

REFERENCE ELECTRODE RECOMMENDATIONS

THE SMARTEST LC-EC APPLICATIONS FOR NEUROSCIENCE ANALYSIS EVER MASTERMINDED

Monoamines and the metabolites

Noradrenalin

Dopamine

Serotonin

5-hydroxyindole acetic acid (5-HIAA)

*3,4-dihydroxyphenylacetic acid
(DOPAC)*

homovanillic acid (HVA)

OPA derivatized amines and amino acids

GABA and Glutamate

4-aminobutyrate (GABA)

Glutamate (Glu)

Choline and Acetylcholine

Choline (Ch)

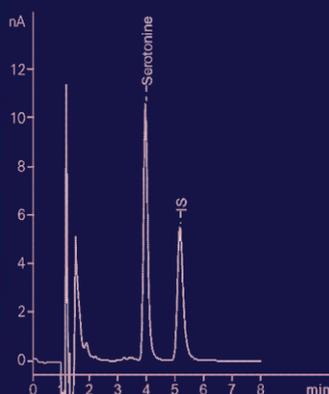
Acetylcholine (ACh)

Markers for oxidative stress

3-nitro-L-Tyrosine

8-OH-DPAT

Glutathione and other thiols



INTRODUCTION

A reference electrode (REF) is an essential but in many cases poorly understood element of an electrochemical flow cell. To meet the great variety in application needs, Antec Leyden supplies 3 different REFs for her flow cells. This document provides you with the guidelines how to select the most suitable REF for a specific application.

- Salt bridge ref – traditional Ag/AgCl
- ISAAC – in situ, for operational convenience
- HyREF – inert, maintenance free, for extreme conditions

Summary

Different type of reference electrodes are described, and recommendations for use for specific applications.

In an electrochemical cell with a three electrode configuration the REF warrants a stable, well-defined and reproducible working potential. It is used as a reference point against which the potential of the working electrode (measuring electrode) can be set.



Fig. 1. Flow cell with salt bridge, HyREF and ISAAC REF (from left to right).

In an electrochemical flow cell the actual cell potential is the sum of:

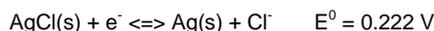
$$E_{\text{actual}} = E_{\text{REF}} + E_{\text{cell}}$$

where E_{cell} is the potential that has been set with the controller and E_{actual} is the actual cell potential.

In other words, if for some reason a REF with another potential is applied the cell potential has to change to get the same actual cell potential.

Ag/AgCl type

The standard electrode potential of the Ag/AgCl electrode (in a 1.0 M Cl⁻ solution at 25 °C) for the following half-reaction is defined as E⁰:



The chloride dependency of this REF is described by the following equation:

$$E_{\text{REF}} = E^0_{\text{AgCl}} - RT/F \ln [\text{Cl}^-]$$

where R is the gas constant (8.314 Jmol⁻¹K⁻¹), T is the temperature and F is the Faraday constant (96485 Cmol⁻¹). Note that if the temperature is raised, the solubility of KCl will increase, resulting in a slight decrease of the REF potential.

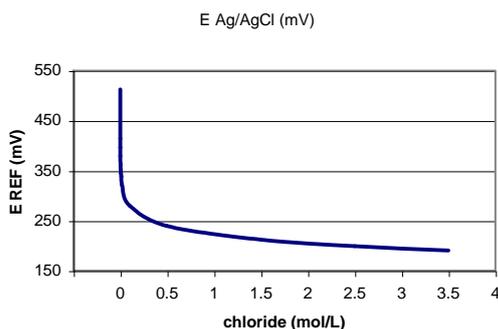


Fig. 2. Dependence of the Ag/AgCl REF potential on the chloride concentration.

ISAAC REF

'We consider the ISAAC REF as the prime choice in combination with our flow cells'

Characteristics

- Very easy to use
- Well-defined and reproducible

ISAAC (*in situ* Ag/AgCl) is integrated in the flow cell inlet block (see Fig. 1). The electrode is in direct contact with the mobile phase. For a constant and well-defined electrode potential the ISAAC requires 2 mM chloride in the mobile phase. If samples with high chloride concentration are analysed (such as microdialysates) 8 mM chloride is recommended. This will counteract a possible chloride effect resulting in REF potential fluctuation in the unretained chloride peak of a chromatogram.

Limitations

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.

