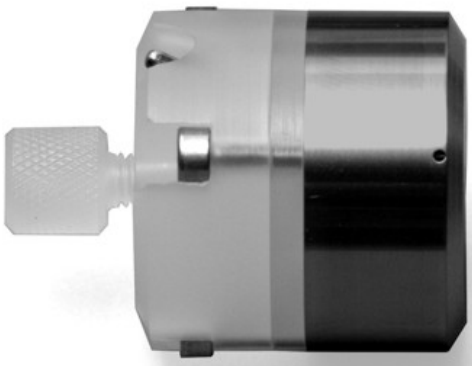
ANTEC LEYDEN

Sencell
Electrochemical Flow Cell

user manual



Edition 1, November 2006 115.0010

Table of contents

C H A P T E R 1

The electrochemical flow cell

Introduction

The Sencell has been developed for ultra-trace analysis in standard, microbore and capillary LC-EC. The flow cell has a unique accelerated wall-jet configuration, facilitated by an integrated 30 µm flow path over the working electrode. The total volume of the flow path is 0.03 mL and a FEP spacer is not used anymore.
The Sencell is available with a glassy carbon working electrode of 0.5, 1, 2, 3 and 5 mm diameter. Effective cell volume, determined by the WE area and the thickness of the flow path, can be as small as 6 nL. As a standard the ISAAC reference electrode is applied, for applications with a high pH or high modifier percentage a HyREF is available.

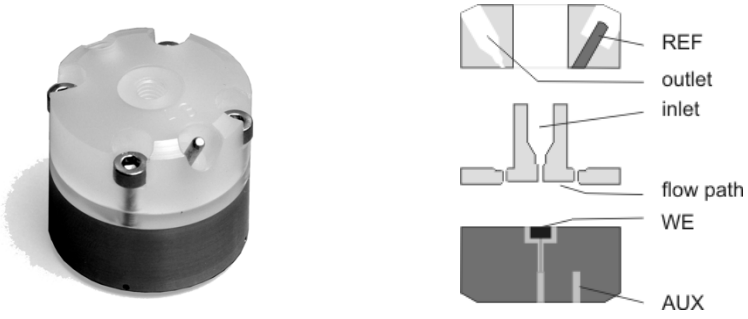


Fig. 1. Sencell. The upper part, the REF block, is separated from the working electrode block by means of an inlet connector with integrated flow path.

Electrochemical detection

Electrochemical detection is based on oxidation or reduction of a substance on the working electrode surface. The reactive substances move from the bulk solution to the stagnant layer on the working electrode. At the surface the electron transfer reaction takes place after which the reaction products move away to the bulk.

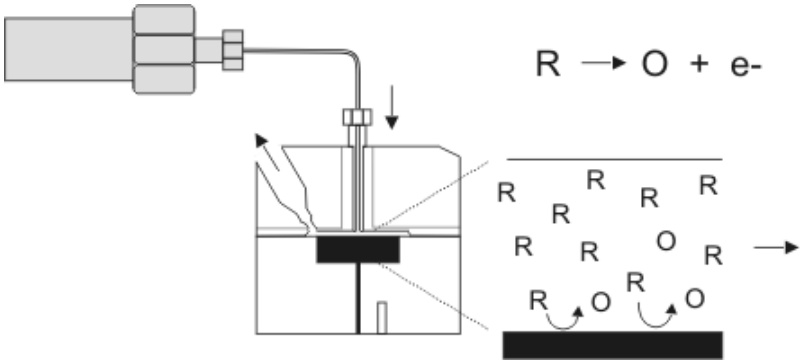


Fig. 2. Principle of electrochemical detection.

The electrons are transferred and measured by a very sensitive electronic circuit called the I/E converter (Fig. 3). This circuit is capable of amplifying very small electric currents to a measurable signal.

Three-electrode configuration

In the Sencell a three-electrode configuration is used (Fig. 3). The working potential is set between the working electrode (WE) and the auxiliary electrode (AUX). The AUX is kept at a precisely defined reference electrode (REF) potential by means of the so-called voltage clamp. This is an electronic feed back circuit that compensates for polarisation effects at the electrodes.
At the WE, which is kept at virtual ground, the electrochemical reaction takes place, i.e. electrons are transferred at the WE. This results in an electrical current to the I/E converter, which is a special type of operational amplifier. The output voltage can be measured by an integrator or recorder.

