direct determination of radioactive Cs or with ICP-MS for stable Cs measurements [12].

Following this work, a specific DGT device for Cs was developed by using agarose as diffusive gel and copper-based potassium hexacyanoferrate (KCuF) as binding agent. However, commercial agarose gel contains impurities in Cs higher than the environmental concentration, thus, requiring specific purification treatment. The elaboration of the treatment is the main objective of this study.

3. Materials and Methods

The study of the Cs impurity in agarose and the elaboration of an impurity removal process were conducted due to the trace concentration of Cs in natural waters and the high uncertainty on the initial Cs content of commercial agarose. Due to the trace amounts of Cs in natural water, the containers and flasks are also tested for its Cs content and its interaction with Cs. All containers used are cleaned using HCl and rinsed using ultrapure water.

3.1 Methodology

Because there little to no available information on the commercial agarose contamination with Cs, an initial determination of its Cs content is required. According to the measured content, the agarose samples to be used in DGT devices may undergo either one or several cleaning steps. Additionally, a determination of the remaining Cs content takes place between two successive cleaning steps until the required purification degree is reached. The methodology followed in this research is presented in the figure 3.
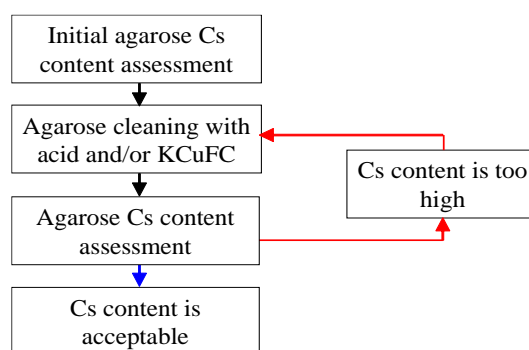

Figure 3. Research Methodology.

3.2 Equipment and materials

In order to prepare the diffusion gel layer of DGT devices, high purity electrophoresis grade agarose was purchased from Alfa Aesar Company. Cs concentration was measured using Induced Coupled Plasma-Mass Spectrometer (ICP-MS) (Xseries 2, THERMOELECTRON). The detection limit in Cs is $(1.5 \pm 0.2) \times 10^{-10}$ mol·L^{-1}. The acid used to digest was ultrapure HNO_3 10 mol·L^{-1}. The two types of acids used in the course of the cleaning steps are taken from ultrapure HCl (37%) or HNO_3 (70%) solutions. The liquid gel and KCuFC exchanger resin were separated using a filter syringe membrane supplied by Sartorius with a pore size of 0.22 µm.

3.3 Agarose digestion

To assess the quantity of Cs in agarose powder, agarose powder was directly digested in HNO_3 4 mol·L^{-1} at room temperature. 0.5 g of agarose powder was digested in 5 ml of HNO_3 4 mol·L^{-1}. The duration of the digestion process is 18 hours.

3.4 Agarose cleaning with Acid

Initially, the cleaning steps consisted in mixing the agarose powder with HCl solutions by using a ratio of agarose mass to acid volume of 62.5 g·L^{-1}. The concentrations of HCl were 0.1 mol·L^{-1}, 1.0 mol·L^{-1} and 2.5 mol·L^{-1}. The contact time was from 1 to up to 18 hours at room temperature. After the cleaning, all trace of acidity was removed by rinsing several times (usually 5-7 times) with ultrapure water. The acid-washed agarose powder was ultimately dried at 35° C in an oven. The cleaned agarose was digested in HNO_3 4 mol·L^{-1} at room temperature for a duration of 18 hours. The solution of digestion is afterward analyzed by ICP-MS.

3.5 Agarose cleaning with KCuFC

According to the targeted degree of purity, the acid-cleaned agarose powder or raw commercial agarose powder may undergo an extra cleaning step. This polishing step pertains to the removal of Cs by adsorption onto KCuFC resin. This is partly because, unlike with Chelex-100, KCuFC resin efficiency is almost pH independent. In practice, the agarose powder is mixed with KCuFC and 20 mL of ultra-pure water (contact time of 1 week). After 1 week, the mixture is heated and gently agitated until the agarose dissolution is complete (at 80 °C). The KCuFC and the agarose solution are then separated by either allowing the resin to settle at the bottom of the vial or by filtration. In the latter case, a syringe filter of 0.22 µm filter was used. The filtrates are after analyzed by ICP-MS. It is important to keep the filtration rate and the temperature of agarose solution high enough to avoid gel formation.

