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Review

Strategies for the liquid chromatographic–mass spectrometric analysis of non-polar compounds

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Abstract

Electrospray ionization and atmospheric pressure chemical ionization (APCI) have evolved recently as very useful tools for the liquid chromatographic–mass spectrometric (LC–MS) analysis of polar substances. Non-polar compounds, however, are difficult to analyze with these atmospheric pressure ionization techniques due to their soft ionization mechanism. Recently, new approaches have been introduced which are likely to overcome this obstacle, at least partly. On-line electrochemical conversion of the analytes to more polar reaction products, atmospheric pressure photoionization, atmospheric pressure electron capture negative ion-MS and coordination ionspray-MS are four techniques which are presented in detail, compared and discussed critically with respect to their current status and future perspectives. Particular focus is directed from a chemical viewpoint on the substance groups which are accessible by each of the new approaches.

Keywords: Liquid chromatography–mass spectrometry; Mass spectrometry; Reviews; Electrochemical conversion; Atmospheric pressure photoionization; Atmospheric pressure electron capture negative ion-MS; Coordination ionspray; Non-polar compounds

Contents

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1. Introduction

In the last 15 years, the coupling of liquid chromatography and mass spectrometry (LC–MS) has evolved into an extremely powerful analytical technique. The use of atmospheric pressure ionization (API) techniques, mainly comprising electrospray ionization (ESI) [1,2] with different variations [3,4] and atmospheric pressure chemical ionization (APCI) [5,6], allows both the transfer of the LC effluent into the gas phase and the ionization of the analytes. With protonation (in the positive ion mode) and deprotonation (in the negative ion mode), acid–base reactions are the most frequently observed ionization mechanisms. These reactions will, of course, be observed mainly by strongly polar analytes. The addition of alkali ions such as Na^+ or of other ions such as NH_4^+ in the positive ion mode or of Cl^-, formate or acetate in the negative ion mode favors the ionization of rather polar compounds. Therefore, many pharmaceuticals, peptides and proteins are today easily accessible by LC–MS using API interfaces. Owing to the significance of these techniques, a large number of reviews cover the use of LC–API-MS for the analysis of polar or charged compounds. These include reviews on technological aspects as well as on applications in particular fields of research.

Niessen has summarized the state of the art of LC–MS with special focus on ionization techniques and mass analyzers [7]. Gelpi has presented the latest developments in the interfacing of liquid-phase separations and mass spectrometry [8]. The technical aspects of the combination of LC and MS will therefore not be covered in this review.

The typical ionization mechanisms of ESI and APCI indicate that it will be difficult to analyze non-polar compounds with ESI- or APCI-MS. Substances which are not prone to undergo acid–base reactions will be detected by ESI-MS only in exceptional cases, e.g. when other adducts with polar ions are formed. This, however, is not likely for non-polar compounds. This is supported by experimental data, where these substances were determined only with poor limits of detection or not at all. Some expansion of the applicability range of APCI to less polar compounds can be achieved by removing ammonium acetate or formate from the mobile phase. Using normal-phase LC in combination with APCI-MS would be another promising strategy. However, the use of normal-phase LC is associated with a large number of problems, including limited compatibility with water in the samples, which results in limited reproducibility. In most cases, the use of normal-phase LC will therefore not be an option. One could now argue that the determination of low-polarity analytes could generally be carried out better by gas chromatography (GC) with mass spectrometric detection using electron ionization (EI). There are, nevertheless, at least two major reasons that can be used to explain why it is useful to expand the applicability of LC–MS to less polar analytes:

Not all non-polar substances are accessible by GC–MS with electron ionization. There are limits caused by the low volatility of the analytes, by their thermolability and by the size of the molecules. ESI and APCI mostly result in mass spectra with the pseudomolecular ion as base peak and little or no fragmentation. In combination with a tandem mass analyzer (e.g., triple quadrupole or ion trap), additional structural information may be gathered, although the possibilities for library searching are limited in comparison with GC–EI-MS. Tandem mass spectrometry will also increase the selectivity of the analysis, being advantageous for the determination of trace concentrations in very complex matrices. The capabilities of different mass analyzers have been described in detail in Ref. [9] and will therefore not be covered in this review.

It is therefore desirable to expand the applicability of LC–MS with API techniques to the determination of rather non-polar compounds. In the last few years, several interesting approaches for this purpose have been suggested. A selection of these is summarized within this review. In the following, the background and applications of four different techniques, comprising: the combination of on-line electrochemical conversions with mass spectrometry; atmospheric pressure photoionization (APPI); electron capture negative ion APCI-MS; and coordination ionspray-MS (CIS-MS) will be presented and critically discussed.

2. Coupling electrochemistry to API-MS

2.1. General aspects of electrochemistry–MS

With respect to the LC–MS analysis of non-polar

compounds, the electrochemical conversion of the analytes to more polar or even charged analytes, which are then easily accessible by ESI- or APCI-MS, is the primary goal. Additionally, this technique allows the on-line observation of electrochemical reactions by mass spectrometry, which is advantageous compared with the previous off-line techniques. Within this review, the major focus will be the on-line approach, and particular focus will be directed to those applications where coupling to liquid-phase separation techniques has either been carried out or is in principle possible. However, those key articles which introduced relevant techniques in conjunction with other LC–MS interfaces are also summarized.

The first publication on the on-line coupling of electrochemistry and MS goes back to the early 1970s, where the combination of a porous electrode with the gas inlet system of a mass spectrometer was reported by Bruckenstein and Gadde [10]. Similar techniques were used frequently for the analysis of volatile species in the following years. These have been reviewed exhaustively by Chang et al. [11], Vielstich et al. [12] and Brajter-Toth et al. [13] and will therefore not be subject to this review.

2.2. The electrospray interface as electrochemical reactor

The on-line coupling of electrochemistry with mass spectrometry for the determination of non-volatile compounds in solution was first described by Hambitzer and Heitbaum in 1986 [14], who coupled a three-electrode electrochemical flow cell to a thermospray mass spectrometer. Five years later, it was recognized that the high potential at the capillary tip of an electrospray needle itself induces redox reactions. Since then, the electrospray interface has been used intentionally as an ‘‘electrochemical reactor’’ [15,16]. Many important contributions to this field have been made by Van Berkel and co-workers. To exclude possible interferences from the spraying process, they directly connected an ESI source with the detection cell of a diode-array spectrometer, leaving the oxidized species in solution until their detection [17].

