Liquid Chromatography/Electrochemistry/
Mass Spectrometry of Ferrocene Derivatives

J. Georg R. Diehl

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ABBREVIATIONS

Å	Ångström
AED	atomic emission detection
APCI	atmospheric pressure chemical ionization
c	concentration
CDL	curved desolvation line
δ	chemical shift
DMAP	4-(*N*,*N*-dimethylamino)pyridine
ε_r	dielectric constant
ECD	electrochemical detection
EI	electron impact ionization
ES	electrospray
FAB	fast atom bombardment
FCC	ferrocenecarboxylic acid chloride
FCE	ferrocenecarboxylic acid ester
FID	flame ionization detection
Fig.	figure
FTICR	fourier transform ion cyclotron resonance
GC	gas chromatography
IR	infrared
LC	liquid chromatography
LC/MS	liquid chromatography/mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
MRM	mutiple reaction monitoring
MS	mass spectrometry
NIST	National Institute of Standards and Technology
NMR	nuclear magnetic resonance
ODS	octadecyl silica
PAH	polycyclic aromatic hydrocarbon
PB	particle beam
PEEK	polyetheretherketone

RSD	relative standard deviation
SIM	selected ion monitoring
TIC	total ion current
TOF	time of flight
TSP	thermospray

1. INTRODUCTION AND SCOPE

1.1 GENERAL REMARKS

In analytical chemistry, methods and techniques are developed for the qualitatitve and/or quantitative determination of components in a mixture. Analytical methods are based on the physical and chemical properties of the components which are seldom specific for a selected analyte and can also be influenced by other interfering components in the mixture. In order to determine a single compound or a group of compounds, the different substances and substance classes have to be separated from each other. Nowadays separation is mainly performed by means of chromatography in which compounds are resolved from each other according to differences in partioning between a stationary phase and a liquid or gaseous mobile phase. Liquid chromatography (LC) can be applied for the analysis of non- or semi-volatile compounds that may not be stable at higher temperatures. LC is mostly combined with spectroscopic detection techniques like UV/vis spectroscopy or fluorescence spectroscopy, but electrochemical and especially mass spectrometric detection are also widely used.

The main reason for the widespread employment of liquid chromatography coupled to mass spectrometry (MS) is the combination of an effective separation technique with a highly selective and sensitive detection method [1]. However, some problems with the hyphenation of such different techniques are still remaining which are mostly caused by the difficulty of coupling a separation technique taking place in the liquid phase with a detection technique that relies on the formation of gas-phase ions. Different ionization interfaces have been developed to overcome this obstacle. The two most commonly employed ionization methods are electrospray (ES) ionization and atmospheric pressure chemical ionization (APCI) [2-4]. The ability of LC/MS in the determination of polar anlytes has been demonstrated in many applications that range from environmental to bioanalytical fields [5]. The amount of energy that is transferred to the analytes during the electrospray and the APCI processes is relatively small when compared to other ionization techniques like electron impact ionization. Therefore, ES and APCI are called "soft" ionization methods. The relatively small amount of energy that is transferred results in ES and APCI mass spectra that are characterized by little fragmentation and base peaks which correspond to pseudomolecular ions. These pseudomolecular ions are typically

formed by protonation in the positive mode and deprotonation in the negative mode but coordination of the analyte with other ions may also be used [6]. This is the reason why polar substances are succesfully ionized with good ionization yields whereas less polar analytes which are not as easily protonated or deprotonated are less accessible to the ES or APCI processes. The low ionization efficiencies of these less polar components cause losses in sensitivity that can lead to the inability of LC/ES-MS or LC/APCI-MS to determine these components in low concentrations. The scope of LC/MS on polar substances is, however, unfortunate considering that analytes of lower polarity are best suited for separation by reversed-phase LC.

One approach to overcome this limitation has been the use of electrochemical reactions that produce more polar or even ionic molecules. Whereas some groups employed additional electrochemical flow cells in their hyphenation of electro-chemistry to MS, others utilized the electrochemical potential in the electrospray interface itself [7-10].

1.2 SCOPE OF THIS THESIS

Although the coupling of an electrochemical flow cell with MS gave promising results, no attempts to use this setup after a liquid chromatographic separation had been reported at the begin of the dissertation. The objective of the research described in this thesis is to explore the potential of the hyphenation of liquid chromatography with on-line electrochemistry and mass spectrometry. To achieve this, a commercially available electrochemical flow cell was inserted between LC and MS of a single quadrupole LC/MS system and later also of a triple quadrupole LC/MS/MS system. A ferrocene containing label was then used to introduce an electrochemically active functionality into the analyte molecules. Although different fuels and oils were examined as samples for possible applications, synthesized standards were employed for most of the experiments. Because the sample preparation for the oil samples including the derivatization step had already been reported in the literature [11,12], additional investigations concerning the sample preparation were considered as out of the scope of the thesis. Furthermore, it was not the aim to develop a fully validated method because the technique was completely new and the focus of the thesis was the overall applicability.

Chapter 2 gives an overview of the different coupling methods of electrochemistry to mass spectrometry that have been reported in the last 30 years. After a brief historic introduction it is divided into three parts which are ordered by increasing degree of complexity of the experimental setup. The first part summarizes the papers on oxidation or reduction reactions in the electrospray interface itself whereas the second part describes several approaches to electrochemistry/MS using additional electrochemical cells which are coupled to all kinds of different ionization interfaces. The last section of the overview covers the hyphenation of chromatographic methods to electrochemistry/ mass spectrometry.

After a brief introduction about the history of ferrocene as the first organometallic sandwich complex **chapter 3** describes the synthesis and characterization of the derivatizing agent ferrocenecarboxylic acid chloride and the standard derivatives of several alcohols and phenols.

Chapter 4 shows the first implementation of the LC/electrochemistry/MS coupling. After an electrochemical characterization of the standards by cyclic voltammetry, the oxidation of the ferrocene function in the "coulometric" flow cell was observed by ES- and APCI-MS. An optimization of the signal intensity was performed for better sensitivity followed by LC/electrochemistry/MS experiments employing both interface techniques. The dependency of the mass spectrometric response on the applied cell potential could be demonstrated. Analytical figures of merit were obtained by calibration measurements with the synthesized standards.

The potential for fast liquid chromatographic separations coupled to electro-chemistry/MS was explored in **chapter 5**. Mixtures of alcohol or phenol derivatives were separated on short guard columns in time scales of 1-1.5 min and detected by MS following on-line ionization in the "coulometric" flow cell in the first part of the chapter. The second part describes the use of a graphite-based in-line filter element as stationary phase for simple chromatographic problems. This method was characterized by a simple experimental setup.

Chapter 6 covers the hyphenation of the developed LC/electrochemistry system with a tandem mass spectrometer. MS/MS experiments were carried out to elucidate the fragmentation pathway of the alcohol and phenol derivatives. Precursor ion scans could be used in the analysis of mixtures because a common fragment ion was observed for each of the substance classes. Multiple reaction monitoring was utilized in a sensitive determination of different standards.

Several gasoline and diesel samples have been investigated by LC/electrochemistry/ MS and LC/electrochemistry/MS/MS in **chapter 7**. A large number of alcohols and alkylphenols were detected in these samples after the derivatization step with ferrocenecarboxylic acid chloride. Only a sum parameter could be obtained for analytes of the same molecular mass because of the large number of structural isomers that were not chromatographically resolved. Besides the alcohol and alkylphenols, unknown compounds were found in the samples that could not be identified although tandem MS was employed.

Chapter 8 describes the quantitative determination of phenol and the C1 to C9 alkylphenols in two oils that are used as standard reference materials. Quantitation was performed by external calibration and the obtained sum parameters for the groups of alkylphenols were compared to literature data.

General conclusions and some remarks concerning the advantages and drawbacks as well as the future perspective of LC/electrochemistry/MS are found in **chapter 9** which concludes the thesis.

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2. ON-LINE ELECTROCHEMISTRY/MASS SPECTROMETRY AND RELATED TECHNIQUES – STATE OF THE ART

2.1 SUMMARY

This chapter summarizes publications about the on-line coupling of electrochemistry to mass spectrometry. After a brief historic introduction, it is divided into three parts that are sorted by ascending degree of complexity of the experimental setup. The first section deals with the use of the electrospray ion source as an electrochemical reactor for oxidation or reduction reactions. It is followed by the second part which covers the hyphenation of different kinds of electrochemical flow cells with various ionization interfaces. The last section focuses on the on-line coupling of chromatographic techniques with electrochemical flow cells and mass spectrometry.

2.2 INTRODUCTION

The use of electrochemistry coupled on-line to mass spectrometry to identify the products and intermediates of electrochemical reactions began in the early 1970s, when Bruckenstein and Gadde reported the coupling of a porous electrode with the gas inlet system of a mass spectrometer [1]. Bruckenstein and co-workers [2-5] and later mainly Heitbaum et al. used this technique extensively to identify volatile reaction products [6-18]. Similar techniques were employed in the 1980s and later by Kreysa and Breidenbach [19], Anderson et al. [20-22] and Iwasita and co-workers [23, 24]. This porous electrode interfacing for the detection of volatile species has already been reviewed by Chang et al. [25], Vielstich and co-workers [26] and Brajter-Toth et al. [27] and will therefore not be the subject of this overview.

This chapter focuses on the on-line hyphenation of electrochemistry with mass spectrometry for the determination or identification of non-volatile compounds in solution. This goal was first accomplished by Hambitzer and Heitbaum in 1986 by connecting a three-electrode electrochemical flow cell to a thermospray mass spectrometer [28]. The idea of coupling an electrochemical flow cell to mass spectrometry was then extended to other ionization techniques like fast atom bombardment, electrospray and particle beam ionization. When in 1991 the high potential at the capillary tip of an electrospray emitter was reported to induce redox reactions [29, 30] and the electrospray interface itself began to be considered as an

electrochemical reactor [30], a third type of electrochemistry/mass spectrometry hyphenation was established. This last type is the most simple setup for electrochemistry/mass spectrometry and the papers dealing with this will be considered in the first part of this chapter. The more complex setups where an additional electrochemical flow cell is connected on-line with the mass spectrometer will make up the second part, sorted by the different kinds of ionization interfaces. Finally, the coupling of chromatographic methods to electrochemistry/mass spectrometry is discussed in the third and last part of this chapter.

2.3 REDOX REACTIONS IN THE ELECTROSPRAY IONIZATION INTERFACE

The electrospray process involves three stages: (a) production of the charged droplets at the capillary tip, (b) evolution of the charged droplets due to solvent evaporation and droplet fission caused by Coulombic repulsion of the charges of the droplets and (c) production of the gas phase ions from very small charged droplets by the charge residue model or the ion evaporation model [31] (figure 2.1).

In 1991, Blades, Ikonomou and Kebarle were the first to compare the electrospray (ES) process to an electrolysis cell [30]. They used stainless steel and zinc tipped capillaries for spraying and could detect Fe(II) and Zn(II) ions in the sprayed solution. The observed concentration of Zn(II) matched the Zn(II) production expected on the basis of the measured electrospray current. This demonstrated that indeed an electrochemical oxidation reaction occurs at the liquid-metal interface of the ES capillary tip. The detection of Fe(II) ions leads to the conclusion that the stainless steel capillaries most often used in electrospray are not inert but take part in the electrochemical process.

In the following years, the group of van Berkel has studied electrochemical reactions in the ES interface extensively. They found that the ability to produce radical cations in the ES process expands the utility of ES ionization thus including compounds that are normally not amenable to ES ionization. The structure and the half-wave oxidation or reduction potentials of the analytes are important factors for the formation of radical cations. They also compared ES to a controlled-current electrolytic cell. Further experimental confirmation for this assumption was obtained

by direct connection of an ES source with the detection cell of an UV/vis diode array spectrometer [32].

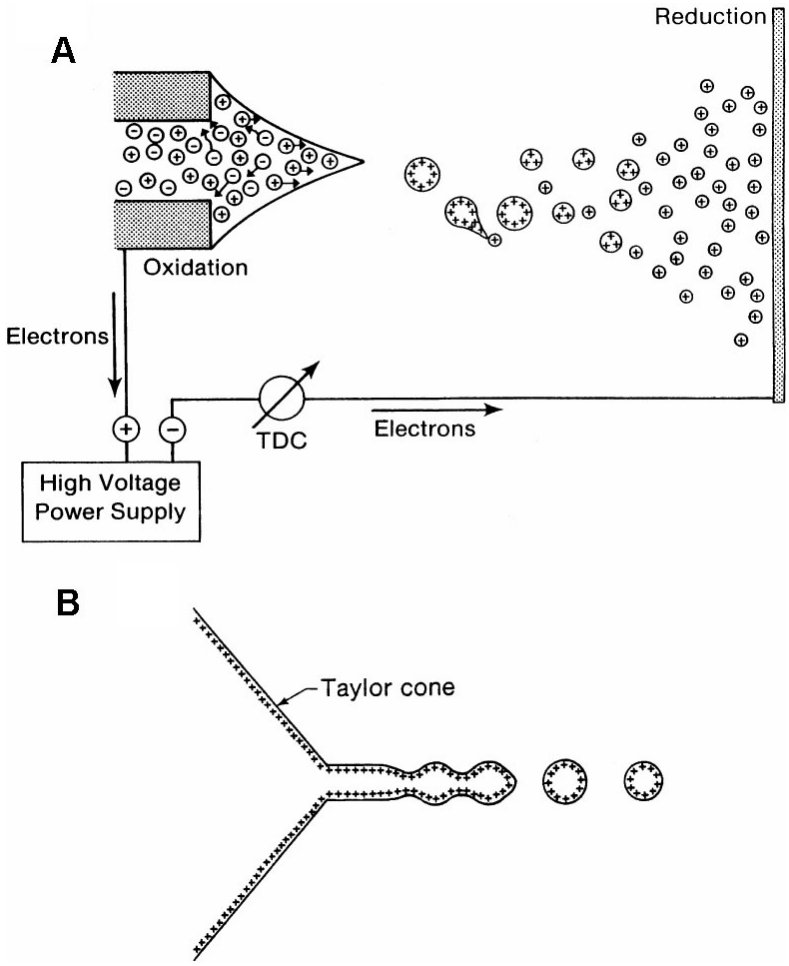

Fig. 2.1 Schematic representation of the mechanisms involved in the formation of gas-phase ions in the electrospray process: (A) The high electric field imposed by the power supply and the needle tip dimension of the metal capillary causes enrichment of positive electrolyte ions at the meniscus of the solution. This net charge is pulled downfield and expands the meniscus into a cone that emits a spray of positively charged droplets. Solvent evaporation reduces the volume of the droplets at constant charge and this leads to Coulomb explosions of the droplets. (B) The least stable point at the tip of the cone extends into a filament which breaks up to charged droplets.

This setup allowed the detection of the electrolysis products while they were still in solution, thereby avoiding any possible interferences from the spraying process. It must also be taken into account that the solvent, in addition to dissolving the analyte and providing a stable spray, has to stabilize and protect the radical cation until it enters the mass spectrometer [33]. The applicability of the method was

demonstrated for different metal porphyrins and PAHs, N,N,N',N',-tetramethyl-1,4-phenylenediamine and the heteroaromatic phenothiazine which all could be detected as radical cations. In [34], van Berkel showed that the electrospray source may serve as a very efficient controlled-current electrolytic flow cell even for species that are relatively difficult to oxidize (E > 1.0 V vs. SCE). The addition of small amounts of electrolyte to the solvent, e.g. lithium triflate, high electrospray voltages and low flow rates result in the highest oxidation yield. Further insight into analyte electrolysis was realized through an examination of the results from off-line chronopotentiometric experiments and mass transport calculations [35]. It could also be demonstrated that an easy way to minimize analyte electrolysis is to exchange the ES stainless steel capillary with a fused silica capillary [36].
Electrochemical reduction in the ES source was observed by Houk et al. when they determined metal cations in an excess of hydrochloric or nitric acid and found a reduction of Fe(III) to Fe(II) [37]. Van Berkel demonstrated the possibility of electrolytic reduction of Ag(I), Cu(II) and Hg(II) and deposition of the respective elements on the surface of the high-voltage contact in the ES source in the negative mode [38]. The deposited metals could be stripped from the surface by operating the ES source in the positive mode. This paper sparked a discussion among six experts in the field of electrospray ionization about the electrochemical processes in ES ionization mass spectrometry [39].

Certain classes of compounds are especially susceptible to electrochemical redox reactions in the interface. The following papers are sorted according to the groups metal complexes, metal porphyrins, fullerenes and stable organic radicals. A section that covers derivatization strategies to include also the non-electroactive substances in the electrospray/mass spectrometry analysis concludes this part of the review.

As can be expected, metal complexes that are easily oxidized or reduced in classical electrochemical approaches may also be subject to electrochemical reactions in the ES interface. Cole and co-workers were able to detect the radical cations of classical metal complexes like ferrocene, ruthenocene and osmocene and different derivatives [40] with ES mass spectrometry. Kane-Maguire et al. tried to oxidize neutral binary metal carbonyls and clusters with ES mass spectrometry but only succeeded with ferrocene and its phenyl-substituted derivative [41]. Ferrocene

derivatives were also the topic of McCarley's group in [42]. They used the oxidative power of the electrospray process for the formation of doubly-charged biferrocenes and oligoferrocenylsilanes with up to four charges per molecule. They demonstrated that higher flow rates resulted in decreased populations for the higher charge states. Eight substituted tris(dithiocarbamato)iron(III) complexes were oxidized in-source by Basic and co-workers [43].

A special kind of metal complexes are the metal porphyrins which is the second class of compounds that are amenable to the electrospray-inherent electrochemical processes. Already in 1991, van Berkel et al. analyzed several free base and metal porphyrins using an electrospray ion trap mass spectrometer [29]. The free base porphyrins were detected as protonated pseudomolecular ions whereas the divalent metal porphyrins were in some cases oxidized and detected as radical cations. This work was expanded to geoporphyrins that are determined by LC/ESI-MS [44]. Chan and co-workers examined neutral lanthanide(III) porphyrin-phthalocyanine heteroleptic sandwich complexes by ES fourier transform ion cyclotron resonance (FTICR) mass spectrometry [45]. They observed intense signals corresponding to the radical cations in all cases. Multiply charged molecular ions were assigned to be formed from successive oxidation of the ligands. Vandell and Limbach succeeded in the selective ionization of one porphyrin over another in a binary mixture [46]. This could be accomplished if the absolute concentration was high and the oxidation potentials differed substantially. Under these conditions, the analyte with lower oxidation potential served as a "redox buffer".

A third class are the fullerenes which are prone to form anions in the negative ion mode. The separation and identification of higher fullerenes that were extracted from carbon soot by LC/ES-MS was reported by Jinno et al. [47]. Peel and co-workers studied the cyano adduct anions of fullerene C_{70} [48] and identified the dianions of the fullerenes C_{84} and C_{90} [49]. Fullerene-functionalized dendritic branches, so-called fullerodendrons, were reduced in the ES source by Gross et al. [50]. Finally, it was demonstrated by the group of Drewello that fullerenes and fullerene derivatives can also be analyzed by nanospray mass spectrometry and that, as in electrospray, molecular ions are detected [51].

Metzger and Griep-Raming investigated the oxidation of several stable organic radicals like 1,3,5-triphenylverdazil [52] and the trityl radical [53] using electrospray as an electrochemical reactor. It was demonstrated in [52] that for 2,2,6,6-tetramethylpiperidine-1-yloxy even with atmospheric pressure chemical ionization the radical cation was detected.

Derivatization techniques play an important role in electrochemistry/mass spectrometry because several classes of substances that are normally not amenable to electrochemical oxidation in the ES source or to the classical ES protonation or deprotonation can be derivatized to include electroactive groups. The group of van Berkel has published a number of papers on different derivatization strategies. In [54], the use of ferrocene-based electrochemically ionizable derivatives of alcohols, sterols and phenols was discussed. The derivatization procedures, electrochemical characteristics of the derivatives and an optimization of ES-MS parameters were presented with reference to ferrocenecarbamate ester derivatives of several alcohols. Tandem MS experiments were shown to provide derivative confirmation, enhanced detection and additional analyte structure information. The applicability of the method was demonstrated by the determination of alcohols in a saw palmetto fruit extract. The same technique was also employed in the identification of several alcohols in the oil of cloves, lemon oil, rose absolut and peppermint oil [55]. A closer investigation of the MS/MS behaviour of different ferrocene carbamate ester derivatives of saturated primary, secondary and tertiary alcohols is given in [56]. The formation mechanisms of the three product ions which are common to all derivatives were elucidated and, by comparing the relative abundances of these ions, it was possible to distinguish among different alcohol structural types. Using ferrocene boronic acid as derivatizing agent, even alkenes can be provided with an electroactive group after their transformation to the respective 1,2-diols [57].

2.4 REDOX REACTIONS IN ADDITIONAL ELECTROCHEMICAL CELLS

2.4.1 Thermospray Ionization

The first electrochemistry/mass spectrometry coupling for the determination of species that remain dissolved after electrolysis was accomplished by Hambitzer and

Heitbaum in 1986 [28]. They constructed a three-electrode assembly for the electrooxidation of N,N-dimethylaniline in neutral solution and connected it to a quadrupole thermospray mass spectrometer. The electrolyte was delivered from the working electrode of the electrochemical cell into the heated capillary tube of the thermospray mass spectrometer by an LC pump (figure 2.2). Different oxidation products of N,N-dimethylaniline could be detected and the system also allowed the recording of mass intensity/potential curves. The method was limited to the analysis of long-lived species due to the system void time of 9 s.

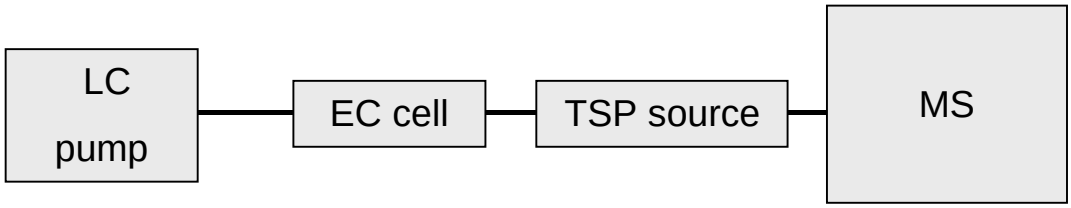

Fig. 2.2 Schematic drawing of the setup that was used by Hambitzer and Heitbaum for the first on-line coupling of an electrochemical flow cell with mass spectrometry for the analysis of electrogenerated products in solution.

In the following years, Brajter-Toth and Yost et al. extended this approach, employing tandem mass spectrometry for the study of the oxidation of the two purines uric acid [58, 59] and 6-thioxanthine [58]. The comparison of daughter ion spectra of standards with the spectra of the electrooxidation products was used for the structural elucidation. Additional advantages compared to the method of Hambitzer and Heitbaum are the utilization of a commercially available "coulometric" flow cell (ESA, Inc.) with large surface area of the working electrode and a significantly lower void time of 500 ms at a flow rate of 2.0 ml min^{-1} [59]. A new thin-layer electrochemical flow cell was developed by Brajter-Toth and Regino and allows easy access to the working electrode for resurfacing and replacement and withstands high back pressures [60]. However, this cell showed relatively low conversion efficiencies of 2% to 32%, depending on the mobile phase used. It was employed in combination with thermospray mass spectrometry to study the oxidation of uric acid.

Getek et al. used a very similar setup in a flow injection experiment to examine the formation of glutathione and cysteine conjugates of acetaminophen [61]. Formation

of the two conjugates occurred only after acetaminophen had been oxidized electrochemically to N-acetyl-*p*-benzoquinoneimine and had been reacted in a mixing tee with either glutathione or cysteine.

The group of Hambitzer revisited electrochemistry/mass spectrometry for the investigation of the anodic oxidation of aniline in diluted sulfuric acid [62, 63] and of N,N-dialkylanilines in neutral aqueous solution [64-66]. The use of a dual beam thermospray ion source enabled the study of reactions in non-aqueous solutions. The setup was tested by elucidating the oxidation process of phenothiazine [64]. Further structural information was obtained by employing deuterated aniline [63].

2.4.2 Fast Atom Bombardment Ionization

Bartmess and Phillips modified the probe commonly used in fast atom bombardment for the first electrochemistry/FAB-MS coupling in 1987 [67, 68]. They inserted a second electrode into the tip of the probe so that a potential difference could be applied to the sample. The most commonly used liquid FAB matrix, glycerol, has a comparatively high dielectric constant (ε_r = 42.5 at 25 °C)and therefore conducts reasonably well. Spectra of 1-bromohexadecane, hexanophenone, anisol, nitro-benzene and other test substances were obtained at a 0 V potential difference and at various oxidizing or reducing potentials. The main problem of the method was the limited solubility of many analytes in the glycerol matrix. This disadvantage could be partly overcome because the modified probe produced less matrix ions and more analyte ions, thus increasing the signal to noise ratio. This was demonstrated for vitamin K_1 and other quinones [69].

2.4.3 Particle Beam Ionization

The first coupling of electrochemistry to particle beam mass spectrometry was realized by the Brajter-Toth group [60]. The already mentioned thin-layer cell was connected to a particle beam mass spectrometer to study the oxidation pathway of dopamine. The effect of the mobile phase composition on the cell conversion efficiency was investigated in [70]. The highest conversion of dopamin solutions was obtained with a mobile phase of 90% methanol and 10% water containing no supporting electrolyte. However, the lack of supporting electrolyte led to a decrease in reproducibility. The electrochemistry/PB-MS system was also used for the real

time characterization of the electrocatalytic oxidation of dimethylaminopyridin by dopamine [71]. A significant signal enhancement was observed for the anodic oxidation of triphenylamine when using tetrabutylammonium perchlorate as supporting electrolyte [72].

2.4.4 Electrospray Ionization

In 1994, Zhou and van Berkel were the first to combine different electrochemical cells with an electrospray mass spectrometer [73, 74]. They used three different cells, a commercially available thin-layer cell (Bioanalytical Systems, Inc.), a commercially available porous graphite electrode cell (ESA, Inc.), and also a self-assembled tubular electrode cell. They showed that in order to couple any of these cells on-line with the electrospray emitter, the cells must be either floated at the high electrospray potential or somehow decoupled from this voltage (figure 2.3). Another problem was the choice of the supporting electrolyte. The high concentrations of non-volatile electrolytes commonly used in electrochemistry experiments can lead to plugging of the electrospray interface and suppression of the analyte gas-phase ion signal because these electrolytes are very prone to gas-phase ion formation. Van Berkel showed that there is a much smaller suppression effect when alkali metal salts like lithium triflate are used as supporting electrolytes. The tubular cell operated in the controlled current mode was employed for electrochemistry/ES-MS to study the electrochemical ionization of the neutral compound perylene. It could be seen that although some amount of perylene radical cation was detected with the cell off, there was a significant signal increase for the radical cation when the cell was turned on. This proves that the electrochemistry/ES-MS approach yields a higher electrolysis efficiency than the inherent electrospray electrolysis process because the latter is current-limited.

Shortly thereafter, Bond et al. studied the oxdiation of copper, nickel and cobalt dithiocarbamate complexes with the hyphenation of a very simple flow cell that consisted of two lengths of platinum microtubing with an electrospray mass spectrometer [75]. The Pt microtubing was connected to a high voltage power supply and a constant potential of 200 V was applied across the two microtubes. Molecular cations of all tested complexes could be observed in both acetonitrile and dichloromethane containing tetrabutylammonium hexafluorophosphate as electrolyte.

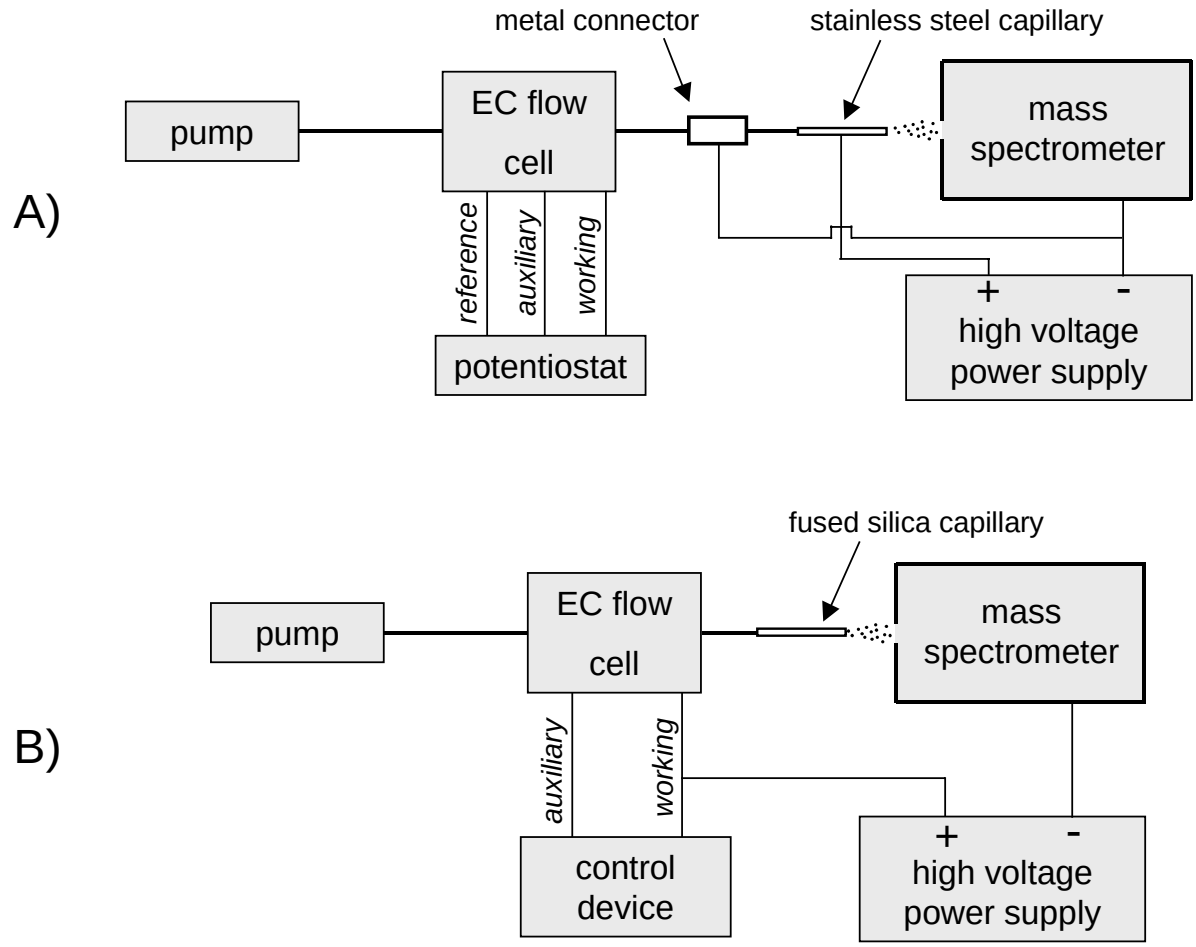

Fig. 2.3 Schematic representation of the possible methods to connect the electrochemical cells on-line with the electrospray emitter. (A) Decoupled mode: a metal connector is connected to the ground of the electrospray high voltage power supply and the eluent is sprayed from a metal capillary that is connected to the high-voltage end of the supply. In this particular setup, a potentiostat is used to control the working electrode potential in the three electrode configuration. (B) Floated mode: the electrochemical cell and the control device are floated at the high voltage of the electrospray source. The control device in this case could be a constant current supply or a constant potential supply.

The group of Cole minimized the time between the electrochemical generation of ions and the mass spectrometric analysis with a low-volume three electrode cell that was constructed inside the electrospray probe [76, 77]. In this setup, a fused-silica layer insulates the microcylinder working electrode from the sample solution until immediately prior to the the electrospray region, thus postponing electrode processes to the latest moment possible. Polycyclic aromatic hydrocarbons could be oxidized and detected as radical cations. The same probe assembly was used in [78]

to study the anodic oxidation of diphenyl sulfide, the reduction of nitrobenzene and the nucleophilic addition of pyridine to an electrogenerated 9,10-diphenylanthracene radical cation.

Electrochemistry/ES-MS was also applied to protein analysis. A simple setup with a 6 cm stainless steel tube as working electrode was used by Lee et al. for the determination of phenylthiohydantoin amino acid derivatives [79]. They employed low flow rates of less than 1 µL min^{-1} and a microscale electrospray nozzle to achieve subfemtomolar detection limits in selected reaction monitoring experiments. The results of the analysis of actual Edman microsequencer samples were consistent with those obtained by LC/UV analysis.

Arakawa et al. utilized a self-assembled electrolytic flow cell coupled to an electrospray mass spectrometer for the oxidation of different ruthenium and osmium complexes [80]. A disadvantage of their setup was the large void volume of the cell which only enabled the analysis of electrolytic products with lifetimes of more than three minutes.