4. Results

4.1 Agarose digestion

The digestion of raw commercial agarose powder using HNO_3 4 mol·L^{-1} gives 2.1×10^{-8} mol·L^{-1} of Cs. This value doesn't seem high but considering the trace amount of Cs in the environment which is below 1×10^{-9} mol·L^{-1}, pretreatment is required before the utilization of the gel. As a matter of fact, the quantity of Cs found in the agarose powder is 56 times higher compared to the average environmental level. Cleaning process is therefore required to be able to distinguish natural Cs in the environment from the Cs impurity in the agarose powder.

4.2 The purity degree of agarose after acidic treatments

The results obtained for agarose cleaning with HCl are displayed in the figure 4 where the x axis represents the ratio of Cs amount in the digested cleaned agarose powder. The contact time is equal to 1 hour because longer durations resulted in pronounced degradation of the agarose samples. As a matter of fact, these turned into a dark mass or black powder when being exposed to concentrated HCl solutions (i.e. 2.5 mol·L^{-1} or 5.0 mol·L^{-1}) for longer periods. The overall decrease in the Cs content is therefore limited: i.e. less than 30%. Furthermore, it appears that the decrease is almost the same for all tested HCl concentrations.

This result shows that the HCl-cleaning method is not quite efficient and calls for longer contact durations, which is susceptible to alter the agarose gelling properties and to promote the formation of charged groups inside the gel matrix. The latter situation may render the interpretation of Cs-DGT measurements complex and thus would call for an exhaustive calibration work of DGT devices. Therefore, we decided to no longer pursue Cs removal from agarose utilizing acids.
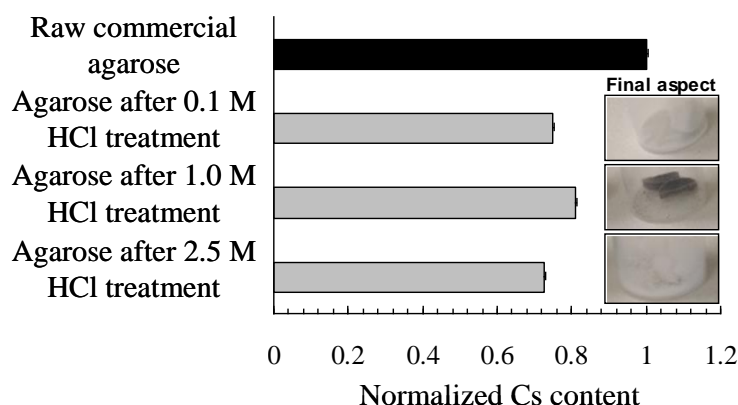

Figure 4. Normalized Cs content of agarose powder in the absence (i.e. raw) or after various treatments with HCl (1-hour cleaning) and subsequent drying. The Cs content of raw commercial agarose is set equal to the unity. The final visual aspect of treated powders is also given as pictures.

Extra cleaning tests conducted with HNO_3 (5 mol·L^{-1}) instead of HCl resulted in complete digestion of the agarose powder. This nevertheless allowed calculating its total Cs content: i.e. 0.1-0.3×10^{-9} mol·g^{-1}. Such low values are encouraging with respect to stable Cs level determination in natural waters by means of agarose gel-containing DGT devices. As a matter of fact, the related Cs contents of the agarose gel formed by using the raw commercial or the HCl cleaned agarose powder are $0.16-0.51 \times 10^{-9}$ mol·L^{-1} and $0.12-0.36 \times 10^{-9}$ mol·L^{-1}, respectively. This result also indicates that agarose powder cleaning is feasible providing an adapted washing agent is used.

4.3 Agarose cleaning using KCuFC

Agarose powder was mixed with KCuFC resin in ultra-pure water the suspension was heated afterwards, the KCuFC resin and the agarose powder were separated by filtration. The results (Figure 5.c) shows that the amount of Cs is reduced by 99% compared to untreated agarose gel (Fig 5.a). Using this method, a significantly higher purity was obtained compared to cleaning with HCl 2.5 mol·L^{-1} (around 6.4 times less Cs). Handling operations are also much more convenient because no acids are used and there is no risk of improper rinsing that can compromise the results.

The cleaning duration is another parameter assessed. The effectiveness of the cleaning process increases with time. Variations of 30 minutes and one week has been tested with the results shown in figure 5.c and 5.d respectively. The effectiveness of the cleaning process only stepped up by a factor of 4 when increasing the cleaning duration by a factor close to 340.