Solvent effects have, of course, to be considered as well. Besides its properties to dissolve the analytes and to stabilize the spray, the formed initial oxidation products, e.g. radical cations, have to be stabilized and protected from further reactions [18]. Using the electrospray source as a controlled-current electrolytical flow cell, Van Berkel et al. were able to detect metal porphyrins, polycyclic aromatic hydrocarbons (PAHs), phenothiazine and other compounds as their radical cations [19].

Not surprisingly, those compounds that are known to be readily oxidized by means of classical electrochemical conversions are also likely to undergo electrochemical oxidation in the electrospray interface. These are mainly compounds with low redox potentials and reversible redox kinetics. Cole et al. presented the oxidation of metallocenes and their derivatives in the electrospray emitter [20], while McCarley and co-workers found doubly charged biferrocenes and oligoferrocenylsilanes with up to four charges per molecule [21]. The separation and identification of higher fullerenes from carbon soot as their respective anions was achieved by Jinno et al. in the negative ion mode, proving that reduction processes may also be observed [22]. Other fullerenes and their derivatives have been investigated by ESI-MS in the negative ion mode by Peel et al. [23,24], Gross et al. [25] and Drewello et al. [26].

2.3. Derivatization for electrochemistry–MS

Derivatization techniques are of great importance in trace analysis using chromatographic techniques, because they allow the introduction of a functional group which can be detected with the selected system. Furthermore, the chemical and physical properties of the analytes may be changed as desired, e.g. with respect to volatility for GC analysis or to adsorption phenomena in reversed-phase LC. On the other hand, derivatization reactions are typically not the first choice, because they are laborious and they may be a potential source of additional problems. It is frequently observed that the yield from derivatization is far from quantitative, which will lead to difficult quantification of the analytes. However, in those cases where direct analysis is either problematic or not possible at all, derivatization is an interesting option. In the case of the derivatizing agents mentioned below, the derivatization yield is high, and strong improvements with respect to selectivity and sensitivity are observed.

The above-mentioned findings concerning the

electrospray interface as electrochemical reactor have led to the development of dedicated ferrocene-based derivatizing agents for the ESI-MS analysis of alcohols by Van Berkel et al. [27]. Fruit extracts [27] and plant oils [28] were investigated using this technique, and the tandem MS capabilities of the ferrocene derivatives were investigated in more detail [29]. Ferrocenecarboxylic acid azide is used as derivatizing agent, and it is converted by heat into ferrocenyl isocyanate, which then reacts with alcohols under formation of the respective urethanes. Ionization occurs in the ESI source without the use of an additional electrochemical cell. The respective reaction scheme is presented in Fig. 1. Ferrocene boronic acid may be used to derivatize alkenes after their oxidation to diols [30] and for the analysis of several neutral mono- and disaccharides [31]. A similar approach was used by Williams et al. for the determination of derivatized estrogens [32].

2.4. Use of external electrochemical cells

All of the authors of the publications summarized in Section 2.3 used the electrospray interface without connection to a separation technique, although this is possible in principle. The major advantage of these methods is the easy setup, as there is no need for any additional equipment. Disadvantages include possible problems in controlling the conditions for the electrochemical conversions, because it is extremely difficult to precisely adjust the redox potential for the electrochemical conversions independently from the spray conditions. Additionally, an (almost) quantitative electrochemical conversion, which would lead to higher signals and would be helpful for easy interpretation of the mass spectra of complex samples, may theoretically only be achieved under extremely low flow-rates. These are hardly compatible with fast separations in reversed-phase LC.

The use of external electrochemical cells is a possibility to overcome these obstacles, although modifications of the equipment have to be carried out. Hambitzer and Heitbaum [14] were the first to couple a three-electrode electrochemical arrangement with thermospray-MS. This approach was expanded to other analytes by Brajter-Toth and Yost, who furthermore applied a commercial ‘‘coulometric’’ cell, which received this name from its manufacturer, because it may provide for quantitative conversions of the analytes under optimized conditions. Uric acid [33,34] and 6-thioxanthine [33] were studied in this way. Regino and Brajter-Toth also used a thin-layer electrochemical cell, which allows easier access to the electrode surface, but which is characterized by a non-quantitative conversion rate [35]. Zhou and Van Berkel [36] connected different electrochemical cells to an electrospray mass spectrometer. The respective experimental arrangements are presented in Fig. 2. A self-assembled tubular electrode cell was used in addition to a commercially available thin-layer cell and a porous glassy carbon electrode. To couple these cells on-line with the electrospray emitter, they must either be used in the ‘‘floated mode’’, with the potential of the electrospray capillary applied to the cell, or completely decoupled from the high potential of the electrospray needle. To minimize the time

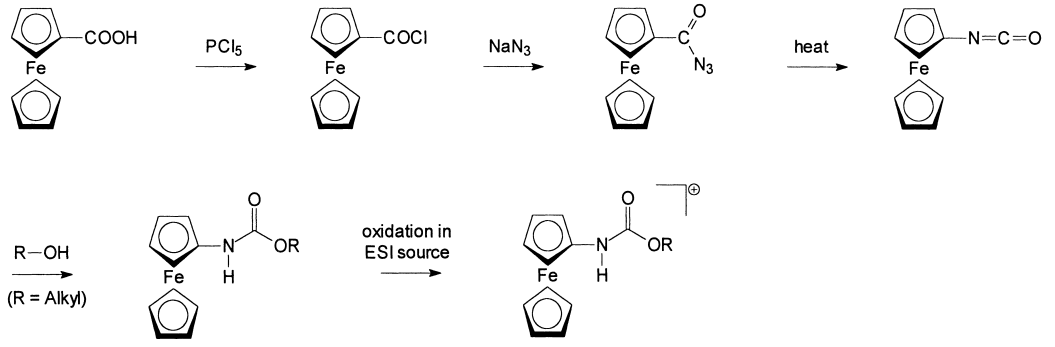

Fig. 1. Reaction scheme for the synthesis of and the derivatization of alcohols with ferrocenecarboxylic acid azide as well as the in-source oxidation of the formed urethanes [27,28].