The investigation of the electrochemical polymerization of aniline using on-line electrochemistry/ES-MS was reported by Deng and van Berkel in 1999 [81]. They connected a three-electrode thin-layer cell combining commercially available and in-house-built parts with a single and a triple quadrupole mass spectrometer. At a working electrode potential of 1.0 V vs. Ag/AgCl, singly protonated oligomers containing as many as 10 aniline units could be observed. Most of the oligomers were observed in more than one redox state ranging from fully oxidized (all imine nitrogens) to fully reduced (all amine nitrogens).

The group of Bruins examined the possible use of electrochemistry/ES-MS as a convenient way to mimic phase I oxidative reactions in drug metabolism [82]. The previously reported N-dealkylation of the dopamine agonist 2-(N-propyl-N-2-thienylethylamino)-5-hydroxytetralin was successfully mimicked by the electrochemical cell. The oxidation of the phenol function was not fully mimicked, as the catechol and p-hydroquinone formed were further oxidized to the corresponding quinones and the 45 s delay between the electrochemical cell and the MS did not allow the detection of short-lived intermediates.

2.5 HYPHENATION WITH CHROMATOGRAPHIC METHODS

The first example of high-performance liquid chromatography used on-line with electrochemistry and mass spectrometry was reported by Yost et al. in 1989 [83]. The products formed during electrooxidation in the porous working electrode flow cell were separated using a C18 reversed-phase column and subsequently analyzed by thermospray tandem mass spectrometry (figure 2.4).

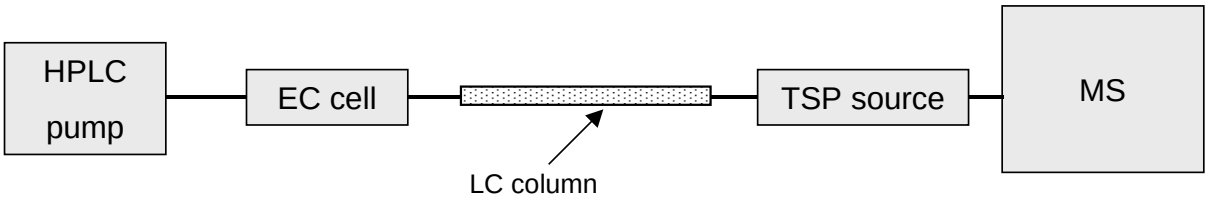

Fig. 2.4 Schematic drawing of the setup that was used by Volk, Yost and Brajter-Toth.

6-Thiopurine was chosen as a model compound to study the electrochemical oxidation of thiopurines. The electrochemistry/LC/TSP-MS chromatograms of 6-thiopurine solutions contained the peak corresponding to the dimerization product bis(purinyl) disulfide and two further peaks which correspond to unknown oxidation products at shorter retention times. These two products could not be identified because their retention times did not match those of the available standards and their low intensities prevented the use of MS/MS for further structural elucidation. In [84], the oxidation pathways of 6-thiopurine and 6-thioxanthine were further investigated by the Brajter-Toth group using the same electrochemistry/LC/TSP-MS system. This system was also employed to compare the enzymatic and the electrochemical oxidation pathways of uric acid [85]. The enzymatic reaction of uric acid with peroxidase and H_2O_2 yielded the same intermediates and products that were observed in the electrochemical oxidation. The high conversion efficiencies in electrooxidation were advantageous for structural elucidation of the products and intermediates using MS/MS scans.

Dewald et al. used a different sequence of electrochemical cell and liquid chromatography in their setup [86]. After their LC separation on a C18 reversed-phase column the three phenolic isoflavones genistein, formononetin and biochanin A were electrochemically detected in a thin-layer flow cell and sequentially in a

thermospray MS that was used as a second detector. The electrochemical cell was not used for ionization purposes. A comparison of conventional LC/EC and LC/MS chromatograms with the sequentially obtained LC/EC-LC/MS chromatograms showed a more stable baseline and less noise in the conventional chromatograms which can be attributed to a non-optimal interface between the instruments.

Iwahashi used a similar setup as Brajter-Toth with liquid chromatographic separation after the electrolysis but exchanged the thermospray interface for an electrospray emitter [87]. After the tryptophan metabolite 3-hydroxy-DL-kynurenine was oxidized in a commercial ESA cell, the oxidative products were separated by reversed-phase LC on a C18 column and determined by UV and ES-MS detection. The mass spectrometric information was used for the identification of the respective peak fractions. The same setup was employed for the study of the oxidation of 3-hydroxyanthranilic acid (3-HAA) [88]. Different 3-HAA dimers, a 3-HAA trimer and a tetramer could be identified by their electrospray mass spectra.

The technique of electrochemically modulated liquid chromatography (EMLC) was coupled on-line with ES-MS by van Berkel's group [89]. With EMLC it is possible to manipulate retention and enhance separation efficiency solely through changes in the potential applied to a conductive stationary phase. The method was used for the separation of mixtures of corticosteroids and benzodiazepines. With the help of the electrospray mass spectra, two electrolysis products of nitrazepam could be identified. The last application of the method was the on-column oxidation of aniline and the separation of the formed products. This demonstrated the potential of EMLC to both generate and separate electrolysis products, which can then be characterized by ES-MS. This use of EMLC might circumvent the need to use a separate electrochemical cell and a conventional LC column in tandem for the same purpose. In the present example, the method was limited to the determination of aniline tetramers and lower oligomers because of the strong adsorption of higher polymerization products onto the porous graphite column.

2.6 CONCLUSION

This overview of current research on the on-line coupling of electrochemistry to mass spectrometry demonstrates the potential of this analytical technique to solve various

problems in biomedical and environmental analysis. The large number of papers from the last two years shows that in the future an increasing impact on the analytical science has to be expected, mainly based on methods employing electrospray ionization. The hyphenation of electrochemistry/mass spectrometry with chromatographic techniques further increases the possible applications of this group of methods. Despite the various applications for electrochemistry/mass spectrometry it has to be stated that there are limitations due to the restriction to electrochemically active analytes or electroactive labels.

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3. SYNTHESIS AND CHARACTERIZATION OF STANDARDS

3.1 SUMMARY

Ferrocenecarboxylic acid chloride was prepared from ferrocenecarboxylic acid and used as a derivatizing reagent for the synthesis of several alcohol and phenol derivatives. These standards and the derivatizing reagent were characterized by IR, EI-MS and, when larger amounts were prepared, by ^{1}H-NMR.

3.2 INTRODUCTION

The synthesis of ferrocene was first accomplished by Kealy and Pauson [1] and, independently, by Miller, Tebboth and Tremaine [2] in the end of 1951. Both groups proposed a ”traditional” structure as a di-σ-complex. In the spring of 1952, Wilkinson, Rosenblum, Whiting and Woodward proposed an aromatic sandwich structure for ferrocene [3]. In the same time period, Fischer and Pfab derived the same sandwich structure for ferrocene from X-ray experiments [4]. This breakthrough openend a new era in the field of organometallic chemistry and led to the Nobel prize for Wilkinson and Fischer in 1973.

Shortly after, the synthesis of several different substituted ferrocenes was reported, among which were the mono- [5] and diacetyl [6] derivatives, the mono- [5] and dicarboxylic [6] acids, the acid chloride, the acid azide and the amine [7].

The ferrocenecarboxylic acid chloride was prepared from ferrocenecarboxylic acid by substitution of the hydroxyl group in the carboxylic acid function by a chlorine atom. As chlorine donor, phosphorus pentachloride was used in the preparation of the ferrocenecarboxylic acid chloride (FCC) by Arimoto and Haven [7]. This method was later modified and oxalyl chloride was used instead [8]. In this work, a modification of this procedure by Rolfes and Andersson [9] was employed for the synthesis of FCC which was used as derivatizing reagent for alcohols and phenols. To obtain the FCC, ferrocenecarboxylic acid was reacted with oxalyl chloride under DMAP catalysis. The FCC was used to derivatize different alcohols and phenols to the corresponding ferrocenecarboxylic acid ester standards (figure 3.1).

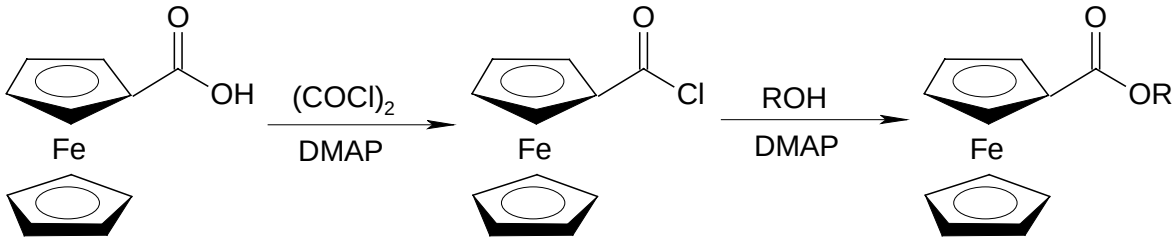


Fig. 3.1 Synthesis of the derivatizing agent ferrocenecarboxylic acid chloride and derivatization of alcohols and phenols to the corresponding ferrocenecarboxylic acid esters.

3.3 EXPERIMENTAL

3.3.1 Chemicals

Ferrocenecarboxylic acid, oxalyl chloride, 4-(*N*,*N*-dimethylamino)pyridine (DMAP) and all alcohols and phenols used were purchased from Aldrich Chemie (Steinheim, Germany) in the highest quality available.

3.3.2 Synthesis of the Derivatizing Reagent

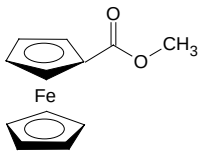
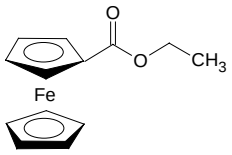
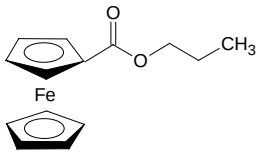
The derivatizing agent ferrocenecarboxylic acid chloride (FCC) was synthesized according to Rolfes and Andersson [9] with slight modifications. At room temperature, a solution of 1.43 ml (2.1 g, 16.6 mmol) of oxalyl chloride in 30 ml of toluene was added to a stirred suspension of 3.0 g (13.0 mmol) of ferrocene-carboxylic acid and a catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine in 35 ml of toluene. A Dimroth condenser with a bubbler was attached to the flask and the reaction mixture was stirred for 1 h at room temperature. The color changed from orange to dark red. Small bubbles of CO_2 could be observed. To complete the reaction, the mixture was heated to 80 °C, left at that temperature for 2 min and then allowed to cool down. The toluene was evaporated under vacuum and the residue was extracted with 2 x 15 ml and 1 x 8 ml of warm pentane (30 °C) which was removed by aspiration with a pasteur pipette. The combined extracts were filtered through a glass frit, which was washed with 5 ml of pentane. The filtrate was left for 3 h at room temperature, then put into a refrigerator at 8 °C for one hour and finally left in the freezer at -18 °C overnight. The FCC precipitated as dark red, cubic crystals. After removal of the pentane layer with a pasteur pipette, the product was dried

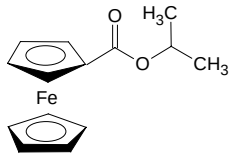
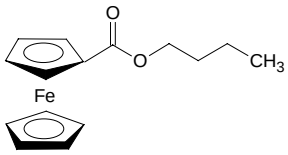
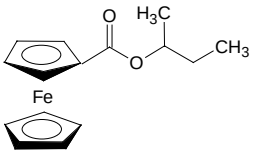
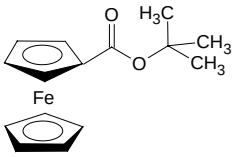
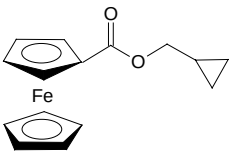
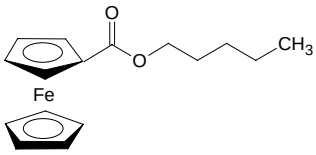
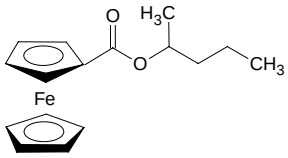
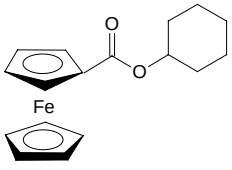
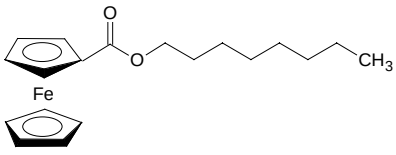
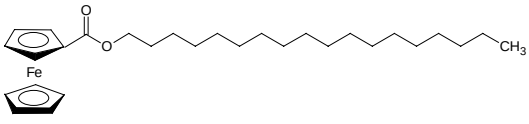
under vacuum and was used for the synthesis of the standards without further purification. The yield was 2.15 g (67%).

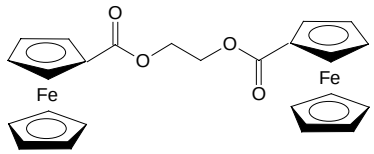
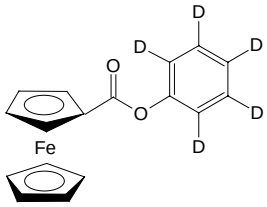
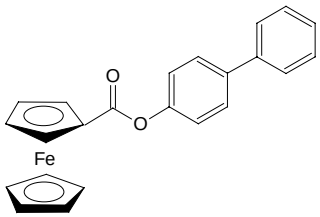
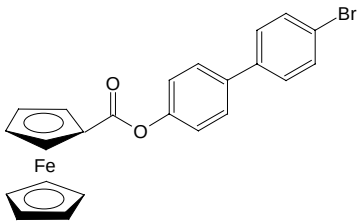
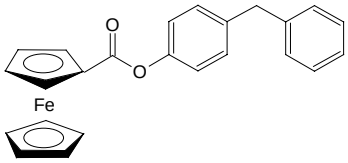
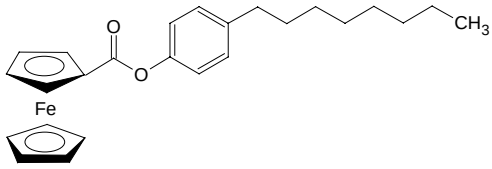
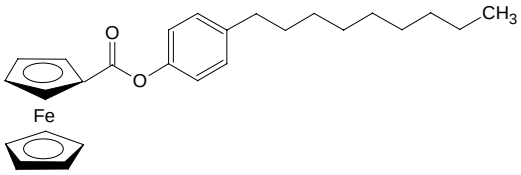
3.3.3 Synthesis of Standards

The derivatives were prepared according to a modification of previously published procedures by Rolfes and Andersson [9, 10]. 50.0 mg (0.2 mmol) FCC and 73.3 mg (0.6 mmol) DMAP were dissolved in 2 ml dichloromethane and added to a solution of 182 µmol alcohol or phenol in 2 ml dichloromethane. The mixture was left to react until the dark red coloration weakened. The DMAP and the excess FCC were removed by filtration on a microcolumn (50 mm × 5 mm i.d. of aluminium oxide). The ferrocenecarboxylic acid esters (FCEs) were eluted with 5 ml of dichloromethane. The solvent was evaporated in a gentle stream of nitrogen. The yield of the orange product was not determined. Table 3.1 shows the standards that were prepared according to this procedure, their molecular and their monoisotopic masses as well as their chemical structures.

Table 3.1: Molecular mass (M), monoisotopic mass (M_i) and chemical structure of the synthesized ferrocenecarboxylic ester (FCE) standards.

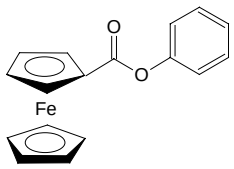
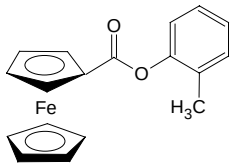
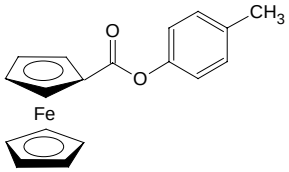
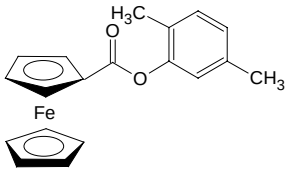
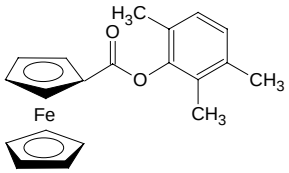
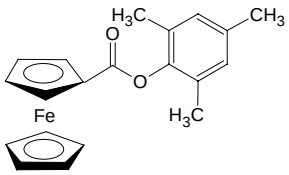
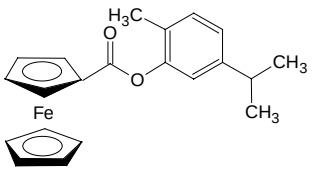
Name	**M [g/mol]**	**M_i [g/mol]**	**Chemical structure**
Methyl FCE	244.07	244.0	
Ethyl FCE	258.10	258.0	
1-Propyl FCE	272.13	272.1	

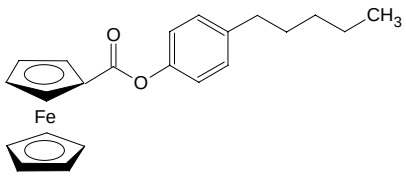
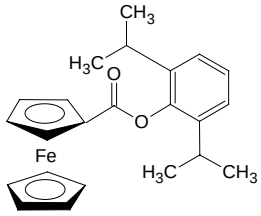
2-Propyl FCE	272.13	272.1	
1-Butyl FCE	286.15	286.1	
2-Butyl FCE	286.15	286.1	
t-Butyl FCE	286.15	286.1	
1-Methylcyclopropyl FCE	284.14	284.1	
1-Pentyl FCE	300.18	300.1	
2-Pentyl FCE	300.18	300.1	
Cyclohexyl FCE	312.19	312.1	
1-Octyl FCE	342.26	342.1	
1-Octadecyl FCE	482.53	482.3	

Glycol diFCE	486.13	486.0	
d_5-Phenyl FCE	311.14	311.0	
Biphenyl FCE	382.24	382.1	
4-Bromo-4'-biphenyl FCE	461.14	460.0	
Benzylphenyl FCE	396.27	396.1	
4-n-Octylphenyl FCE	418.36	418.2	
4-n-Nonylphenyl FCE	432.39	432.2	

Further alkylphenyl FCE standards were kindly provided by F. Wasinski, J. Rolfes and J. T. Andersson from the University of Münster (Münster, Germany). A survey of the employed standards which were provided is given in table 3.2.

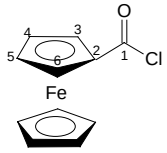
Table 3.2: Molecular mass (M), monoisotopic mass (M_i) and chemical structure of the ferrocene-carboxylic ester (FCE) standards provided by F. Wasinski, Rolfes and J.T. Andersson from the University of Münster, Germany.

Name	**M [g/mol]**	**M_i [g/mol]**	**Chemical structure**
Phenyl FCE	306.14	306.0	
2-Cresol FCE	320.17	320.1	
4-Cresol FCE	320.17	320.1	
2,5-Dimethylphenyl FCE	334.20	334.1	
2,3,6-Trimethylphenyl FCE	348.22	348.1	
2,4,6-Trimethylphenyl FCE	348.22	348.1	
Thymol FCE	362.25	362.1	

4-Pentylphenyl FCE	376.27	376.1	
2,6-Diisopropylphenyl FCE	390.28	390.1	

3.3.4 Characterization of Reagent and Standards

3.3.4.1 Ferrocenecarboxylic acid chloride

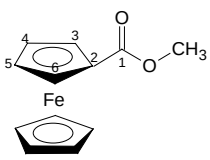


IR ($\tilde{\nu}$/cm^{-1}, KBr): 3111 (w), 3078 (w), 3050 (w), 3012 (w), 2960 (w), 2889 (w), 2756 (w), 2629 (w), 2554 (w), 1751 (m), 1658 (s), 1474 (s), 1435 (w), 1399 (w), 1370 (w), 1286 (s), 1244 (m), 1159 (m), 1107 (w), 1049 (w), 1029 (m), 1005 (w), 936 (m), 915 (w), 830 (m), 782 (m), 742 (w), 694 (w), 598 (w), 564 (w), 509 (m), 487 (m).

^{1}H-NMR (δ/ppm, 300 MHz, $CDCl_3$): 4.35 (s, 5H, $C_5\mathit{H}_5$); 4.66 (dd, 2H, 4-H, 5-H); 4.94 (dd, 2H, 3-H, 6-H).

EI-MS (m/z (%), 70 eV): 248 (100) $[M]^+$; 213 (19) $[M\text{-}Cl]^+$; 185 (16) $[M\text{-}COCl]^+$; 183 (29); 156 (61) $[M\text{-}C_6H_4O]^+$; 129 (8); 121 (7) $[C_5H_5Fe]^+$; 92 (66); 64 (6); 56 (11) $[Fe]^+$.

3.3.4.2 Methyl ferrocenecarboxylic acid ester

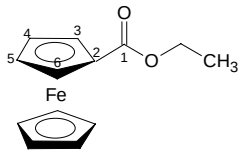


IR ($\tilde{\nu}$/cm^{-1}, KBr): 3086 (w), 2996 (w), 2952 (w), 1711 (s), 1700 (s), 1465 (s), 1429 (w), 1408 (w), 1395 (w), 1374 (m), 1281 (s), 1191 (m), 1139 (s), 1104 (m), 1049 (w), 1025 (m), 998 (m), 962 (m), 898 (w), 871 (w), 839 (w), 822 (m), 772 (m), 595 (w), 533 (m), 505 (m), 485 (m), 461 (m).

^{1}H-NMR (δ/ppm, 300 MHz, $CDCl_3$): 3.80 (s, 3H, OC*H$_3$*); 4.20 (s, 5H, C_5*H$_5$*); 4.38 (dd, 2H, 4-H, 5-H); 4.80 (dd, 2H, 3-H, 6-H).

EI-MS (m/z (%), 70 eV): 244 (100) $[M]^+$; 213 (8) $[M\text{-}OCH_3]^+$; 185 (6) $[M\text{-}CO_2CH_3]^+$; 152 (53) $[M\text{-}C_6H_4O]^+$; 150 (26); 129 (11); 121 (34) $[C_5H_5Fe]^+$; 92 (9); 81 (6); 64 (6); 56 (25) $[Fe]^+$.

3.3.4.3 Ethyl ferrocenecarboxylic acid ester

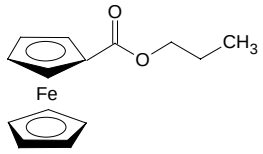


IR ($\tilde{\nu}$/cm^{-1}, KBr): 3111 (w), 2962 (w), 2901 (w), 1695 (s), 1603 (m), 1537 (w), 1519 (w), 1457 (m), 1412 (w), 1377 (m), 1277 (s), 1224 (m), 1170 (m), 1134 (s), 1106 (m), 1092 (w), 1067 (w), 1032 (m), 1001 (m), 988 (m), 945 (w), 916 (w), 858 (w), 847 (w), 825 (m), 807 (m), 778 (m), 755 (w), 535 (m), 501 (m), 486 (m), 467 (m).

^{1}H-NMR (δ/ppm, 300 MHz, $CDCl_3$): 1.36 (t, 3H, OCH_2C*H$_3$*); 4.20 (s, 5H, C_5*H$_5$*); 4.32 (q, 2H, OC*H$_2$*CH_3); 4.38 (dd, 2H, 4-H, 5-H); 4.81 (dd, 2H, 3-H, 6-H).

EI-MS (m/z (%), 70 eV): 258 (100) $[M]^+$; 230 (93) $[M\text{-}C_2H_4]^+$; 213 (7) $[M\text{-}OCH_2CH_3]^+$; 185 (8) $[M\text{-}CO_2CH_2CH_3]^+$; 165 (6); 164 (6); 138 (38); 129 (16); 121 (31) $[C_5H_5Fe]^+$; 94 (5); 56 (17) $[Fe]^+$.

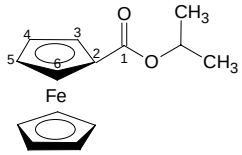
3.3.4.4 1-Propyl ferrocenecarboxylic acid ester



IR ($\tilde{\nu}$/cm^{-1}, KBr): 3092 (w), 2966 (w), 1710 (s), 1646 (w), 1598 (m), 1459 (m), 1375 (w), 1274 (s), 1227 (w), 1135 (s), 1107 (w), 1056 (w), 1025 (w), 1001 (w), 945 (w), 913 (w), 821 (m), 773 (m), 536 (w), 482 (m), 444 (m).

EI-MS (m/z (%), 70 eV): 272 (100) $[M]^+$; 230 (55) $[M\text{-}C_3H_6]^+$; 213 (4) $[M\text{-}OCH_2CH_2CH_3]^+$; 185 (2) $[M\text{-}CO_2CH_2CH_2CH_3]^+$; 165 (2); 138 (13); 129 (3); 121 (34) $[C_5H_5Fe]^+$; 56 (2) $[Fe]^+$.

3.3.4.5 2-Propyl ferrocenecarboxylic acid ester

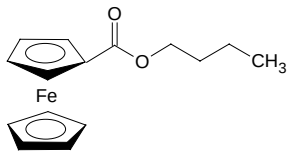


IR ($\tilde{\nu}$/cm^{-1}, KBr): 3095 (w), 2980 (m), 2931 (w), 1709 (s), 1601 (m), 1520 (w), 1459 (m), 1413 (w), 1376 (m), 1278 (s), 1225 (m), 1181 (w), 1146 (s), 1108 (s), 1066 (w), 1026 (w), 988 (m), 934 (m), 824 (m), 807 (m), 775 (w), 750 (w), 540 (w), 503 (m), 486 (m), 449 (w).

^{1}H-NMR (δ/ppm, 300 MHz, $CDCl_3$): 1.33 (d, 6H, OCH(CH_3)$_2$); 4.20 (s, 5H, C_5H_5); .4.38 (dd, 2H, 4-H, 5-H); 4.80 (dd, 2H, 3-H, 6-H); 5.17 (m, 1H, OC*H*(CH_3)$_2$).

EI-MS (m/z (%), 70 eV): 272 (71) $[M]^+$; 230 (100) $[M\text{-}C_3H_6]^+$; 213 (7) $[M\text{-}OCH(CH_3)_3]^+$; 185 (6) $[M\text{-}OCH(CH_3)_3]^+$; 165 (8); 138 (38); 129 (9); 121 (76) $[C_5H_5Fe]^+$; 105 (11); 94 (22); 78 (24); 66 (5), 56 (7) $[Fe]^+$.

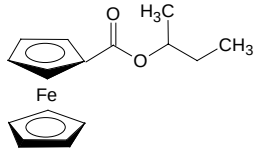
3.3.4.6 1-Butyl ferrocenecarboxylic acid ester



IR ($\tilde{\nu}$/cm^{-1}, KBr): 3091 (w), 2958 (w), 2931 (w), 2871 (w), 1711 (s), 1647 (w), 1599 (m), 1460 (m), 1376 (w), 1275 (s), 1228 (w), 1135 (s), 1059 (w), 1026 (w), 1003 (w), 943 (w), 821 (m), 774 (w), 596 (w), 502 (m), 486 (m), 425 (w).

EI-MS (m/z (%), 70 eV): 286 (100) $[M]^+$; 230 (52) $[M\text{-}C_4H_8]^+$; 213 (3) $[M\text{-}OCH_2CH_2CH_2CH_3]^+$; 185 (2) $[M\text{-}CO_2CH_2CH_2CH_2CH_3]^+$; 165 (2); 138 (12); 129 (4); 121 (14) $[C_5H_5Fe]^+$; 56 (2) $[Fe]^+$.

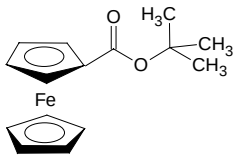
3.3.4.7 2-Butyl ferrocenecarboxylic acid ester



IR ($\tilde{\nu}$/cm^{-1}, KBr): 3096 (w), 2972 (m), 2935 (w), 2878 (w), 1708 (s), 1646 (w), 1599 (w), 1460 (m), 1377 (m), 1276 (s), 1229 (w), 1177 (w), 1145 (s), 1124 (m), 1110 (m), 1054 (w), 1026 (w), 1001 (w), 968 (w), 924 (w), 821 (m), 773 (m), 596 (w), 485 (m).

EI-MS (m/z (%), 70 eV): 286 (100) $[M]^+$; 230 (90) $[M\text{-}C_4H_8]^+$; 213 (8) $[M\text{-}OCH(CH_3)CH_2CH_3]^+$; 185 (7) $[M\text{-}CO_2CH(CH_3)CH_2CH_3]^+$; 165 (4); 138 (26); 129 (7); 121 (22) $[C_5H_5Fe]^+$; 56 (2) $[Fe]^+$.

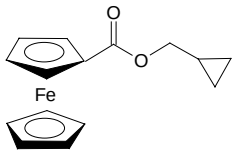
3.3.4.8 t-Butyl ferrocenecarboxylic acid ester



IR ($\tilde{\nu}$/cm^{-1}, KBr): 3093 (w), 2970 (m), 2934 (w), 2880 (w), 1709 (s), 1646 (w), 1598 (w), 1462 (m), 1375 (m), 1275 (s), 1228 (w), 1142 (s), 1124 (w), 1108 (w), 1058 (w), 1018 (w), 1000 (w), 967 (w), 922 (w), 821 (m), 773 (m), 590 (w), 485 (m).

EI-MS (m/z (%), 70 eV): 286 (2) $[M]^+$; 230 (100) $[M\text{-}C_4H_8]^+$; 185 (2) $[M\text{-}CO_2CH(CH_3)_3]^+$; 165 (12); 138 (60); 129 (3); 121 (3) $[C_5H_5Fe]^+$; 92 (4), 73 (10); 56 (7) $[Fe]^+$.

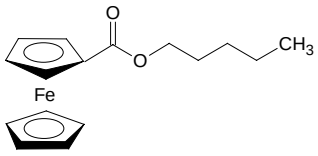
3.3.4.9 1-Methylcyclopropyl ferrocenecarboxylic acid ester



IR ($\tilde{\nu}$/cm^{-1}, KBr): 3116 (w), 3089 (w), 3016 (w), 2952 (m), 1699 (s), 1461 (s), 1399 (w), 1375 (m), 1355 (w), 1275 (s), 1224 (w), 1188 (w), 1132 (s), 1105 (m), 1055 (w), 1032 (m), 1016 (m), 1000 (w), 957 (m), 922 (w), 890 (w), 849 (w), 827 (m), 762 (w), 549 (w), 495 (m).

EI-MS (m/z (%), 70 eV): 284 (66) $[M]^+$; 230 (100) $[M\text{-}C_4H_6]^+$; 213 (4) $[M\text{-}OCH_2CH(CH_2CH_2)]^+$; 185 (5) $[M\text{-}CO_2CH_2CH(CH_2CH_2)]^+$; 165 (4); 162 (5); 138 (25); 129 (12); 121 (12) $[C_5H_5Fe]^+$; 56 (9) $[Fe]^+$.

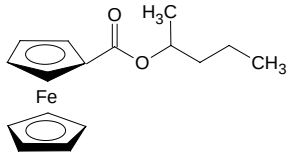
3.3.4.10 1-Pentyl ferrocenecarboxylic acid ester



IR ($\tilde{\nu}$/cm^{-1}, KBr): 3105 (w), 2955 (m), 2930 (m), 2865 (m), 1713 (s), 1462 (m), 1412 (w), 1396 (w), 1374 (m), 1353 (w), 1275 (s), 1217 (w), 1135 (s), 1105 (m), 1052 (w), 1026 (m), 1001 (w), 958 (w), 823 (m), 774 (m), 732 (w), 596 (w), 560 (w), 502 (m), 485 (m), 453 (m).

EI-MS (m/z (%), 70 eV): 300 (100) $[M]^+$; 230 (53) $[M\text{-}C_5H_{10}]^+$; 213 (3) $[M\text{-}OCH_2CH_2CH_2CH_2CH_3]^+$; 185 (2) $[M\text{-}CO_2CH_2CH_2CH_2CH_2CH_3]^+$; 165 (2); 138 (11); 129 (3); 121 (6) $[C_5H_5Fe]^+$.

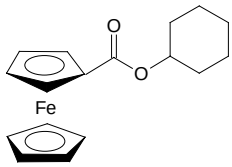
3.3.4.11 2-Pentyl ferrocenecarboxylic acid ester



IR ($\tilde{\nu}$/cm^{-1}, KBr): 3090 (w), 2959 (w), 2931 (w), 2872 (w), 1707 (s), 1646 (m), 1600 (m), 1538 (w), 1459 (m), 1377 (m), 1277 (s), 1226 (m), 1145 (s), 1120 (m), 1064 (w), 1025 (w), 991 (w), 939 (w), 807 (m), 774 (w), 750 (w), 598 (w), 537 (m), 485 (m).

