

A Hyphenation of Stripping Voltammetry with Electrospray Ionization Mass Spectrometry; an Effect of Sodium Perchlorate on Ferrocene Oxidation

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Dedicated to the memory of Dr. habil. Detlef Schröder – one of the tireless investigators and propagators of mass spectrometry

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The coupling of electrospray ionization mass spectrometry (ESI-MS) with an electrochemical (EC) method is a promising technique applicable in electrochemical reaction studies as well as in other analytical fields. The connection of two-electrodes flowing EC cell prior ESI-MS is provided and tested on ferrocene/ferricene system. The base electrolyte used (a mixture of NaClO₄ and HClO₄) causes particular oxidation of ferrocene to ferricene. Consequently, in ESI-MS spectrum are observed two charged ferricene/ferrocene species: protonated ferrocene (Cp₂Fe(II)H)⁺ and ferricene (Cp₂Fe(III))⁺. In the on-line connection of EC with ESI-MS the ferricene species is electrochemically reduced (in accumulation step of differential pulse anodic stripping voltammetry via insertion of negative potential) to ferrocene species, which is detected as (Cp₂Fe(II)H)⁺ using ESI-MS. The yield of electrochemical reduction of ferricene to ferrocene is almost 30 %, whereas the inserted potential is -1000 mV.

Keywords: ESI-MS; Anodic stripping voltammetry; Electrochemistry; Ferrocene; Ferricene; Base electrolyte

1. INTRODUCTION

Hyphenated techniques bring an additional value (information) to the research process. Very common hyphenation is the hyphenation of mass spectrometry (MS) techniques with other techniques,

e.g., hyphenation with various chromatographic separation techniques (gas chromatography, liquid chromatography, high performance liquid chromatography, etc.) [1,2]. On the other hand very common is the hyphenation of mentioned chromatographic separation techniques with electrochemical (EC) detection [3-5].

Several published papers have dealt with the on-line tandem connection of EC flow cells with MS. With a respect to a successful analyte analysis in such hyphenation, there exist two concepts: quantification of flow-electrolysis products using mass spectrometry [6-10] and data collection from two different detection methods in a flow system [11].

The first system based on hyphenation of EC and MS, which was utilized for determination of species generated during electrochemical reaction, was called DEMS (differential electrochemical mass spectrometry [12]). This technique was advantageous especially for determination of uncharged molecules [13,14]. The potential of such hyphenation in the context of analytical questions as well as reaction mechanisms derives from the possibility of measurements directly in the electrochemical cell [6,15]. However, the required presence of supporting electrolytes can cause interferences of the complexes of interest with more abundant ions stemming from the supporting electrolytes. This interference can be avoided using a volatile electrolyte, such as ammonium acetate or electrolytes, which do not form complexes overlapping with the m/z ratios of the metal complexes under study [8]. The problem with the limited efficiency of electrolysis could be solved by an increase of the working electrode surface or by lowering of the flow, but the lower flow is connected with smaller inner cell volume [16].

In order to gain complementary insight into the nature of the complexes formed, electrospray ionization mass spectrometry (ESI-MS) has been used to assess the types of metal complexes [17-22]. In many cases, ESI-MS data show good correlations with solution phase studies [23-25], and ESI-MS conditions are often considered to mimic biological environments [17-19,26-28]. The stoichiometry of the metal complexes can be derived from the MS spectra by the mass to charge ratios (m/z) in conjunction with the characteristic isotope patterns.

Hyphenation of two different analytical methods EC and ESI-MS seems to be very promising target at present [11,29-32]. It enables to enlarge the field of application of both, EC as well as ESI-MS techniques, simultaneously used e.g. to gain insight into electrochemical processes of metals with low molecular weight organic acids [15,33,34] or pesticides [20,35] in solutions. This hyphenation exhibits some general problems and limitations; the product vs. analyte ratio depends on the solution flow, its trajectory, the electrode surfaces [6,7,15,36], transport of isolated product to the mass spectrometric detector [15] as well as on concentration and solubility of a base electrolyte.

EC methods have been famous in the field of qualitative and quantitative analysis for more than ninety years [37]. They are highly suitable for trace analysis of plenty elements [38] and organic/inorganic compounds, mostly under physiological conditions (e.g., [39-41]). Moreover, the EC techniques are famous due to the possibility of speciation [42-45], particularly, direct current voltammetry (DCV) for assessment of stability constants [44,46].

ESI-MS technique was designed to detect “softly” produced intact ions of large and complex molecules [47]. The application of ESI-MS can be found in analytical chemistry [15] as well as in

physical chemistry [48]. The soft ionization of molecules allows to determine stoichiometry of molecules as well as their reactions and moreover to determine the stability of the molecules [49].