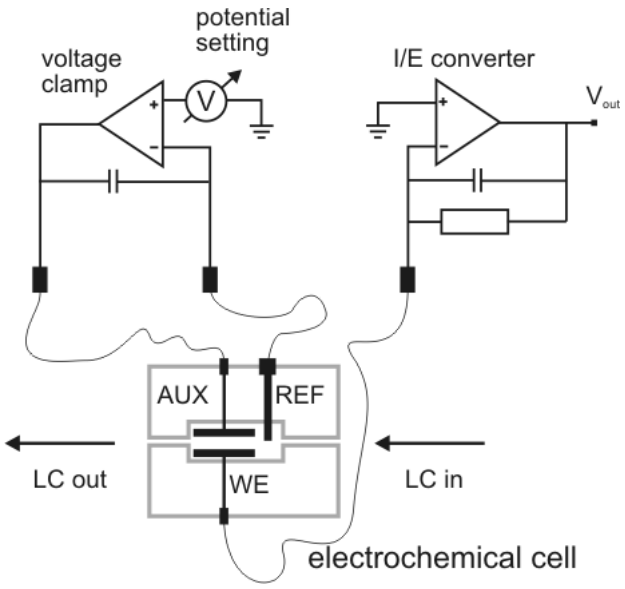


Fig. 3. Schematic representation of an electrochemical cell with a three-electrode configuration.

Essentially, for the oxidation or reduction reaction it would be sufficient to use only two electrodes. However, the three-electrode configuration has several advantages over a two-electrode configuration.
If the working potential would be applied only over an AUX versus the WE (without REF), the working potential would continuously change due to polarisation effects at the electrodes, resulting in highly unstable working conditions.
If the working potential would be applied only over the REF versus the WE (without AUX), the working potential would be very well defined. However, the potential of a REF is only well defined if the current drawn is extremely low (pico-amperes) resulting in a very limited dynamic range.
A three-electrode configuration, combines the best of both electrodes. The REF stabilises the working potential and the AUX can supply high currents. This results in the tremendous dynamic range of a three-electrode system.

Working electrode

Electrochemical detection puts high demands on the WE material. The WE should be made of a (electro-)chemically inert material. Furthermore, to avoid an irregular flow profile over the electrode, it should have a very well defined surface. Finally, it is important that the analyte of interest can be oxidised (or reduced) with favourable I/E characteristics. This in fact me-

ans that a high signal must be obtained at a low working potential. For most applications glassy carbon will be the WE material of choice.

Table I. Working potential limits and application area for different WE materials.

WE material	potential limits (V)				major application
	alkaline		acidic		
Glassy carbon	-1.50	+0.60	-0.80	+1.30	catecholamines
Gold	-1.25	+0.75	-0.35	+1.10	carbohydrates
Platinum	-0.90	+0.65	-0.20	+1.30	alcohols, glycols
Silver	-1.20	+0.10	-0.55	+0.40	halides, cyanide

Another consideration in choosing a WE is the oxidation or reduction of mobile phase constituents or WE material, that occurs when the potential exceeds the limits as given in Table I. At high positive working potentials the water in the mobile phase electrolyses and results in a strong increase of the background current and noise. Formation of metal oxides, resulting in an increase in background current is a limiting factor for metal electrodes. Glassy carbon and platinum have the highest positive potential limits and are therefore often used in oxidative ECD. For negative potentials the use of platinum electrodes is limited by the ease of reducing hydrogen ions to hydrogen gas.

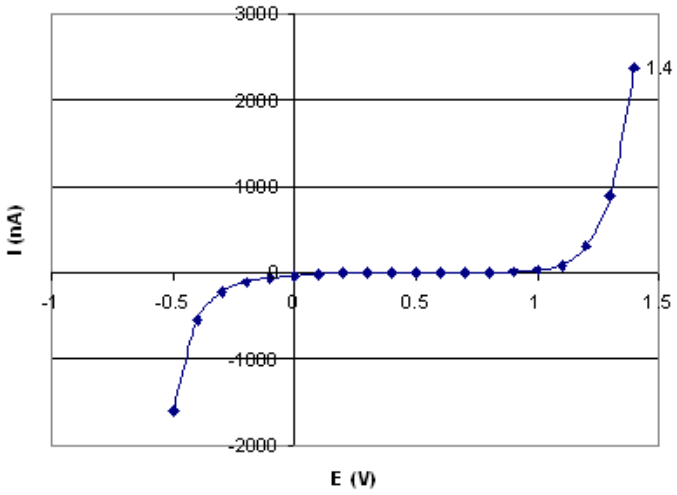


Fig. 4. Example of the working range of a flow cell with glassy carbon working electrode. The background current (I-cell) increases strongly above a working potential (E-cell) of 1.3 V and below -0.5 V.