EI-MS (m/z (%), 70 eV): 300 (73) $[M]^+$; 230 (100) $[M\text{-}C_5H_{10}]^+$; 213 (5) $[M\text{-}OCH_2CH_2CH_2CH_2CH_3]^+$; 185 (3) $[M\text{-}CO_2CH_2CH_2CH_2CH_2CH_3]^+$; 165 (4); 138 (17); 129 (4); 121 (25) $[C_5H_5Fe]^+$.

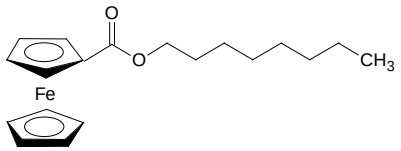
3.3.4.12 Cyclohexyl ferrocenecarboxylic acid ester



IR ($\tilde{\nu}$/cm^{-1}, KBr): 3114 (w), 2934 (m), 2855 (m), 1695 (s), 1602 (m), 1536 (w), 1518 (w), 1459 (m), 1413 (w), 1379 (m), 1317 (w), 1280 (s), 1258 (m), 1223 (m), 1139 (s), 1105 (w), 1052 (w), 1011 (w), 987 (w), 944 (m), 914 (w), 892 (w), 836 (w), 807 (m), 771 (m), 748 (w), 660 (w), 599 (w), 525 (w), 502 (m), 482 (m), 458 (w).

EI-MS (m/z (%), 70 eV): 312 (100) $[M]^+$; 230 (92) $[M\text{-}C_6H_{10}]^+$; 213 (6) $[M\text{-}OCH(CH_2CH_2CH_2CH_2CH_2)]^+$; 185 (4) $[M\text{-}CO_2CH(CH_2CH_2CH_2CH_2CH_2)]^+$; 165 (5); 138 (27); 129 (5); 121 (4) $[C_5H_5Fe]^+$; 56 (2) $[Fe]^+$.

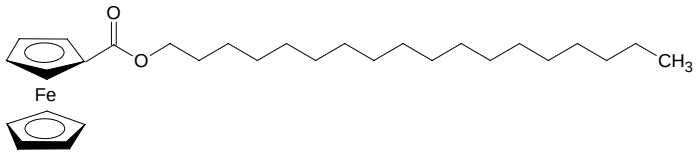
3.3.4.13 1-Octyl ferrocenecarboxylic acid ester



IR ($\tilde{\nu}$/cm^{-1}, KBr): 3094 (w), 2954 (m), 2927 (m), 2856 (m), 1714 (s), 1601 (m), 1520 (w), 1461 (m), 1412 (w), 1377 (m), 1275 (s), 1225 (m), 1138 (s), 1106 (w), 1065 (w), 1027 (w), 988 (w), 951 (w), 918 (w), 808 (m), 775 (w), 748 (w), 536 (w), 503 (m), 485 (m), 441 (w).

EI-MS (m/z (%), 70 eV): 342 (100) $[M]^+$; 230 (57) $[M\text{-}C_8H_{16}]^+$; 213 (5) $[M\text{-}OCH_2(CH_2)_6CH_3]^+$; 185 (4) $[M\text{-}CO_2CH_2(CH_2)_6CH_3]^+$; 165 (3); 138 (14); 129 (5); 121 (10) $[C_5H_5Fe]^+$.

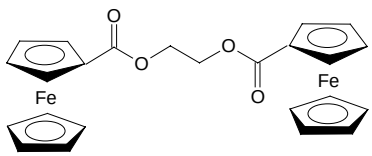
3.3.4.14 1-Octadecyl ferrocenecarboxylic acid ester



IR ($\tilde{\nu}$/cm^{-1}, KBr): 3112 (w), 3090 (w), 2953 (m), 2917 (s), 2850 (s), 1705 (s), 1603 (m), 1536 (w), 1519 (w), 1472 (m), 1456 (m), 1411 (w), 1375 (m), 1348 (w), 1274 (s), 1223 (m), 1147 (s), 1104 (w), 1067 (w), 1039 (w), 987 (m), 957 (w), 912 (w), 848 (w), 826 (m), 807 (m), 773 (m), 749 (w), 717 (w), 557 (w), 539 (w), 506 (m), 487 (m), 458 (w).

EI-MS (m/z (%), 70 eV): 482 (100) $[M]^+$; 230 (4) $[M\text{-}C_{18}H_{36}]^+$; 213 (2) $[M\text{-}OCH_2(CH_2)_{16}CH_3]^+$.

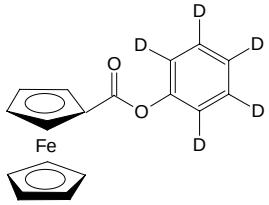
3.3.4.15 Glycol ferrocenecarboxylic acid diester



IR ($\tilde{\nu}$/cm^{-1}, KBr): 3438 (s), 2921 (w), 2852 (w), 1719 (s), 1634 (m), 1460 (m), 1378 (w), 1270 (s), 1140 (s), 1106 (m), 1029 (m), 812 (m), 774 (w), 593 (w), 536 (m), 503 (m), 485 (m).

EI-MS (m/z (%), 70 eV): 486 (100) $[M]^+$; 350 (12); 328 (4); 304 (10); 257 (57) $[M-(C_5H_5)Fe(C_5H_4CO_2)]^+$; 241 (6); 213 (5) $[(C_5H_5)Fe(C_5H_4CO)]^+$; 185 (8) $[M-(C_5H_5)Fe(C_5H_4CO_2CH_2CH_2CO_2)]^+$; 129 (7); 121 (5) $[C_5H_5Fe]^+$.

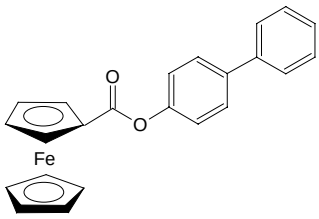
3.3.4.16 d_5-Phenyl ferrocenecarboxylic acid ester



IR ($\tilde{\nu}$/cm^{-1}, KBr): 3101 (w), 2922 (w), 2276 (w), 1722 (s), 1635 (w), 1558 (w), 1454 (m), 1411 (w), 1370 (m), 1272 (s), 1149 (s), 1102 (s), 1056 (w), 1017 (m), 1002 (w), 961 (w), 913 (m), 841 (m), 823 (m), 766 (w), 634 (w), 555 (m), 524 (w), 500 (m), 486 (m), 460 (m).

EI-MS (m/z (%), 70 eV): 311 (100) $[M]^+$; 219 (28) $[M-C_6H_4O]^+$; 213 (75) $[(C_5H_5)Fe(C_5H_4CO)]^+$; 191 (14); 185 (44) $[(C_5H_5)Fe(C_5H_4)]^+$; 129 (25); 121 (24) $[C_5H_5Fe]^+$; 70 (9); 56 (17) $[Fe]^+$.

3.3.4.17 Biphenyl ferrocenecarboxylic acid ester

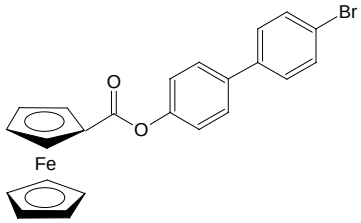


IR ($\tilde{\nu}$/cm^{-1}, KBr): 3092 (w), 3029 (w), 2995 (w), 2921 (w), 2824 (w), 1894 (w), 1724 (s), 1603 (m), 1535 (w), 1514 (m), 1483 (m), 1448 (m), 1412 (w), 1375 (m), 1347 (w), 1273 (m), 1224 (m), 1197 (s), 1163 (m), 1100 (s), 1069 (w), 1029 (w), 1005 (w), 987

(m), 943 (w), 910 (w), 868 (w), 831 (m), 806 (m), 764 (m), 702 (w), 529 (w), 502 (m), 482 (m), 457 (w).

EI-MS (m/z (%), 70 eV): 382 (100) $[M]^+$; 290 (12) $[M\text{-}C_6H_4O]^+$; 262 (5); 219 (28); 213 (78) $[(C_5H_5)Fe(C_5H_4CO)]^+$; 185 (19) $[(C_5H_5)Fe(C_5H_4)]^+$; 141 (4); 129 (9); 121 (8) $[C_5H_5Fe]^+$; 56 (2) $[Fe]^+$.

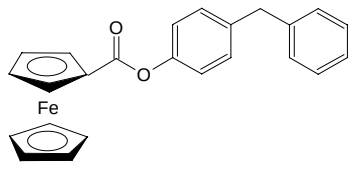
3.3.4.18 4-Bromo-4'-biphenyl ferrocenecarboxylic acid ester



IR ($\tilde{\nu}$/cm^{-1}, KBr): 3086 (w), 3031 (w), 2994 (w), 2901 (w), 2824 (w), 1926 (w), 1896 (w), 1733 (m), 1602 (s), 1536 (m), 1516 (m), 1480 (m), 1449 (s), 1375 (m), 1347 (w), 1269 (s), 1223 (s), 1200 (s), 1166 (m), 1103 (s), 1069 (m), 1029 (w), 999 (m), 987 (s), 943 (w), 913 (m), 807 (s), 764 (w), 748 (w), 626 (w), 529 (m), 514 (m), 485 (m), 458 (w).

EI-MS (m/z (%), 70 eV): 460 (100) $[M]^+$; 368 (5) $[M\text{-}C_6H_4O]^+$; 247 (3); 213 (68) $[(C_5H_5)Fe(C_5H_4CO)]^+$; 185 (28) $[(C_5H_5)Fe(C_5H_4)]^+$; 139 (9); 129 (16); 121 (8) $[C_5H_5Fe]^+$; 56 (4) $[Fe]^+$.

3.3.4.19 Benzylphenyl ferrocenecarboxylic acid ester

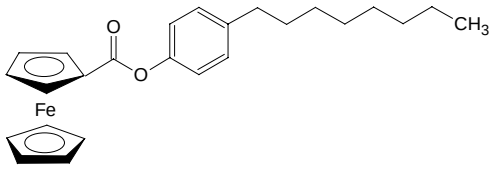


IR ($\tilde{\nu}$/cm^{-1}, KBr): 3081 (w), 3026 (w), 2924 (w), 1913 (w), 1808 (w), 1717 (s), 1602 (m), 1508 (m), 1450 (m), 1414 (w), 1374 (m), 1298 (w), 1270 (s), 1198 (s), 1168 (m), 1105 (s), 1023 (m), 987 (w), 944 (w), 913 (w), 857 (w), 842 (w), 821 (m), 805 (m),

778 (w), 761 (w), 730 (m), 697 (m), 596 (w), 555 (w), 524 (w), 501 (m), 485 (m), 453 (m).

EI-MS (m/z (%), 70 eV): 396 (100) $[M]^+$; 304 (9) $[M\text{-}C_6H_4O]^+$; 290 (12); 238 (5); 213 (67) $[(C_5H_5)Fe(C_5H_4CO)]^+$; 185 (17) $[(C_5H_5)Fe(C_5H_4)]^+$; 129 (8).

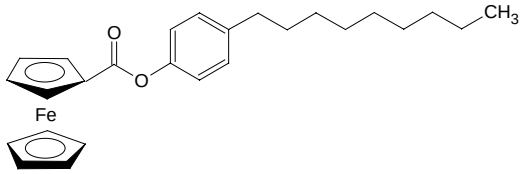
3.3.4.20 4-n-Octylphenyl ferrocenecarboxylic acid ester



IR ($\tilde{\nu}$/cm^{-1}, KBr): 3116 (w), 3091 (w), 3034 (w), 2969 (w), 2947 (w), 2923 (m), 2851 (m), 1905 (w), 1717 (s), 1602 (m), 1509 (m), 1467 (w), 1449 (m), 1414 (w), 1376 (m), 1270 (s), 1196 (s), 1169 (m), 1105 (s), 1022 (w), 1004 (w), 987 (w), 941 (w), 914 (w), 845 (w), 823 (m), 805 (m), 763 (w), 717 (w), 572 (w), 529 (m), 497 (m), 456 (w).

EI-MS (m/z (%), 70 eV): 418 (100) $[M]^+$; 326 (4) $[M\text{-}C_6H_4O]^+$; 227 (10); 213 (65) $[(C_5H_5)Fe(C_5H_4CO)]^+$; 185 (11) $[(C_5H_5)Fe(C_5H_4)]^+$; 129 (5).

3.3.4.21 4-n-Nonylphenyl ferrocenecarboxylic acid ester



IR ($\tilde{\nu}$/cm^{-1}, KBr): 3116 (w), 3084 (w), 3051 (w), 3032 (w), 2954 (m), 2921 (s), 2849 (m), 1901 (w), 1803 (w), 1716 (s), 1603 (m), 1536 (w), 1509 (m), 1466 (w), 1451 (s), 1413 (w), 1376 (m), 1349 (w), 1272 (s), 1223 (m), 1207 (s), 1169 (s), 1110 (s), 1067 (w), 1034 (m), 1019 (m), 1002 (w), 987 (w), 942 (w), 917 (m), 848 (m), 827 (m), 795 (m), 763 (m), 720 (w), 595 (w), 528 (m), 515 (m), 500 (m), 483 (m), 459 (w).

EI-MS (m/z (%), 70 eV): 432 (100) $[M]^+$; 340 (3) $[M\text{-}C_6H_4O]^+$; 227 (13); 213 (56) $[(C_5H_5)Fe(C_5H_4CO)]^+$; 185 (16) $[(C_5H_5)Fe(C_5H_4)]^+$; 129 (7).

3.4 CONCLUSION

The derivatizing agent ferrocenecarboxylic acid chloride (FCC) was used to synthesize several different alcohol and phenol standard derivatives that were characterized by IR, EI-MS and ^{1}H-NMR.

3.5 REFERENCES

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4. LC/ELECTROCHEMISTRY/MS OF FERROCENECARBOXYLIC ACID ESTER STANDARDS

4.1 SUMMARY

The first hyphenation of liquid chromatography, electrochemical on-line oxidation and mass spectrometry is described. Ferrocenecarboxylic acid esters of various alcohols and phenols have been separated by reversed-phase LC and were subsequently oxidized (ionized) "coulometrically" prior to single quadrupole MS analysis using electrospray (ES) ionization and atmospheric pressure chemical ionization (APCI) interfaces. The dependency of the ionization on the electrochemical pretreatment is demonstrated. Limits of detection for selected derivatives range from 3 nM to 0.4 μM depending on the individual compound and the selected interface.

4.2 INTRODUCTION

The hyphenation of liquid chromatography and mass spectrometry 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]. Owing to increased robustness of the instrumentation, LC/MS has become a widely used analytical technique in research and routine analysis [1].

However, some problems are still remaining which are mainly caused by the difficulty of coupling a separation taking place in the 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 (ES) ionization and atmospheric pressure chemical ionization (APCI) [1, 2]. LC/MS measurements with ES 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 ES ionization and APCI. Ionization typically occurs by protonation or deprotonation, but coordination of the analyte with other ions may also be used [3]. Analytes of lower polarity are less accessible to the ES or APCI [4] processes, thus resulting in low ionization efficiencies and losses in sensitivity. The scope of LC/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, several attempts for the efficient ionization of less polar analytes have been reported (see chapter 2 for details). Cole et al. used the electrospray interface for the electrochemical oxidation (ionization) of metallocenes [5]. Van Berkel and co-workers have reported the determination of alcohols in saw palmetto fruit extract [6] and of alcohols and phenols in the oils of cloves, lemon, rose and peppermint [7] 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 [8]. Brajter-Toth et al. used a combination of an electrochemical cell and particle beam mass spectrometry [9]. The coupling of electrochemistry and thermospray-MS was applied by Brajter-Toth et al. for oxidative studies on uric acid [10]. Another approach suggested by van Berkel et al. was the on-line coupling of different electrochemical flow-cells with ES-MS, either floated at or decoupled from the electrospray high voltage [11]. Although the coupling of an electrochemical flow cell with MS gave promising results, no attempts for using this system after LC separation have been reported yet. This might be due to the limited compatibility of the selected conditions for electrospray with the commonly used LC conditions.

Since the derivatization of alcohols [6, 7] and phenols [12, 13] 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, a new LC/electrochemistry/MS technique for the determination of ferrocene derivatives is proposed [14].

4.3 EXPERIMENTAL

4.3.1 Chemicals

Ammonium formate, tetrabutylammoniumhexafluorophosphate, 4-(*N*,*N*-dimethyl-amino)pyridine 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 LC was acetonitrile LiChroSolv gradient grade from Merck (Darmstadt, Germany).

The derivatizing agent ferrocenecarboxylic acid chloride was synthesized according to Rolfes and Andersson [12] with slight modifications (see section 3.3).

4.3.2 Instrumentation

4.3.2.1 Electrochemical Instrumentation

Cyclic voltammetry was performed on a Perkin Elmer 263A potentiostat with Powersuite software. A three electrode configuration was employed. The electrodes used were a glassy carbon working electrode, a platinum wire counter electrode and an Ag/AgCl (in 3 M NaCl solution) reference electrode.

The electrochemical system from ESA, Inc. (Chelmsford, MA, USA) which was used for on-line LC/electrochemistry/MS consisted of a GuardStat potentiostat and a model 5021 conditioning cell. The conditioning cell contains a porous glassy carbon "coulometric" working electrode, a Pd counter electrode, and a Pd/H_2 reference electrode. The utilized electrochemical conditioning cell is characterized by a low void volume and the large surface area of the porous glassy carbon working electrode which allows quantitative oxidation or reduction reactions (figure 4.1). The term "coulometric" is used by the manufacturer because of the quantitative electrochemical conversion. The electrochemically correct term for the cell would rather be an amperometric cell with quantitative conversion because no measurement of charge is carried out. Therefore, the term "coulometric" will be used in this thesis in inverted commas to emphasize that there is no typical coulometry involved.

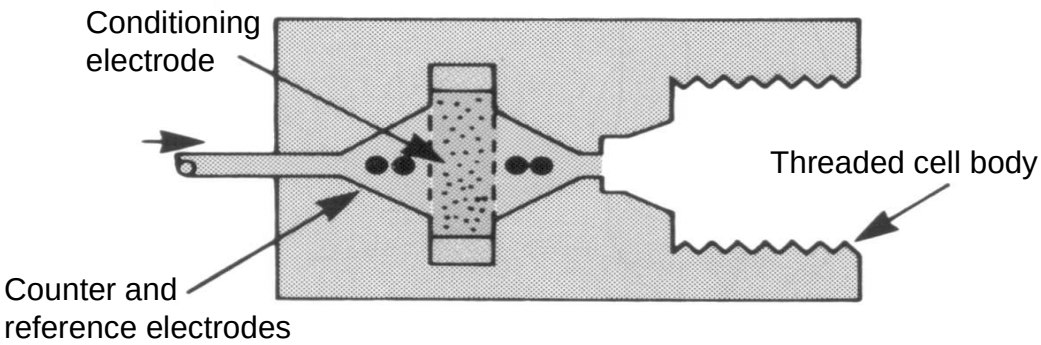

Fig. 4.1 Schematic drawing of the "coulometric" flow cell that was used for on-line LC/electrochemistry/MS experiments (from [14]). The cell contains a porous glassy carbon working electrode, a Pd counter electrode, and a Pd/H_2 reference electrode. The large surface are of the working electrode allows quantitative oxidation or reduction reactions.

4.3.2.2 LC-MS Instrumentation

The LC-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 (ES) ionization and atmospheric pressure chemical ionization (APCI) probes and Class 8000 Version 1.20 software.

4.3.2.3 LC/Electrochemistry/MS setup

For the hyphenation of the existing LC-MS system with on-line electrochemistry, a "coulometric" flow cell that was controlled via a potentiostat was inserted between the outlet of the UV/vis detector and the inlet of the ionization interface of the mass spectrometer (figure 4.2).

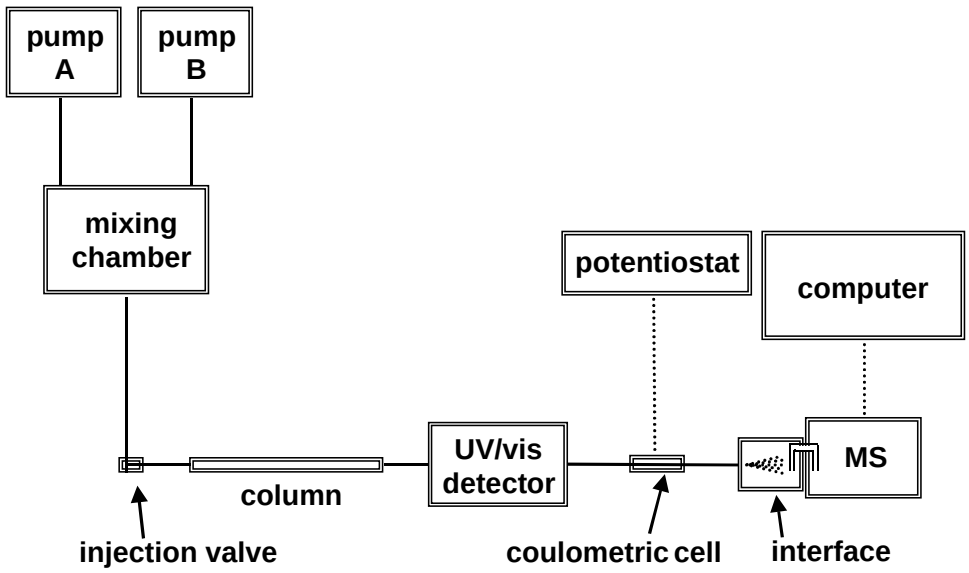

Fig. 4.2 Schematic assembly of the LC/electrochemistry/MS system.

The connection between the flow cell and the interface was kept as short as possible to reduce peak broadening and loss of ions during transport. To prevent electrical connection between interface and "coulometric" cell via the eluent, appropiate ground connection had to be assured as discussed by van Berkel et al. [11]. This was accomplished by replacing part of the PEEK connection between flow cell and MS with a stainless steel capillary that was linked to a stainless steel union which was in contact with the grounded part of the mass spectrometer.

4.3.3 Synthesis of Ferrocenecarboxylic Acid Esters (FCEs)

The derivatives were synthesized according to a modification of a procedure by Rolfes and Andersson [12, 13]. The procedure is described in detail in section 3.3. Further alkylphenyl FCE standards were kindly provided by F. Wasinski, J. Rolfes and J. T. Andersson from the University of Münster (Münster, Germany).

4.3.4 Conditions for Cyclic Voltammograms

2-5 mg of ferrocenecarboxylic acid ester standard and 387 mg of the supporting electrolyte tetrabutylammoniumhexafluorophosphate were solved in 10 ml of dry acetonitrile to form a 1 mM solution of the analyte. After 5 min of stirring under an argon atmosphere, the stirrer was turned off and cyclic voltammograms were recorded with a scan speed of 100 mV/s. Starting at 0 mV, the potential was raised to 1500 mV, lowered to -500 mV and brought back to the starting potential of 0 mV.

4.3.5 LC Conditions

Since the ES interface tolerates only LC flow rates of 0.3 ml/min or less and the APCI interface works best with flow rates of at least 0.6 ml/min, columns of different inner diameters and different LC flow rates and injection volumes had to be used for optimum performance. All separations were carried out 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 ES 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. Different gradients at flow rates of 0.3 ml/min (2.1 mm id column for ES ionization) and 0.6 ml/min (3.0 mm id column for APCI) with the following profiles were used:

Gradient A (injection volume 10 µl)

t [min]	0.01	1	14	14.5	17	18	22
c(CH_3CN) [%]	65	65	75	90	90	65	stop

Gradient B (injection volume 10 µl)

t [min]	0.01	3	8	18	20	25	25.5
c(CH_3CN) [%]	60	60	90	90	60	60	stop

Gradient C (injection volume 5 µl)

t [min]	0.01	3	8	23	25	28	28.5
c(CH_3CN) [%]	60	60	90	90	60	60	stop

4.3.6 MS Conditions

Conditions A: APCI as heated nebulizer

When using the APCI interface as a heated nebulizer, the following parameters were used: nebulizer gas flow 2.5 l/min, APCI temperature 350 °C, APCI probe voltage 0 V, curved desolvation line (CDL) temperature 300 °C, CDL voltage –35 V, deflector voltages +35 V, detector voltage 1.7 kV.

Conditions B: Conventional APCI

When using the APCI interface for classical ionization without electrochemical pretreatment, the following parameters were used: nebulizer gas flow 2.5 l/min, APCI temperature 500 °C, APCI probe voltage +5.0 kV, CDL temperature 300°C, CDL voltage -35 V, deflector voltages +37.5 V, detector voltage 1.7 kV.

Conditions C: APCI as heated nebulizer II

When using the APCI interface as a heated nebulizer with a very small probe voltage, the following parameters were used: nebulizer gas flow 2.5 l/min, APCI temperature 350 °C, APCI probe voltage 0.10 kV, CDL temperature 300 °C, CDL voltage –35 V, deflector voltages +35 V, detector voltage 1.7 kV.

Conditions D: ES with electrochemical pretreatment

When using the ES interface after on-line electrochemistry, the following parameters were used: nebulizer gas flow 4.5 l/min, ES probe voltage 2.5 kV, CDL temperature 300 °C, CDL voltage –35 V, deflector voltages +35 V, detector voltage 1.7 kV.

4.4 RESULTS AND DISCUSSION

4.4.1 Cyclic Voltammetry

To study the electrochemical behaviour of the ferrocenecarboxylic acid esters, cyclic voltammetry was performed in acetonitrile. As a test analyte for the alcohol derivatives, methyl FCE was chosen. The redox system shows a reversible one-electron oxidation of the ferrocene derivative to the ferrocinium cation (figure 4.3). The half-wave potential for methyl FCE is found to be 700 mV vs. Ag/AgCl.

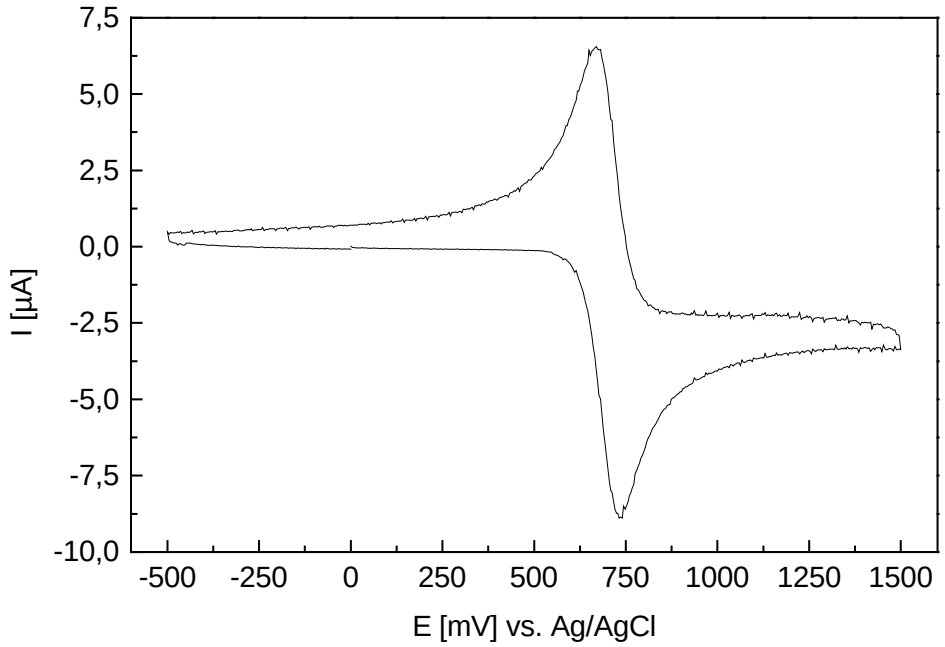

Fig. 4.3 Cyclic voltammogram of methyl FCE in dry acetonitrile.

The cyclic voltammogram of the test analyte for the phenol derivatives, phenyl FCE, also demonstrated the reversibility of the oxidation (figure 4.4). The half-wave potential of phenyl FCE is 742 mV vs. Ag/AgCl. As it can be expected for the aromatic esters because of the inductive effect of the aromatic ring, it is larger than the half-wave potential of the aliphatic methyl FCE.

Since both groups of ferrocene derivatives can be oxidized at rather low positive potentials, it seems to be possible to use both as analytes for a hyphenation of electrochemistry with LC-MS.

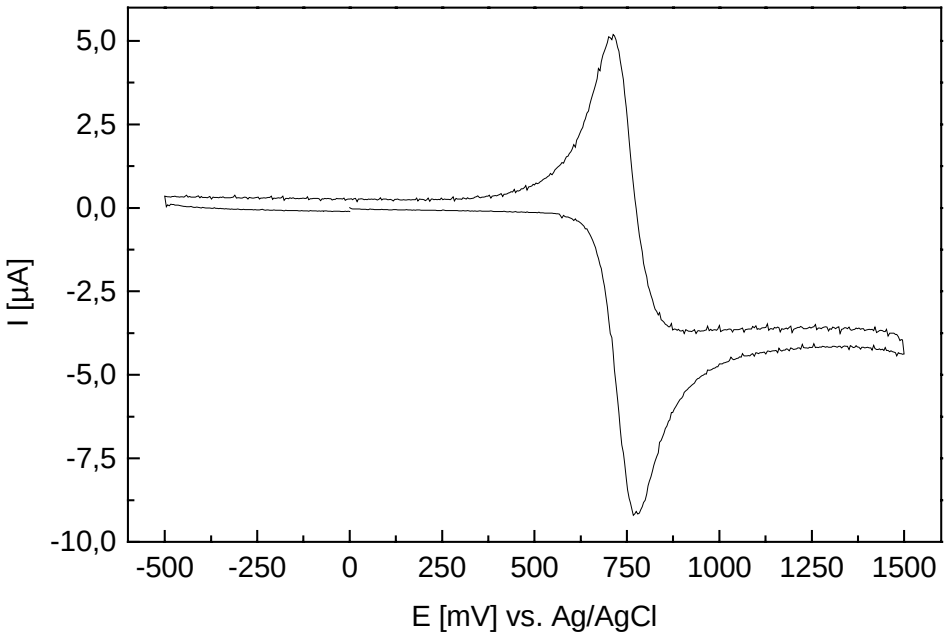

Fig. 4.4 Cyclic voltammogram of phenyl FCE in dry acetonitrile.

4.4.2 Electrochemistry/MS

To show the successful oxidation of the ferrocene function of the FCE standards, it was necessary to first record a mass spectrum using classical ionization conditions. This was carried out in the positive APCI mode for 4-n-nonylphenyl FCE with MS conditions B and without the electrochemical flow cell (figure 4.5). As expected, the base peak of the spectrum at m/z = 433 corresponds to the protonated analyte. No fragmentation was observed.

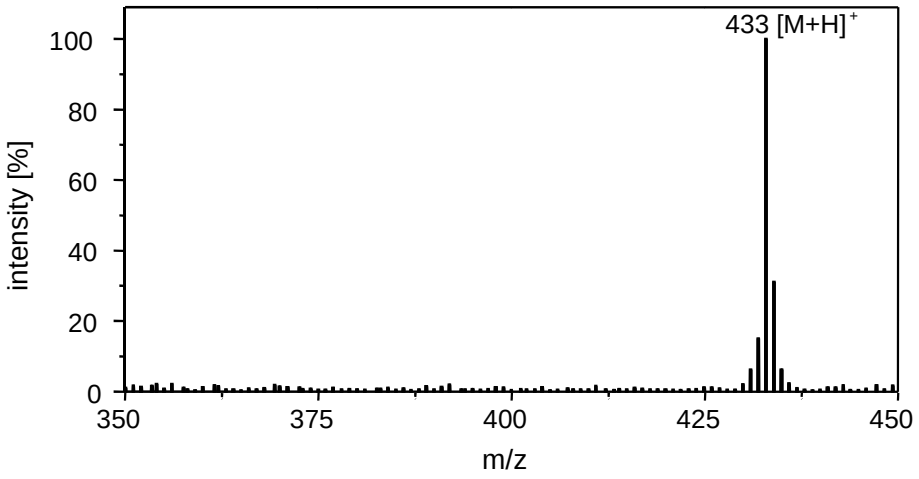

Fig. 4.5 APCI(+) mass spectrum of a 10 µM solution of 4-n-nonylphenyl FCE (no electrochemical cell, APCI probe voltage = 5.0 kV).

The same 4-n-nonylphenyl FCE solution was then analyzed by electrochemistry/ mass spectrometry employing the "coulometric" flow cell at a cell potential of 700 mV vs. Pd/H_2, thus ensuring electrochemical oxidaton of Fe(II) in the ferrocene function to Fe(III) as shown in figure 4.6.

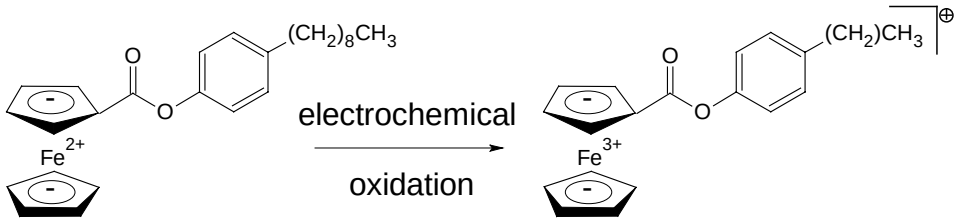

Fig. 4.6 Electrochemical oxidation of 4-n-nonylphenyl FCE to the corresponding ferrocinium radical cation.

