

Liquid chromatography with post-column electrochemical treatment and mass spectrometric detection of non-polar compounds

Georg Diehl, André Liesener and Uwe Karst*

Anorganisch-Chemisches Institut, Westfälische Wilhelms-Universität Münster,
Wilhelm-Klemm-Str. 8, Münster 48149, Germany. E-mail: uwe.karst@uni-muenster.de;
Fax: +49 251 8333169; Tel: +49 251 8333182

Received 13th November 2000, Accepted 18th January 2001
First published as an Advance Article on the web 5th February 2001

The first hyphenation of high performance liquid chromatography (HPLC), electrochemical online oxidation and mass spectrometry (MS) is described. Ferrocenecarboxylic acid esters of various alcohols and phenols have been synthesized, separated by reversed-phase HPLC and oxidized (ionized) coulometrically prior to single quadrupole MS analysis using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) interfaces. The dependence of the ionization on the electrochemical pretreatment is demonstrated. Limits of detection for selected derivatives range from 4×10^{-9} to 4×10^{-7} mol dm⁻³ depending on the individual compound and the selected interface.

Introduction

The hyphenation of high performance liquid chromatography (HPLC) and mass spectrometry (MS) enables the selective and sensitive determination of various groups of analytes, because it combines the advantages of an effective separation technique and a highly selective detection method.¹ Due to increased robustness of the instrumentation, HPLC-MS has become a widely used analytical technique in research and routine analysis.¹

However, some problems remain which are mainly caused by the difficulty of coupling a separation taking place in liquid phase with a detection technique that relies on the formation of gas phase ions. Different designs of interfaces have been developed to overcome this obstacle. Currently, the most common interfaces are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI).¹ HPLC-MS measurements with ESI and APCI have been reported to show excellent results for the determination of ionic or polar analytes, since these either are already ionized or can easily be ionized under the comparably soft conditions used for both ESI and APCI. Ionization typically occurs by protonation or deprotonation, but coordination of the analyte with other ions may also be used.² Analytes of lower polarity are less accessible to the ESI or APCI³ processes resulting in low ionization efficiencies and losses in sensitivity. The scope of HPLC-MS on polar analytes is, however, unfortunate considering that analytes of lower polarity are best suited for separation by reversed phase liquid chromatography.

To overcome this limitation, only few attempts for the efficient ionization of less polar analytes have been reported. Cole *et al.* used the electrospray interface for the electrochemical oxidation (ionization) of metallocenes.⁴ Van Berkel and co-workers have reported the determination of alcohols in saw palmetto fruit extract⁵ and of alcohols and phenols in the oils of cloves, lemon, rose and peppermint⁶ using electrospray as an electrochemical reactor following a derivatization step with ferrocene-based reagents. Hambitzer and Heitbaum connected an electrochemical cell to thermospray-MS to study the

electrooxidation of *N,N*-dimethylaniline.⁷ Brajter-Toth *et al.* used a combination of an electrochemical cell and particle beam mass spectrometry.⁸ The coupling of electrochemistry and thermospray-MS was applied by Brajter-Toth *et al.* for oxidative studies on uric acid.⁹ Another approach suggested by Van Berkel *et al.* was the online coupling of different electrochemical flow cells with ESI-MS, either floated at or decoupled from the electrospray high voltage.¹⁰ Although the coupling of an electrochemical flow cell with MS gave promising results, no attempts for using this system after HPLC separation have been reported yet.

Since the derivatization of alcohols^{5,6} and phenols¹¹ with ferrocene-based reagents can easily be accomplished and the resulting products should be well suited for electrochemical oxidation as well as for reversed phase liquid chromatography, we propose a new HPLC-electrochemistry-MS technique for the determination of ferrocene derivatives.

Experimental

Chemicals

Ammonium formate and all alcohols and phenols used were purchased from Aldrich Chemie (Steinheim, Germany) in the highest quality available. Formic acid was obtained from Fluka (Buchs, Switzerland). Solvent for HPLC was acetonitrile LiChroSolv gradient grade from Merck (Darmstadt, Germany).

Ferrocenecarboxylic acid chloride (FCC) was synthesized according to Rolfes and Andersson¹¹ and was characterized by ¹H-NMR, EI-MS and IR.