Furthermore, using gravity to separate the KCuFC resin from the agarose solution is not feasible due to the viscosity of the agarose solution (figure 5.b). It also resulted in a higher amount of Cs in the gel, which may indicate the contamination of Cs from a previous test or a memory effect of the vials used. Nevertheless, this result also shows the high selectivity of the exchange resin to uptake the Cs and its high sensitivity.

The effect of cleaning process reveals that the combination of time duration, heat, the use of KCuFC resin and filtration is the best condition to decrease the impurity of Cs in the gel. To compare the final concentration of Cs with natural Cs in the environment, water sample was collected in the Loire River Estuary at Mandin station near the city of Saint-Nazaire, France, the results showed that the total level of Cs impurity in agarose gel is 2.2 times lower than the value of natural Cs in the

environment. This procedure shows that there is now a possibility to follow the natural Cs in water after treatment of the gel.
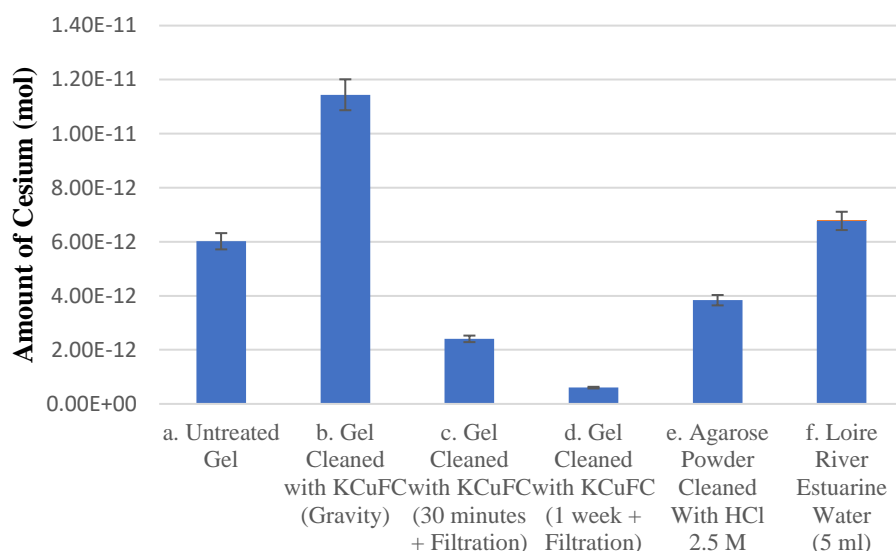

Figure 5. Comparison of various cleaning methods normalized to 2 ml of agarose gel.

The method used for cleaning the agarose gel has several advantages; yet a drawback from using this method is that due to the initial viscosity of the agarose solution. Indeed, whereas the diffusion gel in DGT devices are typically prepared with a ratio of 15 $g_{agarose} \cdot L_{water}^{-1}$, such solutions quickly clog the filter after around 1 ml of filtrate. Instead, a ratio of 5 $g_{agarose} \cdot L_{water}^{-1}$ was used in our research. By using this lower ratio, the filter can be used for up to 5 ml of solution. This results in a gel that is less dense and more fragile, but it may also let Cs ions diffuse easily.

Besides, special care should be done to prevent the formation of air bubbles when the syringe used for filtering the agarose solution is almost empty. The bubbles can cause the filtered gel to sputter, which can lead to air being trapped inside the gel. Air bubbles formed inside the gel may impede Cs diffusion towards the resin. This impedance will lower the amount of Cs in the resin and will lead to an inaccurate calculation of Cs concentration in the water.

5. Conclusions

Agarose gel is a good diffusive medium with a diffusive coefficient similar to polyacrylamide gel. A good thing about agarose is its simplicity in its manipulation and its non-toxicity compared to polyacrylamide. Taking all these results as a whole, it appears that acceptable Cs levels (i.e. with respect to ambient Cs concentration in natural waters) are achievable by the removal of preexisting Cs in the agarose powder before utilization.

In order to reach an even higher degree of purity (in the range of a few tens of 10^{-12} mol·g^{-1}), a series of cleaning stages is maybe required. As an example, the agarose powder could undergo an initial cleaning by adding 0.1 mol·L^{-1} HCl solution and mixing at room temperature for at least 1 hour. After discarding the HCl solution and multiple rinsing with ultrapure water, a polishing of the acid cleaned agarose may be undertaken. This would take place by mixing the agarose powder with KCuFC resin and ultra-pure water. By following these steps, the final Cs content of the agarose gel would be 10-50×10^{-12} mol·L^{-1}. This is below the detection limit of our ICP-MS device as well as stable Cs concentrations of estuarine and riverine waters (i.e. typically less than 5,000 and 100×10^{-12} mol·L^{-1}).

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