Since the electrochemical oxidation results in preformed ions, it is unnecessary to further ionize the analyte. The ions only have to be transferred to the gas phase to enable mass spectrometric detection. Therefore, the APCI probe voltage was set to 0 V for the next experiment to ensure that the ions which are observed in the mass 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 a heated nebulizer interface (MS conditions A). This experiment was not possible with the ES interface, because the spraying process of electrospray depends on a high voltage at the ES capillary. The base peak in the spectrum of m/z = 432 corresponds to the molecular ion of 4-n-nonylphenyl FCE (figure 4.7). Again, no fragmentation could be observed.

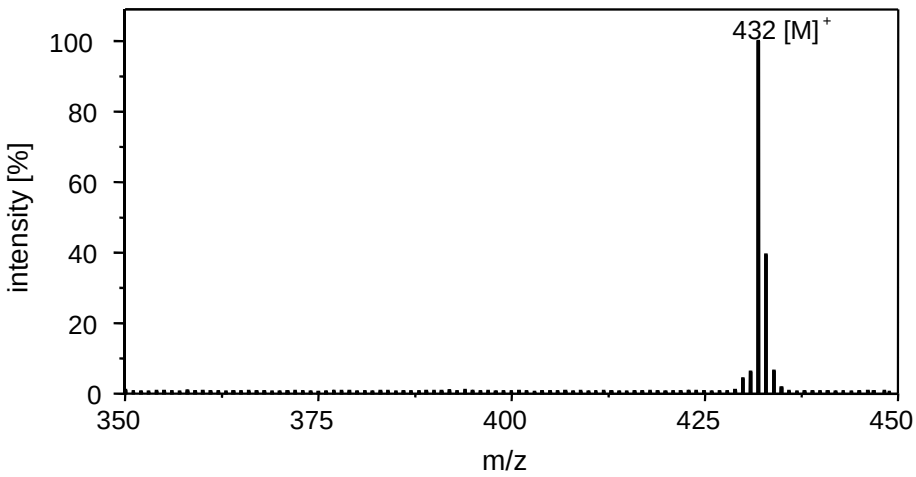

Fig. 4.7 Electrochemistry/APCI(+) mass spectrum of a 10 µM solution of 4-n-nonylphenyl FCE (cell voltage = 700 mV vs. Pd/H_2, APCI probe voltage = 0 V).

The appearance of the molecular ion peak shows that the oxidation of the Fe(II) in the ferrocene function to the Fe(III) in the corresponding ferrocinium ion was successfully accomplished by electrochemical oxidation in the "coulometric" flow cell. Comparing this spectrum to the mass spectrum that was obtained under conventional APCI conditions (figure 4.5), it can be clearly seen that there is an increase in the signal to noise ratio when using the on-line electrochemical oxidation. This raises expectations for a good sensitivity of the method, as it will be demonstrated later.

Figure 4.8 shows an electrochemistry/MS spectrum of methyl FCE using the electrospray interface and MS conditions D.

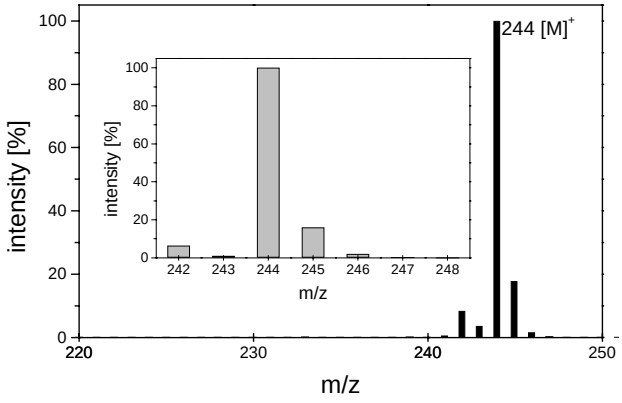

Fig. 4.8 Electrochemistry/ES(+) mass spectrum of a 10 µM solution of methyl FCE (cell voltage = 700 mV vs. Pd/H_2, ES voltage = +2.5 kV); inserted: calculated isotope pattern for $C_{12}H_{12}O_2Fe$.

The base peak of the spectrum at m/z = 244 corresponds to the molecular ion of methyl FCE. The isotope pattern in the spectrum is in accordance with the calculated isotope pattern. This demonstrates that in addition to the APCI interface in the heated nebulizer mode, the ES interface can additionally be used for electro-chemistry/mass spectrometry.

4.4.3 Optimization of the Signal Intensity

4.4.3.1 Optimization of the Interface Parameters

Before a chromatographic method was established, the interface parameters of the APCI interface were optimized for optimum sensitivity. 4-Methylphenyl FCE (p-cresol FCE), 2,5-dimethylphenyl FCE (2,5-DMP FCE), 2,4,6-trimethylphenyl FCE (2,4,6-TMP FCE) and 2-i-propyl-5-methylphenyl FCE (thymol FCE) were selected as test analytes. A mixture of these four alkylphenyl FCEs was separated by reversed-phase liquid chromatography employing gradient A and determined by electrochemistry/MS. The integrated peak areas were plotted against the interface parameters APCI temperature, APCI probe voltage and CDL temperature.

The APCI temperature was varied from 250 °C to 450 °C. Figure 4.9 shows curves that have maxima between 375 °C and 400 °C for the low molecular weight standards p-cresol FCE and 2,5-DMP FCE and curves which have maxima at approximately 325 °C for 2,4,6-TMP FCE and thymol FCE that possess a larger molecular weight.

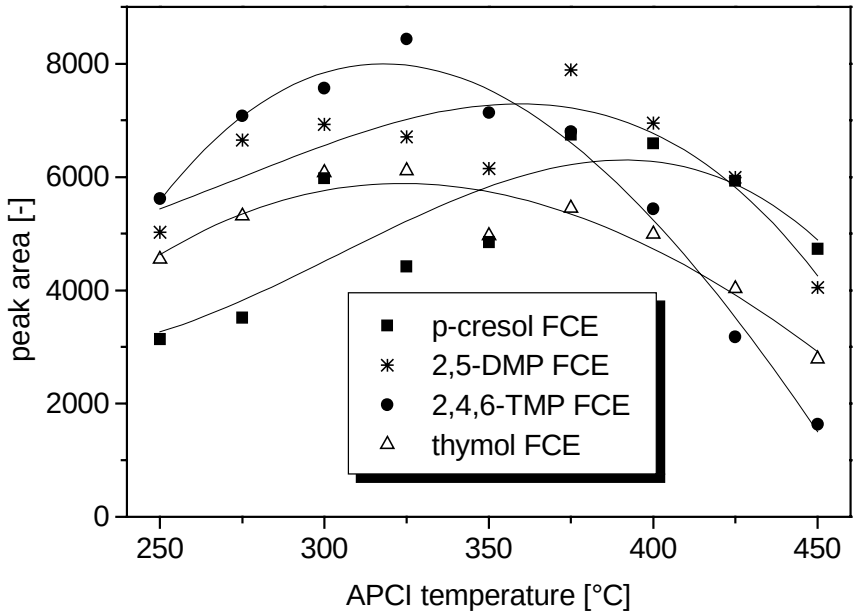

Fig. 4.9 Dependency of the peak areas of p-cresol FCE, 2,5-DMP FCE, 2,4,6-TMP FCE and thymol FCE on the APCI temperature.

This can be explained by two effects. On the one hand, a high APCI temperature improves the spray formation at the APCI capillary tip. Since a good nebulization is very important for an efficient ion transport into the mass spectrometer, a higher temperature should result in a larger signal. On the other hand, a high APCI temperature also means that more energy is transferred to the analyte molecules while they are in the capillary. This can be a problem in the case of unsufficient stability of the analytes. The larger alkylphenyl FCEs are thermally not as stable as the alkylphenyl FCEs of lower molecular weight. This explains that their maximum peak area is obtained at lower APCI temperatures. As a compromise for further LC/electrochemistry/MS experiments with mixtures of all kinds of FCE standards, an APCI temperature of 350 °C will be used.

The effect of the variation of the APCI probe voltage on the peak areas of the FCE standards contained in the test mixture is demonstrated in figure 4.10. All examined FCE standards show a similar behaviour when the APCI probe voltage is varied between 0 V and 0.25 kV.

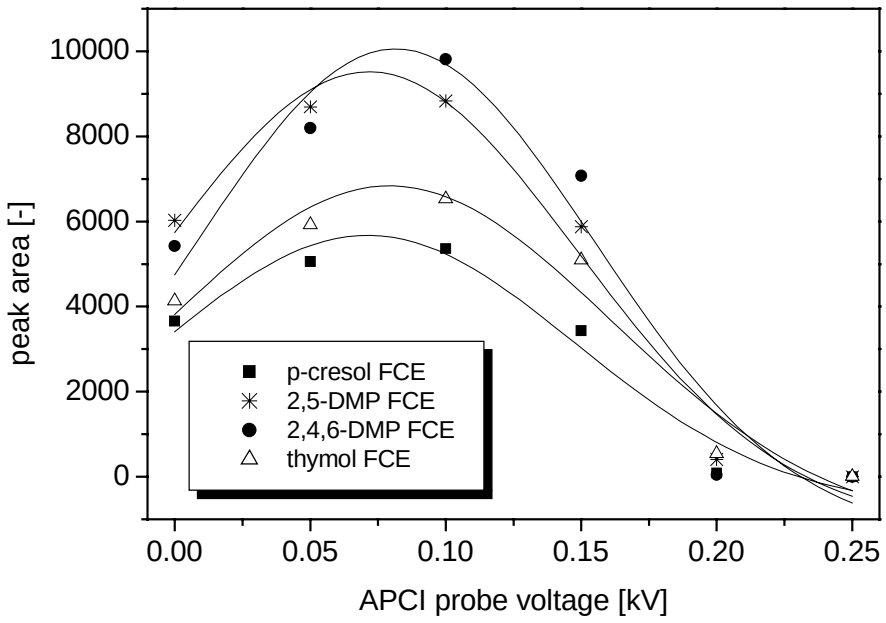

Fig. 4.10 Dependency of the peak areas of p-cresol FCE, 2,5-DMP FCE, 2,4,6-TMP FCE and thymol FCE on the APCI probe voltage.

The maximum peak area for all alkylphenol derivatives is found at an APCI probe voltage of 0.10 kV. The increases in the peak areas between mesurements with a

voltage of 0 V and a voltage of 0.10 kV are between 45% and 80%. A possible explanation for this unexpected phenomenon is that the electrochemical oxidation in the flow cell was not quantitative and that unoxidized analyte is ionized at the APCI needle resulting in higher peak areas at 0.10 kV than at 0 V probe voltage. But this does not seem feasible considering the low probe voltage of 0.10 kV that normally should not result in a coronar discharge at the APCI needle. Also, if this was the case, there should not be a drop of the peak areas in the further run of the peak area/probe voltage curve. Nonetheless, for further LC/electrochemistry/MS experiments an APCI probe voltage of 0.10 kV was used. At higher voltages, the peak areas decrease which may be contributed to the decomposition of the preformed ions which are not sufficiently stable when subject to further energy transfer.

The variation of the CDL temperature was performed in the range from 250 °C to 300 °C. The effect of this variation is shown in figure 4.11. For all examined FCE standards, a linear behaviour can be observed.

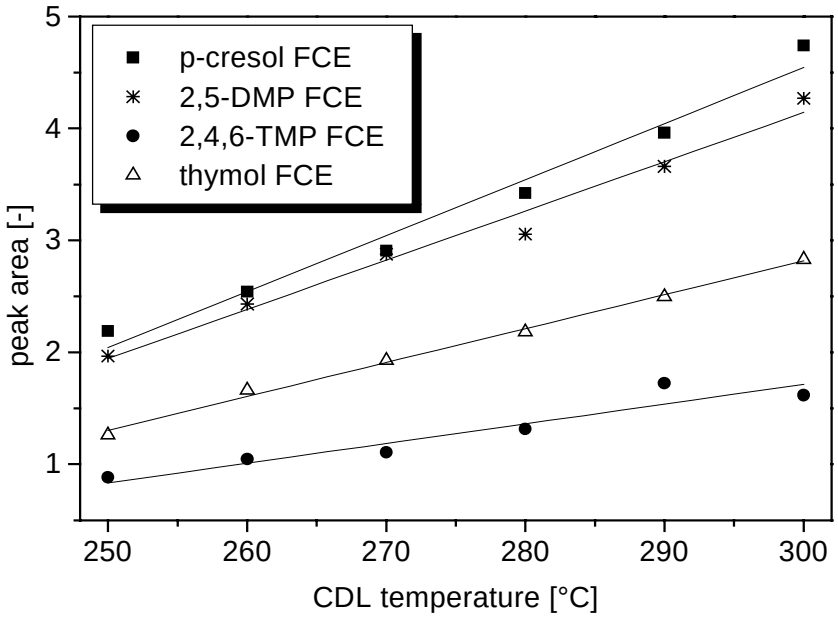

Fig. 4.11 Dependency of the peak areas of p-cresol FCE, 2,5-DMP FCE, 2,4,6-TMP FCE and thymol FCE on the CDL temperature.

The signal intensity increases at higher CDL temperatures for all of the four studied standards. This is consistent with the theory that the condensation or adsorption of

the ions in the CDL region decreases at elevated temperatures thus allowing more ions to reach the mass analyzer. The optimum performance was observed at 300 °C which is the highest possible temperature that can be set for the CDL using this particular instrument.

4.4.3.2 Optimization of the Cell Potential

The reference electrode of the "coulometric" flow cell is a Pd/H_2 electrode. The potential of this pseudo reference electrode strongly depends on the pH and the composition of the eluent that flows through the cell. It is therefore difficult to compare absolute values of half-wave potentials obtained by cyclic voltammetry with potential settings of the "coulometric" flow cell during electrochemistry/MS. Additionally, cyclic voltammetry is a diffusion-controlled process whereas electrode reactions in the "coulometric" flow cell are hydrodynamic processes. It is therefore necessary to optimize the electrochemical cell potential under the regular electrochemistry/MS conditions.

A 10 µM solution of the three alcohol derivatives methyl FCE, ethyl FCE and 2-propyl FCE, the three two ring phenol derivatives biphenyl FCE, 4-bromo-4'-biphenyl FCE, benzylphenyl FCE and the two long chain alkyl phenol derivatives 4-n-octylphenyl FCE and 4-n-nonylphenyl FCE in acetonitrile was separated by means of LC employing gradient B and analyzed with electrochemistry/MS at different cell potentials utilizing the APCI interface and MS conditions A. Using the interface as a heated nebulizer, the resulting peak areas could be fully attributed to an oxidation in the flow cell. The cell potential was varied between 0 and 1000 mV vs. Pd/H_2. The resulting peak area/cell potential curves could be divided into three types. The aliphatic alcohol derivatives showed no oxidation at a cell potential of 0 and 200 mV vs. Pd/H_2 and almost constant peak areas at potentials of 400 mV and higher (figure 4.12).

In agreement with the results of the cyclic voltammetry experiments, the three two-ring phenol derivatives are oxidized at higher potentials than the alcohol derivatives (figure 4.13). Their peak area/cell potential curves show an optimum cell potential of 600 mV vs. Pd/H_2. At higher potentials, the peak areas significantly decrease which can be explained by further oxidation of the ferrocinium product, possibly towards the aromatic system.

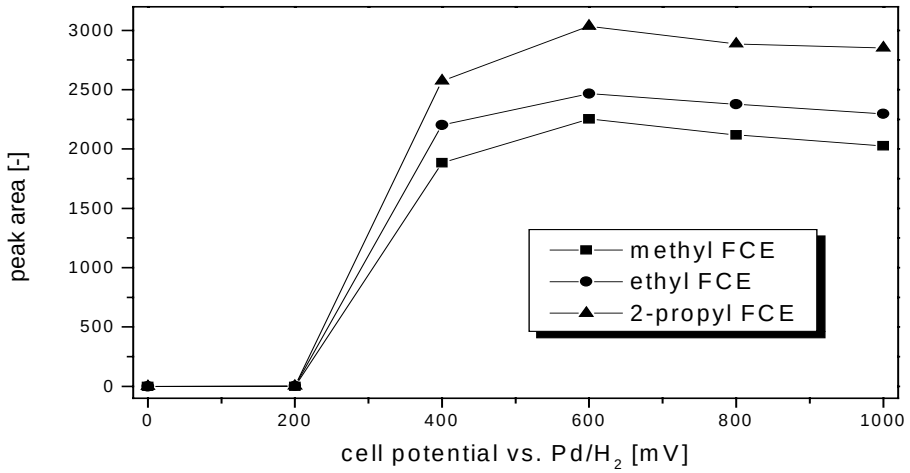

Fig. 4.12 Mass intensity/cell potential curves of the three aliphatic alcohol derivatives methyl FCE, ethyl FCE, 2-propyl FCE.

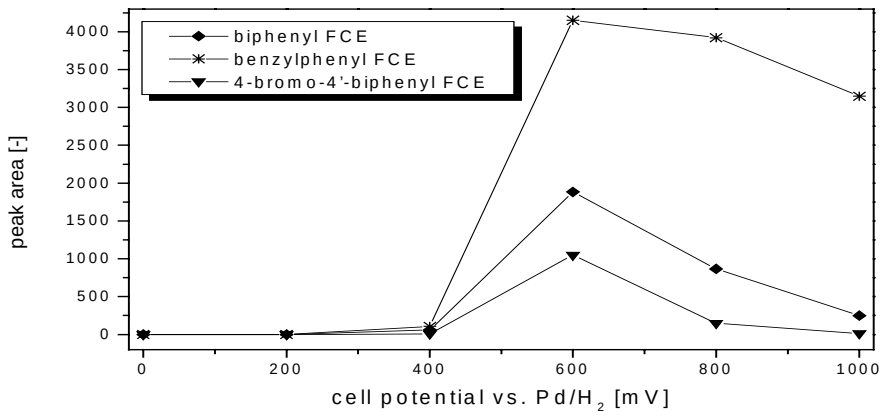

Fig. 4.13 Mass intensity/cell potential curves of the three two ring phenol derivatives biphenyl FCE, benzylphenyl FCE, 4-bromo-4'-biphenyl FCE.

The third type of peak area/cell potential curve is presented in figure 4.14. The peak areas of the long-chain alkyl phenol derivatives are largest at a cell potential of 800 mV vs. Pd/H_2 and decrease again at higher potentials.

Considering that a wide variety of both phenols and alcohols are present in typical real samples, it was concluded that the optimum cell potential for a complex mixture of alcohols and phenols is 700 mV vs. Pd/H_2.

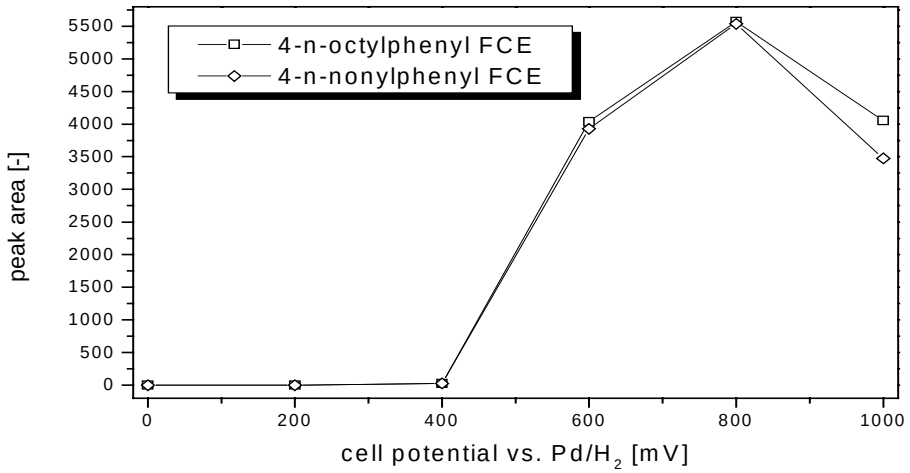

Fig. 4.14 Mass intensity/cell potential curves of the two long chain alkylphenol derivatives 4-n-octylphenyl FCE, 4-n-nonylphenyl FCE.

As a result of the optimization of APCI temperature, APCI voltage, CDL temperature and "coulometric" cell potential, the following values for these four parameters were used in most of the quantitative determinations:

Table 4.1: Optimum values for the examined parameters

APCI temperature	350 °C
APCI voltage	0.1 kV
CDL temperature	300 °C
"coulometric" cell potential	700 mV vs. Pd/H_2

4.4.4 LC/Electrochemistry/MS

When establishing a chromatographic method for LC/electrochemistry/MS, a number of considerations have to be made concerning the choice of the mobile phase. First, the analytes must be easily soluble in the eluent at all gradient concentrations. The organic content and the pH of the mobile phase have to fulfil the requirements for the selected stationary phase. Additionally, there has to be a certain electrolyte concentration in the eluent in order to guarantee high electrochemical conversion efficencies in the "coulometric" flow cell. Furthermore, it is necessary to select

electrolytes which do not strongly suppress other ions. Finally, to prevent a decrease in mass spectrometric performance due to clogging, all components of the mobile phase should be volatile.

In this work, a binary mixture of acetonitrile as organic component and a formic acid/ammonium formate buffer (20 mM, pH ≈ 3) was used for all liquid chromatographic separations. The stationary phase employed was a base deactivated ODS material. It was possible to accomplish a baseline separation of several alkylphenyl FCE standards (figure 4.15). The 10 µM mixture containing phenol and alkylphenol derivatives of alkyl chainlengths between 1 and 9 was separated using gradient C. After the on-line electrochemical oxidation and electrospray nebulization, detection was performed in the selected ion monitoring (SIM) mode with MS conditions D.

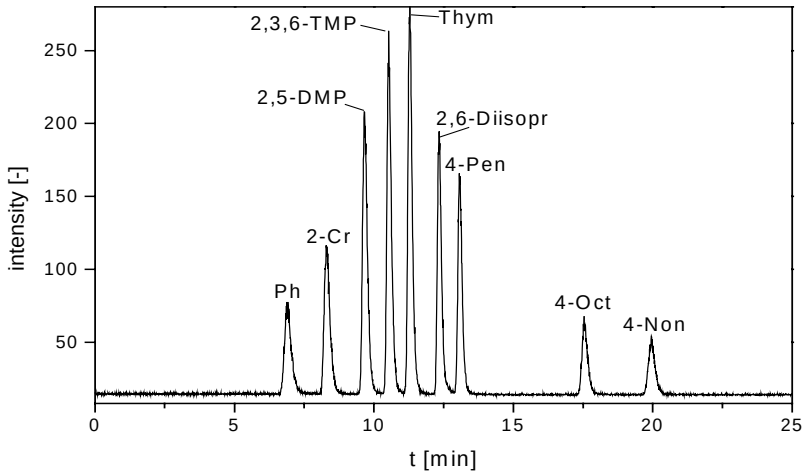

Fig. 4.15 LC/electrochemistry/ES(+)/MS chromatogram, recorded in the SIM mode (m/z = 306.0, 320.1, 334.1, 348.1, 362.1, 376.1, 390.1, 418.2, 432.2); Ph = phenyl FCE, 2-Cr = 2-cresol FCE, 2,5-DMP = 2,5-dimethylphenyl FCE, 2,3,6-TMP = 2,3,6-trimethylphenyl FCE, Thym = thymol FCE, 4-Pen = 4-pentylphenyl FCE, 2,6-Diisopr = 2,6-diisopropylphenyl FCE, 4-Oct = 4-n-octylphenyl FCE, 4-Non = 4-n-nonylphenyl FCE).

It is important to mention that it is not possible to separate all the different structural isomers of the alkylphenol derivatives with liquid chromatography. The higher plate numbers in gas chromatography allow the separation of some of the isomers. This was demonstrated for the three cresol isomers and for some of the C2 alkylphenyl FCEs in [13] employing gas chromatography with atomic emission detection.

The simultaneous separation of different alcohol and phenol derivatives utilizing gradient B is demonstrated in figure 4.16. The chromatograms (raw data, no smoothing of the peaks) of a 10 μM solution were recorded as total ion current (TIC) in SIM mode using MS conditions A. For these measurements, different potentials ranging from 0 mV to 1000 mV vs. Pd/H_2 were applied to the "coulometric" flow cell.

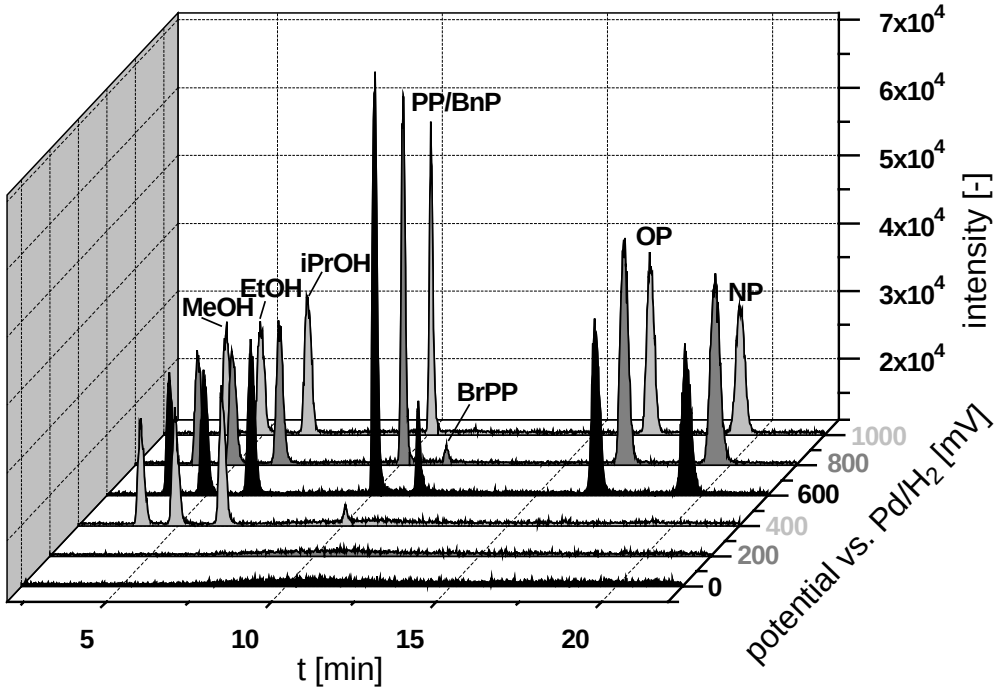

Fig. 4.16 LC/electrochemistry/APCI(+)/MS chromatograms at different "coulometric" cell potentials, recorded in the SIM mode (m/z = 244.0, 258.0, 272.1, 382.1, 396.1, 460.0, 418.2, 432.2), APCI potential 0 V (MeOH = methyl FCE, EtOH = ethyl FCE, iPrOH = 2-propyl FCE, PP = biphenyl FCE, BnP = benzylphenyl FCE, BrPP = 4-bromo-4'-biphenyl FCE, OP = 4-n-octylphenyl FCE, NP = 4-n-nonylphenyl FCE).

No peaks are detected at potentials below 400 mV. Beginning with a potential of 400 mV, molecular ions of the alcohol derivatives as well as of the coeluting biphenyl and benzylphenyl FCEs produce clearly detectable peaks. With a potential of 600 mV, all compounds in the mixture are oxidized to the corresponding ferrocinium ions and can be seen in the chromatogram. It can be observed that the peak areas of biphenyl, benzylphenyl and 4-bromo-4'-biphenyl FCE decrease at higher potentials than 600 mV.

4.4.5 Quantitative Determinations

Calibration data were recorded with the LC/electrochemistry/MS system and both the ES and the APCI interface respectively. To obtain data for APCI, gradient B and MS conditions C were employed whereas in the electrospray mode gradient C and MS conditions D were chosen. In all cases, SIM traces were integrated for quantitation purposes. The limit of detection (LOD) was determined as a signal to noise ratio of 3 and the limit of quantification (LOQ) as a signal to noise ratio of 10. The linear concentration range was derived from the calibration graphs. Since different methods are used to determine LOQ and linear range, the LOQ is higher for some derivatives than the lower limit of the linear concentration range. The relative standard deviation (RSD) for three subsequent analyses was determined at 0.5 μM for APCI and 5 μM for ES.

The calibration functions exhibited excellent linearities in the lower concentration ranges, but the peak areas in the higher concentration range were higher than expected when using the APCI mode (table 4.2). This can be explained on the basis of insufficient oxidation in the flow cell at higher concentration levels and the increased LC flow rate used for APCI. This reduces the linear concentration range for the APCI mode compared to the ES mode. For ES, linear ranges of four decades are observed for selected analytes (table 4.3).

Analytical figures of merit are also provided in table 4.2 for the APCI mode and table 4.3 for the ES mode. Obviously, ES allows slightly 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. The reproducibility of both methods ranges from 0.4% to 8.3%, except for the detection of 4-bromo-4'-biphenyl FCE in the APCI mode. A possible explanation for the large standard deviation in case of the 4-bromo-4'-biphenyl FCE are further electrochemical reaction of this derivative although these do not seem to affect the ES analysis. 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 use of 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 introducing precursor ion scan ES-MS/MS experiments of different ferrocene derivatives without prior separation [6, 7].

Table 4.2: Analytical figures of merit for selected FCEs using the APCI interface (LOD = limit of detection, LOQ = limit of quantification, RSD = relative standard deviation), detection in the SIM mode.

Analyte	**LOD** [nM]	**LOQ** [nM]	**Linear range** [nM]	**RSD** (n = 3; c = 0.5 μM)
Methyl FCE	10	30	20-3000	3.7%
Ethyl FCE	20	60	20-3000	5.2%
1-Propyl FCE	10	30	10-1000	2.0%
Phenyl FCE	10	30	10-2000	2.4%
2-Cresol FCE	10	30	20-2000	2.1%
4-Cresol FCE	10	30	10-2000	2.0%
2,5-Dimethylphenyl FCE	5	20	6-2000	0.7%
2,3,6-Trimethyphenyl FCE	6	20	10-2000	3.7%
2,4,6-Trimethylphenol FCE	8	20	10-1000	2.0%
Thymol FCE	8	25	20-2000	3.5%
4-Pentylphenyl FCE	6	20	6-1000	1.4%
2,6-Diisopropylphenyl FCE	4	15	5-1000	2.5%
Biphenyl FCE	20	60	20-9000	5.6%
4-Bromo-4'-biphenyl FCE	40	100	70-9000	24.9%
Benzylphenyl FCE	10	30	20-3000	4.7%
4-n-Octylphenyl FCE	10	30	20-9000	6.2%
4-n-Nonylphenyl FCE	10	30	20-9000	7.0%

Table 4.3: Analytical figures of merit for selected FCEs using the ES interface (LOD = limit of detection, LOQ = limit of quantification, RSD = relative standard deviation), detection in the SIM mode.

Analyte	**LOD** [nM]	**LOQ** [nM]	**Linear range** [nM]	**RSD** (n = 3; c = 5 μM)
Methyl FCE	400	1000	500-50000	4.9%
Ethyl FCE	300	900	500-100000	2.4%
Phenyl FCE	15	50	50-50000	8.3%
2-Cresol FCE	6	20	20-50000	2.1%
2,5-Dimethylphenyl FCE	5	20	20-50000	2.3%
2,3,6-Trimethylphenyl FCE	6	20	10-50000	0.4%
Thymol FCE	5	20	10-10000	1.1%
4-Pentylphenyl FCE	6	20	10-50000	3.8%
2,6-Diisopropylphenyl FCE	4	15	10-50000	1.7%
Biphenyl FCE	10	30	10-50000	5.4%
4-Bromo-4'-biphenyl FCE	4	10	5-100000	4.8%
Benzylphenyl FCE	3	10	5-50000	4.8%
4-n-Octylphenyl FCE	4	10	5-10000	3.7%
4-n-Nonylphenyl FCE	8	20	10-50000	1.8%

4.5 CONCLUSION

A powerful new hyphenated technique based on the combination of LC, electrochemical ("coulometric") oxidation and ES- or APCI-MS has been developed. Simple and commercially available instrumentation has been used to set up the analytical system.

There are two major advantages of this technique when compared to the electrochemical oxidation in the ES interface [6, 7]. 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 ES capillary.

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

The additional coupling of LC to electrochemistry/MS adds selectivity because of the chromatographic separation. Interfering preformed ions, for example, elute before the more unpolar analytes and do not interfere or suppress the analytes mass signals.

Further improvement of the method can be expected when using triple quadrupole mass spectrometers and tandem MS techniques. Van Berkel et al. have successfully demonstrated the possibility of precursor ion scan ES-MS of different ferrocene derivatives without prior separation [6, 7]. This will also allow to gain more mass spectrometric information which will be important in the analysis of complex real samples.