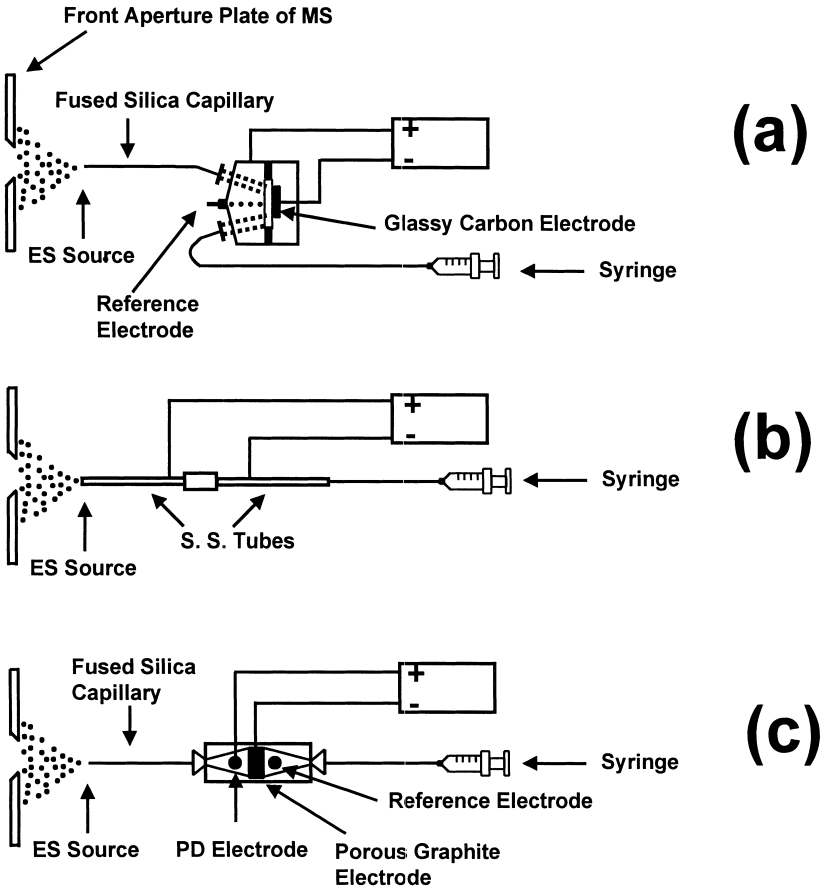

Fig. 2. Scheme of electrochemical flow cells used on-line with ESI-MS: (a) thin-layer electrode cell, (b) tubular electrode cell, (c) porous graphite electrode cell. Reprinted, with modifications, from Ref. [36], with permission.

between electrochemical oxidation and mass spectrometric detection of the analytes, Cole et al. constructed a low-volume three-electrode cell within the electrospray probe [37,38]. This arrangement allowed the detection of polycyclic aromatic hydrocarbons as their radical cations. Other redox reactions, including the anodic oxidation of diphenyl sulfide, the reduction of nitrobenzene and the nucleophilic addition of pyridine to the electrogenerated 9,10-diphenylanthracene radical cation, were observed using the same setup [39]. The oligomerization of aniline using on-line electrochemistry–MS was studied by Deng and Van Berkel et al. [40]. In a thin-layer cell, which was operated at a potential of 1.0 V vs. Ag/AgCl, protonated oligomers consisting of up to 10 monomer molecules were generated. Kertesz and Van Berkel used the same arrangement to study the electropolymerization of methylene blue [41]. With the oxidation of dopamine in an aqueous/organic phase, Deng and Van Berkel expanded the range of applications to the bioanalytical field [42]. Metabolic oxidation reactions of a dopamine antagonist were mimicked by Bruins et al. in an electrochemistry–MS system [43].

The use of electrochemical cells provides for more defined conditions than the approaches used for direct oxidation in the ESI interface. However, it has to be considered that the conversion rate in the electrochemical cell will depend on the type of cell and the flow-rates used. This is one of the most crucial factors when coupling electrochemical conversions on-line with MS. Two different approaches

are attractive for the combination of LC, electrochemistry and MS. To identify the very complex product mixtures generated in the electrochemical cell, the sequence of electrochemistry with subsequent LC–MS is advantageous. In this case, follow-up reactions may occur due to the long transfer time before detection of the products. On the other hand, quantitative information on non-polar analytes can best be obtained by LC–electrochemistry–MS, as non-polar analytes may best be separated under reversed-phase conditions, and the formed more polar species are easier accessible to API-MS detection. While the latter approach also allows for the quasi-simultaneous detection of the reaction products of several analytes during one LC run, the earlier approach is preferred in the case of a complex product mixture.

2.5. Combining electrochemistry with LC and MS

Both approaches, LC–electrochemistry–MS and electrochemistry–LC–MS have been described in the literature. The first report on LC–electrochemistry–MS was described by Volk et al. in 1989 [44]. Using 6-thiopurine as an example, the oxidation of thiopurines was studied using a glassy carbon working electrode with a large surface area, and subsequent reversed-phase LC separation with thermospray-MS detection. Further investigations on the electrochemical reaction pathways of 6-thiopurine and 6-thioxanthine were published later by the same authors using the detection system described above [45]. The same experimental arrangement was also used to compare the electrochemical oxidation of uric acid with its enzymatic oxidation using hydrogen peroxide as oxidant and peroxidase as biocatalyst [46]. Iwahashi and Ishii described a similar approach for the electrochemistry–LC–MS detection of the tryptophan metabolite 3-hydroxy-*dl*-kynurenine. Again, a porous glassy carbon cell was used to ensure high analyte turnover, but detection was performed by ESI-MS [47]. The same instrumentation was also used to study the electrochemical oxidation of 3-hydroxyanthranilic acid [48]. Bruins et al. have recently described the use of a system which allows for the LC–MS–MS observation of products of the Fenton reaction [49]. Hydroxyl radicals are generated electrochemically in a porous glassy carbon cell, reacted with xenobiotics, and the products are characterized in the LC–ESI-MS–MS instrument. The respective reaction pathways, including the electrochemical generation of hydroxyl radicals and subsequent chemical reactions, are presented in Fig. 3 [49].