The hyphenation of EC with ESI-MS allows investigation of electrochemical properties of substances from the kinetic point of view. The field of application of such hyphenated techniques seems to be very wide, e.g., the generation and identification of reactive metabolites [50], investigation and generation of a wide variety of electrode reaction intermediates (e.g., [51,52]). To the most important field of on-line EC-ESI-MS application belongs the possibility to study the reactions *in situ*. Nevertheless, such systems are too complicated; therefore, the simpler redox system has to be chosen for the characterization of basic properties of the investigated system. We applied one electron redox system ferrocene ($\text{Cp}_2\text{Fe(II)}$) /ferricene ($\text{Cp}_2\text{Fe(III)}^+$) in this paper, because this system exhibits high electrochemical reversibility [53].

2. EXPERIMENTAL PART

2.1. Reagents and Materials

The sample solution was prepared from solid ferrocene (to 10^{-4} mol L⁻¹) and base electrolyte (BE) (NaClO_4 and HClO_4 [53]), in pure 99.98 % ethanol (all from Sigma-Aldrich, Czech Republic). The solvent of ferrocene was 99.98 % ethanol and the solvent of BE was pure water. The consequent solvent of all measured samples was 95 % ethanol. More diluted concentrations of BEs were prepared (NaClO_4 10^{-3} mol L⁻¹ and HClO_4 10^{-4} mol L⁻¹) to be suitable for ESI-MS [54]. Prior to the measurements the sample was degassed using N_2 and afterwards was injected continually (0.5 mL h⁻¹) from a Hamilton syringe (Thermo Fisher Scientific, USA) via syringe pump (KD Scientific, USA).

The preconcentration step (120 s long in our case) of anodic stripping voltammetry was used to show the impact of inserted accumulation potential (-1200 mV to -200 mV in 100 mV step, polarization rate 10 mV s⁻¹) to ferrocene speciation visible in MS spectra. The changes of ferrocene speciation during oxidation/reduction were recorded in MS spectra.

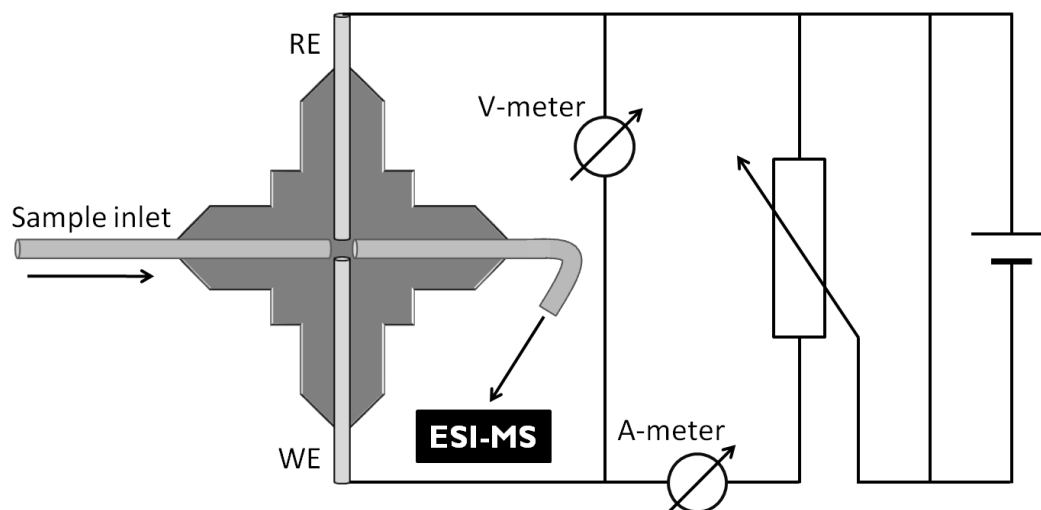
2.2. Apparatus

The ESI-MS experiments were performed with a Finnigan LCQ Advantage ion-trap mass spectrometer (ThermoFinnigan, USA) fitted with an electrospray ionization source operated in positive and negative-ion mode [54]. The sample solutions of ferrocene were introduced into the ESI source via a fused-silica capillary at a flow rate of 0.5 mL h⁻¹. Nitrogen was used as the nebulizer gas. The operating conditions were set as follows: spray voltage 5.0 kV, capillary voltage 0 V and tube lens offset 0 V for the most of measurements shown in this study, heated capillary temperature 250°C, sheath gas flow rate and auxiliary gas flow rate 10–50 arbitrary units. The ionization conditions in ESI are critically influenced by the settings of the capillary voltage and the tube lens offset.

