

1 Introduction

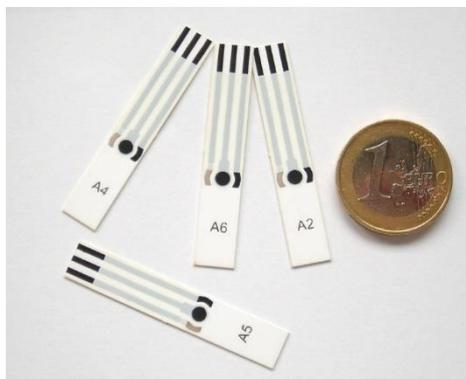


Figure 1 Italsens IS-HM with Euro coin for scale

This application note describes how to quantify Cu, Pb, and Cd in an aqueous solution by stripping voltammetry and the Analytical Mode of PSTrace.

Pollution is a known issue for decades. Of course, a focus of interest is unhealthy or outright toxic substances in resources we use for creating everyday goods or even consume ourselves. Spoiled water is the cause of many illnesses and other health issues. Due to this, the monitoring of drinking water is an important task to keep people healthy. While in surface water bacteria are the main threat, in almost all urban areas water is consumed after flowing through pipelines, which could release metal ions into the water. This or the direct pollution of groundwater can lead to heavy metals in drinking water. Many heavy metals are biologically active and cause illnesses. A well-known example is lead poisoning. In this application note, we will show how to detect different metals in an aqueous solution with the IS-HM1 sensor.

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-HM1 in analyzing Pb, Cd, and Cu concentrations of a polluted river water sample.

Also, by providing a step-by-step introduction to the Analytical Mode of PSTrace and PStouch the user is afterward familiar with this convenient mode of PSTrace and PStouch.

1.2 Alternative use of this application note

The demonstrated technique works also for other heavy metals if they deposit properly on graphite surfaces or form mercury 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 HM1 sensor from Italsens is an excellent electrode to measure the ppb levels of heavy metals quickly such as Pb, Cd, and Cu in aqueous solutions and cheaply. [1] The Italsens mercury salt modified graphite sensors (IS-HM1) are three-electrode sensors, with a mercury salt on graphite working as well as 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 before 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 a matching flow cell. For stripping voltammetry and trace analysis, we recommend a simple cell that allows stirring during the deposition step of the measurement.

2 Experimental part

For the experiment, you need a properly cut IS-HM1, a potentiostat with the usual equipment, a magnetic stirrer, and the solutions from 2.2 and 2.3.

2.1 Equipment required

- Potentiostat of [PalmSens](#) series or [EmStat](#) series
- [PSTrace](#) software or [PStouch](#)
- [Stirrer switch box, magnetic stirrer, and magnetic stirring bar](#)
- Sensor: [ItalSens IS-HM1](#) sensors
- [PalmSens SPE Connector](#) or another suitable connector
- Simple cell (beaker) or flow-through cell of matching dimensions
- Distilled or ultra-pure water
- Plastic or glass containers for the standard solutions

The best results are achieved when automated stirring is used. Thus, we recommend a potentiostat with an AUX port, e.g. PalmSens4, and a stirrer with a switch box.

2.2 Reagents

- 6 M HCl,
- 1 g/L solution of AA/ICP metal standards for Pb
- 1 g/L solution of AA/ICP metal standards for Cd
- 1 g/L solution of AA/ICP metal standards for Cu

2.3 Solutions

Make 10 mL solutions of 5 ppm of each metal by pipetting 10 μ L of HCl, into a bottle. Then add 50 μ L of stock metal solution and clean water, making a total volume of 10 mL.

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 damaged, please take care not to touch them with bare hands. Cut away the access plastic around one SPE and place it in the connector. The markings next to the contacts indicate the width required for the PalmSens Sensor Connector. You must cut the sensor narrow enough that it fits into the connector but take care not to damage the electrodes or the lines during cutting.

2.5 Conducting the Experiment

2.5.1 Conditioning the sensor

Perform repeatedly an LSV with the sensor until the prominent peaks are almost gone and the curves of each scan are stable and overlap. All necessary files can be [downloaded in the HM1conditioning bundle](#).