The first report on LC–electrochemistry–MS was described by Dewald et al., who separated phenolic isoflavones by reversed-phase LC, and used a thin-layer cell for electrochemical and a thermospray-MS for subsequent mass spectrometric detection [50]. In this work, the electrochemical cell was not used to improve the MS detection. The method suffered from non-optimum interfacing between the two detectors. Although it is advantageous to obtain data from the two detectors simultaneously, the limited compatibility of gradient elution with electrochemical detection is also valid for this method. A porous glassy carbon cell was used for the post-column oxidation of various phenols to the respective fluorescent bisphenols [51]. Based on this oxidation, a LC–electrochemistry–fluorescence method for the quantification of phenols was developed, and oligomers of ethylphenol with two, three and four monomers were observed by on-line LC–electrochemistry–APCI(−)-MS.

Recently, Karst et al. [52] presented a method for the determination of ferrocene-labelled alcohols and phenols, in which the analytes were derivatized with ferrocenecarboxylic acid chloride according to literature procedures [53,54]. The derivatives were separated by reversed-phase HPLC and subsequently oxidized in a porous glassy carbon electrode under formation of the charged ferrocinium products. The respective chemical reactions are presented in Fig. 4. The ferrocinium derivatives were detected in a ‘‘heated nebulizer’’ or thermospray mode using a commercial APCI interface with the corona discharge switched off. The method has also been combined with very fast separations using reversed-phase columns of only 20 mm length [55]. In this way, a series of nine phenol derivatives was separated in less than 1.5 min, and a series of six alcohol derivatives in less than 1 min. It can be expected that this and similar labelling strategies will further broaden the applicability of LC–electrochemistry–MS.

Three decades after the first coupling between

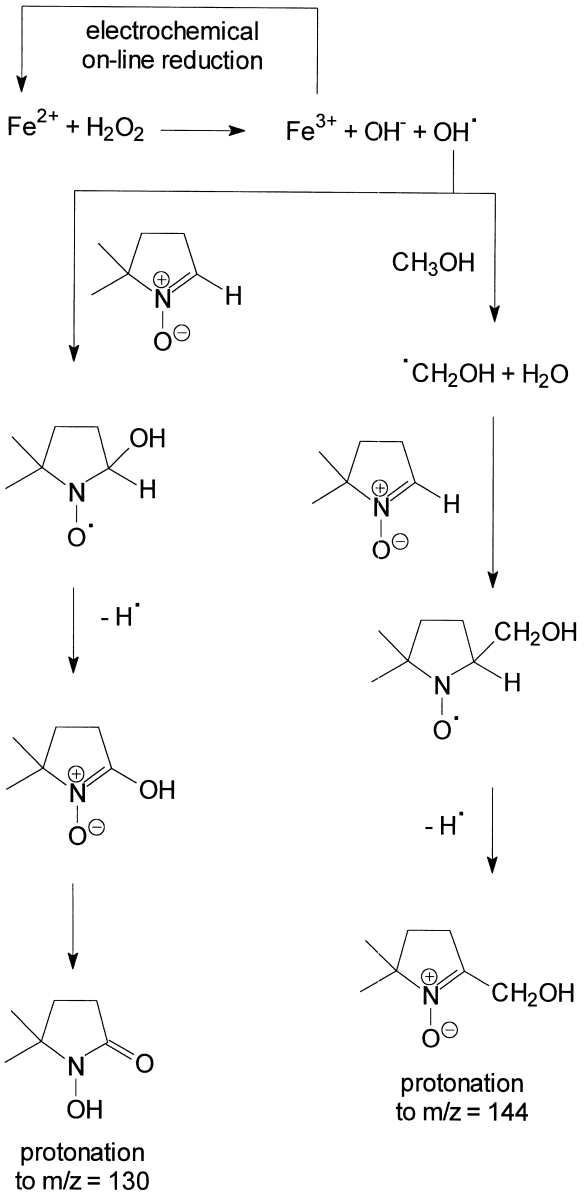

Fig. 3. Reaction scheme for the electrochemically assisted Fenton reaction with subsequent radical scavenging by 5,5-dimethyl-1-pyrrolidone-*N*-oxide (DMPO) and LC–MS analysis of the protonated products [49].

electrochemistry and MS, this field of research is currently experiencing a dynamic development, as described in this article and, with different focus, in previous reviews [11–13,56]. It can be expected that bioanalytical applications of electrochemistry and MS will strongly gain importance in the near future. The groups of Bischoff and Bruins, for example, have recently presented a method for the specific electrochemical cleavage of peptides with on-line mass spectrometric detection [57]. This and related methods are likely to be used either directly or coupled with liquid chromatography in proteomics and related fields.

3. Atmospheric pressure photoionization (APPI)

As an alternative ionization technique to API-MS for non-polar compounds, atmospheric pressure photoionization (APPI) has recently been introduced by Bruins et al. [58]. The APPI interface can be considered as a modified APCI source, with the corona discharge being replaced by a gas discharge lamp, which emits photons in the vacuum UV region of the electromagnetic spectrum. The setup of the APPI interface is presented in Fig. 5. When the energy of the photons is higher than the first ionization potential of a species in solution, their absorbance leads to single-photon ionization. As the common LC solvents are characterized by high first ionization potentials, selective ionization of the analytes may occur [58]. The addition of a dopant, an additive to the mobile phase that is first ionized itself and then leads to the ionization of the analytes in further reactions, resulted in greatly enhanced signals [58]. Toluene and acetone were added post-column to the eluent using a syringe pump. Different non-polar compounds were successfully analyzed using this approach, including polycyclic aromatic hydrocarbons (naphthalene and anthracene), drugs (carbamazepine, caffeine) and various other organics (testosterone, diphenyl sulfide) [58]. The structures of some substances which were successfully analyzed by APPI-MS are depicted in Fig. 6. Mechanistic studies were carried out by Koster and Bruins [59], who concluded that, in the case of proton-accepting, reversed-phase eluents, proton transfer to the analyte will take place via a protonated solvent cluster. In the case of normal-phase eluents, which cannot accept protons, radical cation formation by charge transfer is favored.