The design of the cell (Scheme 1, [11]) (inspired by [54]) was provided regarding to sample flow rate suitable for ESI-MS (0.5 mL h⁻¹). The cell body was built from a peak cross (P-729 PEEK

Cross 0.020" thru-hole with F-300 Fittings; Upchurch Scientific Rheodyne, IDEX Health & Science, USA), with the sweep volume 0.72 μL . The sample inlet and outlet were via capillary PEEK Tubing 0.25 mm (IDEX Health & Science). The voltammetric cell works in two electrodes connection of computer controlled polarographic/voltammetric analyzer PC-ETP (Polaro-Sensors, Czech Republic) with Multielchem 2.1 software (J. Heyrovský Institute of Physical Chemistry of the AS CR, v.v.i., Czech Republic) [55] and POLAR.PRO software v. 5.1 (Polaro-Sensors, Czech Republic). Working electrode (WE) was a platinum wire and reference electrode (RE) was a silver wire (both 1 mm in diameter, Goodfellow Corp., USA).

It has to be noted that Ag electrode is not considered as ideal reference electrode because of its possible polarization during electrochemical reactions. However, the utilization of an electrode of the second kind in the EC cell is impossible from the construction point of view. Only the Ag/AgCl electrode can be installed into peak-cross holes. Nevertheless, the required presence of Cl^- ions is undesirable due to evocation of clustering during the electrospray process. Moreover, the utilization of Ag electrode as the "pseudoreference" electrode takes the polarizability of the Ag electrode advantageous to electrochemical "cleaning" of the Pt electrode via insertion of a potential of opposite value that was applied for desirable electrochemical reaction.



Scheme 1. Design of the voltammetric flow cell (WE – working electrode, RE – reference electrode). Adopted from [11]. This figure is reproduced with kind permission of the Best Servis, Czech Republic.

3. RESULTS AND DISCUSSION

3.1. Impact of capillary voltage and tube lens offset on signal intensity

The final signal of charged species is critically influenced by capillary voltage (CV) and tube lens offset (TLO). The values of CV might also significantly change the oxidation/reduction state of the species originated from electrochemical reduction occurred in EC cell during anodic stripping voltammetry (ASV) [11,54].

As ferrocene is considered as protonated Cp_2FeH^+ m/z 187 with Fe(II) and ferricene is considered as Cp_2Fe^+ m/z 186 with Fe(III) (Fig. 1); CV and TLO values were optimized to highest intensity of both $\text{Cp}_2\text{Fe(II)H}^+$ and $\text{Cp}_2\text{Fe(III)}^+$, respectively. Optimal values for $\text{Cp}_2\text{Fe(II)H}^+$ were lower (CV = 0 V, TLO = 20 V) than for $\text{Cp}_2\text{Fe(III)}^+$ (CV = 20 V, TLO = 40 V) in solution without BE (Fig. 2a). On the other hand, in solution with BE (10^{-3} mol L $^{-1}$ NaClO $_4$, 10^{-4} mol L $^{-1}$ HClO $_4$) were CV and TLO values similar for both (CV = 0 V, TLO = 20 V, Fig. 2b). To minimize the oxidative impact of positive voltage to the sample CV = 0 V and TLO = 0 V were chosen for coupling with ASV.

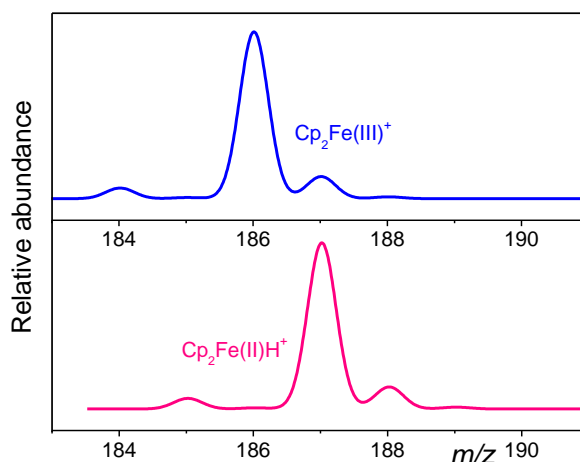


Figure 1. Simulation of isotope patterns and m/z values of ferricene ($\text{Cp}_2\text{Fe(III)}^+$, top) and protonated ferrocene ($\text{Cp}_2\text{Fe(II)H}^+$, bottom).

3.2. Impact of BE on abundance of $\text{Cp}_2\text{Fe(II)H}^+$ and $\text{Cp}_2\text{Fe(III)}^+$ species

The presence of BE in the sample might induce the creation of ferrocene species clusters with BE [11]. Also, the increase of solution conductivity can affect the electrospray ionization effect. Therefore, it was necessary to investigate impact of BE on ferrocene species. Regardless, BEs are considered as unaffected the electrochemical reaction.