Enter the *Scientific mode* of PStouch or PStouch to start the analysis. In PStouch this can be either done by selecting it in the *General Settings* from the *Tools* menu of PStouch or by using the dropdown box top left-hand side of the PStouch screen. In PStouch the app mode selection is found if you touch the PStouch logo in the upper left corner or the menu in the top right corner. Furthermore, you need to tell the software, that you intend to use a magnetic stirrer if you want to use the automated one. Open in PStouch the *Tools – General Settings* menu. Then check the *stirrer present* box and choose the stirrer you use. In PStouch, use the button in the upper right corner to open the menu and choose *Settings*. There you can set the stirrer *ON*.

The electrode requires conditioning before use, and this is done by running a standard method in 20 mM HCl (~ 33 μ L of 6 M HCl in 10 mL). The most convenient way to do this is using a script. This is a convenient tool to perform more complex processes automatically. This time we want to perform repeatedly the same measurement, to condition the sensor. Open the script window in the *Script – Open Script window* menu and load the script named **HM1condition** (Figure 2). You can [download this script including the required pssession files](#) with the **HM1conditioning bundle**. The default data and method path is the PSData folder in *My Documents*. Check the paths of the two method files of the script and the data output ensuring that they are all correct. If they are not correct the blocks of that method will be red instead of blue. The names of the two required Methods are HM1cond.psmethod (first block) and HM1prescans.psmethod (block connected to the repeat block). Unfortunately, scripting is not possible with PStouch. The conditioning needs to be done manually by starting the methods repeatedly. Fill the cell with the 20 mM 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. If you made changes in the script save it and then press Run. The conditioning takes ca. 15 min. For a manual setting of the parameters, the parameters are listed chapter in 3.1.1.

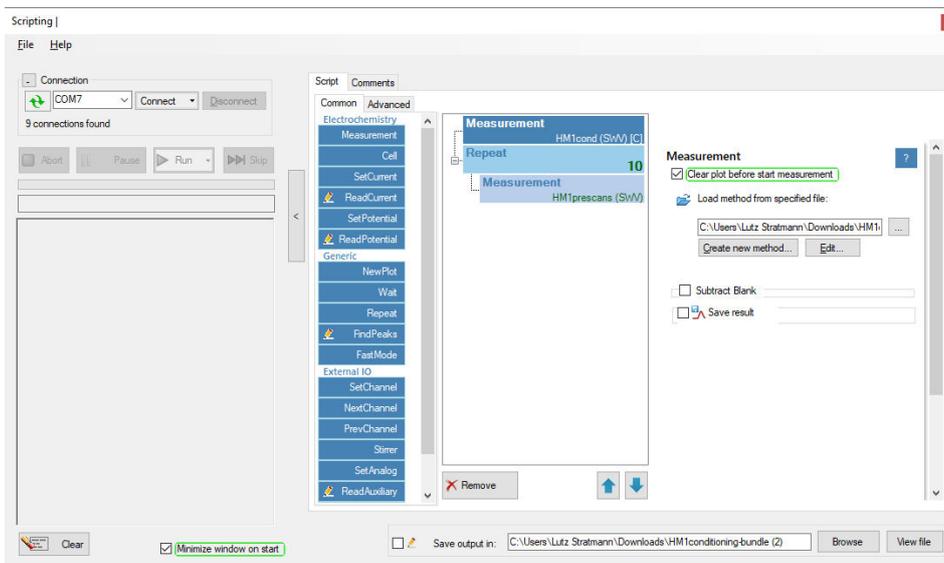


Figure 2 The scripting window

Typical scans from the conditioning stage are shown in Figure 3. Notice please that the peaks are decreasing with each scan until they almost vanish completely. When finished rinse the cell and electrode with some 20 mM HCl solution and clean water.