Reference electrodes

The Sencell is available with an ISAAC (in situ Ag/AgCl) reference electrode and a HyREF reference electrode.

ISAAC reference electrode

The ISAAC reference electrode is in direct contact with the mobile phase which must contains chloride ions. The chloride concentration determines the potential, therefore each time a fresh mobile phase is prepared it should contain **exactly the same concentration** of chloride ions.
The standard electrode potential of the Ag/AgCl electrode (in 1.0 mol/L Cl^- solution) for the following half-reaction is defined as E^0:

$AgCl(s) + e^- <=> Ag(s) + Cl^-$ $\qquad E^0 = 0.222$ V

The potential of the REF is dependent from the chloride concentration as described by the following equation:

$$E_{cell} = E^0_{AgCl} - \frac{RT}{F} \ln [Cl^-]$$

where R is the gas constant (8.314 $Jmol^{-1}K^{-1}$), T is the absolute temperature (293 K) and F is the Faraday constant (96485 $Cmol^{-1}$).

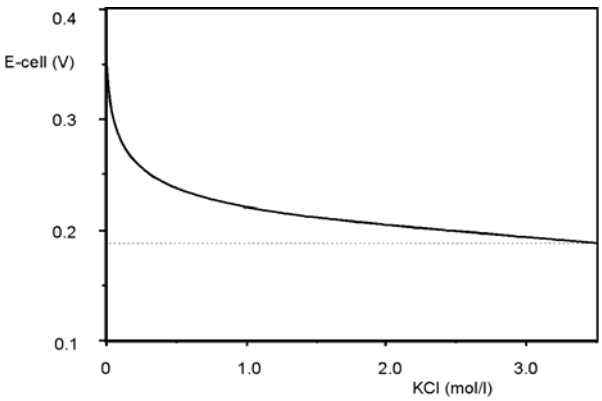


Fig. 5. Dependence of the Ag/AgCl REF potential on the chloride concentration.

The potential of the ISAAC at 2 mmol/L KCl is 379 mV. The potential difference (dE) between the saturated KCl Ag/AgCl reference electrode and the ISAAC is 189 mV. If an application is running at 800 mV (*vs.* Ag/AgCl

with sat'd KCl), the potential setting using the ISAAC should be 611 mV (*vs.* Ag/AgCl in 2 mmol/L KCl).

Table II. Potential of the Ag/AgCl reference electrode, dE is the potential difference with $E_{Ag/AgCl}$ in saturated KCl.

Cl^- (mmol/L)	E Ag/AgCl (mV)	dE (mV)
3500	190	0
2500	199	8
1500	212	21
500	240	49
100	280	90
20	321	130
10	338	148
8.0	344	154
6.0	351	161
4.0	361	171
2.0	**379**	**189**
1.0	396	206
0.5	414	224

In a few of situations the ISAAC cannot be used: in case of a silver working electrode, the addition of Cl^- to the mobile phase will cause formation of an AgCl coating on the working electrode leading to inactivation. Ammonium and sulphide react with silver forming a complex, if high concentrations of these ions are present in buffer or sample the ISAAC should not be used.
The addition of chloride to the mobile phase has a few restrictions. For example, the ISAAC is not recommended at a *high working potential* (> 1.2 V *vs.* Ag/AgCl in 2 mmol/L KCl) because Cl^- is oxidised and contributes to the background current. In *ion chromatography* the addition of Cl^- may lead to undesired chromatographic changes. At *high pH* or *high modifier concentrations* the ISAAC is less suitable and a HyREF is recommended.