In the solution without the BE (Fig. 2a) the intensity of $\text{Cp}_2\text{Fe(II)H}^+$ was more than one order of magnitude higher than the intensity of $\text{Cp}_2\text{Fe(III)}^+$, whereas the absolute intensity of $\text{Cp}_2\text{Fe(II)H}^+$ has not exceeded the value $1.5 \cdot 10^5$ and $\text{Cp}_2\text{Fe(III)}^+$ the value $8.0 \cdot 10^3$. Contrary to this, the ratio between $\text{Cp}_2\text{Fe(II)H}^+$ and $\text{Cp}_2\text{Fe(III)}^+$ is the opposite (the ferricene form $\text{Cp}_2\text{Fe(III)}^+$ dominates) in presence of the BE (Fig. 2b).

Moreover, the intensity of both $\text{Cp}_2\text{Fe(II)H}^+$ and $\text{Cp}_2\text{Fe(III)}^+$ increased in the presence of the BE; the intensity of $\text{Cp}_2\text{Fe(II)H}^+$ was approx. 10^5 and $\text{Cp}_2\text{Fe(III)}^+$ 10^6 . The increase of the signal intensity may be caused by the increase of the solution conductivity. However, the intensity of $\text{Cp}_2\text{Fe(II)H}^+$ was maintained on approximately the same value ($\sim 10^5$) in both solutions. The increase of total intensity was caused by the increase of $\text{Cp}_2\text{Fe(III)}^+$ intensity in solution without BE ($\sim 10^{3-4}$) to solution with BE ($\sim 10^6$). This result was probably caused by the oxidation of $\text{Cp}_2\text{Fe(II)}$ (inactive in ESI-MS) to ESI-MS active $\text{Cp}_2\text{Fe(III)}^+$. The oxidation agent in this case was BE anion (ClO_4^-), see further.

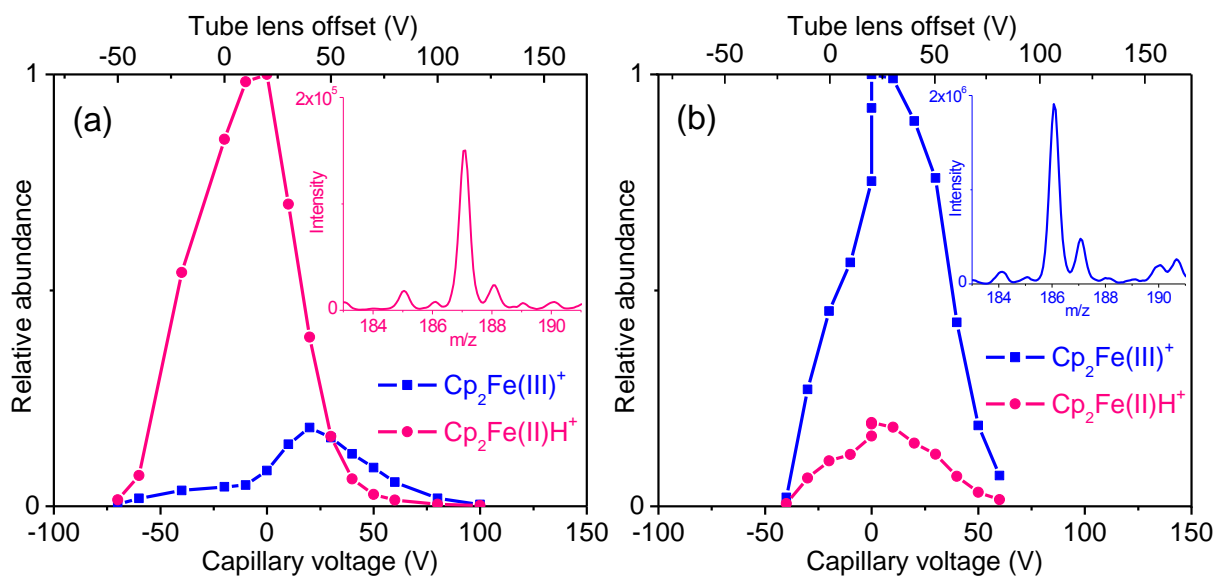


Figure 2. Dependence of relative abundance of Cp₂Fe(II)H⁺ (*m/z* 187) and Cp₂Fe(III)⁺ (*m/z* 186) on capillary voltage (CV) and tube lens offset (TLO). Mass spectra of CV = 0 V and TLO = 0 V is shown on expanded mass scale; 95 % ethanol, ferrocene 10⁻⁴ mol L⁻¹. (a) Solution without BE. (b) Solution with BE (10⁻³ mol L⁻¹ NaClO₄, 10⁻⁴ mol L⁻¹ HClO₄).