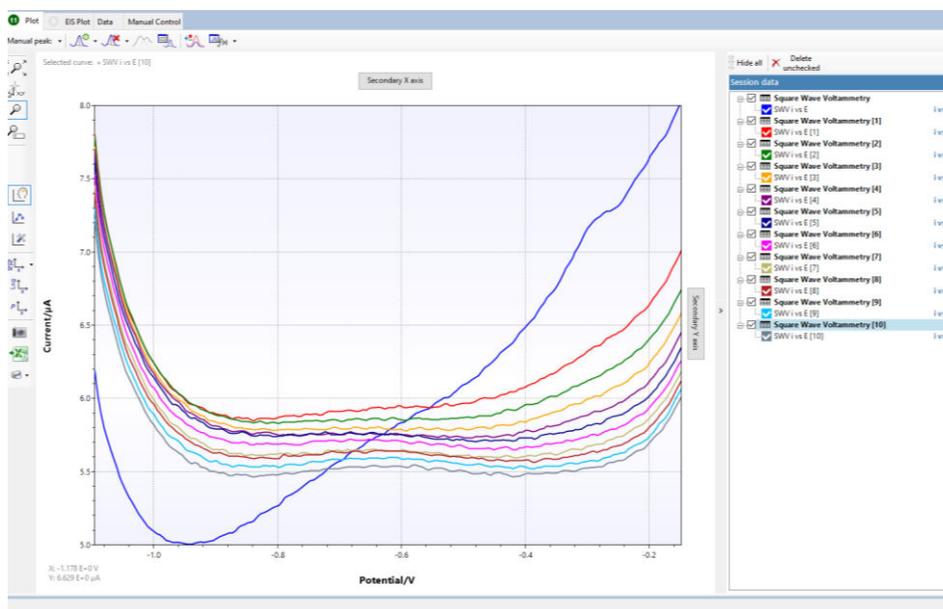


Figure 3 The scans of the conditioning step

2.5.2 Preparing the Analysis in the Analytical Mode

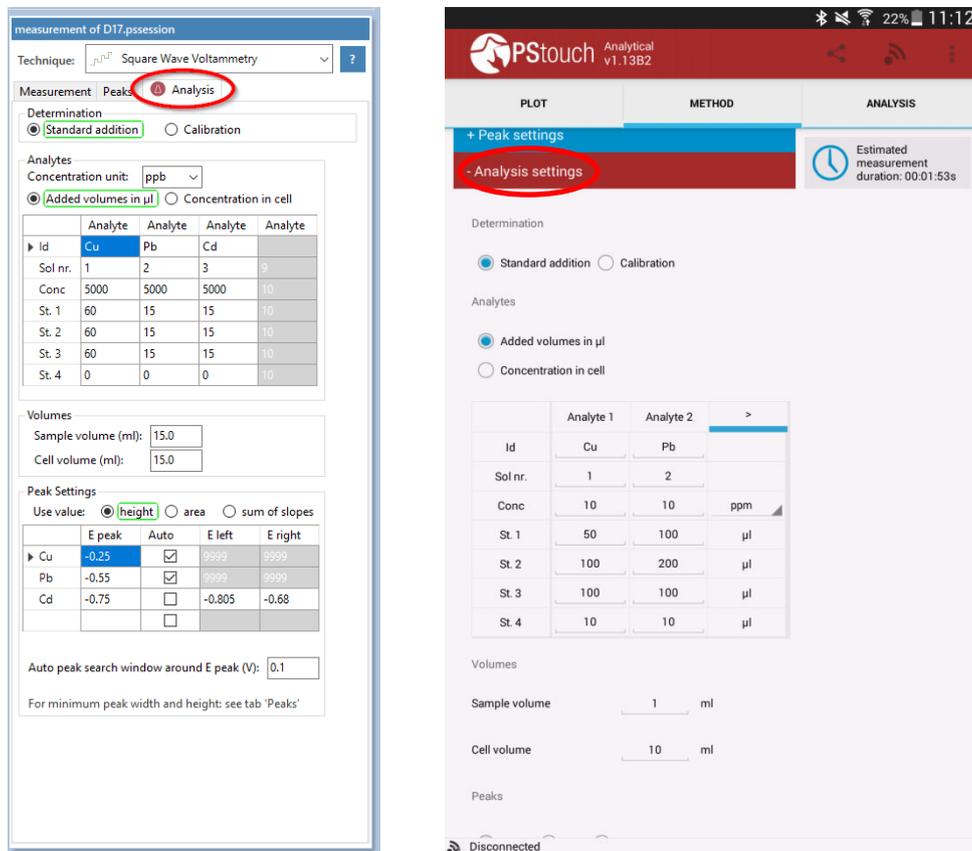


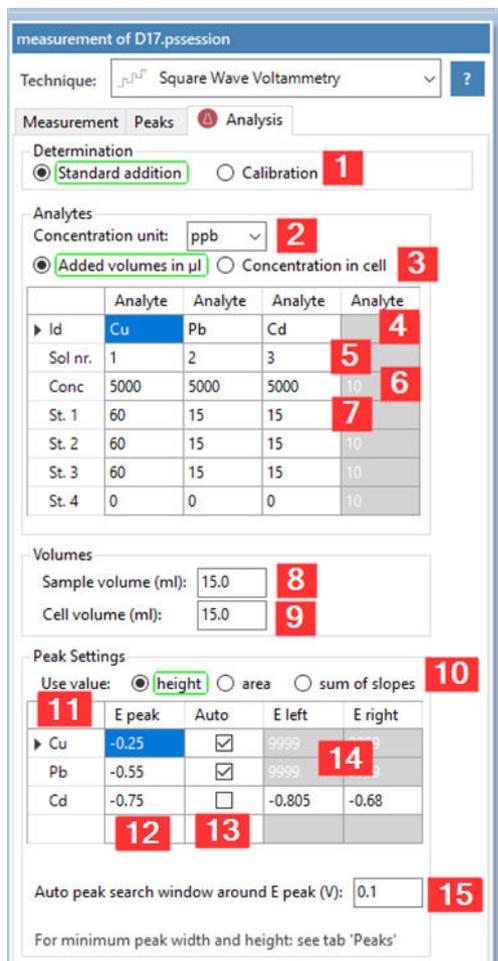
Figure 4: Analysis settings for PSTrace (left) and PStouch (right)

Perform SWV with the parameters from the file [HM1 standard addition river water.psmethod](#) or from chapter 3.1.2. Repeat the measurement after each standard addition and declare for each curve if it is the sample or standard addition. Afterward, read your results in the Analysis Results tab.

This analysis will be performed with the standard addition method. The standard addition method starts by measuring the sample and then measuring the sample again after a known amount of the analyte(s) has 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. Load the analysis file [HM1 standard addition river water.psmethod from our website](#). To load a complete example including all curves in PSTrace click in the Analytical mode in the menu on *Data -Load Data* and [load the HM1 standard addition river water file from our website](#). In the method editor, click on the Analysis tab as shown in Figure 4. Here all the settings for the standard additions are filled out. If you use the provided files, these filled will be prefilled. The magnitude of the additions might need to be adjusted depending on your river water sample. In this example, the Cd has a forced left and right peak to help with low Cd measurements. This can be changed back to Auto. If peaks are visible, the Auto option is a good choice, but if peaks are very small and/or broad it is difficult for the software to find the peak. A

forced peak border is then the mean of choice. The parameters of the measurement are again listed in 3.1.2.

2.5.3 Settings in the Analysis tab



- Find the unknown concentration through *Standard addition* method or with a *Calibration* curve
- Select the unit of concentration