Synthesis of ferrocenecarboxylic esters (FCEs)

The derivatives were synthesized according to ref. 11. The amounts of 50 mg (2×10^{-4} mol) FCC and 73.3 mg (6×10^{-4} mol) 4-dimethylaminopyridine (DMAP) were dissolved in 2 ml dichloromethane and added to a solution of 1.82×10^{-4} mol alcohol or phenol in 2 ml dichloromethane. The mixture was left to react until the dark red colouration weakened. The DMAP and the excess of FCC were removed by separation on an aluminium oxide microcolumn (30 mm \times 5 mm id). The ferrocenecarboxylic acid esters were eluted with 3 ml dichloromethane, dried under nitrogen and characterized by EI-MS.

HPLC-MS instrumentation

The HPLC-MS system from Shimadzu (Duisburg, Germany) consisted of a SCL-10Avp controller unit, DGU-14A degasser, two LC-10ADvp pumps, SUS mixing chamber (0.5 ml), SIL-10A autosampler, SPD-10AV UV/vis detector, LCMS QP8000 single quadrupole mass spectrometer with electrospray ioniza-

tion and atmospheric pressure chemical ionization probes and Class 8000 Version 1.11 software.

Electrochemical instrumentation

The electrochemical system from ESA, Inc. (Chelmsford, MA, USA) consisted of GuardStat potentiostat and model 5021 conditioning cell. The conditioning cell contains a glassy carbon coulometric working electrode, a Pd counter electrode, and a Pd/H₂ reference electrode. All potentials in this study are given vs. Pd/H₂.

HPLC conditions

Since the ESI interface tolerates only HPLC flow rates of 0.3 ml min⁻¹ or less and the APCI interface works best with flow rates of 0.6 ml min⁻¹, columns of different inner diameter and different LC flow rates and injection volumes had to be used for optimum performance. All separations were performed using Discovery C18 columns (Supelco, Deisenhofen, Germany) equipped with guard columns of the same material with the following dimensions: 5 µm particle size, 100 Å pore size, 2.1 mm id (for ESI experiments) and 3.0 mm id (for APCI experiments), 20 mm length (guard column) and 150 mm (analytical column). Eluent A of the mobile phase was a solution of 250 mg ammonium formate and 0.6 ml formic acid in 1 l deionized water (pH ≈ 3). Eluent B was acetonitrile. A binary gradient at flow rates of 0.25 ml min⁻¹ (2.1 mm id column for ESI) and 0.6 ml min⁻¹ (3.0 mm id column for APCI) with the following profile was used:

<i>t</i> /min	0.01	3	8	18	20	25	25.5
[CH ₃ CN] (%)	60	60	90	90	60	60	stop

The injection volume was 5 µl (2.1 mm id column) and 10 µl (3.0 mm id column).

MS conditions

For all measurements, curved desolvation line (CDL) voltage -35 V, CDL temperature 300 °C, deflector voltages 35 V and detector voltage 1.7 kV were used. The ESI parameters were probe voltage +2.5 kV and nebulizer gas flow-rate 4.5 ml min⁻¹. APCI experiments were carried out with probe voltage 0 V, nebulizer gas flow-rate 2.5 ml min⁻¹ and probe temperature 350 °C.

Results and discussion

The online coupling of the electrochemical cell to HPLC-MS was accomplished by inserting a coulometric flow cell for quantitative oxidation between the UV/vis detector and the interface of the MS system (Fig. 1). The connection between the

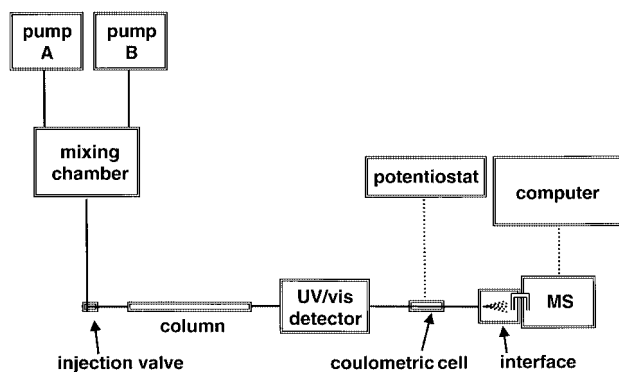


Fig. 1 Schematic assembly of the HPLC-electrochemistry-MS system.

flow cell and the interface was kept as short as possible to reduce loss of ions during transport. To prevent electrical connection between interface and coulometric cell *via* the eluent, appropriate ground connection has to be assured as discussed by Van Berkel *et al.*¹⁰ The MS parameters were adjusted to the conditions imposed by HPLC binary gradient elution.