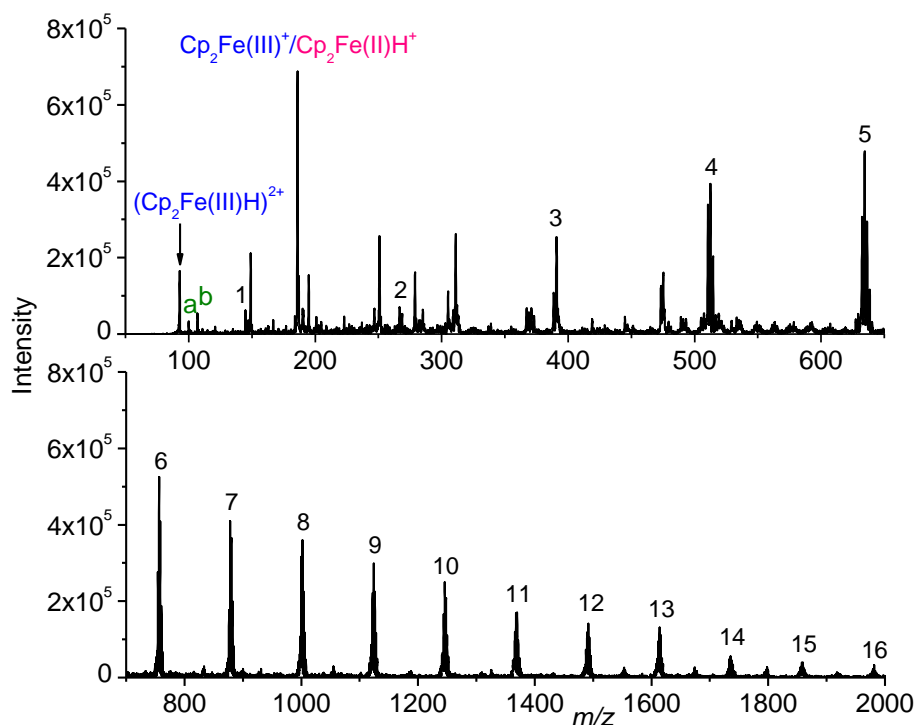
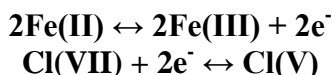


Figure 3. ESI-MS spectra of ferrocene (10⁻⁴ mol L⁻¹) in BE (NaClO₄ 10⁻³ mol L⁻¹, HClO₄ 10⁻⁴ mol L⁻¹) in off-line regime (without connection with EC). Numbers represent *x* in [Na(NaClO₄)_{*x*}]⁺, *a* stands for [H(NaClO₃)]⁺, and *b* stands for [Na(NaClO₃)]⁺

The oxidation mechanism is summarized in Scheme 2. Fe(II), while is oxidized, losses one electron. The reduction of Cl(VII)O₄⁻ to Cl(V)O₃⁻ requires to gain 2 electrons, that is why two Fe(II) are necessary.



Scheme 2. The scheme of $\text{Cp}_2\text{Fe(II)}$ oxidation to $\text{Cp}_2\text{Fe(III)}^+$ by ClO_4^- .

Ferricene creates two complexes $\text{Cp}_2\text{Fe(III)}^+$ (m/z 186) and $(\text{Cp}_2\text{Fe(III)H})^{2+}$ (m/z 93), ferrocene is represented as protonated $\text{Cp}_2\text{Fe(II)H}^+$ (m/z 187) in the spectra (Fig. 3). Other major patterns in the spectra mostly represent BE clusters $[\text{Na}(\text{NaClO}_4)_x]^+$, where x is 1 to 16 in the range m/z 50 to 2000. In the spectra are also present species containing NaClO_3 coming from ferrocene oxidation, $[\text{H}(\text{NaClO}_3)]^+$ and $[\text{Na}(\text{NaClO}_3)]^+$.

From previous discussion it is evident that the ratio $\text{Cp}_2\text{Fe(II)H}^+/\text{Cp}_2\text{Fe(III)}^+$ depends on the concentration of the BE (a mixture of NaClO_4 and HClO_4 , Fig. 4a). The intensity of $\text{Cp}_2\text{Fe(II)H}^+$ in solution without BE was almost 1/3 (31.3 %) of the intensity in solution with BE (the highest concentration). The shift in intensities is evident in the case of $\text{Cp}_2\text{Fe(III)}^+$, where the intensity without BE was less than 0.5 % (0.42 % exactly) of the intensity in presence of the BE (Fig. 4b). The shift in $\text{Cp}_2\text{Fe(II)H}^+/\text{Cp}_2\text{Fe(III)}^+$ ratio is caused mainly with the increase $\text{Cp}_2\text{Fe(III)}^+$ signal with BE addition.