HyREF reference electrode

The HyREF is a hydrogen reference electrode, its potential depends on the pH of the mobile phase. The HyREF is fully comparable with the standard Ag/AgCl REF as to baseline stability and S/N ratio. The HyREF is more user-friendly and in principle this REF is completely free of maintenance. Trapping of air bubbles like in the salt bridge Ag/AgCl type is impossible because of the absence of a salt bridge. Consequently, refilling the REF with saturated KCl is not longer required. Due to the absence of a

salt bridge and its inertness, the HyREF is an excellent alternative for the Ag/AgCl REF, especially in case of high modifier concentrations (i.e. analysis of fat-soluble vitamins) or high pH (analysis of carbohydrates, PAD). Depending on the pH of the mobile phase, the potential setting of the working electrode vs. the HyREF may differ significantly compared to Ag/AgCl.
I/E curves show a shift of more than 200 mV at pH 3.1 (e.g. catecholamines), no shift appears at pH 12 (e.g. PAD of carbohydrates). Therefore, it is advisable first to construct a hydrodynamic (or scanning) voltammogram when using the HyREF. In Table III the potential of the HyREF is measured against the Ag/AgCl (in sat'd KCl) electrode at different pH values.

Table III. Measured cell potential (HyREF - Ag/AgCl) versus pH.

pH	$E_{HyREF - Ag/AgCl}$ (mV)
3.3	232
6.2	130
7.5	90
11.8	0

So, if a salt bridge Ag/AgCl REF is replaced by a HyREF, the pH effect must be taken into account (Table III). The pH vs. voltage relation is described by:

$$E_{HyREF} = E_{Ag/AgCl} - 328 + 29.9\ pH \qquad (1)$$

For example: a working potential of 800 mV (*vs.* Ag/AgCl with sat'd KCl) at pH 3, has to be changed to: $E_{HyREF} = 800 - 328 + 29.9*3 = 561.7$ mV (*vs.* HyREF)

Detection limit

One of the most important parameters used to characterise the performance of a detection system is the signal-to-noise ratio (S/N ratio) from which the concentration detection limit is derived. It enables objective comparison not only between different electrochemical detectors but also between complete analytical methods irrespective what detection system is used.
In literature several ways are described to determined the detection limit. In principle, it does not matter which definition of detection limit is used, as long as the definition is precisely described.

Usually the concentration detection limit (c_{LOD}) for a certain compound is defined as the analyte concentration that results in a signal that is 3 times the peak-to-peak noise:

$C_{LOD} = [C_A . 3 . N_{pp}] / \text{signal}$

where N_{pp} is the peak-to-peak noise and C_A is the concentration of analyte injected.

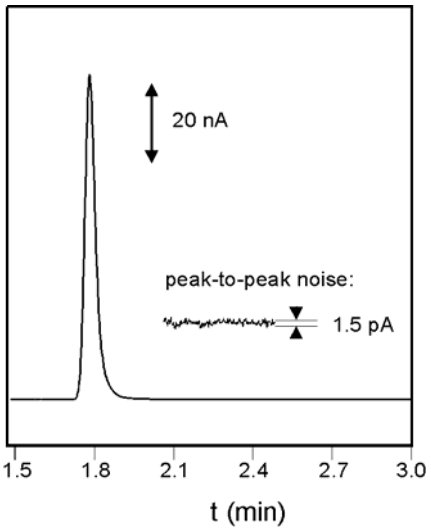


Fig. 6. Typical S/N ratio for norepinephrine measured with a glassy carbon flow cell (peak height: 80 nA, peak-to-peak noise: 1.5 pA). The amount injected is 20 pmol (1.0 µmol/L). The concentration detection limit based on three times the peak-to-peak noise is 55 pmol/L.

In Fig. 6 a typical S/N ratio of a glassy carbon flow cell with 2.74 mm WE is shown. In this example the concentration detection limit for norepinephrine based on three times the peak-to-peak noise is 55 pmol/L.
Expressing the performance of a detection system by only the peak height makes no sense. A system can easily be changed in a way that a larger peak height is obtained. However, if the noise increases similarly, it has the same effect as switching a recorder to a higher sensitivity: peaks appear higher but the S/N ratio is the same.
Expressing the limit of detection in an absolute amount (i.e. in picomoles) without mentioning the injection volume, makes a good comparison between different systems difficult.