- Concentrations are derived from *Added volumes in µL* of a standard solution or a known *Concentration in the cell* is used
- Name of your analyte
- Only for *Added volumes*: Enter different numbers for different solutions e.g., if all analytes in one standard solution enter 1, 1, 1 or if each analyte is in a different standard solution 1, 2, 3
- Only for *Added volumes*: Concentration of the analyte in the standard solution
- For *Added volumes*: *Added Volume in µL* before the measurement of Standard 1, Standard 2, etc.
 - For *Concentration in cell*: *Concentration in cell* during measuring Standard 1, Standard 2, etc.
- Volume of the sample filled into the measurement cell
- Volume in the cell after the sample was diluted
- Property used as signal
- Name of the Analyte
- Expected peak potential
- Check for auto search within search window, remove check for using E left and E right
- Limits for the search of the peak, highest point between limits is declared as peak
- Peak search is performed in E peak ± this value

Figure 5 Analysis tab settings

2.5.4 Performing the measurement

The pH of your sample should be around 2.5. This can be achieved by adding 10 µL of 6 M HCl to 10 mL of your sample. Usually, the volume change that is caused by this addition can be neglected. Fill the cell with your sample and after immersing the connected sensor, make sure the three electrodes are covered with the sample solution. Insert the magnetic stirring bar and position everything on the stirrer.

Press the green Run button to start the analysis of the sample. Repeat scans by selecting *Overlay* from the dropdown box (PSTrace) or in the pop-up menu (PStouch) and pressing run again until you get a stable peak height (< 10 % peak height difference to the former scan). The stirrer should be on and stirring during the deposition and off during the square wave voltammetry. Then select the replicate that you wish to keep and click on it in the curve legend. Designate the curve use (see Figure 6). Your first curve is most likely a blank or the sample.