Syage and co-workers presented low-pressure photoionization (LPPI) and APPI, the latter also in

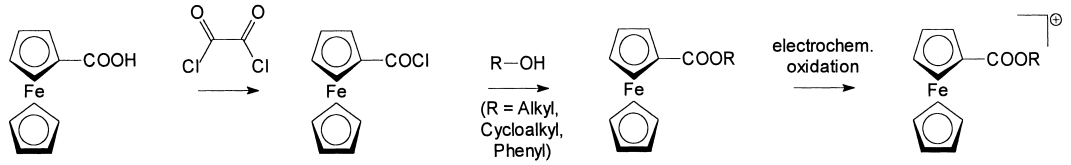

Fig. 4. Derivatization [53,54] and LC–electrochemistry–MS analysis [52,55] of alcohols and phenols using ferrocenecarboxylic acid chloride.

combination with liquid chromatography, as new tools for drug discovery [60,61]. As in the work of Bruins et al. [58], dopants were found to offer increased sensitivity for some classes of compounds, although good results were also obtained in many cases without the presence of a dopant [60].

Kostiainen et al. thoroughly investigated the ionization mechanism and the effect of solvent on the APPI of naphthalenes [62]. A series of seven naphthalene derivatives with electron-donating and electron-withdrawing substituents was investigated in 13 different solvents. Charge exchange and proton transfer were predominantly observed in the positive ion mode, while in the negative ion mode, electron capture or charge exchange were registered for compounds with high electron affinity and proton transfer for compounds with high gas-phase acidity. Again, the ionization efficiency was significantly higher with dopant than without, which was considered as an indication that photoionization of the dopant initiates the ionization process [62]. The group of Kostiainen applied the APPI technique to the analysis of different groups of compounds, including flavonoids [63], steroids [64] and drug metabolites [65]. In all of these cases, APPI-MS was compared with APCI-MS and ESI-MS or ionspray-MS. The best results for these polar analytes were obtained with ESI-MS or ionspray-MS, respectively.

Drug metabolites are also the subject of a recent publication by Yang and Henion [66], who compared APCI-MS and APPI-MS for the determination of idoxifene and its metabolites. While the sensitivity of APPI for idoxifene is six to eight times higher compared with the sensitivity of APCI, the sensitivi-

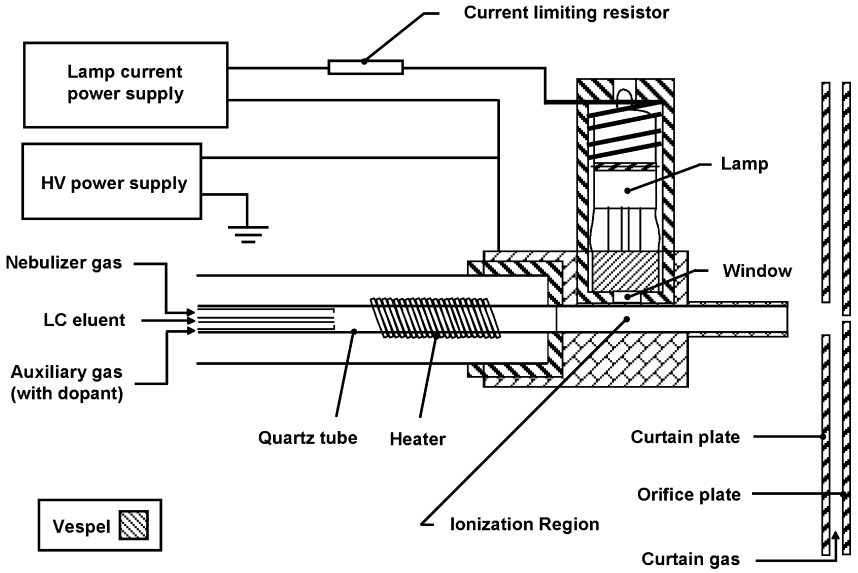

Fig. 5. Setup of the APPI source, including heated nebulizer probe, photoionization lamp and lamp mounting bracket. Reprinted, with modifications, from Ref. [58], with permission.

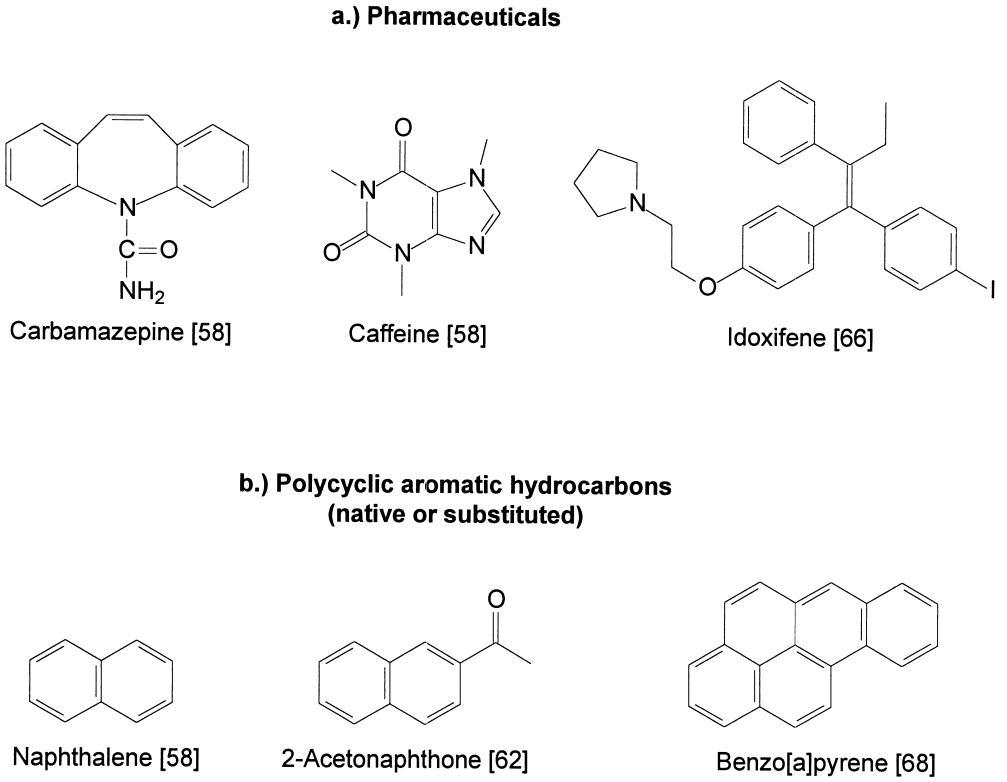

Fig. 6. Structures of selected non-polar substances which are accessible for APPI-MS.

ty for the metabolites differs, but still with better data for APPI-MS. Plante et al. evaluated the APPI interface with respect to the ionization of drugs under varying conditions (kind and concentration of dopants, flow-rates, temperatures and mobile phase composition) in comparison to an APCI and a ‘‘turbo ionspray’’ (modified ESI) interface [67]. With respect to precision and accuracy, the data obtained were comparable to those obtained with the established ionization techniques.