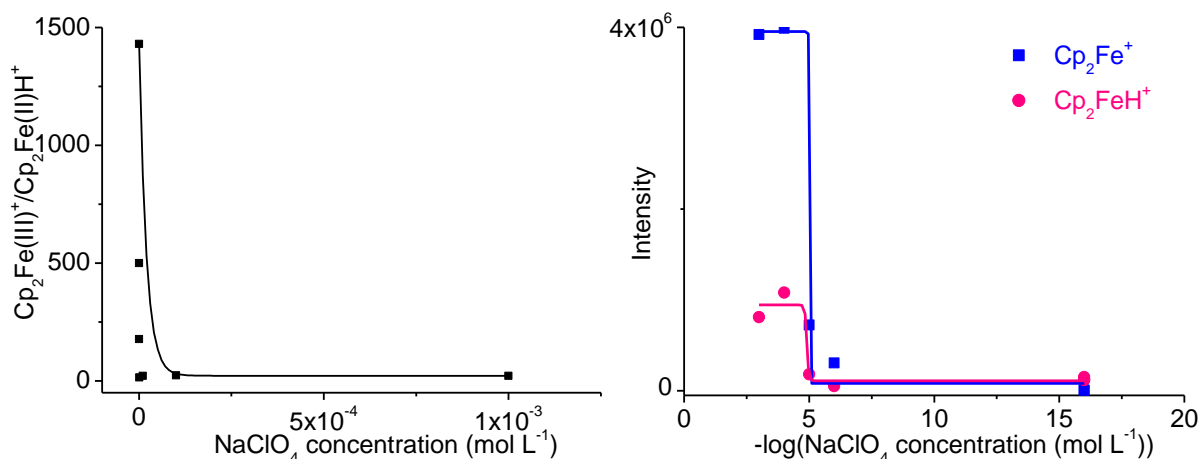


Figure 4. (a) Dependence (exponential decay) of $\text{Cp}_2\text{Fe(III)}^+/\text{Cp}_2\text{Fe(II)H}^+$ ratio on concentration of BE (NaClO_4 , mol L^{-1}). (b) Dependence (Boltzmann sigmoid function) of $\text{Cp}_2\text{Fe(III)}^+$ and $\text{Cp}_2\text{Fe(II)H}^+$ intensity on negative logarithm of NaClO_4 concentration (mol L^{-1}); 95% ethanol, ferrocene $10^{-4} \text{ mol L}^{-1}$, different concentration of BE.

3.3. The hyphenation – influence of inserted potential on $\text{Cp}_2\text{Fe(II)H}^+/\text{Cp}_2\text{Fe(III)}^+$ ratio

Final part of this chapter is devoted to the optimization of electrochemical set up of ASV, namely to the impact of inserted potential during the accumulation step. Moreover, any influence of the electrospray process (ESI) on electrochemical reactions occurred in electrochemical device has to be ruled out.

Consecutive cyclic voltammograms measured in flowing system (Fig. 5a) demonstrate good reproducibility of the measurements (according to the evaluation of the peak height at +430 mV) [11]. The oxidation peak of $\text{Cp}_2\text{Fe(II)}$ to $\text{Cp}_2\text{Fe(III)}^+$ is situated at potential +430 mV, the reduction peak is

not present, because in the flowing system no $\text{Cp}_2\text{Fe(III)}^+$ remains to be consequently reduced. In this point, it is important to be highlighted that the oxidation of $\text{Cp}_2\text{Fe(II)}$ to $\text{Cp}_2\text{Fe(III)}^+$ is caused by addition of the NaClO_4 (used as BE) only, however, the oxidation is enhanced under oxidative electro spray conditions. It means, the oxidation of $\text{Cp}_2\text{Fe(II)}$ to $\text{Cp}_2\text{Fe(III)}^+$ mediated by ClO_4^- is not significant under usual electrochemical conditions. Moreover, the oxidation of $\text{Cp}_2\text{Fe(II)}$ to $\text{Cp}_2\text{Fe(III)}^+$ is also not realizable in electro spray process without ClO_4^- mediation. It means, the enormous oxidation of $\text{Cp}_2\text{Fe(II)}$ to $\text{Cp}_2\text{Fe(III)}^+$ is occurred under electro spray conditions in presence of ClO_4^- only (Scheme 2, Fig. 4).

Differential pulse anodic stripping voltammetry (DPASV) was realized in different accumulation potentials (Fig. 5b) to see the impact of the value of inserted potential. Because oxidation of ferrocene is realized in one-step and on the anodic voltammograms two waves can be seen, it is possible to conclude that two different species are present (oxidized) or that only some part of ferrocene is adsorbed on the electrode surface [11]. The oxidation potential of ferrocene in the system Pt-Ag was in approx. +430 mV, the electrode surfaces were renewed by a reverse scan (not shown).

