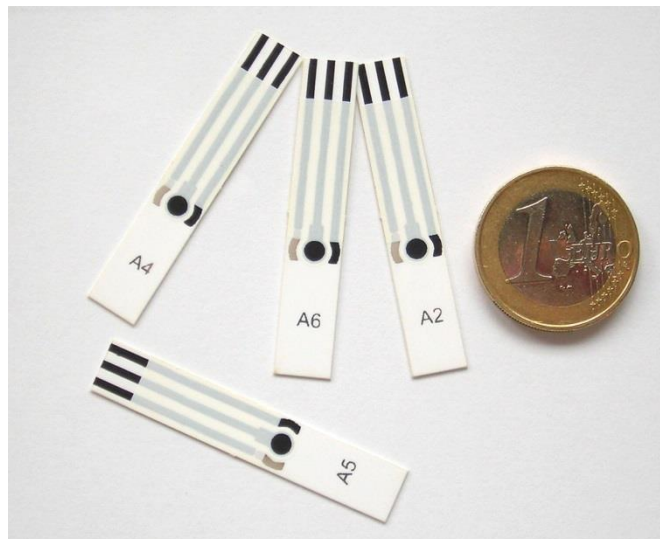


Mercury analysis using the ItalSens Au sensor

Quantification of mercury in aqueous solutions



Last revision: May 27, 2015

© 2014 PalmSens BV

www.palmsens.com

Contents

| | | |
|-------|--|----|
| 1 | Introduction | 3 |
| 1.1 | Goals of this application note | 3 |
| 1.2 | Alternative use of this application note | 3 |
| 1.3 | Sensor used | 3 |
| 2 | Experimental part | 4 |
| 2.1 | Equipment required | 4 |
| 2.2 | Reagents | 4 |
| 2.3 | Solutions | 4 |
| 2.4 | Electrode Preparation | 4 |
| 2.5 | Conducting the Experiment | 4 |
| 2.5.1 | Conditioning the sensor | 4 |
| 2.5.2 | Performing the measurement | 6 |
| 2.5.3 | Preparing the Analysis in the Analytical Mode | 6 |
| 2.5.4 | Auto-Analysis | 7 |
| 3 | Additional Information | 9 |
| 3.1 | Parameters for manual setting of the measurement | 9 |
| 3.1.1 | Conditioning the electrode | 9 |
| 3.1.2 | Stripping Square Wave Voltammetry | 9 |
| 3.2 | Measuring range | 10 |
| 3.3 | Electrode stability | 10 |
| 3.4 | Literature | 10 |

1 Introduction

Pollution is a known issue for decades. A focus of interest is, of course, unhealthy or outright toxic substances in resources we use for creating everyday goods or even consume ourselves. Spoiled water and food is the cause of many illnesses and other health issues. Due to this the monitoring of drinking water and food is an important task to keep people healthy. Mining releases heavy metals into the food chain. A well-known issue is the accumulation of mercury in fish or other animals we consume. A sad fact is that the consequences of mercury pollution could be observed due to careless disposal of mining waste into rivers supplying villages with water. The detection of mercury is not possible with IS-HM sensors, due to the fact that these are based on a mercury salt, but mercury can be detected using the IS-Au sensors. The gold surface will form an alloy with mercury and thus allow stripping voltammetry.

1.1 Goals of this application note

This application note is intended to provide the user with a comprehensive set of instructions for use of the screen printed electrode (SPE) IS-Au for determine Hg concentrations of aqueous solutions. Also by providing a step by step introduction for the Analytical Mode of PStace the user is afterwards familiar with this convenient mode of PStace.

1.2 Alternative use of this application note

The demonstrated technique works also for other heavy metals as long as they deposit properly on gold surfaces or form gold alloys. The detection of heavy metals that have very similar redox potentials will lead to overlapping peaks and thus will make an accurate analysis very difficult. We recommend performing test measurements to ensure a sufficient peak separation.

1.3 Sensor used

The Au sensor from Italsens is an excellent electrode to measure the ppb levels of heavy-metals such as Pb, Cd and Cu in aqueous solutions or for immobilization of molecules via a thiol group. [1, 2] The Italsens gold sensors (IS-Au) are three-electrode sensors, with a gold working electrode, a graphite counter electrode and a silver pseudo-reference electrode. The sensors come printed on polyester stripes with 20 sensors per stripe and need to be cut to the right size prior to use.