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5. FAST LIQUID CHROMATOGRAPHIC SEPARATIONS OF FERROCENECARBOXYLIC ACID ESTERS

5.1 SUMMARY

The possible use of conventional guard columns for fast LC/electrochemistry/MS of various ferrocenecarboxylic acid esters is demonstrated. Separations of mixtures containing up to nine alcohol or phenol derivatives were accomplished during a time of 1 - 1.5 min depending on the number and structure of the individual compounds. In a second part, a graphite-based in-line filter is used as a stationary phase in LC/electrochemistry/MS experiments.

5.2 INTRODUCTION

As described in chapter 4, the determination of analytes of lower polarity using the two most common ionization techniques ES and APCI may be limited by insufficient ionization [1].

Different approaches have been made to overcome this limitation of the ionization process, but only few have employed electrochemical techniques (see chapter 2 for more detail). The first on-line coupling of liquid chromatography with an electrochemical flow cell and ESI- or APCI-MS was accomplished in [9] (see also chapter 4).

In the last years, the evolution of combinatorial chemistry has caused great interest in the development of determination methods that are suitable for high sample throughput. One possible solution for this problem is to reduce the time of the analysis, in this case of the chromatographic run. Several approaches making use of columns with small inner diameter which are packed with gigaporous (perfusive, pore diameter greater than one hundreth of the particle diameter) [12, 13] or micropellicular [14, 15] stationary phases or elevated temperatures [15] have been reported [16, 17]. This study demonstrates that fast LC determinations in a time scale of approximately one minute are also possible when using LC/electro-chemistry/MS. These separations are performed on conventional guard columns.

5.3 EXPERIMENTAL

5.3.1 Chemicals

Ammonium formate was purchased from Aldrich Chemie (Steinheim, Germany). Formic acid was obtained from Fluka (Buchs, Switzerland). Organic solvent for LC was acetonitrile LiChroSolv gradient grade from Merck (Darmstadt, Germany).

5.3.2 Instrumentation

5.3.2.1 Electrochemical Instrumentation

The electrochemical system from ESA, Inc. (Chelmsford, MA, USA) which was used for on-line LC/electrochemistry/MS consisted of a GuardStat potentiostat and a model 5021 conditioning cell. The conditioning cell contains a porous glassy carbon "coulometric" working electrode, a Pd counter electrode, and a Pd/H_2 reference electrode. The utilized electrochemical conditioning cell is characterized by a low void volume and a large surface area of the porous glassy carbon working electrode which allows quantitative oxidation or reduction reactions. The potential used in this study was 0.7 V vs. Pd/H_2 (see section 4.4.3.2).

5.3.2.2 LC/MS Instrumentation

The LC/MS system from Shimadzu (Duisburg, Germany) consisted of a SCL-10Avp controller unit, DGU-14A degasser, two LC-10ADvp pumps, a low void volume mixing tee (Upchurch, Oak Harbor, WA, USA), SIL-10A autosampler, a LCMS QP8000 single quadrupole mass spectrometer with atmospheric pressure chemical ionization (APCI) probe and Class 8000 Version 1.20 software.

5.3.2.3 LC/Electrochemistry/MS Setup

For the hyphenation of the existing LC/MS system with on-line electrochemistry, a "coulometric" flow cell that was controlled via a potentiostat was inserted between the LC column and the inlet of the ionization interface of the mass spectrometer. The connection between the flow cell and the interface was kept as short as possible to minimize peak broadening and loss of ions during transport. To prevent electrical connection between interface and "coulometric" cell via the eluent, appropiate ground connection had to be assured as discussed by van Berkel et al. [8]. This was

accomplished by replacing part of the PEEK connection between flow cell and MS with a stainless steel capillary which was linked to a stainless steel union which was in contact with the grounded part of the mass spectrometer. To protect the porous working electrode from particles, PEEK in-line filters (ESA, Chelmsford, MA, USA), which are inserted between column and electrochemical cell, were used. The schematic setup of the complete system is shown in figure 5-1.

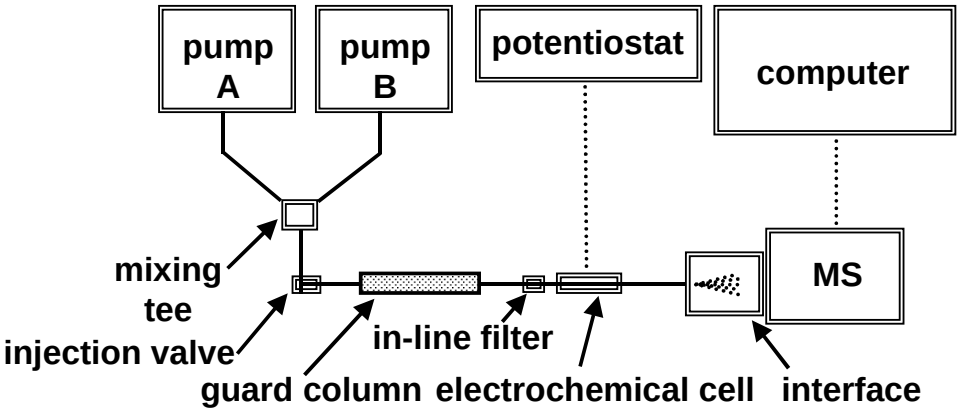

Fig. 5.1 Schematic setup of the LC/electrochemistry/MS system employed for fast separations using guard columns.

For the rapid LC/electrochemistry/MS employing graphite filter elements as stationary phases, the guard column was removed from the setup and the PEEK in-line filter element was replaced by a graphite in-line filter element.

5.3.3 Synthesis of Ferrocenecarboxylic Acid Esters

The derivatives were synthesized according to a modification of a procedure by Rolfes and Andersson [10, 11]. The procedure is described in detail in section 3.3. Further alkylphenyl FCE standards were kindly provided by F. Wasinski, J. Rolfes and J. T. Andersson from the University of Münster (Münster, Germany).

5.3.4 LC Conditions

Separations were performed using Discovery C18 guard columns (Supelco, Deisenhofen, Germany) with the following dimensions: 5 µm particle size, 100 Å pore size, 2.1 mm id, 20 mm length. 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. Different gradients with the following profiles were used:

Gradient A; injection volume 5 µl, flow rate 1.25 ml/min

t [min]	0.01	0.90	0.95	1.00
c(CH_3CN) [%]	40	80	40	stop

Gradient B; injection volume 5 µl, flow rate 1.25 ml/min

t [min]	0.01	0.80	1.00	1.20	1.25
c(CH_3CN) [%]	40	95	95	40	stop

Gradient C; injection volume 5 µl, flow rate 1.25 ml/min)

t [min]	0.01	0.90	1.00	1.10	1.25
c(CH_3CN) [%]	50	90	90	50	stop

Gradient D; injection volume 5 µl, flow rate 1.25 ml/min

t [min]	0.01	0.90	1.35	1.45	1.50
c(CH_3CN) [%]	50	90	90	50	stop

For rapid LC/electrochemistry/MS using graphite filter elements as stationary phases, the separations were performed without the guard column. The gradient with the following profile was used:

Gradient E; injection volume 2 µl, flow rate 0.6 ml/min

t [min]	0.03	0.20	2.20	3.00	3.50	4.00
c(CH_3CN) [%]	20	20	90	90	20	stop

5.3.5 MS Conditions

Employing the APCI interface as heated nebulizer with a small probe voltage, the following parameters were used: nebulizer gas flow 2.5 l/min, APCI temperature

375 °C, APCI probe voltage 0.10 kV, CDL temperature 300 °C, CDL voltage –35 V, deflector voltages +35 V, detector voltage 1.7 kV, sampling rate 10 Hz.

5.4 RESULTS AND DISCUSSION

5.4.1 Fast LC/Electrochemistry/MS of Alcohol Derivatives

To demonstrate that LC/electrochemistry/APCI(+)-MS can also be performed with fast LC separations, a 10 µM mixture of different alkyl ferrocenecarboxylic acid esters of chain lengths C1 to C5 in acetonitrile was analyzed utilizing different gradients. The optimum gradient was found to be gradient A. Figure 5-2 shows the resulting TIC chromatogram. The separation in figure 5-2 was accomplished in just 1 min. Since the first analyte methyl FCE elutes at 17 s, there is still a lot of space in the chromatogram for more polar interferences that elute before the methyl FCE.

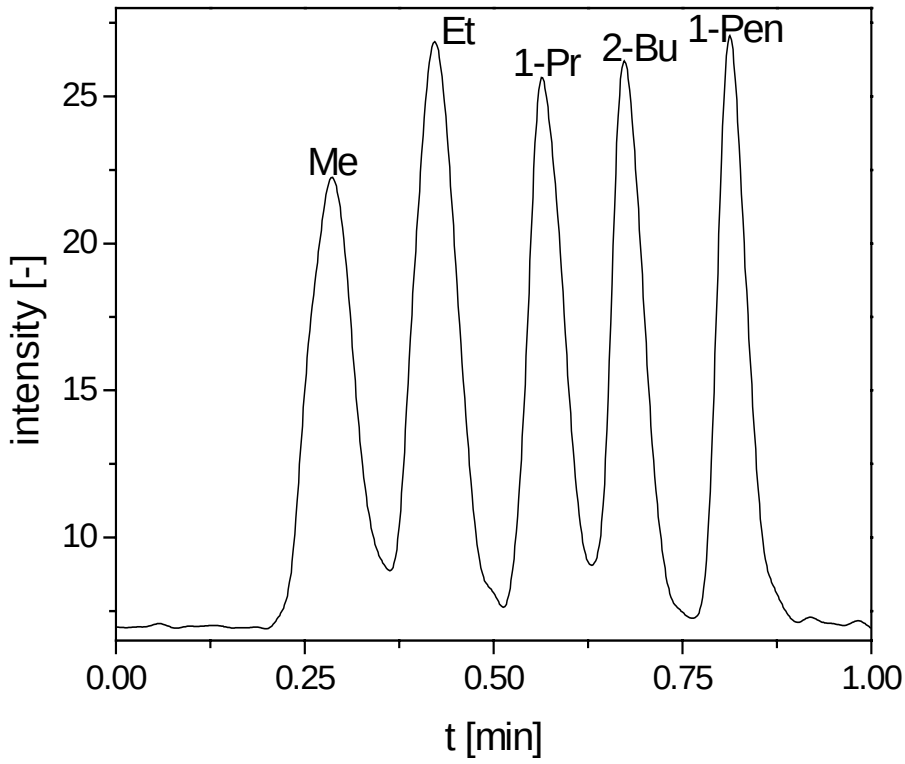

Fig. 5.2 LC/electrochemistry/APCI(+)-MS TIC chromatogram, recorded in SIM mode (m/z = 244.0, 258.0, 272.1, 286.1, 300.1); Me = methyl FCE, Et = ethyl FCE, 1-Pr = 1-propyl FCE, 2-Bu = 2-butyl FCE, 1-Pen = 1-n-pentyl FCE).

Although no baseline separation could be achieved, the separation is sufficient for quantification even in the TIC. The possible plotting of SIM traces adds further selectivity for qualitative of quantitative determinations.

The same mixture of alkohol derivatives but now additionally containing the 1-n-octanol derivative, was separated using the slightly different gradient B and electrochemistry/APCI(+)-MS (figure 5-3).

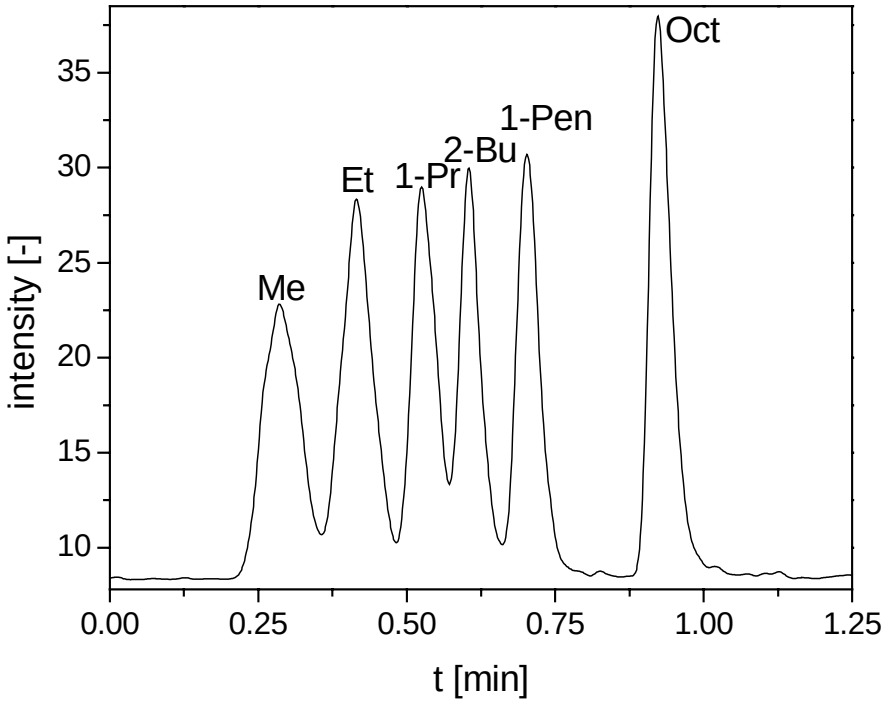

Fig. 5.3 LC/electrochemistry/APCI(+)-MS TIC chromatogram, recorded in SIM mode (m/z = 244.0, 258.0, 272.1, 286.1, 300.1, 342.1); Me = methyl FCE, Et = ethyl FCE, 1-Pr = 1-propyl FCE, 2-Bu = 2-butyl FCE, 1-Pen = 1-n-pentyl FCE, Oct = 1-n--octyl FCE).

As figure 5-3 demonstrates, the separation could be performed in less than 1.25 min. Again, no baseline separation was accomplished, but although the separation efficiency was not as good as in the chromatogram of figure 5-2 it is still sufficient for quantification in TIC or SIM traces.

5.4.2 Fast LC/Electrochemistry/MS of Phenol Derivatives

Since it was possible to employ fast LC/electrochemistry/MS for the determination of several alkohol derivatives, the same approach was used for the determination of different phenol derivatives.

A 10 µM solution of the alkylphenyl derivatives of phenol, 2-cresol, 2,5-dimethylphenol, 2,3,6-trimethylphenol, thymol, 4-n-pentylphenol and 2,6-diisopropylphenol in acetonitrile was separated using gradient C in less than 1.25 min (figure 5-4).

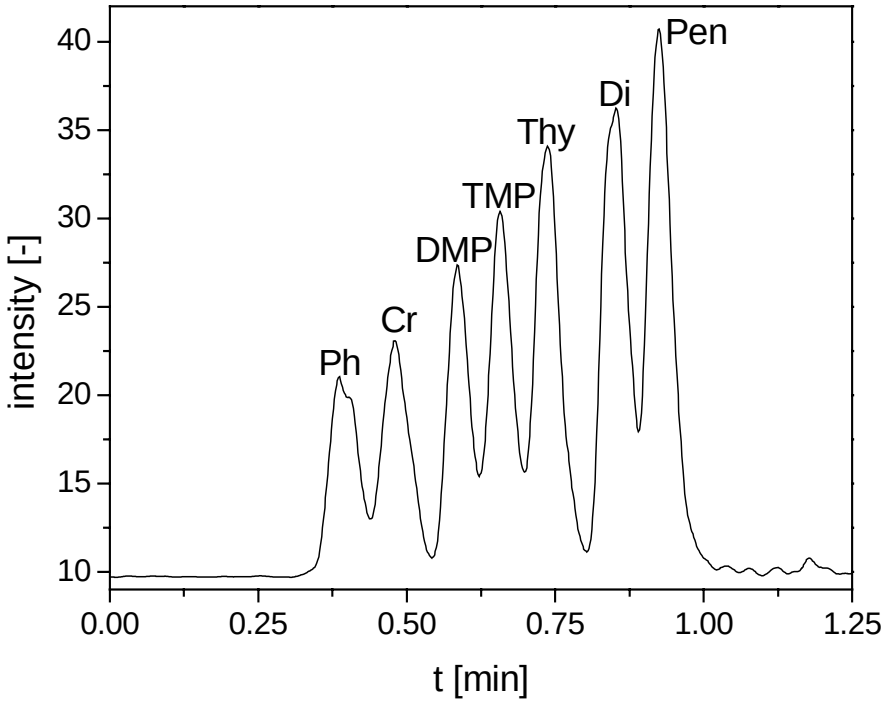

Fig. 5.4 LC/electrochemistry/APCI(+)-MS chromatogram, recorded in SIM mode (m/z = 306.0, 320.1, 334.1, 348.1, 362.1, 376.1, 390.1); Ph = phenyl FCE, Cr = 2-cresol FCE, DMP = 2,5-dimethylphenyl FCE, TMP = 2,3,6-trimethylphenyl FCE, Thy = thymol FCE, Di = 2,6-diisopropylphenyl FCE, Pen = 4-n-pentylphenyl FCE).

As it can be expected because of its more spherical shape, 2,6-diisopropylphenyl FCE elutes before the 4-pentylphenol derivative. As in the previous examples, no baseline separation was achieved. Nonetheless, the peak shape and separation allow the easy identification and quantification when using the SIM traces.
If even more unpolar analytes have to determined, the gradient has to be kept at a high acetonitrile concentration for a longer time. An example for this is shown in figure 5-5.

The mixture of alkylphenol derivatives that was analyzed with fast LC/electrochemistry/APCI(+)-MS also included the long chain 4-n-octylphenyl FCE and 4-n-nonylphenyl FCE. Even including these unpolar substances, an efficient

separation of the 9 standards could be accomplished in less than 1.5 min. In the chromatogramm, it can be seen that there is a gap between the 4-n-pentylphenol and the 4-n-octylphenol derivative caused by a large variation in the polarity of these two compounds.

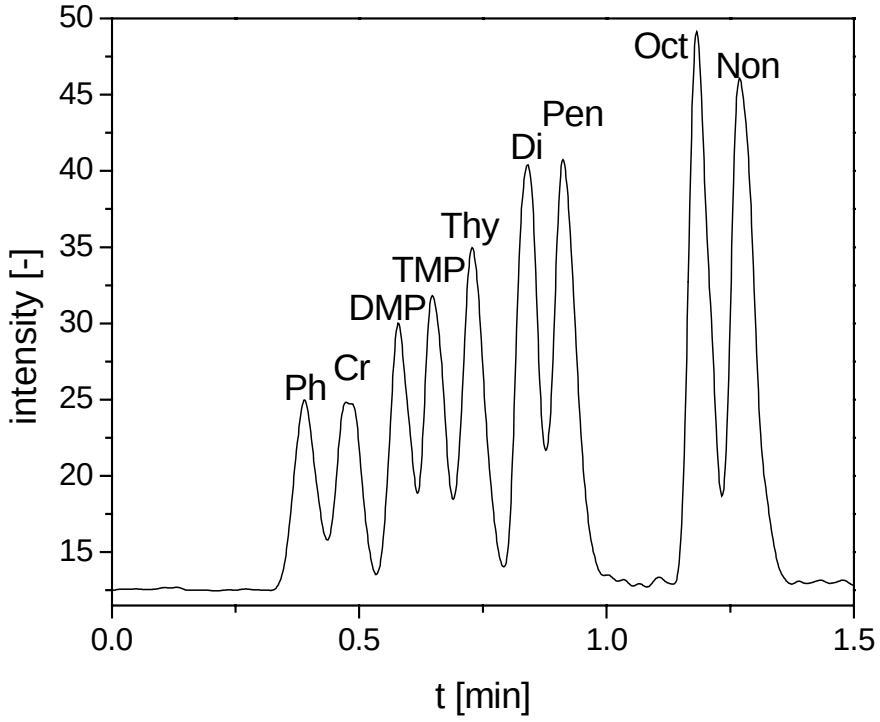

Fig. 5.5 LC/electrochemistry/APCI(+)-MS chromatogram, recorded in SIM mode (m/z = 306.0, 320.1, 334.1, 348.1, 362.1, 376.1, 390.1, 418.2, 432.2); Ph = phenyl FCE, Cr = 2-cresol FCE, DMP = 2,5-dimethylphenyl FCE, TMP = 2,3,6-trimethylphenyl FCE, Thy = thymol FCE, Di = 2,6-diisopropylphenyl FCE, Pen = 4-n-pentylphenyl FCE, Oct = 4-n-octylphenyl FCE, Non = 4-n-nonylphenyl FCE).

The sensitivity of the fast LC/electrochemistry/MS analysis of both the alcohol and the phenol derivatives was comparable to the sensitivity of the LC/electrochemistry/MS determinations using standard length analytical columns (section 4, [9]).

5.4.3 Rapid LC/Electrochemistry/MS using Graphite Filter Elements as Stationary Phases

During work with the conventional (0.5 ml mixing chamber, standard analytical columns of 150 mm length; see section 4 and [9]) LC/electrochemistry/MS system,

when using a UV/vis detector connected in series between the chromatographic column and the electrochemical cell, a considerable time delay between the UV and the MS signal was noticed which could not be traced back to the additional void volume caused by inserting the electrochemical cell between UV detector and mass spectrometer. This time delay increased from the derivatives of the short chain length alcohols to those of the larger phenols like n-nonylphenol. Further investigations have been undertaken to explain this effect and to use it in a LC/electrochemistry/MS system without a dedicated chromatographic column.

To investigate if the observed separation is due to retention on the glassy carbon material of the electrochemical cell or on the graphite material of the in-line filter, the graphite filter material was exchanged by PEEK material which is available from the same manufacturer. Under these conditions, all derivatives elute almost within the void volume, indicating that the graphite material is responsible for the retention.

Different lots of the graphite filters were used to test the reproducibility of the separation. Due to their asymmetric peak shape the retention times of the of very early eluting substances varied by up to 15% between filter elements and up to 6% for different injections on the same filter element. The retention times of several well retained analytes varied only slightly between the filter elements (less than 3%) and for for different injections on the same filter element (less than 1%), thus encouraging experiments with respect to their use as stationary phases. Although the material is characterized by a thickness of only 1 mm, experiments to use the filter instead of a chromatographic column were carried out in the following.

The SIM traces of one of the resulting APCI-MS chromatograms using a graphite in-line filter and gradient E are shown in figure 5-6. It can be seen that although the peak shape is not optimal, a separation of some of the FCEs can be accomplished.

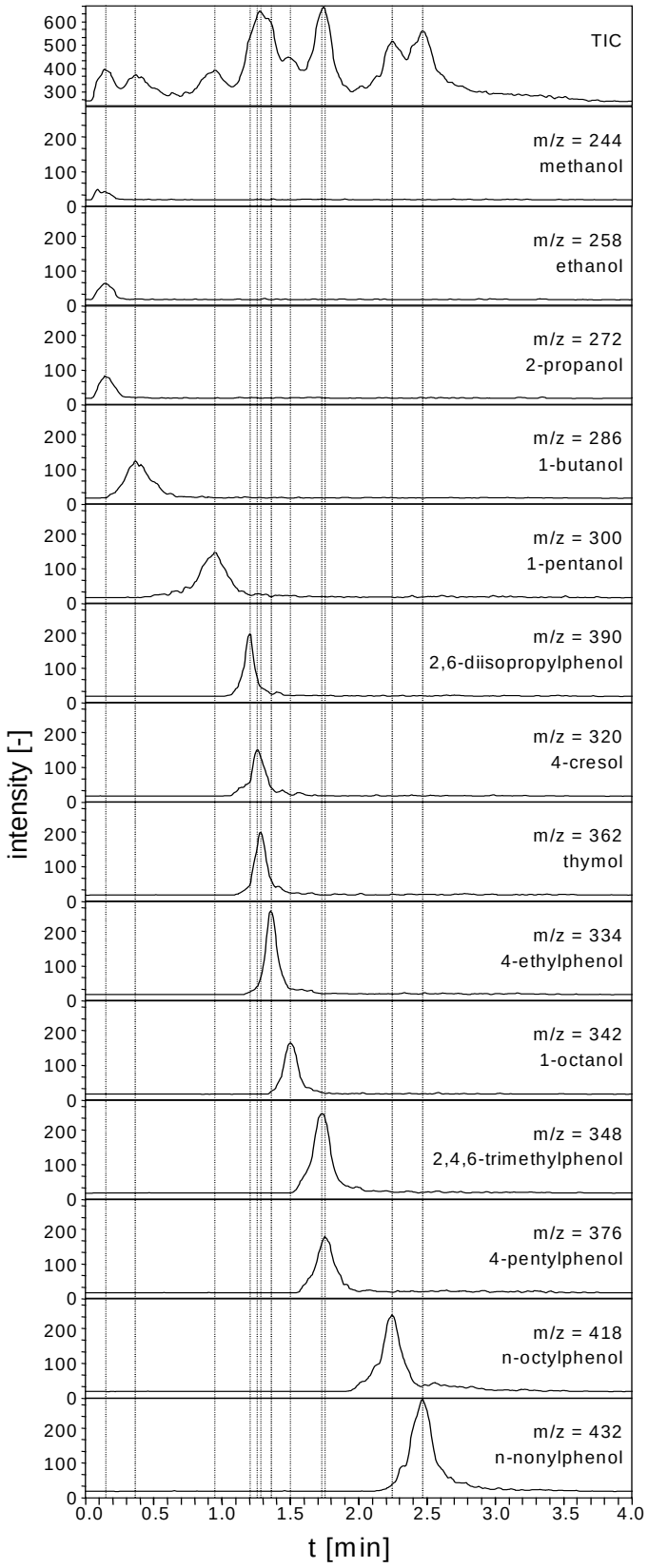

Fig. 5.6 Graphite in-line filter based separation of the FCE derivatives of selected alcohols and phenols employing LC/electrochemistry/APCI-MS, recorded in SIM mode.

The FCEs of the alcohols from methanol to 2-propanol elute first with no separation, followed by 1-butanol and 1-pentanol which are partially separated. Interestingly, the rather large but spherical 2,6-diisopropylphenyl FCE elutes next, a short time before the smaller p-cresol derivative. As expected, the long chain alkylphenol FCEs of n-octyl- and n-nonylphenol elute last. A possible explanation is the interaction of the aromatic structures with the planar graphite material. This interaction is reduced by bulky substituents at the aromatic ring, resulting in an elution of the larger 2,6-diisopropylphenol derivative in front of several of the smaller derivatives.

Calibration experiments resulted in limits of detection in the low nanomolar concentration range, thus being comparable to the published limits of detection when using a chromatographic column [9].

5.5 CONCLUSION

It was demonstrated that fast LC separations employing conventional guard columns can be used with the new technique of LC/electrochemistry/MS. Although no baseline separation was achieved for different mixtures of FCE standards, efficient separation that was well sufficient for identification and quantification could be accomplished in a time scale of 1 -1.5 min.

In the second part, it was shown that the retention of several alcohol and phenol derivatives is caused by the graphite filter element of the in-line filter employed in the electrochemistry/MS setup. The separation without classical chromatographic column and the mass spectrometric detection of different standards was accomplished using the APCI interface. Although the separation on dedicated columns is superior, this approach is characterized by a very simple experimental setup. In combination with mass spectrometric detection, the separation achieved here is sufficient for many analytical problems.

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6. LC/ELECTROCHEMISTRY/MS/MS OF FERROCENECARBOXYLIC ACID ESTERS

6.1 SUMMARY

The fragmentation pathway of ferrocenecarboxylic ester derivatives of several alcohols and phenols was studied using both nanospray and electrospray ion sources and tandem-MS. Common fragmentation products for each substance class enabled the use of precursor ion scans. LC/electrochemistry/ES(+)-MS chromatograms were recorded in selected ion monitoring (SIM) and multiple reaction monitoring (MRM) mode. The limits of detection for all phenol derivatives employed were below 10 nM. Good linearity and reproducibility were reached in calibration experiments.

6.2 INTRODUCTION

The hyphenation of liquid chromatography and mass spectrometry has been become a widely used technique both in research and routine analysis [1]. The most commonly employed ionization methods are electrospray (ES) ionization [2] and atmospheric pressure chemical ionization (APCI). When triple quadrupole mass analyzers are used, easier identification and quantification is possible due to the additional selectivity of MS/MS techniques.

Since both ES and APCI are soft ionization methods that are suitable for analytes of medium or strong polarity but fail to give good ionization yields for analytes of lower polarity that are not easily protonated or deprotonated, several approaches have been reported to overcome this restriction. A few groups have coupled electrochemical techniques to various ionization interfaces in order to use the electrochemical conversion of the analytes for better mass spectrometric performance (see section 2 for details). A recent approach has used the hyphenation of LC/MS with a "coulometric" flow cell for the on-line oxidation of several ferrocene-labeled alcohols and phenols to its corresponding ferrocinium radical cations that were analyzed by mass spectrometry in a single quadrupole mass analyzer [3].

The aim of this study was to elucidate the fragmentation reactions of these ferrocenecarboxylic acid esters and to use this knowledge to develop new determination methods employing tandem-MS techniques.

6.3 EXPERIMENTAL

6.3.1 Chemicals

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

6.3.2 Instrumentation

6.3.2.1 Electrochemical Instrumentation

The electrochemical system from ESA, Inc. (Chelmsford, MA, USA) which was used for on-line LC/electrochemistry/MS/MS consisted of a GuardStat potentiostat and a model 5021 conditioning cell. The conditioning cell contains a porous glassy carbon "coulometric" working electrode, a Pd counter electrode, and a Pd/H_2 reference electrode. The utilized electrochemical conditioning cell is characterized by a low void volume and the large surface area of the porous glassy carbon working electrode which allows quantitative oxidation or reduction reactions.

6.3.2.2 LC and MS Instrumentation

The LC system from Agilent (Waldbronn, Germany) was a HP 1100 with high pressure binary gradient pumping system, vacuum degasser, heated column department, automated liquid sampler and handheld control module.

When the sample was delivered by syringe pump, a Harvard Apparatus (Holliston, MA, USA) pump was employed.

As MS system a Micromass (Manchester, United Kingdom) Quattro LCZ with electrospray probe and home-built nanospray probe was employed in cooperation with H. Luftmann. The software utilized was Masslynx 3.2.

6.3.2.3 LC/Electrochemistry/MS/MS setup

The "coulometric" flow cell that was controlled via a potentiostat was inserted between the LC column and the inlet of the ionization interface of the mass spectrometer. The connection between the flow cell and the interface was kept as short as possible to reduce peak broadening and loss of ions during transport. To prevent electrical connection between interface and "coulometric" cell via the eluent, appropiate ground connection has to be assured as discussed by van Berkel et al. [4]. This was accomplished by replacing part of the PEEK connection between flow cell and MS with a stainless steel capillary which was in electrical contact with the grounded part of the mass spectrometer.

6.3.3 Synthesis of Ferrocenecarboxylic Acid Esters

The ferrocenecarboxylic acid esters (FCEs) were synthesized according to a modification of a procedure by Rolfes and Andersson [5, 6]. The procedure is described in detail in section 3.3. Further alkylphenyl FCE standards were kindly provided by F. Wasinski, Dr. J. Rolfes and Prof. Dr. J. T. Andersson from the University of Münster (Münster, Germany).

6.3.4 LC Conditions

All separations were performed using a Discovery C18 column (Supelco, Deisenhofen, Germany) equipped with a guard column of the same material with the following dimensions: 5 µm particle size, 100 Å pore size, 2.1 mm id, 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 a flow rate of 0.3 ml/min with the following profile was used:

t [min]	0.01	3	8	23	25	28	28.5
c(CH_3CN) [%]	60	60	90	90	60	60	stop

6.3.5 MS Conditions

For nanospray measurements, the following source parameters were used:

Capillary voltage 1.36 kV, cone voltage 41 V, source block temperature 60 °C, desolvation temperature 100 °C.

For electrospray measurements, different source parameters were used:

A) no electrochemical cell: capillary voltage 4.00 kV, cone voltage 34 V, source block temperature 100 °C, desolvation temperature 100 °C.

B) with electrochemical cell: capillary voltage 3.01 kV, cone voltage 32 V, source block temperature 110 °C, desolvation temperature 150 °C.

Multiple Reaction Monitoring (MRM), precursor ion and daughter ion scan experiments were performed with a collision energy of 36 V. Argon was used as collision gas.

Time program for MRM experiments

t [min]	*MRM m/z*
5.00 - 7.20	306.1 → 213.0 306.1 → 185.0
7.21 - 8.80	320.1 → 213.0 320.1 → 185.0
8.81 - 10.20	334.1 → 213.0 334.1 → 185.0
10.21 - 12.00	348.1 → 213.0 348.1 → 185.0 362.1 → 185.0 362.1 → 185.0
12.01 - 15.00	376.1 → 213.0 376.1 → 185.0 390.1 → 185.0 390.1 → 185.0
15.01 - 18.20	418.2 → 213.0 418.2 → 185.0
18.21 - 21.00	432.2 → 213.0 432.2 → 185.0

Time program for SIM experiments:

t [min]	*m/z*
5.00 - 7.20	306.1
7.21 - 8.80	320.1
8.81 - 10.20	334.1
10.21 - 12.00	348.1, 362.1
12.01 - 15.00	376.1, 390.1
15.01 - 18.20	418.2
18.21 - 21.00	432.2

6.4 RESULTS AND DISCUSSION

6.4.1 Elucidation of the Fragmentation Mechanisms

The first experiments to elucidate the fragmentation mechanism of the ferrocenecarboxylic acid esters were performed with a home-built nanospray interface (figure 6.1) and without the "coulometric" flow cell.