There are two major advantages of this technique when compared to the electrochemical oxidation in the ESI interface. The oxidative potential in the electrochemical flow cell can be adjusted precisely to the requirements for the analysis. Analytes that are more easily oxidized than interfering substances could be selectively ionized. The high voltage used in the electrospray interface cannot be adjusted to the requirements of the oxidative process and it is not possible to gain knowledge about the exact oxidative potential within the ESI capillary.

The large surface of the glassy carbon working electrode in the coulometric flow cell enables quantitative turnover rates in the oxidation process resulting in increased sensitivity and a large linear concentration range. The electrochemical set-up in the ESI interface is more similar to a thin layer amperometric cell which has oxidation efficiencies of typically less than 20%. Although the oxidation in the electrospray process might be quantitative at very low concentrations, good linearity cannot be expected.

The additional coupling of HPLC to electrochemistry and MS adds selectivity because of the chromatographic separation.

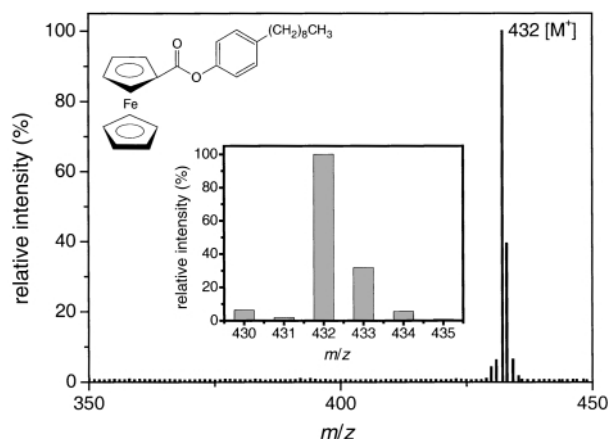


Fig. 2 Electrochemistry (cell voltage = 0.7 V)-APCI (APCI voltage = 0 V) mass spectrum of a 1×10^{-5} mol dm⁻³ solution of 4-n-nonylphenyl FCE. Inserted: calculated isotope pattern for C₂₆H₃₂O₂Fe, chemical structure of 4-n-nonylphenyl FCE.

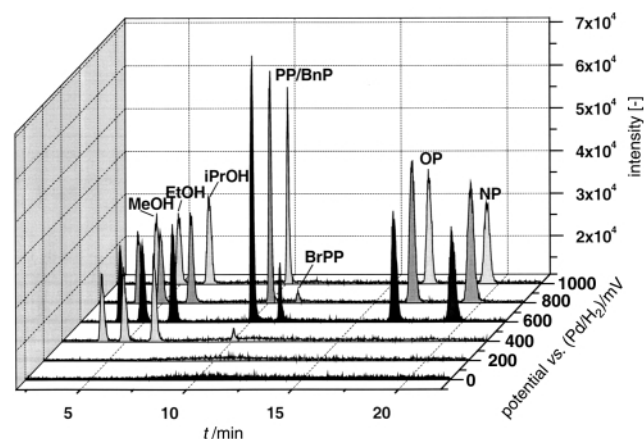


Fig. 3 HPLC-electrochemistry-APCI-MS chromatograms at different coulometric cell potentials, recorded in SIM mode (m/z = 244.0, 258.0, 272.1, 382.1, 396.1, 460.0, 418.2, 432.2), APCI potential 0 V (MeOH = methyl FCE, EtOH = ethyl FCE, iPrOH = i-propyl FCE, PP = 4-biphenyl FCE, BnP = 4-benzylphenyl FCE, BrPP = 4-bromo-4'-biphenyl FCE, OP = 4-n-octylphenyl FCE, NP = 4-n-nonylphenyl FCE).