The sensors can be used by placing a drop of the solution on the sensor surface, by using a simple cell or by using the Italsens flow cell or batch cell. For stripping voltammetry and trace analysis we recommend a simple cell that allows stirring during the deposition step of the measurement.

This application note describes how to quantify Hg in aqueous solution by stripping voltammetry and the Analytical Mode of PStace.

2 Experimental part

For the experiment you need a properly cut IS-Au, a potentiostat with the usual equipment, a magnetic stirrer and the solutions from 2.2 and 2.3.

2.1 Equipment required

- PalmSens or EmStat with PSTrace software (version 3.0 or higher)¹
- Stirrer switch box, magnetic stirrer and magnetic stirring bar
- Sensor: ItaSens IS-Au sensors
- ItaSens sensor cable or another suitable connector
- Simple cell or ItaSens flow through cell
- Distilled or ultra-pure water
- Plastic or glass containers for the standard solutions

2.2 Reagents

- 6 M HCl
- $\text{Hg}(\text{CH}_3\text{COO})_2$ (standard solution or solid)
- 0.5 M H_2SO_4

2.3 Solutions

The used support electrolyte is 0.1 M HCl. It can be made by adding 1.67 mL 6 M HCl to around 10 mL water and fill up to 100 mL with water. For performing the standard addition a Hg standard solution of 1 mM (200.59 mg/L) is needed. This can be produced by diluting an existing standard solution or by dissolving solid mercury acetate. For the latter dissolve 31.87 mg $\text{Hg}(\text{CH}_3\text{COO})_2$ in 100 mL 0.1 M HCl. The volumes of mercury solution added during the measurement are so low that it is also fine use a standard mercury solution without HCl in it.

2.4 Electrode Preparation

To attach the electrode to the sensor cable, take the strip of electrodes and hold it at the end with the writing. Since the electrodes are easily contaminated or destroyed, please take care not to touch it with bare hands. Cut away the access plastic around one SPE and place it in the connector. You must cut the sensor narrow enough that it fits into the connector, but please take care not to damage the electrodes nor the lines during cutting.

2.5 Conducting the Experiment

2.5.1 Conditioning the sensor

Perform repeatedly CVs with the sensor until the SWV is stable and the scans overlap. All necessary files can be downloaded in the **Au conditioning bundle**.

Enter the *Scientific mode* of PSTrace to start the analysis. This can be either done by selecting it in the *General Settings* from the *Tools* menu of PSTrace or by using the dropdown box top left hand side of the PSTrace screen. Furthermore you need to tell the software, that you intend to use a magnetic stirrer. Open in PSTrace the *Tools – General Settings* menu. Then select the check the *stirrer present* box and choose the stirrer you use.

¹ It is also possible to use the Android App PStouch to control your potentiostat, but the App does not offer the Analytical Mode.

The electrode requires conditioning before use and this is done by running a standard method in 0.1 M HCl. The goal is a stable background current in 0.1 M HCl. A series of CVs is performed in 0.1 M HCl to create a stable surface and is checked by SWV. You can download the method file for the CVs at palmsens.com with the **Au conditioning bundle**. The needed method is **Au Pretreatment in HCl.psmethod**. Fill the cell with 0.1 M HCl, insert the magnetic stirring bar, put the cell on the magnetic stirrer, connect the sensor to the potentiostat, immerse the sensor and make sure the three electrodes of the sensors are covered with solution. For manual setting of the parameters are these listed in chapter 3.1.1.

