Chapter 6

Practical aspects of electrochemistry on-line with ESI/MS

6.1 Introduction

In 1994, Zhou and Van Berkel connected a number of different electrochemical cells on-line with electrospray ionization mass spectrometry (ESI/MS). Since then, the number of publications in the field of EC/ESI/MS has been steadily increasing. The ease of use and the many advantages that can be gained from adding an electrochemical cell in front of the electrospray ionization mass spectrometer (ESI/MS) will most certainly lead to an extended use in the future. However, the coupling of electrochemistry on-line with ESI/MS is a fairly new technique and many practical aspects are yet to be explored. In order to make the two techniques compatible, there are a number of things that have to be considered, such as currents between the electrochemical cell and the electrospray needle, signal suppression from the supporting electrolyte and retention of the analytes in the electrochemical flow cell. The aim of this chapter is to provide some solutions to the most common problems that can be encountered in an EC/ESI/MS system.

6.2 Currents between the electrochemical cell and the electrospray needle

An electrochemical cell in an on-line setup close to the electrospray needle will suffer from disturbing currents originating from the applied electrospray potential. A high positive or negative potential is applied at the electrospray needle, and a current will inevitably be flowing between the tip of the electrospray needle and the electrochemical cell if it is kept at ground potential. A number of ways to circumvent this problem were described by Zhou and Van Berkel as they presented their on-line electrochemical cells and will not be discussed in detail here. One setup recommend by Zhou and Van Berkel is to float the electrochemical cell at the same potential as the potential as applied at the electrospray needle. They also described how the introduction of a metal connector at ground between the electrochemical cell and the electrospray needle can solve the problem of interfering currents.
Most efforts to combine electrochemistry with mass spectrometry have been aimed at characterization of short–lived intermediates in electrochemical reactions or to make ions of compounds with low polarity for signal enhancement in electrospray mass spectrometry. In our experiments, we were primarily interested in the end–products of the electrochemical oxidations and were therefore not dependent of a short response time between the electrochemical cell and the ESI/MS. Due to the relatively large distance between the electrochemical cell and the electrospray interface, currents between the electrospray needle and the electrochemical cell did not turn out to be a major problem in our EC/ESI/MS–setup and the electrochemical cell was connected on–line with the ESI/MS without further modifications. Before we came to this conclusion, we performed a number of experiments with the electrospray needle kept at ground potential in order to avoid currents interfering with the electrochemical reaction. A description of the setup is presented in Figure 6.1.

Figure 6.1 Experimental setup for EC/ESI/MS with the electrospray needle at ground. The ring around the electrospray needle represents a large stainless steel nut.

A potential of $-3.5 \text{ kV}$ was applied at the nut and the electrospray needle was connected to ground. Positive ions in the solution will be drawn to the surface of the droplet in the same way as when the needle is kept at a high positive voltage with a surrounding at ground potential. When the positive ions finally end up in the gas phase, they will be drawn towards the negatively charged nut because of the direction of the electric field. However, by applying a high enough flow of nebulizing gas, a sufficient amount of positive ions will be physically pushed against the electric field and end up at the front of the mass spectrometer where they are "sucked in" by the next electric field. Although a part of the ions end up on the nut, the amount of ions arriving at the detector was about 60 % of what was observed in normal electrospray mode. When the mass spectrometer was operated in the negative mode, a potential of $+3.5 \text{ kV}$ was applied to the nut. In this type of setup, the outlet from the electrochemical cell can be directly connected to the electrospray emitter.
and the delay time between the electrochemical reaction and the detection can be minimized.

### 6.3 Signal suppression from the supporting electrolyte

For most organic solvents used in electrochemistry, the addition of supporting electrolytes is necessary for any electrochemical reaction to occur. Aqueous solutions of organic molecules have a moderate conductivity and some electrochemical reactions will take place even without addition of supporting electrolytes. However, in order to achieve a good electrochemical conversion in the electrochemical cell it is essential to add some kind of supporting electrolyte to increase the conductivity.

When choosing the suitable electrolyte for an electrochemical reaction it is of great importance to consider what pH is most favorable for the desired electrochemical reaction. Changing the pH of the solution can completely alter the outcome of the electrochemical reaction. Most tertiary amines are for example not oxidized at all in 0.1 M acetic acid, while they are easily oxidized in neutral or basic media as illustrated for tributylamine in Figure 6.2.
The problem when it comes to coupling electrochemistry on–line with ESI/MS is that most traditional supporting electrolytes for electrochemistry are not suitable for electrospray mass spectrometry. Some examples of common supporting electrolytes are silver perchlorate, tetraalkylammonium perchlorates, tetraalkylammonium p–toluenesulfonates, phosphate buffers and borate buffers. All of these supporting electrolytes will give rise to strong background peaks in the mass spectra or lead to crystallization on the electrospray needle or at the curtain plate. In order to avoid these types of problems it is often necessary to consider other supporting electrolytes than the salts traditionally used.