Table 1 Analytical figures of merit for selected FCEs (LOD = limit of detection, LOQ = Limit of quantification, RSD = relative standard deviation)

Analyte	ESI interface				APCI interface			
	LOD/mol dm ⁻³	LOQ/mol dm ⁻³	Linear range/mol dm ⁻³	RSD (<i>n</i> = 3) <i>c</i> = 5 ×	LOD/mol dm ⁻³	LOQ/mol dm ⁻³	Linear range/mol dm ⁻³	RSD (<i>n</i> = 3) <i>c</i> = 3 ×
				10 ⁻⁶ mol dm ⁻³				10 ⁻⁶ mol dm ⁻³
Methyl FCE	4 × 10 ⁻⁷	1 × 10 ⁻⁶	5 × 10 ⁻⁷ –5 × 10 ⁻⁵	4.9%	1 × 10 ⁻⁸	3 × 10 ⁻⁸	2 × 10 ⁻⁸ –3 × 10 ⁻⁶	2.7%
Ethyl FCE	3 × 10 ⁻⁷	9 × 10 ⁻⁷	5 × 10 ⁻⁷ –1 × 10 ⁻⁴	6.6%	2 × 10 ⁻⁸	6 × 10 ⁻⁸	2 × 10 ⁻⁸ –3 × 10 ⁻⁶	2.4%
Biphenyl FCE	1 × 10 ⁻⁸	3 × 10 ⁻⁸	1 × 10 ⁻⁸ –5 × 10 ⁻⁵	5.4%	2 × 10 ⁻⁸	6 × 10 ⁻⁸	2 × 10 ⁻⁸ –9 × 10 ⁻⁶	3.6%
Benzylphenyl FCE	3 × 10 ⁻⁹	9 × 10 ⁻⁹	5 × 10 ⁻⁹ –5 × 10 ⁻⁵	4.8%	1 × 10 ⁻⁸	3 × 10 ⁻⁸	2 × 10 ⁻⁸ –3 × 10 ⁻⁶	6.0%
4-Bromo-4'-biphenyl FCE	4 × 10 ⁻⁹	1 × 10 ⁻⁸	5 × 10 ⁻⁹ –1 × 10 ⁻⁴	4.8%	4 × 10 ⁻⁸	1 × 10 ⁻⁷	7 × 10 ⁻⁸ –9 × 10 ⁻⁶	20.2%
4-n-Octylphenyl FCE	4 × 10 ⁻⁹	1 × 10 ⁻⁸	5 × 10 ⁻⁹ –1 × 10 ⁻⁵	3.7%	1 × 10 ⁻⁸	3 × 10 ⁻⁸	2 × 10 ⁻⁸ –9 × 10 ⁻⁶	3.8%
4-n-Nonylphenyl FCE	8 × 10 ⁻⁹	2 × 10 ⁻⁸	1 × 10 ⁻⁸ –5 × 10 ⁻⁵	1.8%	1 × 10 ⁻⁸	3 × 10 ⁻⁸	2 × 10 ⁻⁸ –9 × 10 ⁻⁶	6.5%

Preformed ions, for example, will elute before the more unpolar analytes and cannot interfere or suppress the analytes mass signals.

The mass spectrum of 4-n-nonylphenyl FCE recorded with this HPLC-electrochemistry-MS system using the APCI interface is shown in Fig. 2. The APCI probe voltage was set to 0 V for these measurements to ensure that ions which are observed in the spectrum are generated by the oxidative potential of 700 mV in the coulometric cell and not by the APCI process. The interface may therefore be considered as being a heated nebulizer interface. This experiment was not possible with the ESI interface, because the spraying process of ESI depends on a high voltage at the ESI capillary. The base peak in the spectrum of *m/z* = 432 corresponds to the molecular ion of 4-n-nonylphenyl FCE. The isotope pattern in the spectrum fits well to the calculated isotope pattern. The corresponding spectrum recorded with the ESI interface (probe voltage of +2.5 kV) is almost identical and is therefore not shown. The appearance of the molecular ion peak shows that the oxidation of Fe(II) in the ferrocene function to Fe(III) in the corresponding ferrocenium ion was successfully accomplished by electrochemical oxidation in the coulometric flow cell.