Other situations require consideration before using an ISAAC. In ion chromatography the addition of Cl⁻ may lead to undesired chromatographic changes. At a high working potential (> 1.2 V) Cl⁻ is oxidised and contributes to the background current. Under these circumstances another REF is advisable.

Salt bridge REF

'The Antec Leyden salt bridge REF is the most rugged Ag/AgCl electrode on the market'

Characteristics

- Extreme longevity
- 'Standard' in literature

The Antec Leyden salt bridge REF consists of a silver rod, coated with solid AgCl, in a container of (over)saturated KCl. Electrical contact with the other electrodes in the electrochemical cell is made through a wetted cotton wool frit, which has a very low electrical resistance and slows down leakage of KCl. Both factors are responsible for the unsurpassed quality compared to other salt bridge designs.

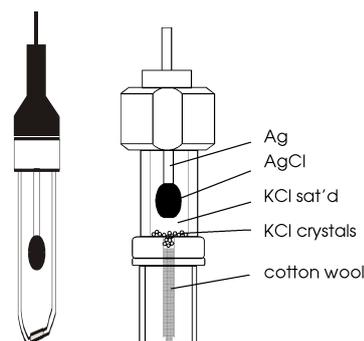


Fig. 3. Schematic representation of the salt bridge Ag/AgCl REF of FLEXCELL (left) and VT-03 (right).

To warrant the potential stability it is important to keep the salt bridge saturated. Due to the inevitable leakage over the cotton frit the reference potential will go up when the salt bridge is not saturated any more (Fig. 1). In the FLEXCELL the salt bridge REF is equipped with a ceramic frit.

Limitations

In case of perchlorate containing mobile phase NaCl must be used, because potassium perchlorate precipitates and will clog the salt bridge frit. At high modifier percentages (>70%) the salt bridge must be filled with LiCl for similar reasons. Air bubbles in the salt bridge destabilise the REF and may result in increased baseline noise. In case of alkaline conditions (pH >11) the salt bridge REF is not recommended.

Hy-REF

'The Hy-REF is tailor-made for extreme LC-EC conditions'

Characteristics

- Very easy to use
- Maintenance free
- Compatible with extreme solvents

The Hy-REF is fully comparable with the Ag/AgCl types as to baseline stability and S/N ratio. It is user-friendly and in principle completely free of maintenance. Also due to its inertness, the Hy-REF is the prime choice in case of high modifier concentrations (i.e. analysis of fat-soluble vitamins), organic mobile phases (straight phase chromatography) or high pH (analysis of carbohydrates by PAD).

Its potential is dependent on the pH of the mobile phase, so the potential setting of the working electrode vs. the Hy-REF may differ significantly from an Ag/AgCl type.

The empirically found relation between a Hy-REF and a salt bridge Ag/AgCl REF is described by:

$$E_{\text{Hy-REF}} = E_{\text{Ag/AgCl sat'd}} - 328 + 29.9 \text{ pH}$$

For example: a working potential of 800 mV (vs. Ag/AgCl with sat'd KCl) at pH 3, must be changed to about 560 mV:

$$E_{\text{Hy-REF}} = 800 - 328 + 29.9 \cdot 3 = 561.7 \text{ mV}$$

It is advisable first to construct a hydrodynamic (or scanning) voltammogram when using the Hy-REF. In Fig. 4 the potential of the Hy-REF is measured against the salt bridge Ag/AgCl electrode at different pH values.

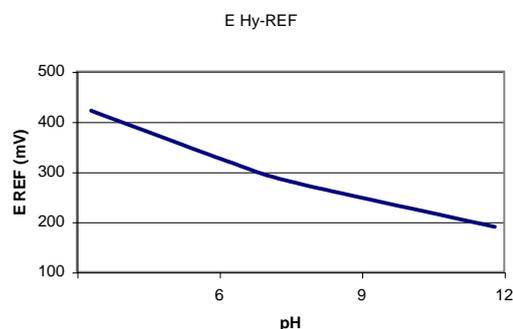


Fig. 4. Dependence of the Hy-REF potential on the hydrogen concentration (pH).

CONCLUSION

For the majority of applications the ISAAC is to be preferred because of its stability and ease of use, the salt bridge Ag/AgCl in cases where the ISAAC cannot be used and last but not least the Hy-REF if the conditions require a specific ruggedness.

References

1. Reference electrodes, theory and practice, D.J.G. Ives and G.J. Janz (Ed), Academic Press, NY 1961