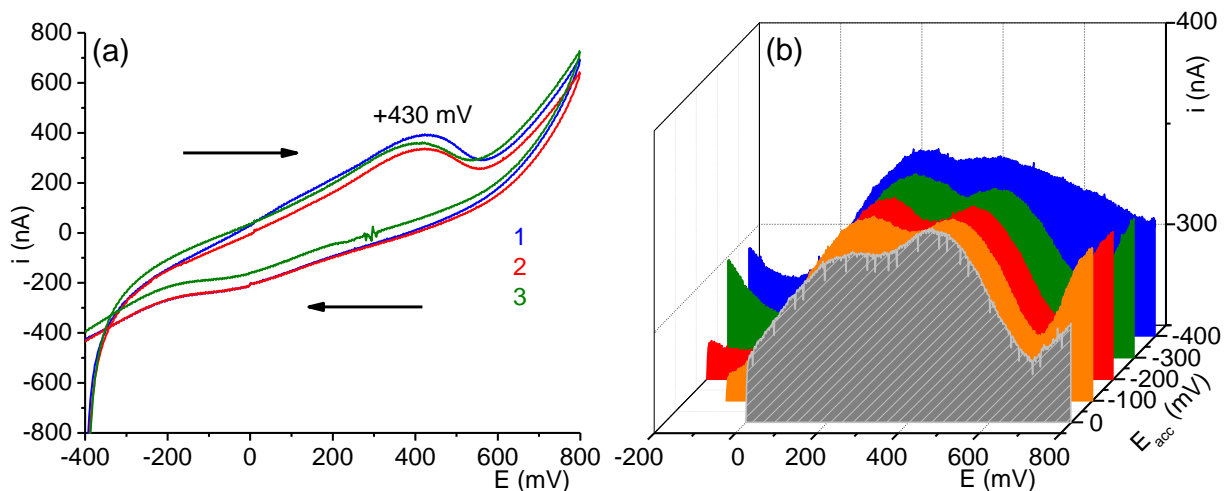


Figure 5. (a) Consecutive cyclic voltammogram of ferrocene in flowing system; flow rate 0.5 mL h^{-1} , scan rate 10 mV s^{-1} . (b) DPASV in different accumulation potentials, accumulation time 120 s, polarization rate 10 mV s^{-1} .

To see the effect of inserted potential using the EC cell working in ASV mode on ESI mass spectrum in off-line regime (the cell disconnected) were measured. While the EC cell was connected in front of the mass spectrometer (on-line regime), the working electrode (WE) may operate as anode (oxidation is occurred, more positive potentials than the standard potential are inserted) or as cathode (reduction is occurred, more negative potentials than the standard potential are inserted).

The efficiency of reduction in EC cell is expressed as percentage contribution of electrochemically reduced $\text{Cp}_2\text{Fe(II)H}^+$, which linearly depends on inserted accumulation potential (Fig. 6):

$$RED_{\text{Cp}_2\text{FeH}^+} = \frac{\text{Cp}_2\text{FeH}^+(\text{on-line}) - \text{Cp}_2\text{FeH}^+(\text{off-line})}{\text{Cp}_2\text{FeH}^+(\text{off-line})} \cdot 100,$$

where $RED_{Cp_2Fe(II)H^+}$ stands for the electrochemical contribution of $Cp_2Fe(II)H^+$ (%), Cp_2FeH^+ (*off-line*) is the intensity of $Cp_2Fe(II)H^+$ in mass spectra measured in off-line regime and Cp_2FeH^+ (*on-line*) is the intensity of $Cp_2Fe(II)H^+$ in mass spectra measured in on-line regime for different negative accumulation potentials. $RED_{Cp_2Fe(II)H^+}$ is exactly the yield of electrochemical reduction of $Cp_2Fe(III)^+$ to $Cp_2Fe(II)$. The yield linearly depends on inserted potential (Fig. 6). The maximum yield (almost 30 %) is reached for potential -1000 mV. The bars in the graph correspond to 3 standard deviations.

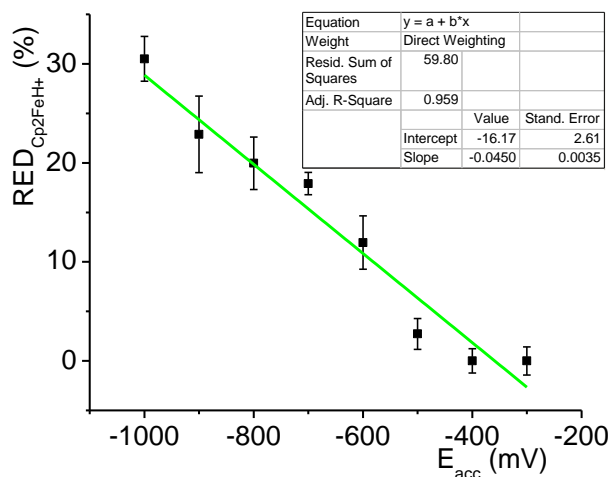


Figure 6. The influence of inserted potential on EC cell demonstrated as the efficiency of electrochemical reduction expressed as percentage amount of electrochemically reduced $Cp_2Fe(II)H^+$; 95% ethanol, ferrocene 10^{-4} mol L $^{-1}$, 10^{-3} mol L $^{-1}$ NaClO $_4$, 10^{-4} mol L $^{-1}$ HClO $_4$. The bars in the graph correspond to 3 standard deviations.