Add an amount of analyte(s) that roughly resembles the amount of analyte(s) you expect in your sample. If the added volume is different than expected, change it in the table of the *Analysis* tab. Perform the

measurement, and select for this curve *Standard 1* in the context menu (PSTrace) or pop-up menu (PStouch) seen in Figure 6. Repeat the measurement. Once you are getting a stable *Standard 1* curve, add your second volume of metal standard solution and record *Standard 2* as described for *Standard 1*. Repeat these steps to add *Standard 3* and maybe *Standard 4*. A typical number of standard additions is three. In PSTrace it is also possible to assign the curves a function before the measurement to the different curve types after they have been recorded. Just select them in the legend window and change the curve assignment in the dropdown menu or pop-up menu.

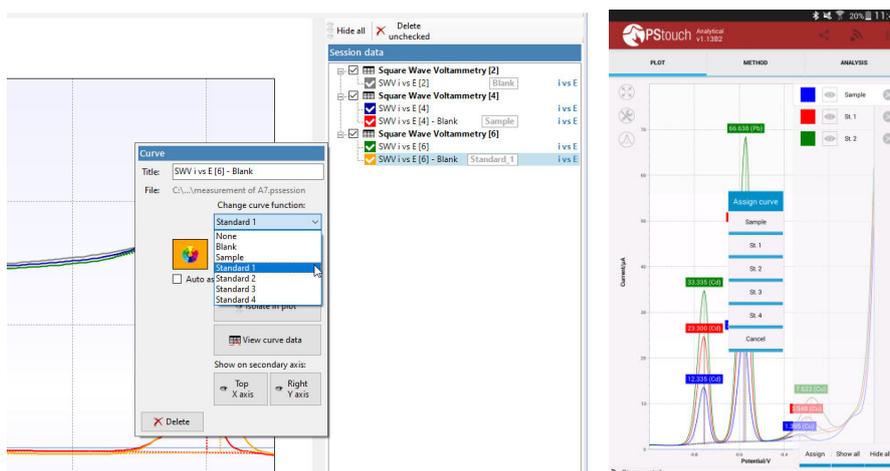


Figure 6 The first scan of the sample and selection of additions for PSTrace (left) and the assignment of curves for PStouch (right).

2.5.5 Auto-Analysis

After completion of all measurements for this analysis click on the *Analytical result* tab (PSTrace) or the *Analysis* tab (PStouch) (Figure 7) to view the calculated data. Points can be included or excluded from individual metal calibration curves by toggling the check box next to that addition as seen in Figure 8. If a mistake was made in the settings of the additions table, it can be corrected in the *Analysis results* table, and the data recalculated by pressing the *Recalculate* button. The values from each calibration or standard addition are summarized in the box *All results* are at the top right 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 *Data – Save Data*.

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 8).