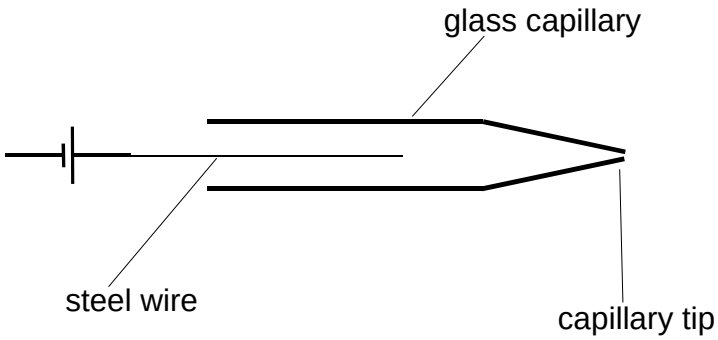

Fig. 6.1 Schematic drawing of the setup of the home-built nanospray interface.

When nanospray mass spectra of a solution of ethyl FCE in acetonitrile/methanol were obtained, the first scans showed a lot of noise with only a small peak of m/z = 259 among many interferences. This peak at m/z = 259 could be attributed to the protonated pseudo molecular ion. Since no molecular ion could be detected in these first scans, no electrochemical oxidation in the nanospray interface itself seemed to take place. However, after 1.9 min, a peak at m/z = 258 began to appear in the still very noisy spectrum, at first reaching the same intensity as the protonated species, and after 3.7 min becoming the base peak (figure 6.2).

The absolute intensity increased from the first scans where a lot of noise was observed and protonation was dominant to the later scans where mainly the oxidation product was observed and almost no noise could be seen. This behavior can be explained with the setup of the particular nanospray source employed. As can be seen in figure 6.1, the steel wire that is connected to the high voltage outlet of the mass spectrometer has a certain distance to the capillary tip. Analyte molecules that are near the capillary tip at the beginning of the experiment do not come into contact with the steel wire and therefore cannot be oxidized.

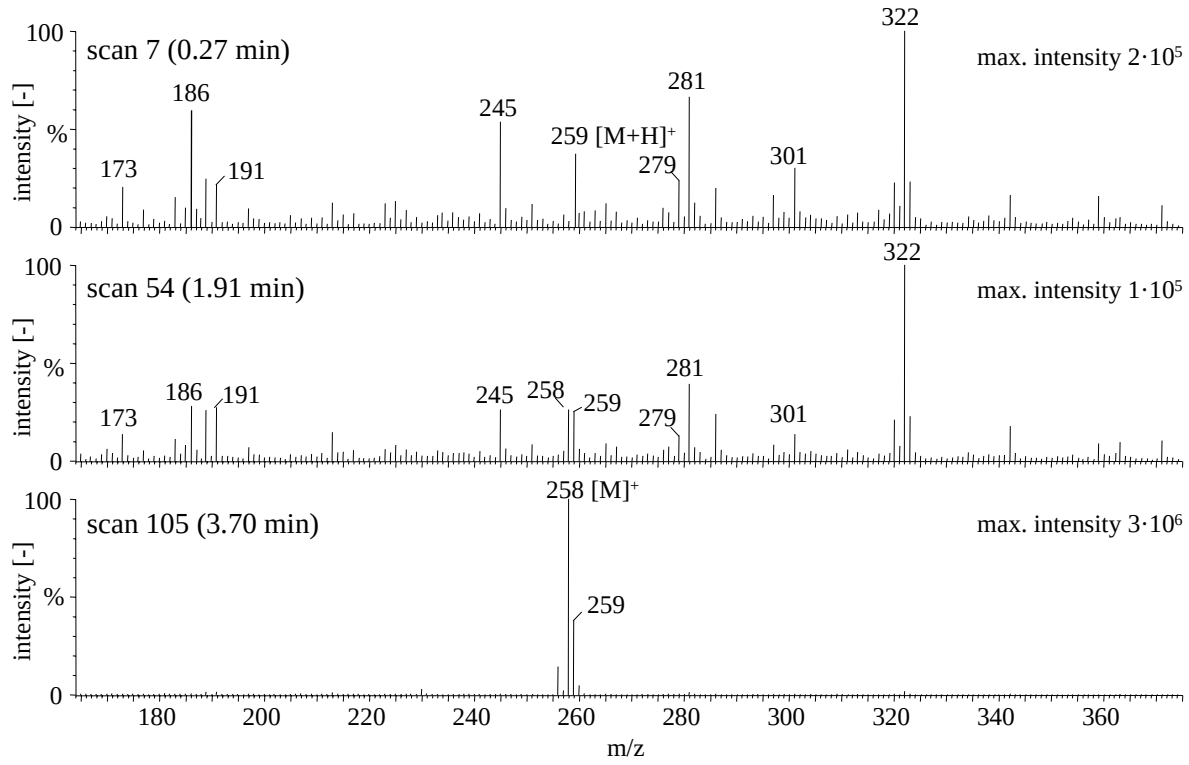

Fig. 6.2 Nanospray mass spectra of ethyl FCE taken at different times during the experiment, sample in acetonitrile/methanol.

This is the case in the upper spectrum of figure 6.2 where only the protonated analyte can be observed. With progression of the experiment, more analyte molecules that had the chance to be oxidized at the steel wire enter the MS resulting first in equal ratio of protonated and oxidized species as in the middle spectrum and finally in a base peak of the molecular ion in the bottom spectrum of figure 6.2. Although the $[M]^+$ peak is the base peak in the last spectrum, the peak at m/z = 259 is higher than what would be expected from the isotope pattern of the molecular ion. This can be attributed a small amount of protonation of the analyte that occurs in parallel with the oxidation process.

Daughter ion spectra of the molecular ion of ethyl FCE and 1-propyl FCE were then recorded by using the second quadrupole of the mass spectrometer as a collision cell. Both compounds gave very simple spectra with only two common fragments of m/z = 230 and m/z = 138 (figure 6.3).

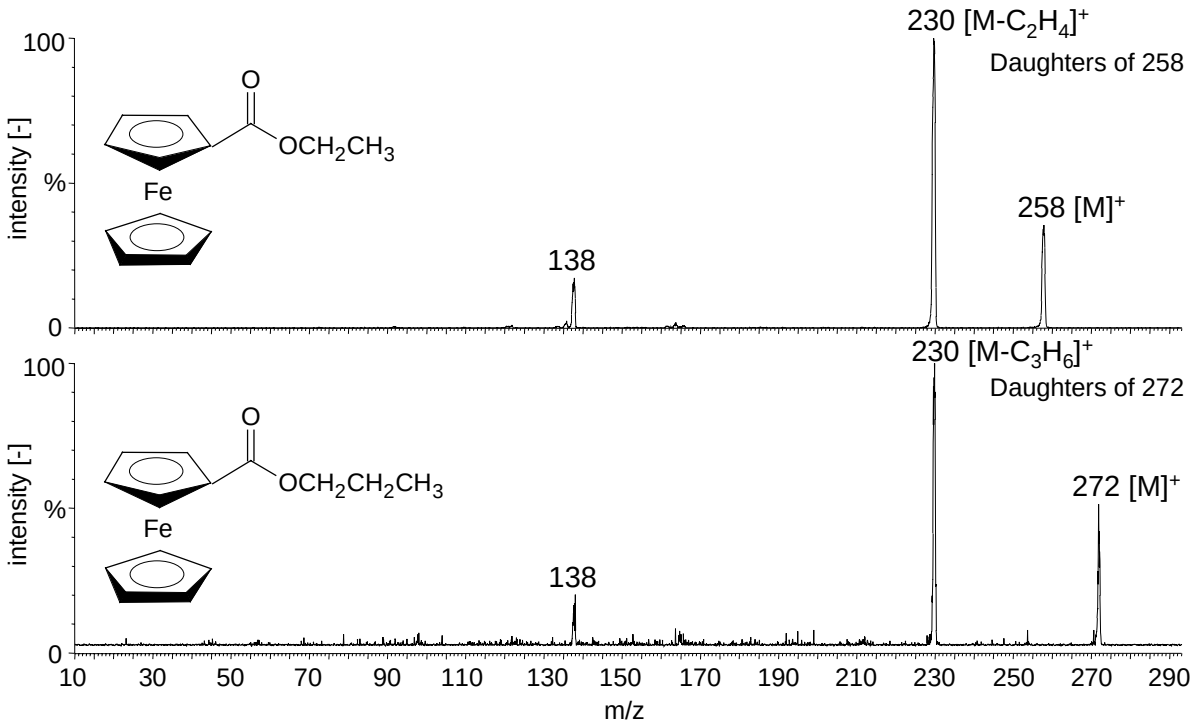

Fig. 6.3 Nanospray daughter ion spectra of ethyl FCE (top, daughters of m/z = 258) and 1-propyl FCE (bottom, daughters of m/z = 272), samples in acetonitrile/methanol, chemical structures of the analytes inserted.

The base peak in the spectra at m/z = 230 results from a neutral loss of the corresponding alkenes ethene and propene (figure 6.4). A possible structure for the second fragment at m/z = 138 is Fe(III) with a hydroxyl and a cyclopentadienyl ligand.

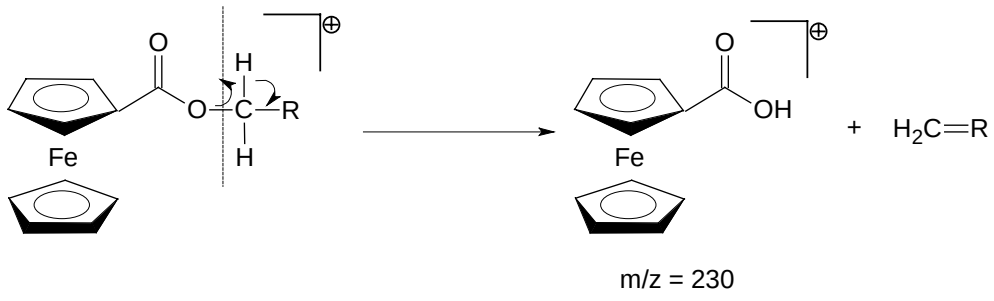

Fig. 6.4 Fragmentation scheme for the aliphatic alcohol derivatives. Neutral loss of the corresponding alkene results in a ferrocenecarboxylic acid fragment ion.

A strong fragmentation with a common daughter ion can also be used for precursor ion scans. In these scans, only precursor ions that produce a selected fragmentation product can be observed in the spectrum.

Figure 6.5a shows a conventional electrospray mass spectrum of a solution of 9 alkyl FCEs in acetonitrile/eluent A that was delivered by a syringe pump at a flow rate of 10 µl/min. This spectrum was recorded with source parameters A and without the "coulometric" flow cell. Although all molecular ions of the nine FCEs are observed in the ES spectrum, many interferences are also detected which makes the spectrum rather complex. Both oxidation and protonation seem to occur as both molecular ions and protonated pseudo molecular ions are observed. In case of the glycol diferrocenecarboxylic acid ester, an intense peak corresponding to a Na^+ attachment is present besides the protonated ion and only little oxidation product is observed. The decrease, in comparison with the nanospray results, of the ratio of oxidation to protonation may be caused by the shorter time span during which the molecules are within the ES capillary even at a flow rate of only 10 µl/min and during which they could be oxidized.

A precursor ion scan of the same mixture of FCEs is shown in figure 6.5b. All precursors of of the fragment with m/z = 230 are detected. As the comparison of the conventional ES spectrum with the precursor ion scan demonstrates, the additional selectivity of the precursor ion scan clearly simplifies the spectrum and results in a much better signal to noise ratio. Of the 9 FCEs, only 7 are observed in the precursor ion scan. Since the glycol derivative is labeled twice with the ferrocene function, a different fragmentation pathway has to be expected. Therefore, it does not produce a fragment with m/z = 230 and cannot be seen in the precursor ion scan. The methyl FCE is also not detected in the precusor ion scan. The fragmentation to a product with m/z = 230 did not occur in this case, because the possible leaving group would be methylene which is energetically unfavourable.

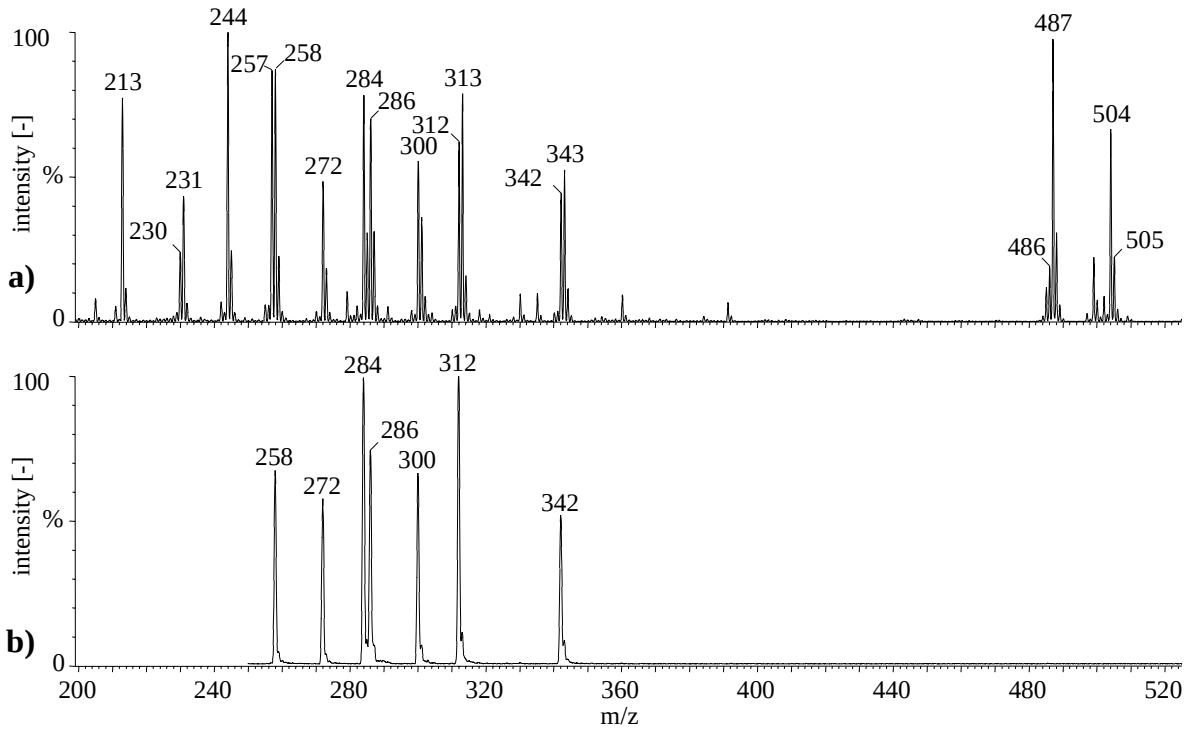

Fig. 6.5 a) ES(+) mass spectrum and b) precursor ion scan (precursors of m/z = 230) of a mixture of 9 alkyl FCEs (methyl FCE (m/z = 244), ethyl FCE (m/z = 258), 1-propyl FCE (m/z = 272), 1-methylcyclopropyl FCE (m/z = 284), 1-n-butyl FCE (m/z = 286), 1-n-pentyl FCE (m/z = 300), cyclohexyl FCE (m/z = 312), 1-n-octyl FCE (m/z = 342), glycol diFCE (m/z = 486)) in acetonitrile/eluent A, sample delivered by a syringe pump at a flow rate of 10 µl/min.

To test if the phenol derivatives fragment in a similar way as the alkohol derivatives, solutions of 2-cresol FCE and 4-n-octylphenyl FCE were analyzed by electrochemistry/ES(+)-MS employing the "coulometric" flow cell at a potential of 700 mV vs. Pd/H_2 and MS conditions B. The LC column was removed for these experiments since there was no need for separation. The daughter ion spectra of m/z = 320 for 2-cresol FCE and m/z = 418 for 4-n-octylphenyl FCE show three common fragment ions at m/z = 129, 185 and 213 (figure 6.6).

The fragment at m/z = 213 corresponds to a cleavage within the ester function next to the carbonyl carbon atom. Further loss of carbon monoxide results in the base peak of both spectra at m/z = 185 corresponding to the ferrocene group (figure 6.7). So far, no possible explanation for the fragment at m/z = 129 was found.

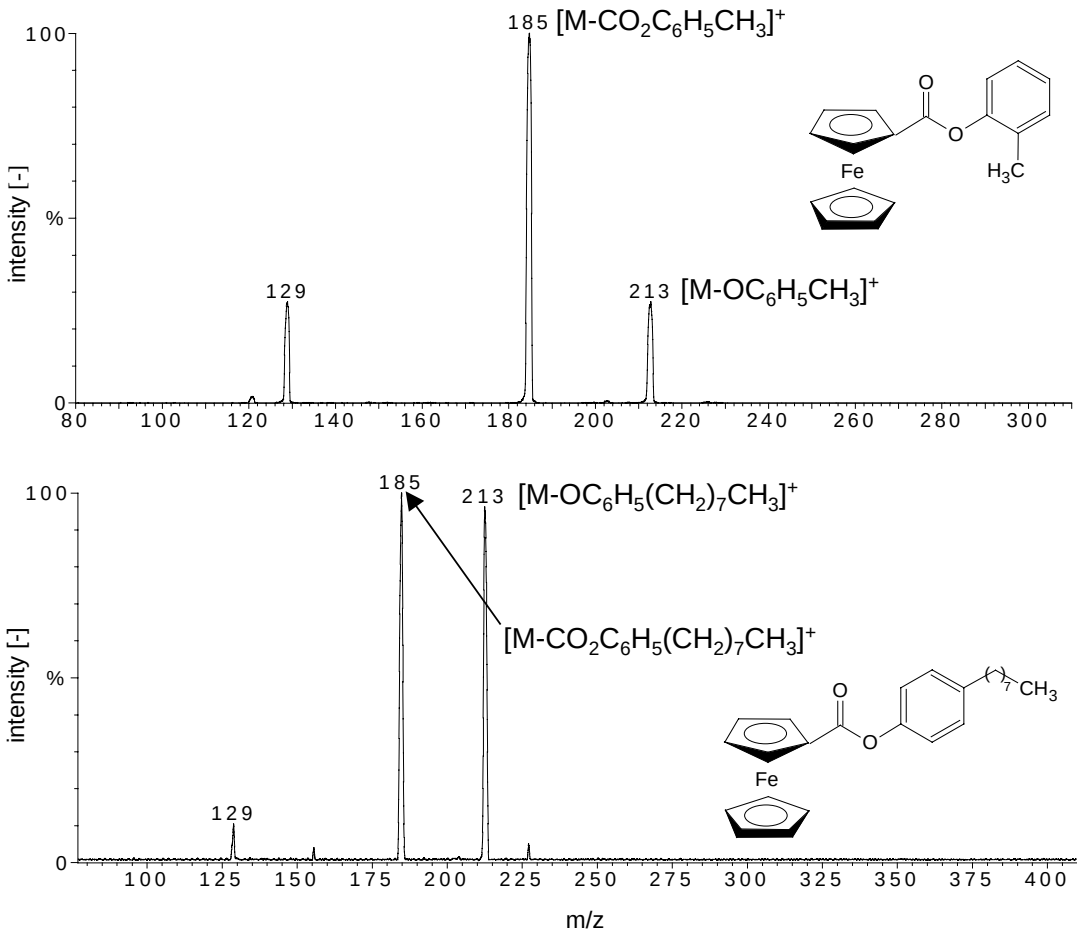

Fig. 6.6 Electrochemistry/ES(+) daughter ion spectra of 2-cresol FCE (top, daughters of m/z = 320) and 4-n-octylphenyl FCE (bottom, daughters of m/z = 418), samples in acetonitrile/eluent A; chemical structures of the analytes inserted.

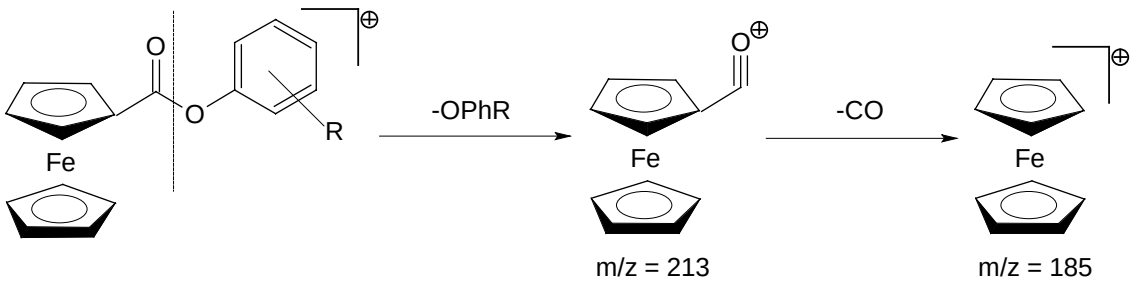

Fig. 6.7 Fragmentation scheme for the phenol derivatives. After the cleavage next to the carbonyl carbon atom which produces the fragment at m/z = 213, neutral loss of carbon monoxide results in a ferrocinium ion at m/z = 185.

Since the phenol derivatives fragment differently than the alcohol derivatives it is possible to distinguish between the two substance groups using selected precusor ion scans. A precusors ion scan of the fragment with m/z = 230 detects the alcohol derivatives (figure 6.5 b)) whereas a precursor ion scan of the fragments with m/z = 213 or 185 selectively shows the phenol derivatives. Such a precursor ion scan of a mixture of 9 alkylphenyl FCEs is shown in figure 6.8. Again it is demonstrated that even the spectrum of a mixture of several different compounds looks quite simple.

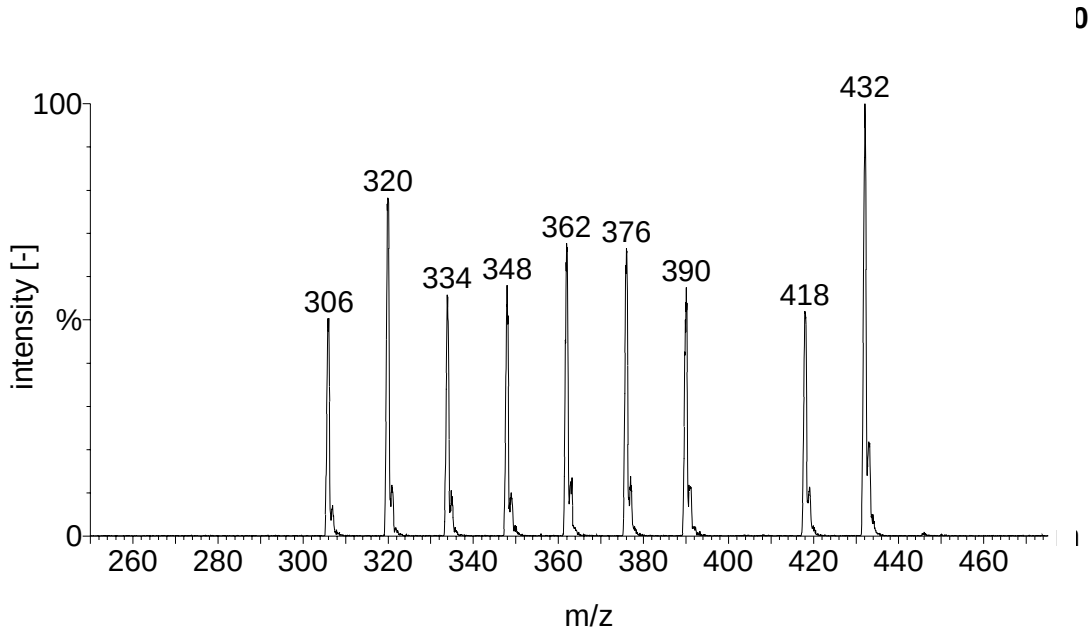

Fig. 6.8 Electrochemistry/ES(+)-MS precursor scan (precursors of m/z = 185) of a mixture of 9 alkylphenyl FCEs (phenyl FCE, 2-cresol FCE, 2,5-dimethylphenyl FCE, 2,3,6-trimethylphenyl FCE, thymol FCE, 4-pentylphenyl FCE, 2,6-diisopropylphenyl FCE, 4-n-octylphenyl FCE, 4-n-nonylphenyl FCE) in acetonitrile/eluent A.

6.4.2 LC/Electrochemistry/MS/MS

The first LC/electrochemistry/ES(+)-MS chromatograms were recorded in the SIM mode using source conditions B. Figure 6.9 shows two chromatograms of a 100 nM solution of 9 alkylphenyl FCEs. The top chromatogram was obtained with the electrochemical flow cell turned off (figure 6.9a). As can be expected, almost no oxidation of the analytes occurs and it is hard to identify any peaks in the chromatogram because they are of the same order of magnitude as the noise. After the cell was turned on, the absolute intensity increased by two orders of magnitude and a well resolved chromatogram was recorded (figure 6.9b). This clearly

demonstrates the usefulness of the LC/electrochemistry/MS setup. The different alkylphenol derivatives elute according to their alkyl substitution with the more spherical 2,6-diisopropylphenyl FCE eluting before the 4-pentylphenyl FCE.

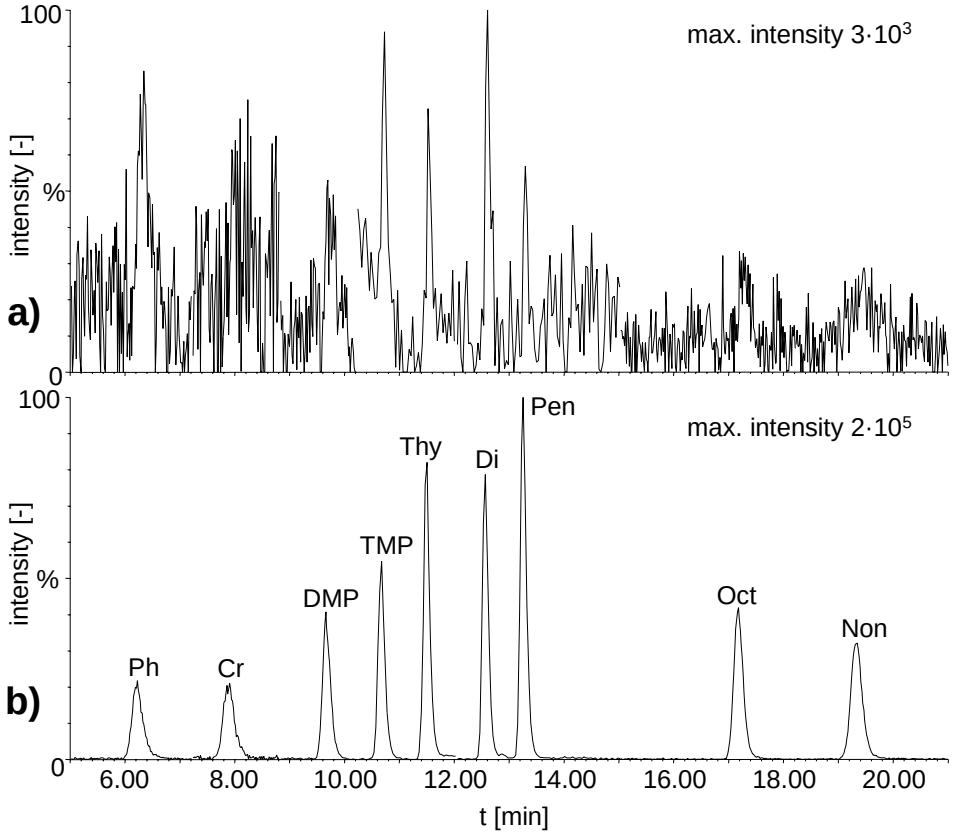

Fig. 6.9 LC/electrochemistry/ES(+)-MS chromatograms of a 100 nM solution of 9 alkylphenyl FCEs (Ph = phenyl FCE, Cr = 2-cresol FCE, DMP = 2,5-dimethylphenyl FCE, TMP = 2,3,6-trimethylphenyl FCE, Thy = thymol FCE, Di = 2,6-diisopropylphenyl FCE, Pen = 4-n-pentylphenyl FCE) in acetonitrile, baseline substracted, recorded in SIM mode with a) electrochemical cell turned off b) electrochemical cell turned on, cell potential 700 mV vs. Pd/H_2.

Quantitative calibration data was then acquired in the MRM mode which was time programmed for the specific fragmentations. Figure 6.10 shows a LC/electrochemistry/MS/MS chromatogram of the same mixture of nine alkylphenol derivatives at a 10 nM concentration. The time program can be recognized in the chromatogram because no baseline subtraction was performed and the intensity scales of the different MRM windows were not adjusted.

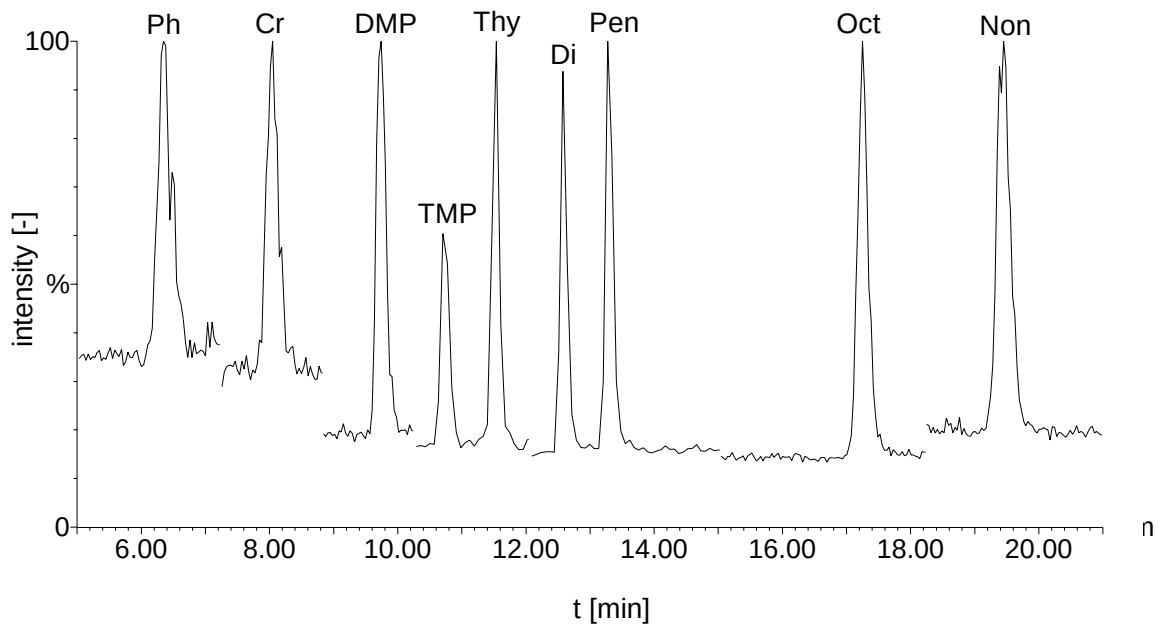

Fig. 6.10 LC/electrochemistry/ES(+)-MS chromatogram of a 10 nM solution of 9 alkylphenyl FCEs (Ph = phenyl FCE, Cr = 2-cresol FCE, DMP = 2,5-dimethylphenyl FCE, TMP = 2,3,6-trimethylphenyl FCE, Thy = thymol FCE, Di = 2,6-diisopropylphenyl FCE, Pen = 4-n-pentylphenyl FCE) in acetonitrile, recorded in MRM mode, no smoothing, no background subtraction, intensity axis scale of different MRM windows not adjusted.

The limits of detection for the alkylphenyl FCEs were then determined as a signal to noise ratio of 3 in a chromatogram of a 1 nM mixture (table 6.1). A subnanomolar detection limit was reached for selected derivatives. The reproducibility was determined at a concentration of 10 nM. The relative standard deviation for two subsequent analysis' was between 3.7% and 18.1% depending on the specific derivative. Similar chromatograms were recorded at concentrations of 100 nM and 10 µM. Plotting peak areas vs. concentration resulted in all cases in linear calibration graphs with a correlation coefficient > 0.999.

When the detection limits of the alkylphenol derivatives using LC/electrochemistry/MS/MS in the MRM mode are compared to the limits of detection that were obtained using a single quadrupole mass spectrometer (table 4.2 and 4.3), it is demonstrated that the additional selectivity of the MRM experiment clearly lowers the sensitivity of the method.

Table 6.1: Analytical figures of merit for selected alkylphenyl FCEs (LOD = limit of detection, RSD = relative standard deviation), detection in the MRM mode.