Further proof for this assumption is provided in Fig. 3 showing chromatograms (raw data, no smoothing of the peaks) of the separation of a 1 × 10⁻⁵ mol dm⁻³ mixture of different FCEs recorded as total ion current (TIC) in the selected ion monitoring (SIM) mode. For these measurements, different potentials ranging from 0 to 1000 mV vs. Pd/H₂ were applied to the coulometric flow cell. No peaks are detected at potentials below 400 mV. Beginning with a potential of 400 mV, molecular ions of the short chain FCEs as well as of the coeluting 4-biphenyl and 4-benzylphenyl FCEs produce clearly detectable peaks. At a potential of 600 mV, all compounds in the mixture are oxidized to the corresponding ferrocenium ions and can be seen in the chromatogram. It can be observed that the peak areas of 4-biphenyl, 4-benzylphenyl and 4-bromo-4'-biphenyl FCE are lowered for higher potentials than 600 mV. The optimum potential for this mixture of FCE derivatives was found to be 700 mV vs. Pd/H₂. Therefore, a cell voltage of 700 mV was used for all following experiments.

Calibration data were then recorded with the HPLC-electrochemistry-MS system and both ESI and APCI interfaces. The calibration functions exhibited excellent linearities in the lower concentration ranges, but smaller than expected peak areas for higher concentrations when using the APCI mode (Table 1). This can be explained by insufficient oxidation in the flow cell at higher concentration levels and the increased HPLC flow rate used for APCI. This reduces the linear concentration range for the APCI mode compared to the ESI mode. For ESI, linear ranges of four decades are observed for selected analytes.

Analytical figures of merit for both interfaces are also provided in Table 1. Obviously, ESI allows for lower limits of detection and larger linear concentration ranges than APCI for the phenol derivatives, whereas the short chain aliphatic alcohol FCEs can be detected at lower concentrations in the APCI

mode. In the ESI mode, it was obvious that the limits of detection differed strongly between the derivatives of alcohols and phenols. To investigate if this effect is due to the different composition of the mobile phase in the course of the applied gradient, thus resulting in different spray conditions in the interface, isocratic elution was applied as well. However, the same results were obtained as for gradient elution. The reproducibility of both methods ranges from 1.8 to 6.6% (*n* = 3), except for the detection of 4-bromo-4'-biphenyl FCE in the APCI mode and could be further lowered by the use of an internal standard. Although the developed method is characterized by excellent detection limits even for the use of a single quadrupole mass spectrometer, it should be possible to even lower those limits of detection by using a triple quadrupole mass spectrometer coupled to liquid chromatography. Van Berkel *et al.* have successfully demonstrated the possibility to further reduce the limits of detection by using precursor ion scan ESI-MS - MS experiments of different ferrocene derivatives without prior separation.^{5,6}

A powerful new hyphenated technique based on the combination of HPLC, electrochemical (coulometric) oxidation and ESI- or APCI-MS has been developed. Simple and commercially available instrumentation has been used to set up the analytical system. Further research will be directed to the analysis of real samples of endocrine disruptors with alkylphenol structure. Other work shall focus on the investigation of subnanomolar concentrations of ferrocene derivatives by using HPLC-electrochemistry-MS with triple quadrupole instrumentation in the precursor ion scan mode.

Acknowledgement

Financial support of this work by the Fonds der Chemischen Industrie (Frankfurt/Main) is gratefully acknowledged.

References

- W. M. A. Niessen, *J. Chromatogr. A*, 1999, **856**, 179.
- E. Bayer, P. Gfrörer and C. Rentel, *Angew. Chem. Int. Ed.*, 1999, **38**(7), 992.
- G. J. Van Berkel and K. G. Asano, *Anal. Chem.*, 1994, **66**(13), 2096.
- X. Xu, S. P. Nolan and R. B. Cole, *Anal. Chem.*, 1994, **66**(1), 119.
- G. J. Van Berkel, J. M. E. Quirke, R. A. Tigani, A. S. Dilley and T. R. Covey, *Anal. Chem.*, 1998, **70**(8), 1544.
- J. M. E. Quirke, Y.-L. Hsu and G. J. Van Berkel, *J. Nat. Prod.*, 2000, **63**, 230.
- G. Hambitzer and J. Heitbaum, *Anal. Chem.*, 1986, **58**(6), 1067.
- M. C. S. Regino and A. Brajter-Toth, *Anal. Chem.*, 1997, **69**(24), 5067.
- K. J. Volk, R. A. Yost and A. Brajter-Toth, *Anal. Chem.*, 1989, **61**(15), 1709.
- F. Zhou and G. J. Van Berkel, *Anal. Chem.*, 1995, **67**(20), 3643.
- J. Rolfes and J. T. Andersson, *Anal. Commun.*, 1996, **33**(12), 429.