As a general rule, it is always recommended to start with a supporting electrolyte that is also suitable for the ESI/MS. For aqueous solutions, we recommend either ammonium acetate or ammonium formate. For lower pH:s, diluted acetic acid or formic acid can be used alone or added to the ammonium acetate/formate solutions. Ammonia can be added for electrochemical reactions requiring higher pH:s. The disadvantage of electrolytes based on ammonium acetate/formate is that ammonia will sometimes react as a nucleophile with the electrochemically generated intermediates. If such reactions occur, a different supporting electrolyte has to be chosen. We have testes a number of different salts as supporting electrolytes for the electrochemical oxidation of lidocaine (see chapter 2). The results are presented in Table 6.1.
### Table 6.1 Effects of different electrolytes on the oxidation of lidocaine.

<table>
<thead>
<tr>
<th>Salt</th>
<th>Lidocaine onset of oxidation (mV)</th>
<th>Background noise (pos. ions)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ammonium acetate</td>
<td>230</td>
<td>low</td>
<td>100 % conversion. pH may be modified with acetic acid and ammonia.</td>
</tr>
<tr>
<td>LiCF₃SO₃</td>
<td>320</td>
<td>very high</td>
<td>100 % conversion. pH may be modified with LiOH and HCF₃SO₃.</td>
</tr>
<tr>
<td>lithium chloride</td>
<td>350</td>
<td>medium</td>
<td>100 % conversion. pH may be modified with LiOH and HCl.</td>
</tr>
<tr>
<td>lithium fluoride</td>
<td>470</td>
<td>medium</td>
<td>100 % conversion. pH may be modified with LiOH and HF.</td>
</tr>
<tr>
<td>lithium formate</td>
<td>260</td>
<td>high</td>
<td>100 % conversion. pH may be modified with LiOH and HCOOH.</td>
</tr>
<tr>
<td>lithium perchlorate</td>
<td>320</td>
<td>high</td>
<td>100 % conversion. pH may be modified with LiOH and HClO₄.</td>
</tr>
<tr>
<td>sodium chloride</td>
<td>320</td>
<td>high</td>
<td>100 % conversion. pH may be modified with NaOH and HCl. Crystallizes on the curtain plate.</td>
</tr>
<tr>
<td>sodium formate</td>
<td>350</td>
<td>very high</td>
<td>100 % conversion. pH may be modified with NaOH and HCOOH.</td>
</tr>
<tr>
<td>sodium dihydrogen citrate</td>
<td>410</td>
<td>very high</td>
<td>100 % conversion. pH may be modified with NaOH and citric acid.</td>
</tr>
</tbody>
</table>

All salts were 10 mM in 80 % water and 20 % acetonitrile. 10 µM lidocaine was used to test the conversion and ion suppression. The flow rate was kept at 25 µl/min. Lithium forms clusters with acetonitrile. Otherwise rather clean spectra. pH modified to 7 with LiOH and formic acid. Sodium forms clusters with acetonitrile. Otherwise rather clean spectra. The background noise in the negative mode is also very high. The experiments were performed with the dissolved salts only and pH was not adjusted.

Lidocaine was oxidized to 100 % in the presence of all supporting electrolytes tested, but the background noise and signal suppression in the positive mode was higher than for ammonium acetate for all salts in Table 6.1. Some negative ions, such as trifluoromethane sulfonate, perchlorate and dihydrogencitrate also give rise to high background noise and
signal suppression in the negative mode. The background noise in the positive mode comes from positively charged clusters of neutral molecules with Li\(^+\) or Na\(^+\). If acetonitrile is added as organic modifier to the aqueous solution, clusters of acetonitrile and Li\(^+\) or Na\(^+\) will give an increased background noise when the mass spectrometer is operated in positive mode. The cluster formation is generally more pronounced with sodium than with lithium. The use of a different organic modifier, such as methanol, might decrease these effects. It should be noted that methanol, like ammonia, can react as a nucleophile with electrochemically generated intermediates.