Impey and co-workers [68] investigated polycyclic aromatic hydrocarbons (PAHs), a group of substances which should be ideal candidates for APPI-MS. Excellent results with very low limits of detection were obtained for normal-phase LC, while satisfactory results were achieved with reversed-phase LC and addition of a dopant. Ambient aerosol samples were investigated using this technique. Cormia et al. [69] used a similar approach, but achieved similar results for most analytes when comparing reversed-phase and normal-phase separation conditions. Methanol gave excellent results as a constituent of the mobile phase, and the addition of toluene as dopant proved to be essential.

Ubiquinones and menaquinones were subject to a comparative study of APPI and APCI by Van Berkel et al. [70]. For ubiquinones, both techniques proved to be equally sensitive. For menaquinones, however, sensitivity for APPI was three times higher. As in other studies, toluene was used as a dopant. Kertesz and Van Berkel [71] observed a reduction of the oligomers formed in the on-line electropolymerization of aniline and of other substances under suitable conditions in both APCI and APPI. It is assumed that reactive species, possibly hydrogen radicals, are involved in a surface-assisted process, leading to the reduced compounds.

Fat-soluble vitamins were subject to another study by Miller et al. [72]. Vitamin A and vitamin E were directly ionized in the APPI source without the need

for a dopant. Depending on the analytes, the $[M+H]^+$ or the $M^{\cdot+}$ (vitamin E) or the $[M-H_2O+H]^+$ are observed. Further reactions, which may complicate the interpretation of the spectra, may also be observed.

Very recently, the similarity of the APPI and APCI sources, the need for comparisons between the two techniques and the missing possibility to predict the performance differences of APCI and APPI for a given analytical task has led to the development of a combined source [73].

Summarizing, APPI-MS is a very young and promising technique which is likely to help overcome the problems associated with APCI and, especially, ESI in the analysis of compounds with very low polarity. However, there are only very few reports in the literature on APPI, and its performance in the analysis of real samples is still to be proven, because the recent publications in this field are mainly focused on the analysis of model compounds or on mechanistic effects. Furthermore, more thorough comparisons between APPI, APCI and ESI, in which the optimum experimental parameters are determined individually for each of the techniques, are needed to allow fair conclusions on the performance of the interfaces. The determination of PAHs is likely to become a key application for the APPI source, as the APCI performance is very low, while excellent results have already been obtained with APPI. It can be expected that, after a period of a few years, APPI will have reached the same degree of maturity that APCI and ESI have reached today and that a large fraction of those compounds which are currently considered to be optimal candidates for GC–MS will be accessible by APPI. Therefore, APPI is likely to become a powerful complementary technique to APCI and ESI for the low end of the polarity scale.

4. LC–atmospheric pressure electron capture negative ion-MS

When selecting the most selective and sensitive ionization technique for mass spectrometry, electron capture (EC) is certainly among the leading candidates. In combination with gas chromatography, attomole amounts of the analyte can be detected. The major prerequisite for EC is the presence of an electrophoric group in the molecule to be analyzed, and this is mainly introduced using dedicated derivatizing techniques based on polyhalogenated reagents. For analytes bearing polar functionalities, derivatization not only improves the detectability, but also the volatility by transferring hydrogen-bond-forming functional groups into esters, ethers and related compounds. Recent developments in electron capture MS, with particular focus on GC–MS, are summarized in a review by Giese [74].

Only a few attempts have been made to use electron capture mass spectrometry in combination with liquid chromatography. The earlier approaches were performed using the particle beam ionization (PBI) interface. L-Tryptophan and L-kynurenine were derivatized with pentafluorobenzyl bromide by Boni et al. [75], separated by normal-phase LC and detected by PBI-MS. The same general strategy was applied by Wang et al. [76], who used 4-pentafluorobenzyl-1,2,4-triazoline-3,5-dione as electron capture derivatizing agent for dienophiles. Cappiello et al. determined four widely used explosives, 2,4,6-trinitrotoluene (TNT), pentaerythritol tetranitrate (PETN), nitroglycerine (NG) and 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX), by microflow LC–particle beam-NICI-MS [77].

Blair et al. reported recently that, under certain conditions, the corona discharge in commercial APCI interfaces may also be used as a source for low energy electrons [78], which are generated by displacement of electrons from the nitrogen sheath gas. In this way, the known applications of GC–NICI-MS may also be transferred to LC–APCI-MS. Blair et al. used pentafluorobenzyl bromide to derivatize various hydroxy-functionalized biomolecules and drugs. The respective reaction scheme is presented in Fig. 7. The reversed-phase and normal-phase LC separation of the derivatives with subsequent electron capture atmospheric pressure chemical ionization mass spectrometry led to limits of detection in the attomole range, thus coming close to the limits of detection reported for GC–NICI-MS. In a comparison between negative ion APCI-MS of the underivatized analytes and the new technique, the latter provided an increase in sensitivity of two orders of magnitude [78]. Under dissociative electron capture (loss of the pentafluorobenzyl group), very clear spectra with a