Working electrode diameter

The size of the WE is an important factor in LC-EC, it affects both the signal and the noise. For the Sencell several *glassy carbon* WE diameters are available (0.5, 1, 2, 3 and 5 mm). In a *standard* LC system the signal and the noise increases linearly with the WE diameter. This means that he S/N ratio remains more or less the same. In case of *micro*-LC an increase of the WE diameter will increase the noise more than the signal. Therefore, in micro-LC a decrease of the WE diameter will result in a better S/N ratio.

Table IV. Sencell recommendations.

Diameter	(mm)				
Column ID	≥ 4.5	3	2	1	≤ 0.5
Sencell WE ID*	5	3	2	1	0.5

* WE diameters are rounded numbers. See Table V for the real numbers.

The choice for a flow cell is primarily based on the HPLC column diameter (Table IV). This way the best possible detection limit for a standard, microbore or capillary column is warranted.
The recommended combinations are giving the best S/N ratios. It should be kept in mind that other combinations are possible that still result in acceptable sensitivities for many applications. All Sencells are individually tested and meet our high standards of quality and detection sensitivity.

Thickness of flow path

The thickness of the flow path over the electrode affects the linear flow velocity in the cell. A thinner flow path results in a higher linear flow velocity. The signal increases while the noise remains more or less constant. Several authors have described the relation between the layer thickness (i.e. flow path thickness) in a thin layer flow cell and the measured current (S) as $S = k\, b^{-2/3}$ where b is the flow path thickness and k a constant. Also for the Sencell the relation between S and $b^{-2/3}$ results in a straight line.

Table V. Effective flow cell volume*

WE diameter (mm)	5.45	2.74	2.00	1.00	0.51
flow path (µm)	cell volume (µl)				
30.0	0.70	0.18	0.09	0.024	0.0061
60.0	1.40	0.35	0.19	0.047	0.0123
120.0	2.80	0.71	0.38	0.094	0.0245

*Effective cell volume is the WE surface area x the flow path thickness.

Decreasing the flow path thickness is limited by an increased pressure drop over the flow cell which eventually will lead to an obstruction of the flow. The minimum flow path thickness available is 30 µm. Applying these small flow paths should be done with care. Over-tightening of the bolts may cause an excessive pressure built up over the flow cell and increase the noise considerably.

C H A P T E R 2

Installation

Sencell with HyREF or ISAAC

The flow cell is assembled properly when it arrives. The force on the bolts is pre-set to 50 Ncm (“a little bit beyond fingertight”). Familiarise yourself with this force, since over-tightening of the bolts strongly deteriorates the S/N ratio and eventually the cell itself.

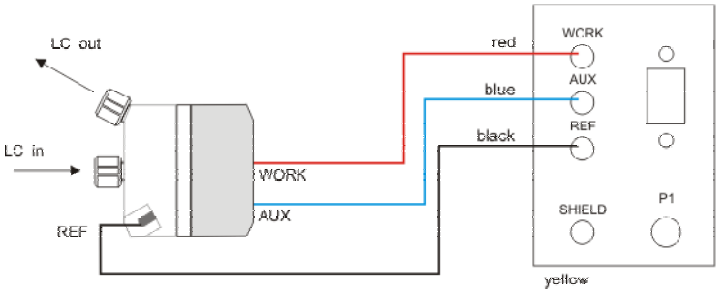


Fig. 7. Installation of flow cell (Intro or DECADE). WORK, AUX and REF are connected using the red, blue and black cell cable. LC out should be on top to prevent entrapment of bubbles.

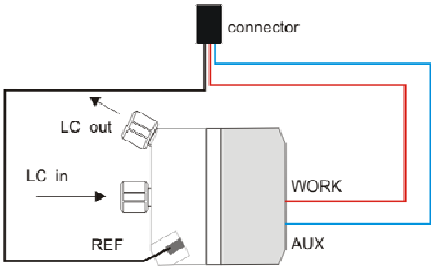


Fig. 8. Installation of flow cell (DECADE II). WORK, AUX and REF are connected using the red, blue and black cell cable. LC out should be on top to prevent entrapment of bubbles.

Avoid contact of the metal working electrode block with other metal parts because it will lead to high currents and instable conditions.