All of the salts presented in Table 6.1 can thus be used as supporting electrolytes. If the compound under investigation is readily oxidized in ammonium acetate/formate but undesired reactions occur with ammonia, the best choice is generally to use a combination of acetic acid/formic acid and sodium or lithium acetate/formate. For electrochemical conversions requiring high pH, sodium or lithium hydroxide can be added. If acetonitrile is added as organic modifier, it is preferable to use lithium acetate/formate because in general the cluster formation with acetonitrile is less pronounced for lithium than sodium.

In our experiments, we always worked with water based solutions. For certain electrochemical reactions, the presence of water is undesirable and dry organic solvents are preferred. Zhou and Van Berkel suggested lithiumtrifluorosulphonate as a suitable electrolyte for organic solvents like acetonitrile or dichloromethane.\(^2\)

In conclusion, the choice of supporting electrolyte will always be a trade–off between good electrochemical conversion and good conditions for the ESI/MS. If the oxidized/reduced mixture is separated on an HPLC–column, most salts will elute within the unretained front and get separated from the reaction products. Therefore, the use of HPLC–separation between the electrochemical cell and the ESI/MS allows the use of a broader range of supporting electrolytes.

### 6.4 Retention in the electrochemical flow cell

Many species dissolved in solution have a tendency to adsorb on the electrode surface. In an electrochemical flow cell this will lead to retention of the analyte and thus a delayed arrival at the mass spectrometer. The adsorption is dependent on the type of compound investigated, the solvent composition, the electrode material and the potential applied to the working electrode. Due to electrostatic interactions, negatively charged species will adsorb at positive potentials while positively charged species will adsorb on the electrode at negative potentials. The pre–concentration of tamoxifen described by Pretty, Deng, Goeringer and Van Berkel relies on the fact that positively charge ions are accumulated on the working electrode at a negative potential and then rapidly released as a more positive potential is applied.\(^3\)
In aqueous solution, neutral organic molecules interact with the electrode primarily because of hydrophobic interactions and adsorb to the greatest extent on the uncharged working electrode.\textsuperscript{4,5} This phenomenon is particularly pronounced for organic compounds with non–polar groups and thus with low solubility in water. As a positive or negative potential is applied, the dipole charges of the surrounding water molecules are attracted towards the electrode and displace the neutral organic molecules on the surface. In pure aqueous solution, adsorption on the working electrode can be a great cause of retention in an electrochemical flow cell. The addition of an organic modifier, such as acetonitrile, to the solution makes the neutral molecules less prone towards adsorption and will often solve the problem.

### 6.5 The electrochemical cell

Several home–made electrochemical cells have been presented in the literature over the last decade. They have all been constructed to fit the purpose of each investigation and have indeed served their purpose at the time. We have also experimented with some different cell design, but have come to the conclusion that the flow–through cells provided by ESA are best suited for our applications.

![Diagram of the ESA Coulochem cell 5011](image)

**Figure 6.3** The ESA Coulochem cell 5011\textsuperscript{6}

The ESA Coulochem cell 5011, presented in Figure 6.3, was used for the majority of our experiments. It contains two flow–through porous graphite working electrodes connected in series, each with independent palladium reference and counter electrodes. In our experiments, we connected the cell to a home made potentiostat and used both working electrodes for oxidation. The large surface area allows a high electrochemical conversion at moderate flow rates (<100 µl/min). At higher flow rates, the conversion efficiency goes down. It should be noted that our electrochemical cells had all been used extensively in
analytical applications before we received them and that the conversion in a new cell might be good also at higher flow rates. The ESA Coulochem 5020 guard cell was used for the EC–Fenton experiments (chapter 3). It is constructed in the same way as the 5011 cell but contains only one large porous working electrode and is constructed to withstand high pressure. This can come in handy for certain applications since it can be connected prior to an HPLC column. The ESA Coulochem cell 5011 is not constructed to withstand high pressure and can only be used post–column or in a setup like the one described in chapter 4, where the electrochemical products are collected in an injection loop at low pressure.

In conclusion, the most important consideration for setting up an EC/ESI/MS system is a definition of the purpose of the system. If the main aim of the setup is to detect short–lived electrochemical intermediates, the delay time between the EC cell and the ESI/MS has to be minimized. It will then be of great importance to set up the system in such a way that currents between the electrochemical cell and the electrospray needle can be avoided. If, on the other hand, the main aim is to characterize the end–products of an electrochemical reaction, a larger distance can be allowed between the electrochemical cell and the ESI/MS. The installation of an HPLC column between the electrochemical cell and the ESI/MS allows the use of a broad range of supporting electrolytes.

6.6 References