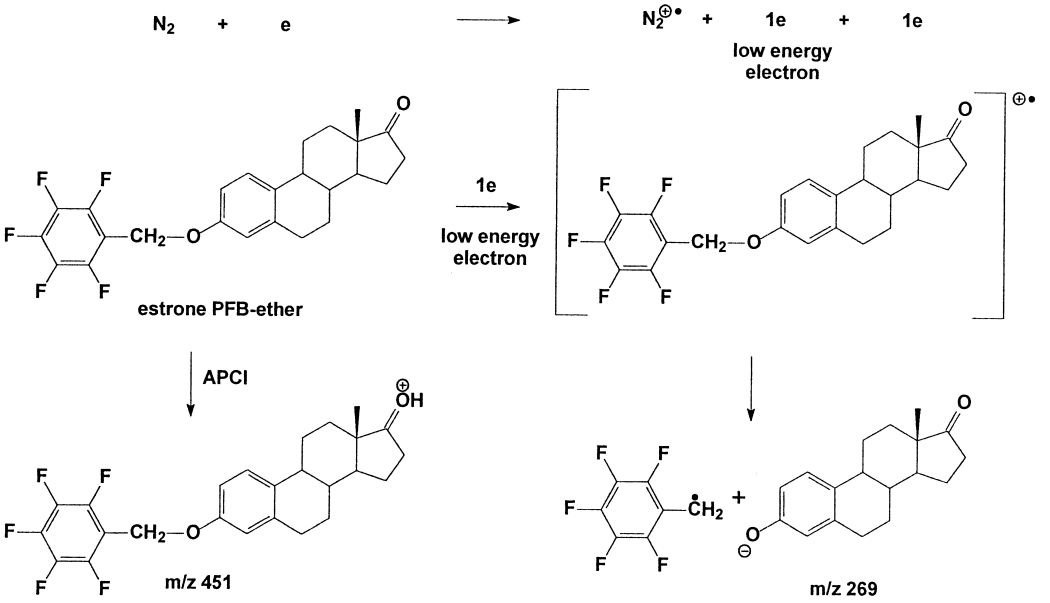

Fig. 7. Proposed mechanism for electron capture ionization using a commercial APCI interface. Reprinted, with modifications, from Ref. [78], with permission.

distinct base peak at a mass which corresponds to the $[M-H]^-$ of the underivatized analytes are obtained, as presented in Fig. 8. The authors proved by APCI(+) experiments that the observed effect is indeed based on electron capture. The structural integrity of the analytes was maintained during the ionization process.

This technique was then used by the same authors and other groups for various new applications. Impey et al. [79] analyzed prostaglandins, estradiol and D-group vitamins using the same methodology, while Fujiwara et al. [80] developed a respective method for the determination of thromboxane in biological samples, with the LC separation performed in the normal-phase mode. Blair et al. again increased the range of applications by a method for the determination of estrogen metabolites [81]. In this work, normal-phase LC was again used with a silica column and a binary isopropanol–hexane gradient.

It can be expected that the range of applications for this methodology will be strongly increased in the next few years. The technique is especially interesting in those cases where multifunctional analytes are derivatized to very large products, which are problematic when analyzed by GC–NICI-MS.

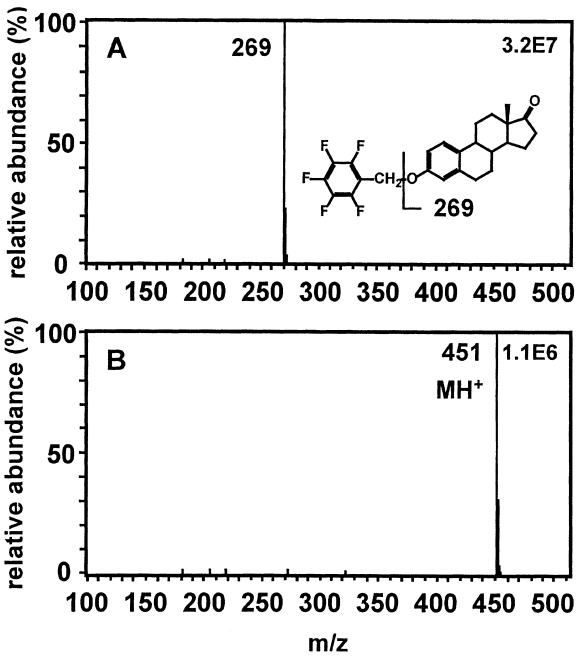

Fig. 8. Mass spectra of the pentafluorobenzyl derivative of estrone using an APCI-MS instrument. (A) Dissociative electron capture in the negative ion mode. (B) Protonation in the positive ion mode. Reprinted from Ref. [78], with permission.

Even before the pioneering work of Blair et al. on dedicated electron capture derivatizing agents for use with the APCI interface [78], other authors observed radical anions of nitroaromatics, but obviously without realizing the mechanistic background. Snow et al. [82] determined different nitro-based explosives by LC–MS and detected a significant response for the molecular anion of 2,4,6-trinitrotoluene (TNT) in the negative ion mode. With increasing needle voltage, a strong increase of the abundance of the TNT molecular anion was observed. However, quantification was performed for the stronger peak of the isopropanolate adduct $TNT+C_3H_7O^-$. The authors state that, for nitroaromatics, the signals using the APCI interface are generally much stronger compared with the signals using the ESI interface. Palloch and Pelzing [83] also observed the radical anion of TNT, but with lower abundance than the ion of deprotonated TNT. No comments were made on the formation of the M^- peak. Very recently, Keely et al. [84] studied the ionization of different explosives using APCI-MS and ESI-MS more exhaustively. The analytes were transferred into the interface either by infusion of by direct injection, but in all cases without chromatographic separation. In this work, the limit of detection for TNT using APCI-MS surpassed that using ESI-MS by three decades, with both ionization techniques being applied in the negative ion mode. For TNT, Keely et al. [84] detected the radical anion as base peak, and some ions of lower abundance. However, the mechanism of ion formation was also not discussed in this case.

Karst et al. described unambiguous electron capture effects in the determination of native nitroaromatics and of substances derivatized with nitroaromatics [85] using LC–APCI-MS in the negative ion mode. The electron capture effect was found to be in strong competition with other ionization mechanisms. As soon as easy deprotonation was possible for the nitroaromatics, this was favored in comparison with electron capture. This was demonstrated for the analysis of amines after their derivatization with 4-chloro-7-nitrobenzooxadiazole (NBD chloride). For the derivatives of primary amines, deprotonation at the amino-N occurred. For the derivatives of secondary amines, deprotonation is no longer possible, resulting in a strong signal for the electron capture product, the radical anion. The respective mass spectra for the derivatives of a primary and a secondary amine are presented in Fig. 9. It should be mentioned that, in this case, non-dissociative electron capture was observed, while dissociative electron capture occurred, for example, in the case of *N*-methylhydrazino-functionalized aldehydes and ketones [85].