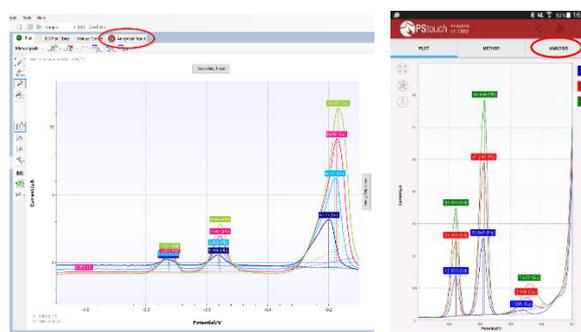


Figure 7 The final analytical scans and where to find the analytical results

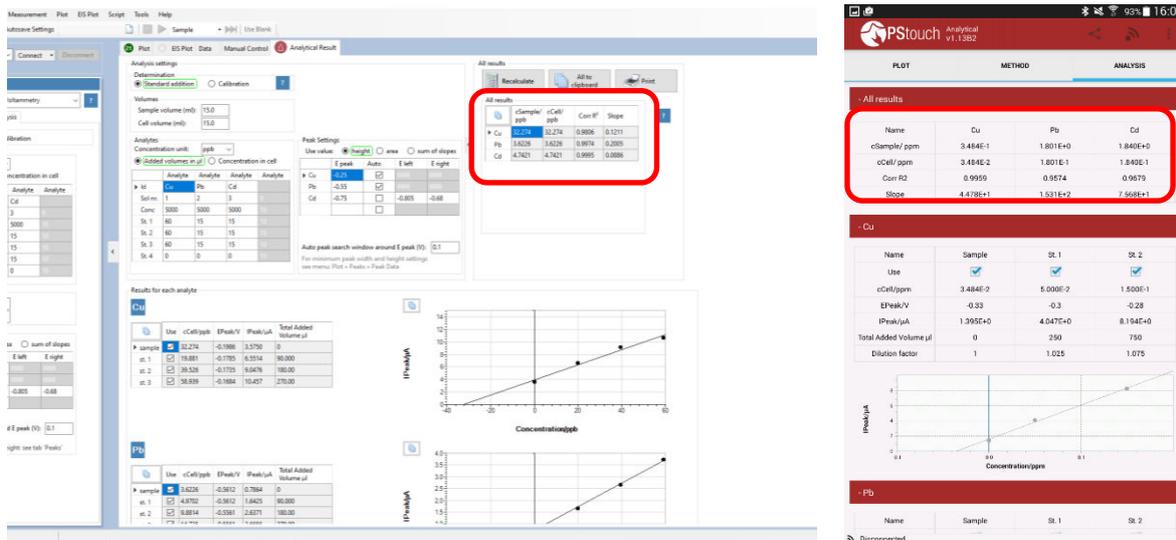


Figure 8 The calculated results for each analyte and summarized (red box)

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. The script used to perform the conditioning is comprised of two methods. First, the following method with a long period for deposition is performed.

Parameter	Value
Technique	Square Wave Voltammetry
Current Range	1 μ A-100 μ A
E cond	-0.18
t cond	30
E dep	-1.1
t dep	300
t eq	10
E begin	-1.1
E end	-0.15
E step	0.005
Amplitude	0.025
Frequency	15.0

Afterward, the following method is repeated **ten times**.

Parameter	Value
Technique	Square Wave Voltammetry
Current Range	1 μ A-100 μ A
E cond	-0.18
t cond	30
E dep	-1.1
t dep	0
t eq	10
E begin	-1.1
E end	-0.15
E step	0.005
Amplitude	0.025
Frequency	15.0

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	1 μ A-100 μ A
E cond	-0.12
t cond	45
E dep	-1.15
t dep	120
t eq	10
E begin	-1.1

E end	-0.1
E step	0.005
Amplitude	0.025
Frequency	25.0

3.2 Measuring range

Table 1 the typical LOD for 120s deposition. Calculated using 3*standard deviation of repeat measurements of 1ppb of each metal

Metal	LOD (ppb)	~Slope (nA/ppb)	Linear range (ppb)
Cu	<1	30	1-1000
Pb	<1	115	1-1000
Cd	<1	60	1-1000

This example is set for a typical river water sample, 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. Just adjust t dep in the method editor. For example, set it to 60 s and the Limit of Detection (LOD) the measurement will be less sensitive in low concentrations.

As a good working practice, the first addition should double the peak height of the sample peak height. So, the added volumes of the additions in Figure 5 might need to be changed. Keep the added volumes as small as possible (<50 µL/10 mL) to avoid significant changes in the cell volume.

3.3 Electrode stability

In between measurements when in solution the sensor is held at a standby potential. This is to maintain the mercury film of the electrode while in solution. The electrode can be repeatedly taken out and the sample changed without any effect on the electrode performance. Care must be taken to not run the electrode in a solution without acid or no salt. The stability of results is excellent throughout a typical day and will also work the following day. The best way to monitor electrode stability/performance is by using the gradient of the calibration slopes which are shown in the table as a guide. To store overnight, store dry but repeat the conditioning steps.

Be aware that applying potentials more positive than -0.1V will destroy the electrode!

The electrode is sufficiently stable to calibrate in the morning, obtain the sensitivity (S) and use the same S for all measurements (concentration of metal in the sample = I_{peak}/S). One-point standard additions are also acceptable if you are happy with the electrode stability.

3.4 Interferences

The IS-HM1 sensor is best suited to aqueous environments but the results can be seriously impacted by surfactants (soaps) and oils. Oils can be overcome by only sampling below the water surface. Surfactants are best dealt with by diluting the sample.

3.5 Synthetic river water

It is sometimes desirable or easier to work with synthetic water. Smith et al describe how to replicate a typical river water sample. [2] For this application note, it is sufficient to use clean water acidified with HCl.

3.6 Literature

- [1] Palchetti, I.; Laschi, S.; Mascini, M.; *Miniaturised stripping-based carbon modified sensor for in field analysis of heavy metals*; *Analytica Chimica Acta* 530, **2005**, 61-67
- [2] Smith, E.J.; Davison, W.; Hamilton-Taylor, J.; *Methods for preparing synthetic freshwaters*. *Water Res.*; 36; **2002**; 1286–1296