The ISAAC reference electrode requires 2 mmole/l chloride ions (KCl or NaCL) in the mobile phase. Add and equilibrate before installation of the ISAAC. See manual electrochemical detector for optimisation of working potential

1. Connect the column outlet to the flow cell inlet, using small-bore PEEK tubing (0.3 mm ID) and one of the fingertights supplied. **Use only our factory supplied fingertights in the flow cell, others may cause serious damage!** Tighten it such that the tubing is not or slightly indented by the fitting.
2. Do not over-tighten the fingertight. Over-tightening affects the flow pattern through the tubing (turbulence) and may strongly decrease the flow cell performance.
3. Connect 0.5 mm ID PEEK tubing to the outlet of the flow cell. Use only our factory supplied fingertights in the flow cell, others may cause serious damage! Again (see above), do not over-tighten the fingertight.
4. Turn on the HPLC pump. Keep some tissues at hand as you probably will spill some mobile phase during this mounting procedure.
5. Fill the flow cell, by keeping it in an angle of about 45° with the outlet (LC out) on top to force the air through the outlet.
6. Position the flow cell in its clamp in the controller with the REF at the lower side and the outlet at the upper side. This excludes trapping of air bubbles.
7. Connect the cell cable as illustrated in Fig. 7.

Never switch ON the flow cell when:
- the cell cable is not correctly connected
- the cell is only partly (or not at all) filled with buffer
- the outside of the flow cell is wet, particularly the part between the auxiliary and working electrode connection
because substantial damage to the working electrode or electronics may occur.

The maximum detection stability is attained when not only the flow cell, but also the HPLC column is incorporated in the controller. The controller has an integrated Faraday cage and an accurately thermostatted oven compartment which ensures stable working conditions. Installing the flow cell and column within such a controlled environment is the minimum requirement for high-quality LC-EC trace analyses.

Fig. 9. Installation of the Sencell in a DECADE II.

Avoid contact of the metal working electrode block with other metal parts because it will lead to high currents and instable conditions.

Sencell micro

The micro flow cell is assembled properly when it arrives. The force on the bolts is pre-set to 50 Ncm (“a little bit beyond fingertight”). Familiarise yourself with this force, since over-tightening of the bolts strongly deteriorates the S/N ratio and eventually the cell itself.

Columns with 1 mm ID: A 1 mm ID column can be used with narrow bore PEEK tubing. The micro flow cell can be installed in a similar way as described on page 13 (ISAAC REF).

Fused silica (FS) columns (< 1 mm ID): The micro flow cell has an inlet block that is specially designed for coupling with FS columns (Fig. 10). The capillary column can be inserted all the way to the working electrode resulting in zero dead volume.

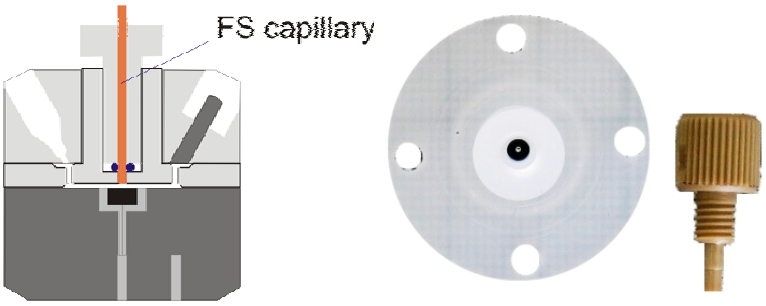


Fig. 10. Sencell micro flow cell. Right: the inlet (top view) and fingertight.

Before installing the fused silica column, make sure the column outlet is perfectly flat. If not, use the supplied polishing disc (Fig. 11) to polish the column outlet. Keep the pump running, to avoid particles from clogging the column outlet during the polishing procedure.

Polishing procedure FS column outlet

1. Take the green polishing disc and clean with demi water. The disc must be completely wet. No polishing slurry is used, as the disc already contains grinding particles!
2. Switch on the LC pump and make sure liquid comes out the FS column outlet. **Do not use corrosive or harmful solvents during polishing!** See column manufacturer recommendations.
3. Take the FS column and position it in the column holder block. This ensures the column is at right angles to the polishing disc. Carefully make circular movements until the outlet is perfectly flat. Use a magnifying glass or microscope to observe the result.
4. When done, clean and dry the disc and store free from dust and dirt. Switch your system to the required mobile phase.