Analyte	LOD [nM]
Phenyl FCE	1.0
2-Cresol FCE	1.7
2,5-Dimethylphenyl FCE	0.7
2,3,6-Trimethylphenyl FCE	1.6
Thymol FCE	1.3
4-n-Pentylphenyl FCE	0.8
2,6-Diisopropylphenyl FCE	0.8
4-n-Octylphenyl FCE	0.8
4-n-Nonylphenyl FCE	1.0

6.5 CONCLUSION

The fragmentation mechanisms of several alkyl and alkylphenyl FCEs were elucidated employing daughter ion scans. Since common fragment ions were observed for both the alcohol and the alkylphenol derivatives, precursor ion scans were used for the determination of mixtures of FCE standards without prior separation. LC/electrochemistry/ES(+)-MS chromatograms were recorded in the SIM and MRM modes for quantification purposes. Using the MRM mode, limits of detection below 1 nM were observed for selected alkylphenol derivatives. Overall, the hyphenation of electrochemistry with LC/MS/MS was succesfully accomplished incorporating the advantages of higher selectivity and sensitivity when compared to LC/MS.

6.6 ACKNOWLEDGEMENT

I kindly thank Dr. H. Luftmann of the Institut für Organische Chemie (University of Münster, Münster, Germany) for the opportunity to use the LC/MS/MS equipment and for his advice concerning many mass spectrometric questions.

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7. ANALYSIS OF GASOLINE AND DIESEL SAMPLES USING LC/ELECTROCHEMISTRY/MS

7.1 SUMMARY

Seven gasoline and four diesel samples were analyzed by LC/electrochemistry/ APCI(+)-MS. In the resulting complex chromatograms, selected mass traces were used to identify several groups of alcohols and alkylphenols. Only a sum parameter for alcohols and phenols of the same molecular mass could be obtained because of the large number of structural isomers that were not chromatographically resolved in the mass traces. Apart from the alcohols and phenols, several other compounds were found in the samples. Many of these unknown compounds could be arranged in four series with a mass difference of 14 mass units. Tandem-MS experiments were performed to identify the unknown substances, but only little information was gained.

7.2 INTRODUCTION

Alkylphenols have several natural and anthropogenic sources and are thus found in a variety of different matrices. They are released into the atmosphere and subsequently into rivers, lakes, soil, flora and fauna by the combustion of oil and coal [1]. The most important factor for human exposure to phenol and cresols is cigarette smoke [2] but tea, coffee and wine also contain phenolic substances [3]. Another source for phenols is the decomposition of a group of widely used non-ionic surfactants, the alkylphenylpolyethoxylates, during waste water treatment. The main decomposition product are nonylphenols which show endocrine disrupting properties. They can be found ubiquitously [4]. Because of their toxicity a number of alkylphenols is regulated by the U.S. Environmental Protection Agency [5].

The hyphenation of liquid chromatography with electrochemistry and mass spectrometry after a derivatization step that introduces an electrochemically active function has been sucessfully employed for the determination of alcohols and phenols in standard mixtures [6] (see chapter 4). When using MS/MS techniques, additional selectivity was gained (see chapter 6). This novel hyphenated method will now be utilized for the qualitative determination of several alcohols and alkylphenols in four diesel and seven gasoline fuel samples.

7.3 EXPERIMENTAL

7.3.1 Chemicals

Ammonium formate and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) were purchased from Aldrich Chemie (Steinheim, Germany). Formic acid and dichloromethane were obtained from Fluka (Buchs, Switzerland). Alumina (chromatography grade, 90 mesh, neutral) from Fluka was activated at 450 °C for 12 h before use. Solvent for LC was acetonitrile LiChroSolv gradient grade from Merck (Darmstadt, Germany). Silanized glass wool (pesticide grade) was purchased from Supelco (Deisenhofen, Germany).

7.3.2 Instrumentation

7.3.2.1 Electrochemical Instrumentation

The electrochemical system from ESA, Inc. (Chelmsford, MA, USA) which was used for on-line LC/electrochemistry/MS is described in detail in section 4.3.2.1. For all experiments, the cell potential was set to 700 mV vs. Pd/H_2.

7.3.2.2 LC/MS Instrumentation

The LC-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 atmospheric pressure chemical ionization (APCI) probe and Class 8000 Version 1.20 software.

7.3.2.3 LC/MS/MS Instrumentation

The LC system from Agilent (Waldbronn, Germany) was HP 1100 with high pressure binary gradient pumping system, vacuum degasser, heated column department, automated liquid sampler and handheld control module. The MS/MS system analyzer was Micromass (Manchester, United Kingdom) Quattro LCZ with electrospray probe. The software utilized was Masslynx 3.2.

7.3.2.4 LC/Electrochemistry/MS and LC/Electrochemistry/MS/MS setup

For LC/Electrochemistry/MS experiments, the setup described in section 4.3.2.3 was employed, whereas for LC/Electrochemistry/MS/MS measurements, the setup of section 6.3.2.3 was used.

7.3.3 Derivatization Procedure for Gasoline and Diesel Samples

The derivatization reagent ferrocenecarboxylic acid chloride (FCC) was synthesized according to a procedure by Rolfes and Andersson [7]. The procedure is described in detail in section 3.3.2.

The derivatization of the gasoline and diesel samples was performed according to a modification of a procedure by Rolfes and Andersson [8]. In a 2 ml screw-capped sample vial, 200 µl of a 85 mM (21.1 mg/ml) solution of FCC and 300 µl of 165 mM (20.1 mg/ml) solution of DMAP in dichloromethane were added to 50 µl of the gasoline or diesel sample. After a few minutes the reaction mixture was transferred by Pasteur pipette to a microcolumn (23 mm $\times$ 5 mm i.d. of alumina). The resulting ferrocenecarboxylic acid esters were eluted with 4.5 ml of dichloromethane, while the DMAP, the excess FCC and the more polar compounds of the samples remained on the column. After evaporation of the dichloromethane, the residue was redissolved in 200 µl of a mixture of 30 % dichloromethane and 70% n-pentane, transferred to a second microcolumn (23 mm $\times$ 5 mm i.d. of alumina), and the hydrocarbons from the sample matrix were eluted with 3 ml of the 30% dichloromethane mixture. Subsequently, the FCEs were eluted with 4.5 ml of dichloromethane. The solvent was removed and the residue was redissolved in 1.2 ml of acetonitrile for LC/electrochemistry/MS analysis.

7.3.4 LC Conditions

Since the electrospray interface tolerates only low flow rates and the APCI interface works best with flow rates of at least 0.6 ml/min, 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 ES

experiments) and 3.0 mm id (for APCI experiments), length 20 mm (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 0.3 ml/min (2.1 mm id column for ES ionization, injection volume 5 µl) and 0.6 ml/min (3.0 mm id column for APCI, injection volume 10 µl) with the following profile was used:

t [min]	0.01	3	8	23	25	28	28.5
c(CH_3CN) [%]	60	60	90	90	60	60	stop

7.3.5 MS Conditions

The APCI interface was used as heated nebulizer with the following parameters: nebulizer gas flow 2.5 l/min, APCI temperature 375 °C, APCI probe voltage 0.10 kV, CDL temperature 300 °C, CDL voltage –35 V, deflector voltages +35 V, detector voltage 1.7 kV.

7.3.6 MS/MS Conditions

For all electrospray tandem-MS measurements the following parameters were used: capillary voltage 3.01 kV, cone voltage 32 V, source block temperature 110 °C, desolvation temperature 150 °C, collision energy 36 V. The collision gas was argon.

7.4 RESULTS AND DISCUSSION

7.4.1 Gasoline Samples

Seven different gasoline samples were analyzed by LC/electrochemistry/APCI(+)-MS after derivatization with ferrocenecarboxylic acid chloride. A typical total ion current (TIC) chromatogram is shown in figure 7.1. This chromatogram of the gasoline Suhner UB 95 was recorded in scan mode to enable the detection of a large variety of alcohols and phenols.

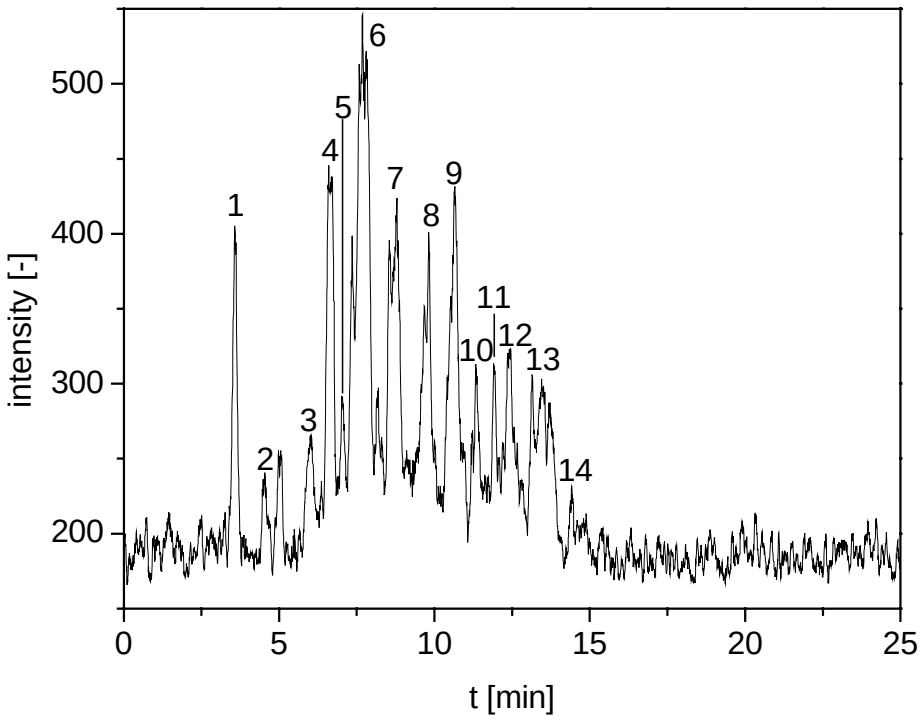

Fig. 7.1 LC/electrochemistry/APCI(+)-MS TIC chromatogram of gasoline Suhner UB 95, recorded in scan mode (m/z = 175-700). The numbered peaks could be identified as the derivatives of 1: methanol; 2: ethanol; 3: C3 alcohols; 4: phenol; 5: cresols, 6: cresols; C4 alcohols; 7: C2 phenols, C5 alcohols; 8: C3 phenols, C4 phenols; C6 alcohols; 9: C7 alcohol; 10: C8 alcohol; 11: C9 alcohol; 12: C9 alcohol; 13: C10 alcohol; 14: C10 alcohol.

The strong background noise is a result of the large scan range. Several intense ”peaks” can be observed in the chromatogram. If possible, single compounds (in the case of methanol, ethanol and phenol) or groups of compounds were assigned to the different peaks. Several of the peaks contained coeluting structural isomers of alcohols or phenols of the same molecular mass and some were even formed by coeluting alcohol and phenol isomers of different molecular mass. It is therefore important to note that due to the complexity of the samples that contain various alcohols and phenols of different molecular mass, selected mass traces should be extracted from the TIC chromatogram for further analysis. Even in those mass traces, peaks of single substances can normally not be resolved because too many different isomers are present. This is demonstrated in figure 7.2 which shows selected mass traces of the LC/electrochemistry/APCI(+)-MS chromatogram of the gasoline sample Suhner UB 95.

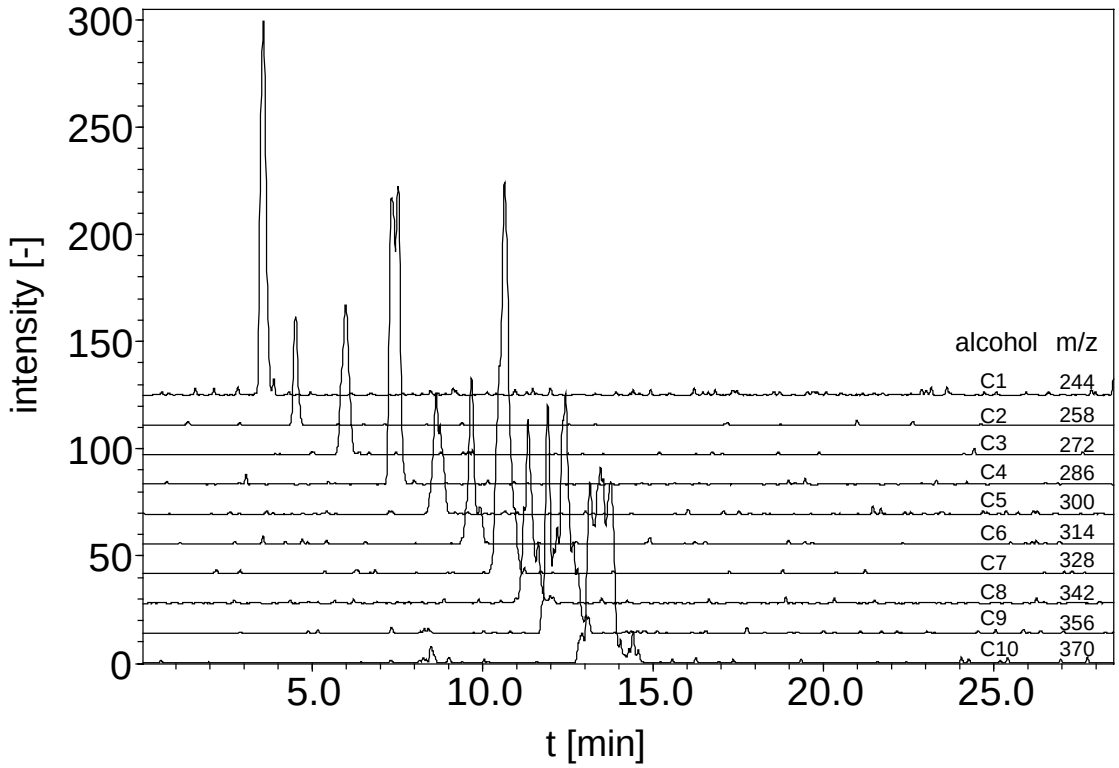

Fig. 7.2 LC/electrochemistry/APCI(+)-MS chromatogram of gasoline Suhner UB 95, recorded in scan mode (m/z = 175-700), displayed are base-shifted extracted mass traces corresponding to the C1 to C10 alcohol derivatives.

The mass traces for m/z = 244 and 258 which correspond to the methanol and ethanol derivatives show a narrow peak whereas the mass traces of the higher alcohol derivatives display broad peaks with peak widths of more than 1 min. This leads to the conclusion that a large number of different isomers of the higher alcohols are present in the sample. These isomers have similar but slightly different retention times. Broad peaks are observed because they cannot be completely separated by LC. Therefore, only a sum parameter can be obtained for these alcohols and phenols. This seems to be inferior to GC separations but is true only for the low molecular weigth compounds. Although it is possible to separate most isomers of the low molecular weight alkylphenyl derivatives up to C3 phenols using gas chromatography [8], GC is not superior to LC when separating higher alkylphenol isomers because of the increasing number of possible isomers. Additionally, for identification of single isomers, standards have to be available to determine the exact retention time. This is a considerable problem as it is reported in [8] that even for the C3 alkylphenols not all isomers were commercially available.

Employing selected mass traces, alcohols and phenols were found in all seven gasoline samples. However, other compounds were also detected in some samples. The molecular masses of these compounds often differed by 14, leading to the conclusion that different chain lengths of the same substance class are observed. Therefore, these unknown substances in the gasoline and diesel samples were ordered into four series. The first series that was two mass units below the alcohol derivatives was called series A. The next series that was again two mass units below series A was called series B. No compounds of a possible series C with two mass units less than series B were found in both the gasoline and the diesel samples. The next group of substances eight mass units lower than the alcohols are the phenols and again two and four mass units below the phenols are series D and E respectively. An overview of the detected compounds in all seven gasoline samples is given in table 7.1.

In the gasoline samples, compounds of series B, D and E were detected. Possible structures for the compounds of series B which are four mass units below the alcohols are all structures that differ from the alcohols by two double bond equivalents. This could be bicyclic alcohols, cyclic alcohols with one double bond or alcohols with two double bonds. Also, phenol derivatives with nine or naphthol derivatives with two double bond equivalents could be considered but these would have a molecular mass that is larger than what was found in the gasoline samples.

A similar argumentation can be used for the compounds of series D which is ten mass units below the alcohols and two mass units below the phenols. This series could contain alcohols with five or phenols with one double bond equivalent, for example phenols with an unsaturated side chain. At this point, it is important to consider that only a C10 or higher alcohol can contain five double bond equivalents. Such a derivatized alcohol would have a molecular mass of 360. Since lower molecular masses than 360 are found in all three gasoline samples that contain compounds of series D, this structure is not possible. The same problem arises for the phenolic structures with one double bond equivalent. In this case, only a C2 or higher alkylphenol could contain one double bond equivalent and this compound would have a mass of 332 in its derivatized form. Although the gasoline samples Coop UB 95 and Elf, oxgygen-free contain only compounds of series D with

molecular masses of 346 and higher, the two-stroke mixture contains substances in this series with masses that are lower than 332. This could either mean that the proposition of the structure is incorrect or that it is only correct for the Coop and Elf samples and the substances in the two-stroke mixture are of another structure. Overall, the structure of the compounds of series D can not be fully elucidated at this point.

For the compounds of series E a similar discussion of possible structures results in alcohols with six double bond equivalents or phenols with two double bond equivalents. For the Elf, oxygen-free sample which contains compounds of series E with masses of 386 and higher, both structures are possible because a derivatized alkylphenol with two double bonds would have a molecular mass of 358 or higher and a derivatized alcohol with six double bonds would have a mass of 386 or higher. Again, the two-stroke mixture does not fit into this pattern because one compound that fits into series E was found in this sample with a mass of 330. A possible explanation would be that this compound is not structurally connected to the substances of series E. This is quite probable since only one compound with a mass that fits into series E was detected in the two-stroke mixture.

To gather more information about the unknown compounds of series B, D and E, tandem mass spectrometry was employed. Four of the seven gasoline samples were analyzed by LC/electrochemistry/ES(+)-MS/MS. Chromatograms were recorded in the precursor ion mode with m/z = 185 as the selected precursor ion (see section 6.4). A TIC chromatogram of the gasoline elf oxygen-free is shown in figure 7.3. Several phenol derivatives are detected starting with phenol itself at a retention time of 6.3 min. The alkylphenol that elutes at 16.6 min was identified as a C9 phenol. When this chromatogram is compared to the APCI chromatogram of figure 7.1, it can be seen that the noise is reduced strongly as an effect of the additional selectivity that is caused by the precursor ion scan.

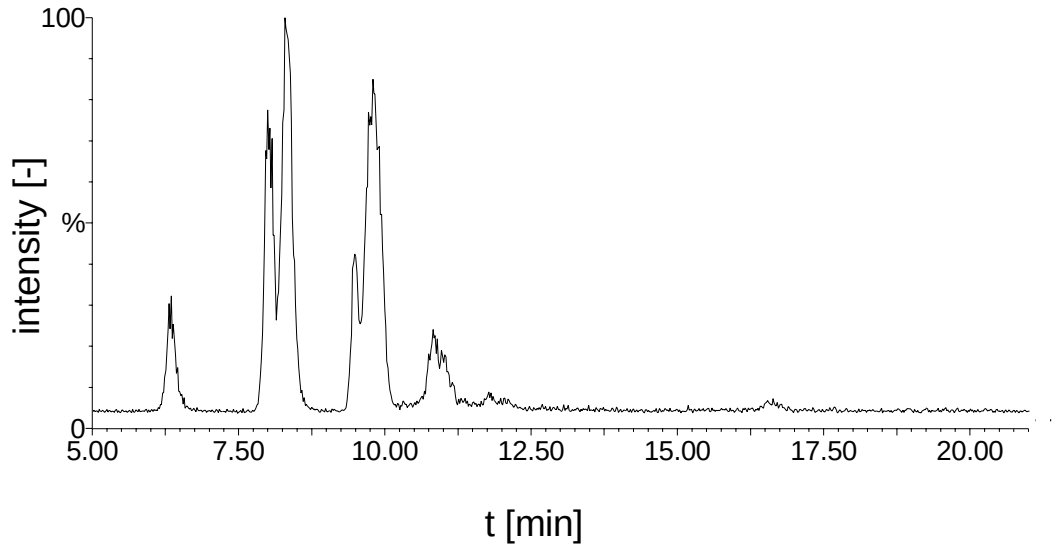

Fig. 7.3 LC/electrochemistry/ES(+)-MS/MS TIC chromatogram of gasoline elf oxygen-free, recorded in precursor ion mode (precursors of m/z = 185).

The fragmentation to the ferrocene ion with m/z = 185 is characteristic for phenol derivatives. Therefore, no alcohol derivatives were observed in the precursor ion scan mode with precursor ion m/z = 185. Additionally, the compounds of series E were not detected. This demonstrates that the substances of series E do not fragment like the phenol derivatives. It is therefore unlikely that they are phenol derivatives with unsaturated side chains as it was discussed above. However, the compounds of series B and D could be detected in the tandem MS experiments which shows that they fragment in a way similar to the phenol derivatives. This result does not match the structures that were discussed above. To completely elucidate the structures further experiments would be necessary. The exact mass measurement of the unknown compounds employing a time of flight (TOF) mass spectrometer might have helped to elucidate the structures but unfortunately, no TOF instrument was available for such experiments.

From table 7.1 it can be seen that the different gasoline samples contain similar phenol fractions. The alcohol contents differ more drastically. It is interesting to note that the elf gasoline that is supposed to be ”oxygen-free” contains rather more than average amounts of alcohols and phenols in high concentrations whereas the elf version with ”no additives” can be considered quite clean.

Table 7.1: Overview of alcohols, phenols and unknown compounds found in 7 derivatized gasoline samples that were analyzed by LC/electrochemistry/APCI(+)-MS.

Gasoline samples	**Alcohols**	**Phenols**	**Series B, m/z =**	**Series D, m/z =**	**Series E, m/z =**
BP UB 95	C1-C7	C0-C4	338, 352	-	-
Suhner UB 95	C1-C11	C0-C4	310-352	-	-
Coop UB 95	C1-C6	C0-C5	310, 366	346-374	-
Shell UB 95	C1-C4	C1-C3	310	-	-
Elf, no additives	C1-C3	C1	-	-	-
Elf, oxygen-free	C1-C4, C11-C14	C0-C4, C9	-	346-374	386-470
Two-stroke mixture	C1-C4	C0-C2	-	304-332	330

7.4.2 Diesel Samples

Four different diesel samples were analyzed by LC/electrochemistry/APCI(+)-MS after derivatization with ferrocenecarboxylic acid chloride. A typical diesel TIC chromatogram is shown in figure 7.4. This chromatogram of the diesel Coop Baar was recorded in scan mode in order to be able to detect a large variety of alcohols and phenols. Again, the strong background noise is a result of the large scan range. The intense peak labeled peak 1 corresponds to the methanol derivative. Starting at 6 min, many different alcohols and phenols coelute until approximately 21 min. This leads to a broad shoulder in the chromatogram. This broad shoulder is typical for the diesel samples and allows an easy distinction between gasoline and diesel samples. An exception is the elf with no additives diesel sample which contains less alcohols and phenols than the other diesel samples and therefore does not show a shoulder in the chromatogram. A few intense peaks rise from the shoulder in figure 7.4 and some of these correspond to single compounds whereas others correpond to groups of different isomers of alcohols or phenols of the same molecular mass. For further qualification, selected mass traces have to be recorded. As with the gasoline samples, even in those mass traces single isomers normally cannot be resolved.

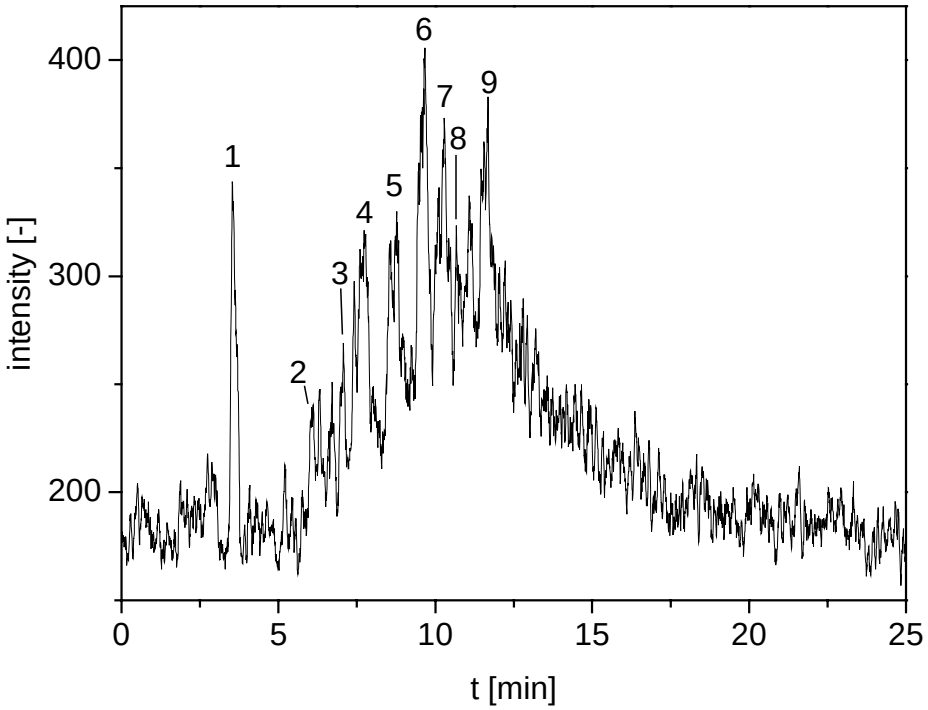

Fig. 7.4 LC/electrochemistry/APCI(+)-MS TIC chromatogram of diesel Coop Baar, recorded in scan mode (m/z = 175-700). The numbered peaks could be identified as the derivatives of 1: methanol; 2: C3 alcohols; 3: cresols; 4: cresols; 5: C2 phenols; 6: C3 phenols; 7: C4 phenols 8: C5 phenols; 9: C6 phenols.

Figure 7.5 shows the mass traces of the C0 to C15 phenols in the LC/electrochemistry/APCI(+)-MS chromatogram of the diesel sample Coop Baar.

It can be seen that the peaks of the low molecular weight alkylphenyl FCEs are quite narrow whereas the peaks of the larger alkylphenol derivatives become wider with increasing molecular weight and the possible number of structural isomers. A maximum in peak height is reached at m/z = 362 corresponding to the C4 alkylphenols. If one would draw a line through the centre of the peaks of the C0 to C9 alkylphenols a diagonal would be seen in the chromatogram because of the base shift of the different mass traces. Interestingly, starting with the C10 alkylphenyl derivatives at m/z = 446, the substitution pattern of the alkylphenols seems to change because the main peaks of these mass traces are observed at shorter retention times when compared to the C8 or C9 alkylphenols.

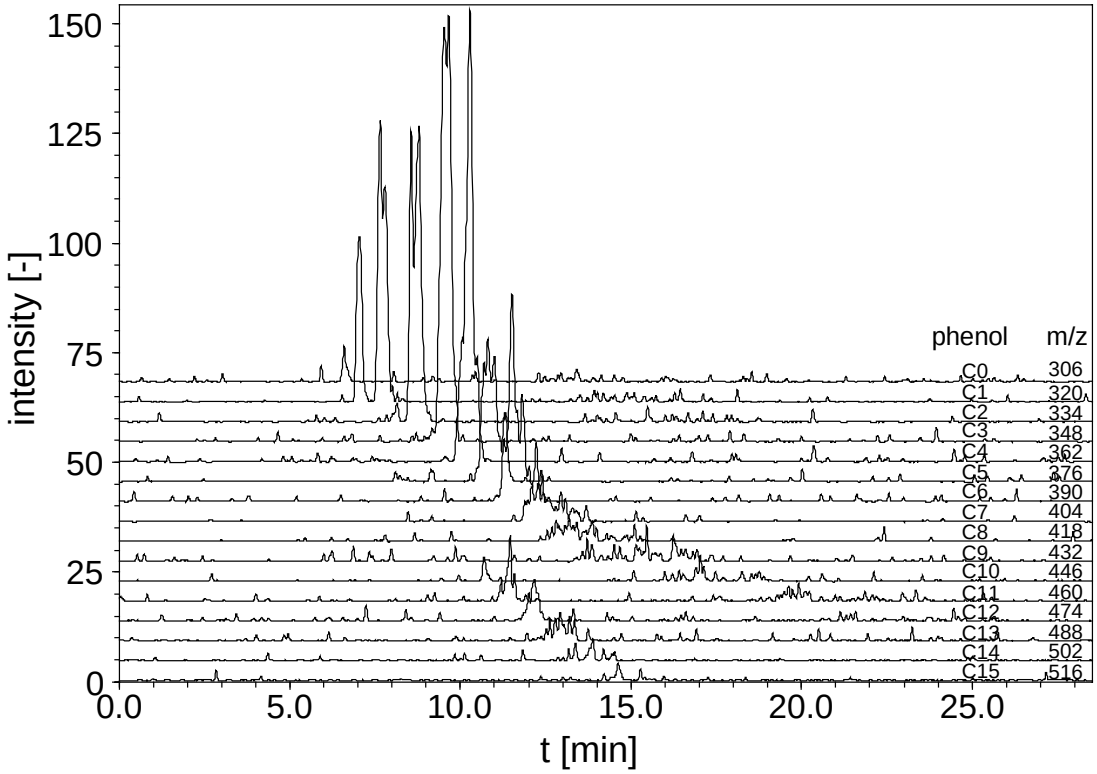

Fig. 7.5 LC/electrochemistry/APCI(+)-MS chromatogram of diesel Coop Baar, recorded in scan mode (m/z = 175-700), displayed are base-shifted extracted mass traces corresponding to the C0 to C15 phenol derivatives.

A second diagonal could now be drawn for the C10 to C15 alkylphenols that would appear to the left of the first diagonal. This can have three possible explanations. In the refining process of the diesel fuel, different isomer groups of the C0 to C9 alkylphenols and the C10 to C15 alkylphenols could have been used because of similar boiling points. Further evidence for this explanation is that the long-chain isomers of the phenols of higher molecular weight that would have extremely high boiling points do not seem to be present in the sample, they would not elute before the C8 and C9 alkylphenols. The second explanation could be that these long-chain isomers of the C10 to C15 phenols were not even present in the crude oil and therefore also cannot be in the refined diesel sample. A third approach is that a these compounds are not phenol derivatives but alcohol or naphthol derivatives with four double bond equivalents. Although this seems unlikely for the alcohols because they would rather elute after the phenols, naphthol derivatives with four double bonds would have molecular masses of 460 and higher and could also possibly elute before the phenol derivatives.

Apart from the alcohols and phenols, other unknown substances were found in the four diesel samples. Some of these could be included in the above mentioned series B and D, but most of them are within series A that is two mass units below the alcohols. The possible structures of the compounds of series B and D have already been discussed above. A possible structure for the compounds of series A would be that of alcohols with one double bond equivalent as it is the case in unsaturated or cyclic alcohols. This idea was again tested with LC/electrochemistry/ESI(+)-MS/MS. As with the gasoline samples, precursor ion scan chromatograms with precursors of m/z = 185 were recorded. All of the compounds of series A were detected in these chromatograms. It is therefore unlikely that these compounds are derived from alcohols since the simple alcohol derivatives did not fragment to a product with m/z = 185. As with the unknown compounds of series B, D and E, further studies employing exact mass measurements are necessary to elucidate the identities of the compounds of series A. Table 7.2 gives an overview of all detected substances in the four diesel samples.

Table 7.2: Overview of alcohols, phenols and unknown compounds found in 4 derivatized diesel samples that were analyzed by LC/electrochemistry/APCI(+)-MS.

Diesel samples	**Alcohols**	**Phenols**	**Series A, m/z =**	**Series D, m/z =**	**Series B, m/z =**
Coop Baar	C1-C5, C8	C0-C15	396-480	-	310
BP Zürich	C1-C5, C8	C0-C13	396-480	-	310
Diesel with ferrocene	C1-C4	C0-C8	410-452	-	310
Elf, no additives	C1-C3	C1-C8	396-452	346-388	-

Although the Diesel with Ferrocene sample was supposed to contain ferrocene as antiknock, no ferrocene was found in the sample. This can be explained by the sample preparation. Ferrocene is quite unpolar and was probably removed with the unpolar fraction of the alkanes. As with the gasolines, the elf with no additives sample contained comparably few alcohols and phenols. However, quite a few unknown compounds of series A and D were found in this sample.

7.5 CONCLUSION

The novel hyphenation of electrochemistry with LC/APCI-MS was employed for the qualitative determination of alcohols and phenols in seven gasoline and four diesel fuels after derivatization with ferrocenecarboxylic acid chloride. The selective and sensitive method allowed the detection of several groups of alcohols and phenols. Compounds of both low and high molecular mass were found. Apart from the alcohols and phenols, the samples also contained other compounds that could be grouped into four series. Although LC/electrochmistry/MS/MS was used in an attempt to identify these groups, the structure of these compounds was not fully elucidated and further studies with exact mass determination using time of flight mass spectrometry will have to be carried out when an appropiate instrument is available.