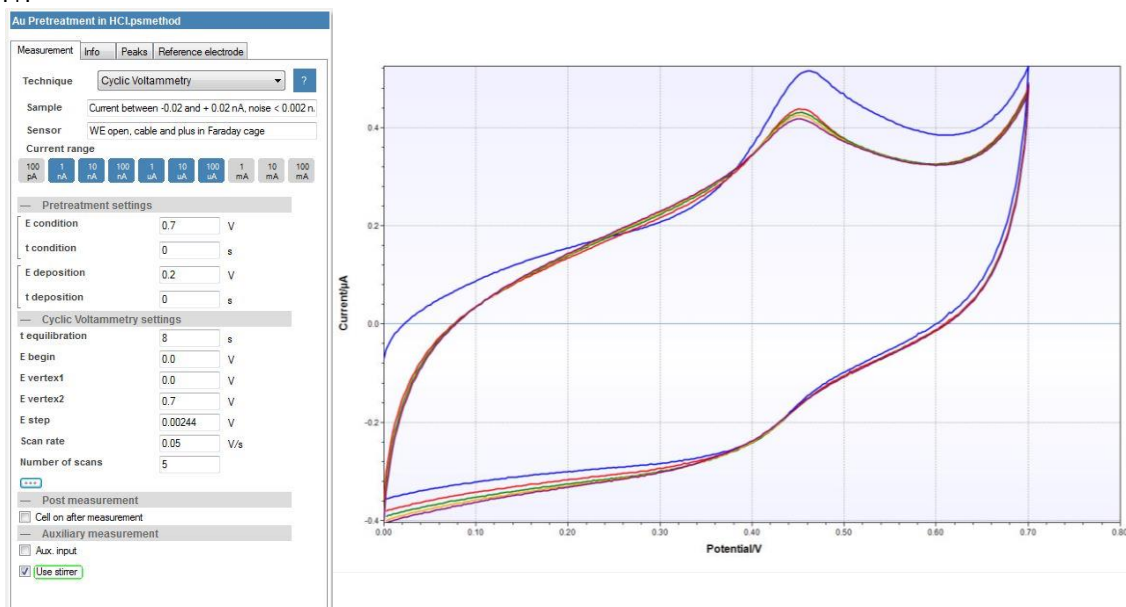


figure 1 conditioning of the IS-Au in HCl: method editor on the left and plot on the right

Typical scans from the conditioning stage are shown in figure 1. After the conditioning step, test the background by loading the method **Hg Detection.psmethod** and perform the measurement by pressing **Run**. The stirrer should be on and stirring during the deposition and off during the square wave voltammetry. After the first measurement is finished change the drop down menu next to the **Run** button to **Overlay**. Perform the measurement again. The difference between the two measurements should be less than 5 %. If it is bigger repeat the conditioning and check again the change of the background between two measurements. If after a few tries no stable background is formed, it might be necessary to clean the gold surface. This is done electrochemically. Exchange the 0.1 M HCl solution for 0.5 M H₂SO₄ and load the method **Au Pretreatment in H2SO4.psmethod** or enter the parameters manually. Perform the measurement. The CVs should look similar to figure 2. Change the solution back to the 0.1 M HCl and repeat the corresponding conditioning and check the stability of the background again. Once you have a stable background save it under the *curve – save curve* menu.

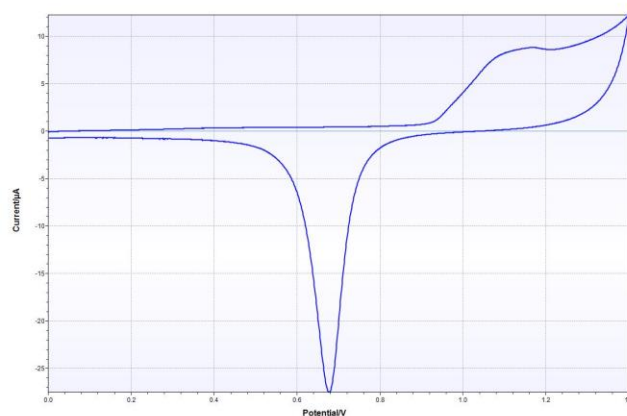


figure 2 CV of a IS-AU in 0.5 M H₂SO₄

2.5.2 Performing the measurement

If your sample needs dilution, because the concentration is higher than the linear range of the IS-Au, i.e. $0.499\ \mu\text{M}$ ($100\ \mu\text{g/L}$), it is a good idea to add your sample to the solution used for conditioning. Otherwise fill the cell with your sample after removing the conditioning solution. Your sample should have a concentration of $0.1\ \text{M HCl}$. This can be achieved by adding $6\ \text{M HCl}$ to the sample solution. After immersing the connected sensor again, make sure the three electrodes are covered with sample solution. Insert the magnetic stirring bar and position everything on the stirrer.

Load the **Hg Detection.psmethod** and press the green *Run* button to start the analysis of the sample. Repeat scans by selecting **Overlay** from the dropdown box and pressing play again until you get a stable peak height ($< 10\%$ peak height difference to the former scan). Then select the replicate that you wish to keep and click on it in the curve legend and save it. An example for the sample measurement can be seen in figure 3 (red curve).

Add a volume of mercury standard solution that contains roughly the amount of mercury that is in the sample to the cell solution. Perform the measurement and save the curve. Add mercury and repeat the measurements of curves until you have a satisfying amount of standard additions. A typical number is three additions. Keep in mind: The concentration of mercury in the cell should after the last standard addition be below $0.499\ \mu\text{M}$ ($100\ \mu\text{g/L}$), because the linear range of the sensor ends there.