Fig. 7 shows the differences among off-line spectra and spectra operated in on-line regime, while oxidation/reduction is occurred. The impact of oxidation/reduction is evident despite of the combined pattern from $Cp_2Fe(II)H^+$ and $Cp_2Fe(III)^+$. The signal in m/z 186 belongs mostly to $Cp_2Fe(III)^+$, the main signal of $Cp_2Fe(II)H^+$ (m/z 187) has to be corrected from the contribution of $Cp_2Fe(III)^+$ with m/z 187, which amounts 13.2 % from the main signal of $Cp_2Fe(III)^+$. While in the solution was none $Cp_2Fe(II)H^+$, the height of the second peak of $Cp_2Fe(II)H^+$ would be 13.2 % of the highest one. In the mass spectra obtained in off-line regime, the second peak amounts to 19 % of the other one, it means the contribution of $Cp_2Fe(II)H^+$ to 187-peak intensity is 5.8 %. In oxidation regime the amount of $Cp_2Fe(II)H^+$ is lower comparing to off-line regime and on the other hand in reduction regime the amount of $Cp_2Fe(II)H^+$ increases due to foregoing electrochemical reduction of $Cp_2Fe(III)^+$. Additionally, the increase of the intensity of the peak (m/z 186) belonging to $Cp_2Fe(III)^+$ in oxidation regime comparing to off-line regime and more visible in reduction regime can be observed (Fig. 7).

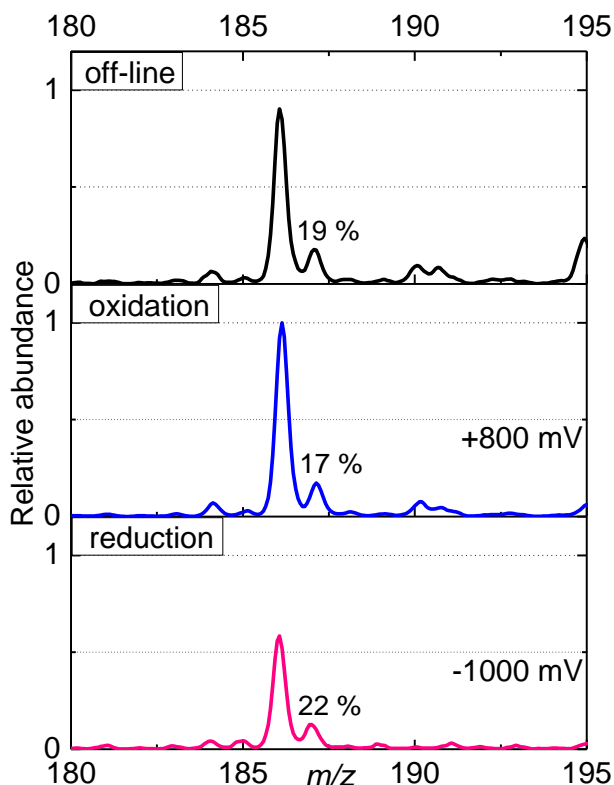


Figure 7. ESI-MS spectra of ferrocene (10^{-4} mol L $^{-1}$) in BE (NaClO_4 10^{-3} mol L $^{-1}$, HClO_4 10^{-4} mol L $^{-1}$) in off-line regime (without connection with EC), in oxidation regime (+800 mV was inserted on WE) and reduction regime (-1000 mV was inserted on WE). The amount of $\text{Cp}_2\text{Fe(II)H}^+$ comparing to $\text{Cp}_2\text{Fe(III)}^+$ is expressed in %.

4. CONCLUSIONS

The coupling of electrochemistry (EC) with electrospray ionization mass spectrometry (ESI-MS) allows to study products of electrochemical reactions. In the first step (using solely ESI-MS) the oxidation of ferrocene to ferricene with the addition of NaClO_4 (10^{-3} mol L $^{-1}$) and HClO_4 (10^{-4} mol L $^{-1}$) as BE was studied and observed under electrospray ionization conditions. Consequently, in on-line connection of electrochemical cell prior to ESI-MS the re-reduction of ferricene to ferrocene on the platinum wire electrode was observed. The yield of the electrochemical reduction of ferricene to ferrocene depended on the inserted potential. The reduction force of the electrochemical cell increased with decreasing potential.

This study serves to optimize the electrochemical cell construction as well as to investigate the impact of BE to simple redox system presented by ferrocene/ferricene couple. The electrochemical cell construction is applicable to study of electrochemical reaction with stable products in detectable amounts. The maximal yield of reduction reaction is almost 30 % in dependence on inserted potential.

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