7.6 ACKNOWLEDGMENT

I thank H. Luftmann of the Institut für Organische Chemie (University of Münster, Münster, Germany) for the opportunity to use the LC/MS/MS equipment and for his advice concerning many mass spectrometric questions. The gasoline and diesel samples were kindly provided by T. Schmidt of the Swiss Federal Institute for Environmental Science and Technology (EAWAG, Duebendorf, Switzerland). Help with the sample preparation and derivatization by B. Roberz and F. Wasinski of the Institut für Anorganische und Analytische Chemie (University of Münster, Münster, Germany) is gratefully acknowledged.

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8. ANALYSIS OF MINERAL OIL SAMPLES USING LC/ELECTRO-CHEMISTRY/MS

8.1 SUMMARY

Two oil samples that are used as standard reference materials were analyzed by LC/electrochemistry/APCI(+)-MS and LC/electrochemistry/ES(+)-MS after a derivatization step with ferrocenecarboxylic acid chloride and a short sample preparation. Phenol and the C1 to C9 alkylphenols were detected in the resulting chromatograms using selected ion monitoring. Quantitation was performed by external calibration and the results were compared to data obtained by the NIST using GC-FID and LC-ECD as well as by Rolfes and Andersson employing GC-AED.

8.2 INTRODUCTION

Several methods for the determination of low molecular weight phenols in organic matrices have been reported. To obtain a sum parameter for all phenols, titrimetric determinations have already been used in the 1950s [1]. IR spectroscopy and NMR spectroscopy were utilized after derivatization with trifluoracetic acid anhydride [2]. Following the isolation of the phenol fraction, high resolution chromatographic techniques have to be used for the analysis of individual alkylphenols. A sample preparation is needed in many cases because of the complexity of the oil samples. Most isolation methods use an aqueous alkaline extraction step that is followed by acidification in combination with back extraction into an organic solvent for GC or LC analysis [3-6]. Other isolation techniques involve solid phase extraction [7], thin-layer chromatography [8] or adsorption column chromatography [9]. Although LC separation with electrochemical detection is easy to perform, due to the limited separating power of liquid chromatography only phenol and the cresols can be determined without coelution [7]. The C2 and C3 alkylphenols can only be sufficiently separated by GC techniques but then a derivatization step is normally necessary.

An interesting approach that involves derivatization of the alkylphenols with ferrocenecarboxylic acid chloride to the corresponding ferrocenecarboxylic acid esters was reported by Rolfes and Andersson [10-12]. They used gas chromatography with atomic emission detection at the iron line for a highly selective and sensitive determination method of the C0 to C3 alkylphenols in oil samples. Since

they kindly provided their derivatized oil samples for further analysis, the newly developed LC/electrochemistry/MS technique for the determination of ferrocene derivatives [13] could be used for the determination of groups of alkylphenols in those samples. Thus, it would be possible to compare the results of the LC/electrochemistry/MS measurements with those of Rolfes and Andersson that were determined using an independent method.

8.3 EXPERIMENTAL

8.3.1 Chemicals

Ammonium formate were purchased from Aldrich Chemie (Steinheim, Germany). Formic acid was obtained from Fluka (Buchs, Switzerland). Solvent for LC was acetonitrile LiChroSolv gradient grade from Merck (Darmstadt, Germany).

8.3.2 Oil samples

Two derivatized mineral oil samples were kindly provided by J. Rolfes and F. Wasinski of the Institut für Anorganische und Analytische Chemie of the University of Münster (Münster, Germany). These samples had been derivatized with ferrocenecarboxylic acid chloride according to [10, 11]. Because the derivatized samples were in toluene which is not suitable for reversed-phase liquid chromatography, the solvent was removed from 200 µl of each sample and the residues were redissolved in 1 ml of acetonitrile for LC/electrochemistry/MS analysis.
The first oil sample is standard reference material (SRM) 1582 from the National Institute of Standards and Technology (NIST). The second oil sampe is SRM NGS-NSO-1 from the Norwegian Petroleum Directorate (NPD).

According to the NIST, SRM 1582 is a typical crude oil matrix for the development of analytical methods. The concentration of an analyte is only published by NIST as a certified value if the concentration was determined by two methods of which every step is based on a different principle. Unfortunately, this is not the case for any alkylphenols in SRM 1582 but the concentration of phenol and 2-cresol is given for information purposes although the value is not certified [14]. In the first method, the acidic, basic and neutral components were separated in a acid-base extraction step

and subsequently analyzed by gas chromatography with flame ionization detection without prior derivatization Quantification was performed using an internal standard. The alternative method employed liquid chromatographic fractionation and subsequent LC with electrochemical detection. The determined values were not certified because only one sample was analyzed by GC and the results varied strongly in comparison to the values that were obtained with the alternative method.

The SRM NGS-NSO-1 of the NPD has been used for interlaboratory comparisons in the determination of alkanes, aromatics and certain biomarkers [15]. So far, no determination of alkylphenols was conducted in these interlaboratory comparisons but since this oil is widely available in large quantities it can be expected that it will be used for further studies in the future and it is therefore a suitable sample to test the developed method.

8.3.3 Instrumentation

8.3.3.1 Electrochemical Instrumentation

The electrochemical system from ESA, Inc. (Chelmsford, MA, USA) which was used for on-line LC/electrochemistry/MS is described in detail in section 4.3.2.1. For all experiments, the cell potential was set to 700 mV vs. Pd/H_2.

8.3.3.2 LC/MS Instrumentation

The LC-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 (ES) and atmospheric pressure chemical ionization (APCI) probes and Class 8000 Version 1.20 software.

8.3.3.3 LC/MS/MS Instrumentation

The LC system from Agilent (Waldbronn, Germany) was a HP 1100 with high pressure binary gradient pumping system, vacuum degasser, heated column department, automated liquid sampler and handheld control module. As MS/MS system a Micromass (Manchester, United Kingdom) Quattro LCZ with electrospray probe was employed. The software utilized was Masslynx 3.2.

8.3.3.4 LC/Electrochemistry/MS and LC/Electrochemistry/MS/MS setup

For LC/Electrochemistry/MS experiments, the same setup as in section 4.3.2.3 was employed, whereas for LC/Electrochemistry/MS/MS measurements, the setup of section 6.3.2.3 was used.

8.3.4 LC Conditions

Since the both the Shimadzu and the Micromass electrospray interfaces tolerate only LC flow rates of 0.3 ml/min or less and the APCI interface works best with flow rates of at least 0.6 ml/min, 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 ES 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 0.3 ml/min (2.1 mm id column for ES ionization, injection volume 5 µl) and 0.6 ml/min (3.0 mm id column for APCI, injection volume 10 µl) with the following profile was used:

t [min]	0.01	3	8	23	25	28	28.5
c(CH_3CN) [%]	60	60	90	90	60	60	stop

8.3.5 MS Conditions

For all single quadrupole MS measurements a curved desolvation line (CDL) voltage of -35 V, a CDL temperature of 300 °C, deflector voltages of 35 V and a detector voltage of 1.7 kV were used. The ES source parameters were a probe voltage of 2.5 kV and a nebulizer gas flow rate of 4.5 l/min. The APCI interface was used as heated nebulizer with an APCI temperature of 375 °C, an APCI probe voltage of 0.10 kV and a nebulizer gas flow rate of 2.5 l/min.

8.3.6 MS/MS Conditions

For all electrospray tandem-MS measurements the following parameters were used: capillary voltage 3.01 kV, cone voltage 32 V, source block temperature 110 °C, desolvation temperature 150 °C, collision energy 36 V. The collision gas was argon.

8.4 RESULTS AND DISCUSSION

8.4.1 Analysis of SRM 1582

The derivatized sample of the crude oil SRM 1582 from the NIST was analyzed using LC/electrochemistry/MS with both the APCI and the ES interface. The resulting SIM traces when using the APCI interface are shown in figure 8.1.

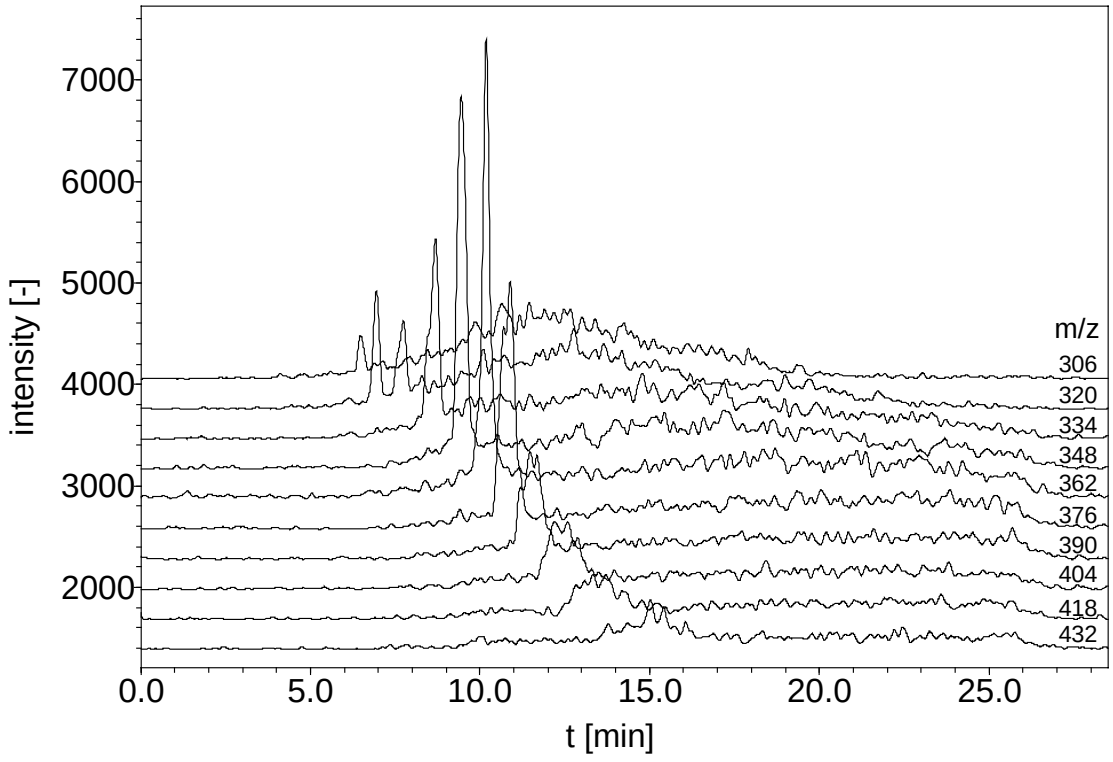

Fig. 8.1 LC/electrochemistry/APCI(+)-MS chromatogram of SRM 1582, recorded in SIM mode (m/z = 306, 320, 334, 348, 362, 376, 390, 404, 418, 432), displayed are base-shifted SIM traces corresponding to the C0 to C9 phenol derivatives.

The concentrations of the alkylphenols were in the low ppm range. At these low concentrations, a broad shoulder in the baseline is observed especially in the lower mass traces. There are different explanations for this observation. It could be caused

by a baseline dependence on the composition of the mobile phase because the nebulization changes with increasing acetonitrile content but this would not explain why this effect seems to affect different SIM traces in different ways. Additionally, only a small amount of this effect was observed in chromatograms of blanks. A second possible explanation would be that there are chemical interferences in the derivatized oil sample. These could be a series of homologues where the smaller homologues elute earlier and the homologues of higher molecular masses elute later which would explain why the shoulder is observed at higher retention times in the higher mass traces. These interferences would have to be something that is either also derivatized by ferrocenecarboxylic acid chloride or that is in another way electroactive because the APCI interface was only used as heated nebulizer and ionization must have occured in the electrochemical flow cell.
This shoulder in the mass traces hindered the integration of the later eluting peaks to some extent. Because of the large number of possible isomers (see section 7.4.1), only a sum parameter could be obtained for the alkylphenols of the same molecular mass. The high molecular weight C8 and C9 alkylphenols eluted in a very broad peak that was difficult to integrate. Therefore, quantitative values for the C8 and C9 phenols are given in italics to emphasize the uncertainty caused by the integration problems. Calibration was performed using external standards. Since selected isomer standards in the calibration experiments are compared to sum parameters in the oil sample measurements it is important to note that the response of the different isomers depends on the electrochemical oxidation efficiency of the ferrocene function which is the same in all isomers. The C7 phenols could not be quantified directly because no C7 phenol standard was available. Instead, they were quantified using the C6 phenol standard. Therefore, the value for the C7 phenols is also given in italics because of the increased uncertainty of this procedure.

Table 8.1 summarizes the alkylphenol concentrations that were obtained using the developed LC/electrochemistry/MS methods and also shows values that were reported by Rolfes and Andersson [11] employing GC-AED as well as the concentrations reported by NIST [14]. For better comparability, the concentrations for the cresol and C2 phenol isomers obtained by Rolfes and Andersson were added to obtain a sum parameter. Since their method allows only the quantification of a few

C3 and higher alkylphenol isomers, no data for the C3 and higher alkylphenols could be displayed.

Table 8.1: Alkylphenol concentrations [µg/g] in crude oil SRM 1582, determined by LC/electrochemistry/APCI(+)-MS, LC/electrochemistry/ES(+)-MS, GC-AED [11, 12] and GC-FID and HPLC-ECD from NIST [14] (not certified). Values for C7, C8 and C9 phenols are given in italics to emphasize their uncertainty due to calibration and integration problems.

	APCI	**ES**	**GC-AED [11. 12]**	**NIST [14]**	
				GC-FID	**HPLC**
phenol	0.26 ± 0.03	0.10 ± 0.06	0.52 ± 0.01	0.26	0.26 ± 0.02
cresols	0.69 ± 0.05	0.81 ± 0.06	1.97 ± 0.10	-	-
C2 phenols	1.74 ± 0.12	2.54 ± 0.49	7.65 ± 0.82	-	-
C3 phenols	2.88 ± 0.14	7.98 ± 2.01	-	-	-
C4 phenols	2.64 ± 0.09	6.88 ± 1.21	-	-	-
C5 phenols	2.96 ± 0.17	9.72 ± 0.72	-	-	-
C6 phenols	1.54 ± 0.08	6.53 ± 0.56	-	-	-
C7 phenols	*(1.22 ± 0.12)*	*(5.00 ± 0.64)*	-	-	-
C8 phenols	*(2.32 ± 0.44)*	*(16.08 ± 4.61)*	-	-	-
C9 phenols	*(1.82 ± 0.40)*	*(16.45 ± 5.15)*	-	-	-

The concentrations that were obtained with the two ionization interfaces differ strongly. In all cases except for phenol, the method utilizing electrospray results in larger concentrations. Andersson and Rolfes found even higher alkylphenol concentrations. The concentration of phenol that was reported by the NIST and the value obtained using LC/electrochemistry/APCI-MS agreed within their uncertainties. For a better method validation, it would be necessary to obtain a standard reference material with certified values for more alkylphenols but this has not been available so far.

8.4.2 Analysis of NGS-NSO-1

The oil sample NGS-NSO-1 from the Oseberg oil field in the North Sea was analyzed by LC/electrochemistry/ES(+) and APCI(+)-MS. Figure 8.2 shows the SIM traces of a chromatogram utilizing the ES interface. This oil sample had higher alkylphenol contents than the SRM 1582 sample. Although the baseline was better than that of the chromatogram in figure 8.1, integration of the broad peaks of the C8 and C9 alkylphenols was still difficult. Therefore, the concentration values of these groups of compounds are again considered uncertain and are thus printed in italics.

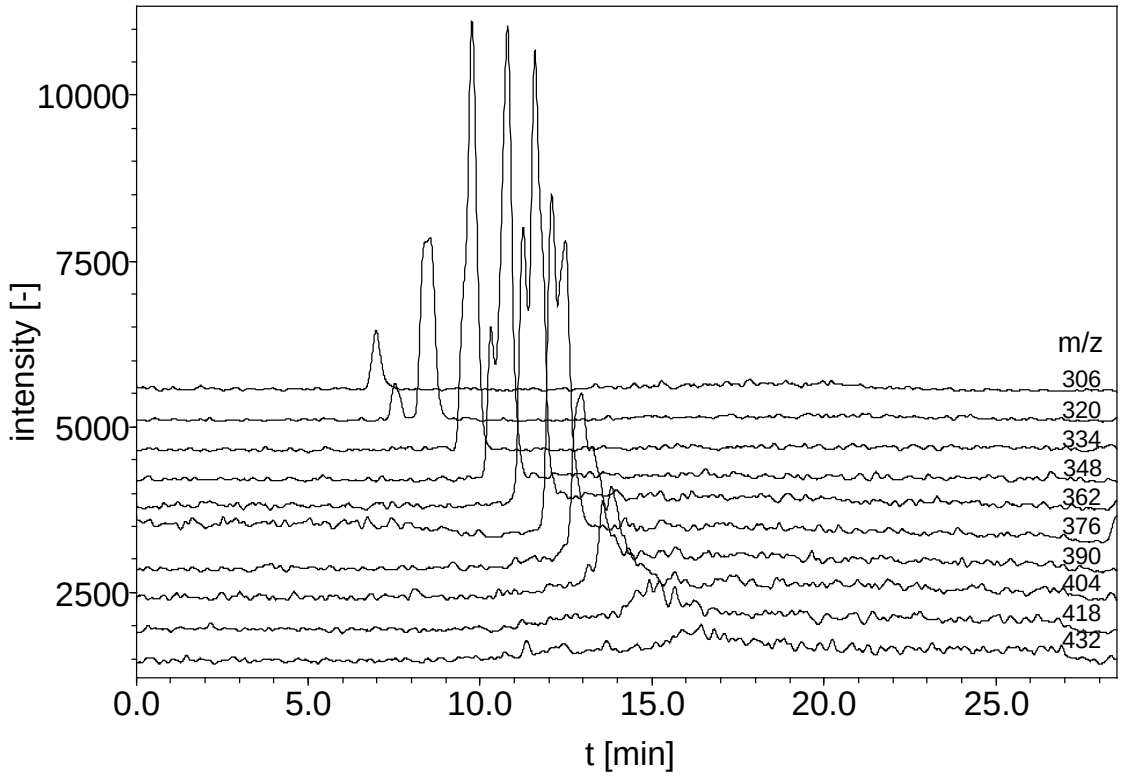

Fig. 8.2 LC/electrochemistry/ES(+)-MS chromatogram of NGS-NSO-1, recorded in SIM mode (m/z = 306, 320, 334, 348, 362, 376, 390, 404, 418, 432), displayed are base-shifted SIM traces corresponding to the C0 to C9 phenol derivatives.

A comparison of the chromatograms of SRM 1582 and NGS-NSO-1 shows that the latter contains a larger fraction of high molecular weight alkylphenols with the C5 phenols being the most abundant. An overview of the determined concentration levels of the alkyphenol groups is given in table 8.2.

Table 8.2: Alkylphenol concentrations [µg/g] in the North Sea oil NGS-NSO-1, determined by LC/electrochemistry/APCI(+)-MS, LC/electrochemistry/ES(+)-MS and GC-AED [12]. Values for C7, C8 and C9 phenols are given in italics to emphasize their uncertainty due to calibration and integration problems.

	APCI	**ES**	**GC-AED [12]**
phenol	4.55 ± 0.23	5.13 ± 1.13	4.88 ± 0.15
cresols	14.06 ± 0.63	14.80 ± 0.89	14.73 ± 0.86
C2 phenols	16.31 ± 0.57	18.64 ± 6.23	21.07 ± 1.18
C3 phenols	19.43 ± 0.54	21.71 ± 4.34	-
C4 phenols	25.51 ± 0.82	27.40 ± 4.66	-
C5 phenols	29.62 ± 0.44	43.95 ± 3.08	-
C6 phenols	14.90 ± 0.73	24.48 ± 1.96	-
C7 phenols	*(12.64 ± 1.13)*	*(21.75 ± 3.33)*	-
C8 phenols	*(19.66 ± 2.91)*	*(19.78 ± 7.91)*	-
C9 phenols	*(21.04 ± 2.97)*	*(12.10 ± 5.44)*	-

The concentrations found using the two different ionization interfaces differed for the NGS-NSO-1 sample only to a small degree. The values reported for the phenols, cresols and C2 phenols by Rolfes and Andersson are also very similar to the concentrations determined by LC/electrochemistry/MS. This demonstrates that at concentrations that are higher than in the crude oil SRM 1582 the developed methods give results that are in overall agreement with other reported data.

8.5 CONCLUSION

The analysis of two derivatized reference material oil samples employing the developed LC/electrochemistry/MS techniques demonstrated the applicability of the new methods although some problems remain. Due to the large number of structural isomers of the alkylphenols and the limited separation efficiency of liquid

chromatography only a sum parameter that includes all isomers of a particular molecular mass can be obtained. This can be a drawback if there is a special interest in a selected isomer but it can also be an advantage if a quick overview of the overall alkylphenol content is needed. The comparison of the results of the developed methods with each other and with reported data showed a large difference for the oil sample SRM 1582 with a very low alkylphenol content but good agreement for the oil sample NGS-NSO-1 which contained larger alkylphenol concentrations. Additionally, the use of LC/electrochemistry/MS allows the determination of alkylphenols of higher molecular mass that are normally not suited for gas chromatographic analysis. Compared to GC-AED, GC-FID and LC-ECD, the detection by MS also allows easier identification of unknowns. Therefore, the method is well suited for identification purposes whereas quantitative results (especially at low concentrations) have to be carefully interpreted.

8.6 ACKNOWLEDGEMENT

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9. CONCLUDING REMARKS AND FUTURE PERSPECTIVE

A new technique hyphenating liquid chromatography, electrochemical on-line oxidation and mass spectrometry was developed for the improved ionization of less polar analytes.

The use of a "coulometric" cell that is inserted between LC and MS has two major advantages when compared to electrochemical oxidation in the ES interface which is the main alternative method for electrochemistry/MS.
The oxidative or reductive potential in the electrochemical cell can be adjusted precisely to the requirements for the analysis whereas the high voltage used in the electrospray interface cannot be adjusted to the requirements of the redox process as it is not possible to gain knowledge about the exact oxidative potential within the ES capillary. Therefore, employing the "coulometric" flow cell, analytes that are more easily oxidized or reduced than interfering substances can be selectively ionized.
The large surface area 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 setup in the ES interface is more similar to a thin layer amperometric cell that has oxidation efficiencies of less than 20%. Although the oxdiation in the electrospray process might be quantitative at very low concentrations, good linearity cannot be expected.

The additional selectivity that is obtained by the coupling of LC to electrochemistry/ MS can be used to lower the effect of interferences in complex sample matrices. Interfering preformed ions, for example, elute before the more unpolar analytes and cannot interfere or suppress the analytes mass signals. Since it is also possible to use fast LC separations with electrochemistry/MS in a time scale of 1 -1.5 min, high throughput analysis may be performed. The simple experimental setup without dedicated column and a graphite in-line filter element as stationary phase could be employed for very simple chromatographic problems where the mass spectrometric detection gives sufficient selectivity.

On-line electrochemistry coupled to tandem mass spectrometry even allows the simultaneous analysis of mixtures using precursor ion scans without prior chromatographic separation. The precursors ions scans can be used for both the alkyl and the

phenyl FCEs because the fragmentation results in common daughter ions for each of the substance classes. LC/electrochemistry/tandem MS in the multiple reaction mode can be employed for highly sensitive and selective determinations.

The applicability of the method could be demonstrated for the qualitative determination of alcohols and phenols in several gasoline and diesel fuels. The limited separating power of liquid chromatography is the reason that only sum parameters of the different alcohol and phenol derivatives can be obtained but an advantage of the LC technique is the ability to determine higher alkylphenols that cannot be analyzed by normal gas chromatographic techiques. A drawback of the simple fragmentation to common daughter ions is the limited ability of to elucidate the structure of unknown compounds by MS/MS. Therefore, it was not possible to identify the compounds that were found in the gasoline and diesel samples besides the alcohols and phenols and that could be grouped into four series. This might have been possible using exact mass measurements with a time of flight mass spectrometer but no such instrument was available.

The quantitative determination of alkylphenols in derivatized standard reference material samples of two oils showed the same problems with many structural isomers that could not be resolved chromatographically resulting in a sum parameter for each group of alkylphenols with the same molecular mass. This can be a disadvantage if there is a special interest in a selected isomer but it can also be an advantage if a quick overview is needed. The comparison of the results of the developed methods with each other and with literature data demonstrated the applicability for oils with higher alkylphenol content but the use of an internal standard may be necessary at very low alkylphenol concentrations. An advantage over the GC based determination methods is the wide range of alkylphenols that can be analyzed because the LC/electrochemistry/MS hyphenation is not restricted to low molecular weigth alkylphenols.

As the overview of current research on the on-line coupling of electrochemistry to mass spectrometry and the large number of papers from the last two years demonstrated, an increasing impact on the analytical science has to be expected in the future. The potential of this technique could be used to solve various problems in

biomedical and environmental analysis. Due to the easy experimental setup, methods employing electrospray ionization as an electrochemical reactor could be favoured although the hyphenation of additional electrochemicall flow cells to LC/MS further increases the possible applications of this group of methods. Despite the various applications for liquid chromatography/electrochemistry/mass spectrometry it has to be stated that limitations due to the restriction to electrochemically active analytes or electroactive labels remain.

SUMMARY

A powerful new hyphenated technique based on the combination of LC, on-line electrochemical ("coulometric") oxidation and ES- or APCI-MS has been developed. Simple and commercially available instrumentation has been used to set up the analytical system.

Ferrocenecarboxylic acid chloride was prepared from ferrocenecarboxylic acid and used as derivatizing reagent for the synthesis of several alcohol and phenol derivatives that were characterized by IR, EI-MS and ^{1}H-NMR. These ferrocenecarboxylic acid esters have been separated by reversed-phase LC and oxidized (ionized) "coulometrically" prior to single quadrupole MS analysis using electrospray ionization and atmospheric pressure chemical ionization interfaces. The dependency of the ionization on the electrochemical pretreatment was demonstrated. Limits of detection for selected derivatives ranged from 3 nM to 0.4 µM depending on the individual compound and the selected interface. To test the applicability of the new hyphenation technique for fast LC separations, conventional guard columns were employed for the separation of mixtures containing up to nine alcohol or phenol derivatives that could be accomplished in a time of 1 - 1.5 min depending on the number and structure of the individual compounds. Nanospray and electrospray ion sources were used in combination with tandem mass spectrometry to study the fragmentation pathway of the ferrocenecarboxylic ester derivatives. Common fragmentation products for each substance class enabled the use of precursor ion scans. LC/electrochemistry/ES(+)-MS chromatograms were recorded in multiple reaction monitoring mode. The limits of detection for selected phenol derivatives were below 1 nM. Seven gasoline and four diesel samples were then analyzed by LC/electrochemistry/ APCI(+)-MS. In the resulting complex chromatograms, selected mass traces were used to identify several groups of alcohols and alkylphenols. Only a sum parameter for alcohols and phenols of the same molecular mass could be obtained because of the large number of structural isomers that were not chomatographically resolved in the mass traces. Apart from the alcohols and phenols, several other compounds were found in the samples. Many of these unknown compounds could be arranged in four series with a mass difference of 14

mass units. Tandem-MS experiments were performed to identify the unknown substances but only little information was gained. Finally, to validate the method two oil samples that are used as standard reference materials were analyzed by LC/electrochemistry/MS after a derivatization step with ferrocenecarboxylic acid chloride and a short sample preparation. Phenol and the C1 to C9 alkylphenols were detected in the resulting chromatograms using selected ion monitoring. Quantitation was performed by external calibration and the results were compared to data obtained by the NIST and another research group employing independent methods.

SAMENVATTING

Dit proefschrift beschrijft de ontwikkeling van een krachtige, nieuwe, gekoppelde techniek gebaseerd op een combinatie van vloistofchromatografie (LC), on-line electrochemische oxidatie en electrospray (ES)- of atmosferische druk chemische ionisatie (APCI)-massaspectrometrie (MS). Er is gebruik gemaakt van eenvoudige en commercieel verkrijgbare instrumentatie om het systeem samen te stellen.

Ferroceencarbonzuurchloride werd gesynthetiseerd uitgaande van ferroceen-carbonzuur en vervolgens gebruikt als derivatiseringsreagens voor de synthese van verschillende alcohol- en fenolderivaten, die dmv. IR, EI-MS en ^{1}H-NMR zijn gekarakteriseerd. Deze ferroceencarbonzuur-esters zijn gescheiden m.b.v. reversed-phase LC en geoxideerd (geïoniseerd) voordat analyse met single quadropool MS in combinatie met ES- en APCI-interfaces kon plaatsvinden. Effect van de electrochemische voorbehandeling op de ionisatie werd aangetoond. De detectielimieten van de geselecteerde derivaten varieerden van 3 nM tot 0.4 µM, afhankelijk van de betreffende verbinding en de gekozen interface.
Om de toepasbaarheid van de nieuwe gekoppelde techniek op snelle LC-scheidingen te testen zijn conventionele voorkolommen gebruikt. De scheiding van de mengsels tot negen alcohol- of fenolderivaten kon in 1 à 1.5 min. worden bewerkstelligd, afhankelijk van het aantal en de structuur van de betreffende verbindingen.
Voor onderzoek naar het fragmentatiepatroon van de ferroceencarbonzuur-esterderivaten zijn nanospray- en electrospray-ionenbronnen gebruikt, in combinatie met tandem-MS.
Door de vorming van de gebruikelijke fragmentatieproducten bij elke klasse van verbindingen kon gebruik worden gemaakt van „precursor ion scans“.
LC/electrochemie/ES(+)-MS chromatogrammen werden verkregen in „multiple reaction monitoring mode“. Detectielimieten waren lager dan 1 nM. Vervolgens zijn zeven benzine- en vier dieselmonsters geanalyseerd mbv. LC/electrochemie/ APCI(+)-MS. In de resulterende chromatogrammen zijn specifieke „mass traces“ gebruikt om de verschillende alcohol- en alkylfenolgroepen te identificeren.

Voor de alcoholen en fenolen kon slechts een somparameter van dezelfde massa worden verkregen vanwege de vele niet-gescheiden structuurisomeren. Behalve de alcoholen en fenolen werden verschillende andere componenten in de monsters gedetecteerd. Vele van deze onbekende verbindingen konden in vier series worden geclassificeerd met een verschil van 14 massa-eenheden. Er zijn enkele tandem-MS experimenten uitgevoerd ter identificatie van de onbekende verbindingen, dit leverde echter weinig informatie op. Tenslotte zijn er 2 olie-monsters ter validatie geanalyseerd dmv. LC/electrochemie/MS, na een derivatiseringsstap met ferroceen-carbonzuurchloride en een korte monstervoorbehandeling.
In de chromatogrammen werden fenol en de alkylfenolen van C1-C9 gedetecteerd dmv. „selected ion monitoring“. Kwantificering vond plaats door externe calibratie en de resultaten werden vergeleken met die van het NIST (National Institute of Standards and Technology) en andere onderzoeksgroepen welke gebruik maakten van onafhankelijke methoden.

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CURRICULUM VITAE

PERSONAL DETAILS

- **Name**: J. Georg R. Diehl
- **Nationality**: German
- **Date of Birth**: 17.01.1974
- **Born in**: Bremen, Germany

EDUCATION

- **1980-1984** Elementary School, Fischerhude, Germany
- **1984-1990** Gymnasium Arnoldinum, Steinfurt, Germany
- **1990-1991** Exchange student, Walton High School, Atlanta, Georgia, USA
- **1991-1994** Städtisches Gymnasium Borghorst, Steinfurt, Germany
  graduation in June 1994 (mark: 1.4)
- **1994-1999** Studies of Chemistry at the University of Münster, Germany
  Intermediate exam in December 1996 (mark: very good, 1.5)
  Graduation (diploma) in March 1999 (mark: with distinction, 1.0),
  Thesis: ”*New Methods for the Determination of Phenothiazines”*
- **4/1999** Begin of the dissertation in Analytical Chemistry at the University of Münster with Prof. Dr. U. Karst
- **10/2001** Continuation of the dissertation at the University of Twente, Enschede, The Netherlands with Prof. Dr. U. Karst
- **1999-2001** Fellowship of the Fonds der Chemischen Industrie, Frankfurt, Germany

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1. Diehl G, Karst U (2000) J Chromatogr A 890: 281-287
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2. Diehl G, Karst U.; 11. Chromatographie-Symposium (Hohenroda, Germany)
   HPLC/Elektrochemie/MS - Eine Kopplungstechnik mit Potential
3. Diehl G, Karst U; Pittcon 2001 (New Orleans, Louisiana, USA)
   Improving HPLC-ESI-MS with Electrochemically Reactive Derivatization Reagents
4. Diehl G, Karst U; InCom 2001 (Düsseldorf, Germany)
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