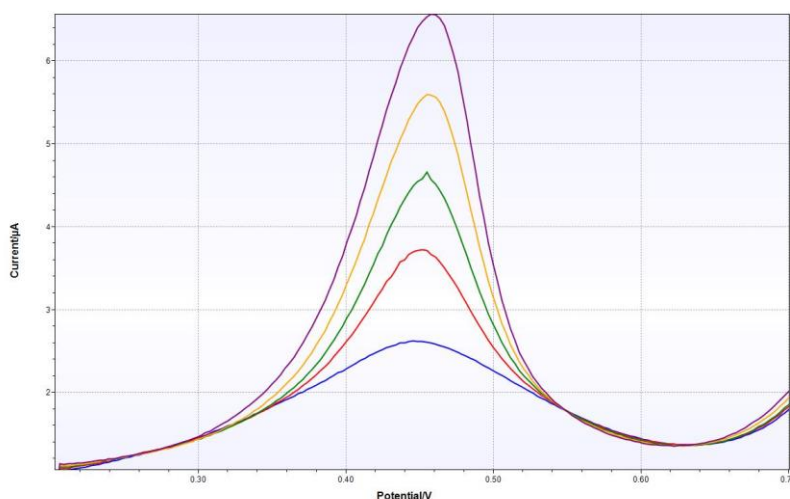


figure 3 raw data of a series of standard additions including sample (red) and background (blue) measurement

Perform SWV with the parameters from the file Hg detection.psmethod or from chapter 3.1.2. Repeat the measurement after each standard addition

2.5.3 Preparing the Analysis in the Analytical Mode

This analysis will be performed with the standard addition method. The standard addition method starts by measuring the sample and then measure the sample again after a known amount of the analyte(s) have been added. If a linear relationship between signal (peak current) and analyte (heavy metal) concentration is given, the concentration of the original sample can be calculated. PSTrace will be used in the Analytical Mode to auto-calculate sample metal concentrations from the standard additions. The experimental part has been already done this way, if you performed the measurement according to chapter 2.5.2.

To load a complete example including all curves in PSTrace click in the Analytical mode in the menu on *Analysis – Load Analysis* and load the **Au standard addition mercury.pds** file from our website.

In the method box click on the Analysis tab as shown in figure 4. Here all the settings for the standard additions will be filled out for you. The volumes and mercury concentration of the additions might need to be adjusted depending on your sample and standard solution. The concentration of the standard solution can be adjusted under *Conc.* The volumes added for each standard addition can be changed under *St. 1* to *St. 4*. The parameters of the measurement are again listed in 3.1.2.

Hg Detection with blank correction.psmethod

Measurement Info Peaks Reference electrode **Analysis**

Determination
☒ Standard addition ☐ Calibration

Analytes
 Concentration unit: uM
☒ Added volumes in uL ☐ Concentration in cell

| | Analyte 1 | Analyte 2 | Analyte 3 | Analyte 4 |
|---------|-----------|-----------|-----------|-----------|
| Id | Hg | | | |
| Sol nr. | 1 | 1 | 1000 | 1000 |
| Conc | 1000 | 10 | 10 | 10 |
| St. 1 | 1.88 | 1.88 | 10 | 10 |
| St. 2 | 1.88 | 1.88 | 10 | 10 |
| St. 3 | 1.88 | 1.88 | 10 | 10 |
| St. 4 | 0 | 0 | 10 | 10 |

Volumes
 Sample volume (ml): 15.0
 Cell volume (ml): 15.0

Peak settings
 Use peak value: ☒ height ☐ area ☐ sum of slopes

| | E peak | Auto | E left | E right |
|----|--------|-------------------------------------|--------|---------|
| Hg | 0.45 | <input checked="" type="checkbox"/> | 9999 | 9999 |
| | | <input type="checkbox"/> | | |
| | | <input type="checkbox"/> | | |

Auto peak search window around E peak (V): 0.1

figure 4 Analysis tab of the method editor showing the experimental setup for the additions.

next to that addition as seen in figure 6. If a mistake was made in the settings of the additions table, they can be corrected and the data recalculated by pressing the *Recalculate* button. The values from each calibration or standard addition are summarised in the box *All results* at the top of the analysis window. On completion of the measurements you can save all the data and curves in one file for use later by clicking *Save Analysis* within the *Analysis* menu.