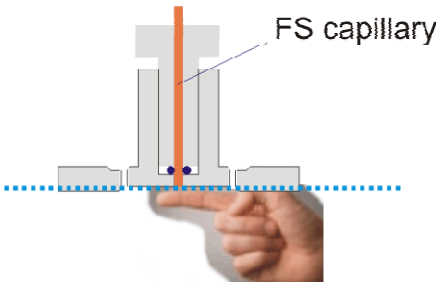
Fig. 11. Green polishing disc for FS column outlet.

Use proper eye and skin protection when working with solvents.

Installation of a FS column

To prevent any damage to the flow cell the following steps should be carried out (Fig. 10):

1. Insert the fused silica capillary into the inlet.
2. Mount this combination carefully in the injection block.
3. Let the fused silica slightly (0.5-1 mm) protrude through the injection hole.
4. Withdraw the FS capillary until the surface feels flat. Then carefully tighten the fitting. **Only small pressure is necessary as the inlet fitting is designed to have a very strong grip on the FS column!**



5. Mount the two flow cell blocks by crosswise tightening of the bolts (max. 50 Ncm).
6. Continue installation as described at point 3 on page 14 (ISAAC REF).

Never switch ON the flow cell when:
- the cell cable is not correctly connected
- the cell is only partly (or not at all) filled with buffer
- the outside of the flow cell is wet, particularly the part between the auxiliary and working electrode connection
because substantial damage to the working electrode or electronics may occur.

C H A P T E R 3

Maintenance

HyREF

The HyREF reference electrode is in principle maintenance free. If not in use it should be stored dry after disassembling the flow cell.

ISAAC

The ISAAC reference electrode requires maintenance, usually not more than once in 3 months. In practice this means that when the flow cell is opened to service the working electrode, the reference electrode should be serviced as well.

Fig. 12. Servicing the ISAAC REF, polishing (left) and coating (right).

Servicing the reference electrode is done by polishing the reference electrode surface until the shining metal appears (Fig. 12 *left*). **Immediately after polishing** the electrode is coated by applying a few drops of the reference electrode solution on the reference surface (Fig. 12 *right*). After 20 min the reference solution is flushed away with distilled water.
If not in use for longer period of time, disassemble the flow cell. The flow cell including the reference electrode should be cleaned with distilled water, dried with a tissue and stored dry.

Polishing

Polishing the reference electrode is done using the factory supplied polishing kit, containing diamond slurry and polishing disc.

1. Shake diamond slurry thoroughly before use!!
2. Rinse the polishing disc with demi water before applying the diamond slurry!

3. Apply a few drops of slurry on the wetted polishing disc, and polish the electrode with a ‘figure 8’ motion for about one minute. Apply only gentle pressure.
4. Clean the electrode with a wetted tissue and check the surface visually, repeat the procedure if necessary until the shining metal REF surface appears.
5. Clean the polishing disc with demi water.
6. Store the polishing disc dust free in its plastic bag.

Working electrode

Cleaning of the working electrode block is necessary if the electrode surface has been electrochemically changed. This may be due to fouling by oxidation (reduction) reaction products. Excessively high currents also may change the electrode surface. This is noticed by a strongly decreased sensitivity after prolonged use.
As a rule of thumb: only polish if the surface of the working electrode lacks its mirror-like finish, which cannot be restored by wiping the electrode surface with a tissue wetted with ethanol or acetone.

Decreased flow cell performance

Use proper eye and skin protection when working with solvents.

Several actions can be taken at decreased flow cell performance. Avoid unnecessary polishing, take the next step only if the previous was not successful.

1. Electrochemical cleaning of glassy carbon WE: In the pulse mode let the potential jump between +1 and -1 V for 10 min. Settings: t1 = 1000 ms, t2 = 1000 ms, t3 = 0 ms, E1 = +1V, E2 = -1V.
2. Wiping the electrode surface with a tissue wetted with ethanol or acetone
3. Polishing the electrode surface

Polishing

1. Shake diamond slurry thoroughly before use!!
2. Rinse the polishing disc with demi water before applying the diamond slurry!

3. Apply a small amount, **a few drops** is sufficient, of slurry on the wetted polishing disc, and polish the electrode with a ‘figure 8’ motion for about one minute. Apply only gentle pressure.
4. Clean the electrode with an ethanol-wetted tissue and check the surface visually; repeat the procedure if necessary.
5. Reassemble the detector cell.
6. Clean the polishing disc with demi water.
7. Store the polishing disc dust free in its plastic bag.

C H A P T E R 4

Index