All the data (raw or calculated) can be exported or printed for further manipulation using the *All to clipboard* and the *Print* buttons at the top of the data screen (figure 6).

2.5.4 Auto-Analysis

Load all the curves you need for the analysis: the background also known as blank, the sample and the measurements after each addition. Assign for each curve a designation. Click on the curve in the legend and select the designation, for example *Sample* for the sample solution, from the drop down menu above the legend.

This way the background should be assigned *blank*, the sample solution *sample* and the curve after the first addition *Standard_1*, the next one *Standard_2* etc. Once all curves are assigned the background needs to be subtracted from the other curves. Click on the curve that needs background substraction, e.g. the sample, and click on the curve in the legend. Click on the wave symbol with the green minus and black B to subtract the blank. Repeat that for each curve. Afterwards press *Autodetect peaks* (peak symbol with green cog) and make sure the Hg peak is detected for each curve. If not, check the *Epeak* set in the method editor window.

After completion of the background correction (figure 5) click on the *Analytical result* tab to view the calculated data (red circle, figure 5). Points can be included or excluded from individual metal calibration curves by toggling the check box

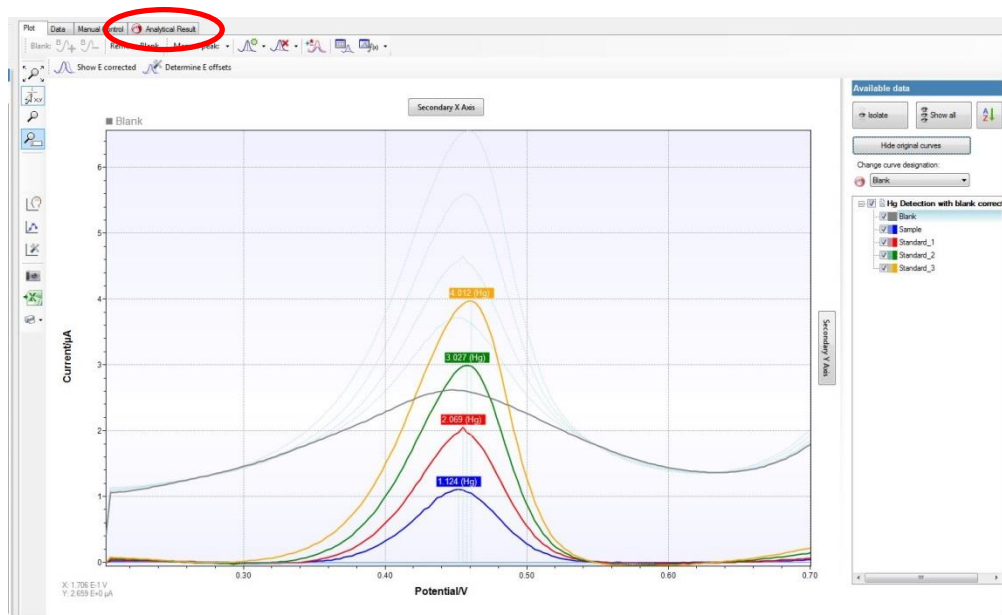


figure 5 The final analytical scans and where to find the analytical results

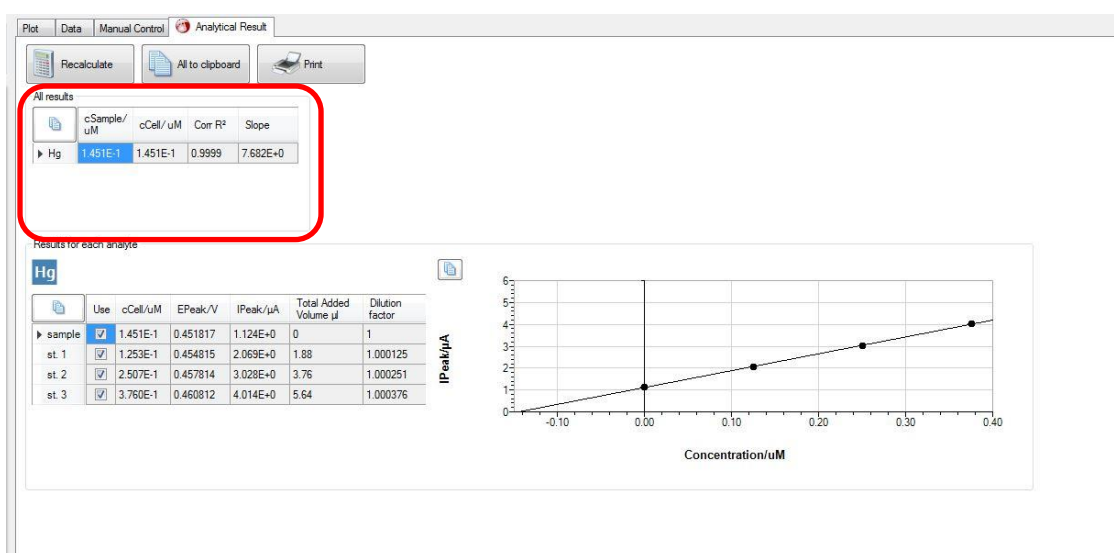


figure 6 The calculated results for each analyte and summarized (red box)

3 Additional Information

3.1 Parameters for manual setting of the measurement

3.1.1 Conditioning the electrode

If the corresponding method file is not available, you can just enter the following parameters by hand.

For the conditioning in 0.1 M HCl:

| Parameter | Value |
|-----------------|--------------------|
| Technique | Cyclic Voltammetry |
| Current Range | 1nA-100µA |
| E cond | 0 |
| t cond | 0 |
| E dep | 0 |
| t dep | 0 |
| t eq | 8 |
| E begin | 0 |
| E vertex1 | 0 |
| E vertex2 | 0.7 |
| E step | 0.00244 |
| Scan rate | 0.05 |
| Number of Scans | 5 |

Electropolishing of the gold surface

| Parameter | Value |
|-----------------|--------------------|
| Technique | Cyclic Voltammetry |
| Current Range | 1µA-100µA |
| E cond | 0 |
| t cond | 0 |
| E dep | 0 |
| t dep | 0 |
| t eq | 8 |
| E begin | 0 |
| E vertex1 | 0 |
| E vertex2 | 1.4 |
| E step | 0.001 |
| Scan rate | 0.05 |
| Number of Scans | 5 |

3.1.2 Stripping Square Wave Voltammetry

If the corresponding method file is not available, you can just enter the following parameters by hand.

| Parameter | Value |
|---------------|-------------------------|
| Technique | Square Wave Voltammetry |
| Current Range | 1nA-100µA |
| E cond | 0.7 |
| t cond | 30 |
| E dep | 0.2 |
| t dep | 120 |
| t eq | 30 |
| E begin | 0.2 |
| E end | 0.7 |
| E step | 0.003 |
| Amplitude | 0.028 |
| Frequency | 15.0 |

3.2 Measuring range

Table 1 the typical LOD for 120s deposition. Taken from reference [1]

| Metal | LOD (ppb) | ~Slope (nA/ppb) | Linear range (ppb) |
|-------|-----------|-----------------|--------------------|
| Hg | <1 | 11 | 0-100 |

This example is set for an artificial sample produced in the lab but concentrations of metals can vary massively from location to location. The electrodes do have a large linear range but if the electrode is saturating then the deposition time needs to be lowered. This is done from the method editor and change t dep. Set it to 60 s and the LOD will be 2 times larger (less sensitive).

As good working practice the first addition should double the peak height of the sample peak height. So the magnitude of the additions in figure 4 might need to be changed. Keep standard addition volumes as small as possible (<50 μL /10 mL), so the standard solutions might need adjusting.

3.3 Electrode stability

The electrode can be repeatedly taken out and the sample changed without any effect on the electrode performance. Care must be taken not to take out the sensor from the connector since the alignment of the contacts of sensor and holder have an impact. To store overnight, store dry but repeat the conditioning steps.

Be aware that applying potentials more positive than 0.7 V in chloride containing solutions will destroy the electrode! The chloride will be oxidized to chlorine. The chlorine forms water soluble gold chloride and thus corrodes the electrode.

3.4 Literature

- [1] Laschi, S.; Mascini, Palchetti, I.; M.; *Gold-based screen-printed sensor for detection of trace lead*; Sensors Actuat B-Chem 114, **2006**, 460-465; DOI: 10.1016/j.snb.2005.05.028
- [2] Meuccia, V.; Laschi, S.; Minunnib, M.; Prettic, C.; Intorrea, L.; Soldania, G.; Mascini, M.; *An optimized digestion method coupled to electrochemical sensor for the determination of Cd, Cu, Pb and Hg in fish by square wave anodic stripping voltammetry*; Talanta.; 77; **2009**; 1143-1148; DOI: 10.1016/j.talanta.2008.08.008