A series of nitrosubstituted and/or halogenated derivatizing agents was recently introduced by Higashi et al. [86,87]. In Ref. [86], a large series of different nitroaromatics and polyhalogenated aromatics was investigated with the goal to find the most suitable backbone molecules for negative ion electron capture-MS in the APCI interface. It was found that the 2-nitro-4-trifluoromethylphenyl moiety was most effective for increasing the sensitivity. Reagents based on this backbone were applied to the analysis of steroids in human plasma. In another study, 2-nitro-4-trifluoromethylphenylhydrazine was successfully applied to derivatize 20-oxosteroids for subsequent electron capture in the APCI interface [87].

In this area, the development of new powerful dedicated derivatizing agents based on polyhalogenated compounds or nitroaromatics can be expected.

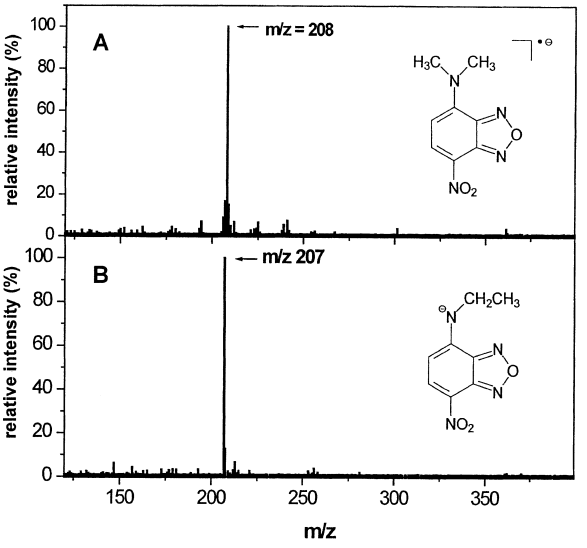

Fig. 9. APCI(−) mass spectra of the 4-chloro-7-nitrobenzooxadiazole (NBD chloride) derivatives of dimethylamine (A) and ethylamine (B). While non-dissociative electron capture is observed in (A), deprotonation occurs in (B). Reprinted, with modifications, from Ref. [85], with permission.

In combination with dissociative or non-dissociative electron capture and tandem mass spectrometry, extremely low limits of detection might be achieved for molecules which currently cannot be analyzed by GC–MS because of their thermolability even after derivatization. These analytes could include, in particular, biomolecules with molecular masses of many hundred up to a few thousand g/mol, which are currently only accessible by MALDI–MS, but not by MS in on-line combination with separation techniques. Additionally, selected established derivatizing agents which have been applied for a long time in LC with UV or fluorescence detection or in GC with selective detectors may now be used in LC–MS. It should, however, be remembered that deprotonation and electron capture are competing processes [85], and that both may occur in parallel for selected compounds. The ratio between these processes may differ from compound to compound, and strong solvent effects have been observed by the present authors for nitroaromatics. Therefore, individual optimization for any group of analytes will be required to make optimum analytical use of electron capture processes in the APCI interface.

5. Coordination ionspray-MS (CIS-MS)

The addition of various anions or cations to a solution of analytes with the purpose of obtaining charged adducts has been a well-known strategy in various mass spectrometric techniques for many years [88]. This method is mainly applied to polar analytes, which readily form adducts with, for example, the alkali cations [89] and transition metal cations [90]. In this section, the focus shall exclusively be directed towards those methods in combination with ESI-MS or APCI-MS, in which ions are added to non-polar compounds to induce the ionization of originally non-charged compounds. One possibility to estimate the usefulness of an added ion to form a complex with the analyte is the application of Pearson's classification of ''hard'' and ''soft'' acids and bases [91]. In a simplified summary of Pearson's classification, hard acids or bases are electron pair acceptors or donors, which have comparably high charge and small size. Soft acids or bases, on the other hand, are characterized by low charge and large size. Pearson concludes that hard acids form stable complexes with hard bases, and weak acids form stable complexes with weak bases. As strongly polar compounds will fall under Pearson's definition for hard acids and bases, this review will focus on complexes of soft acids (large metal cations with little charge) with soft bases (large, non-polar organics).

As a precursor to what today is called ''coordination ionspray'', Henderson and Nicholson [92] added silver nitrate to solutions of neutral metal carbonyl complexes. The respective silver adducts were then detected in the electrospray mass spectrometer. Laakso and Voutilainen [93] analyzed triacylglycerols by LC–MS with silver ions added to the mobile phase. In APCI-MS, protonated and fragmented molecular ions were observed with the highest abundance, and silver adducts were detected only with lower intensities. On the other hand, the separation of equally unsaturated triacylglycerols was improved. Schuyl et al. [94] investigated the same analytes and also detected silver(I) complexes, but again the major focus was the improvement of LC separation by the addition of silver ions.

In 1997, Siu et al. described the formation of complexes of peptides and proteins with silver(I) cations [95]. The latter were directly added to a solution of the analytes, and electrospray analysis was carried out by infusion of the mixture directly into the ESI interface. No separation was carried out within this work. For proteins such as insulin, multiple coordination was observed, resulting in multiple charging of the analytes. According to Pearson's classification, the ''soft'' methionine sulfur acts as a coordination site for the silver(I) ions. Roussis and Prouix [96] used a similar approach to determine the molecular mass distribution of heavy aromatic petroleum fractions by ESI-MS. The addition of Ag^+ led to intense signals of the respective complexes. Although possible in principle, no separation was carried out.

The term ''coordination ionspray-MS'' was introduced by Bayer et al. in 1999 [97]. They investigated a series of truly non-polar compounds and added, after liquid chromatographic separation of the analytes, various ionic reagents to induce coordination and therefore charging of the objects of investigation. While Ag(I) was added to ionize arenes, olefins,

polyolefins and carotinoids, Pd(II) was used to ionize vitamins A, D and E as well as estrogens. The structures of selected non-polar compounds which may be detected based on their adduct formation with Ag(I) ions are depicted in Fig. 10. More-polar compounds could also be analyzed, for example sugars by addition of boric acid, or peptides, sugars and alcohols by addition of lithium halogenides. It should be noted, however, that the more polar analytes may typically be detected by ESI-MS, while the less polar compounds are detected with inferior limits of detection or not at all. The solution of the ions to be coordinated is added by a simple setup in a sheath flow stream. The experimental setup is presented in Fig. 11. A previous paper from the same group described the application of a similar technique to the determination of tocopherols and carotenoids by LC–ESI-MS [98]. Ag(I) ions were added in this case to ionize the analytes. Femtomole amounts of selected carotenoids could be detected. In a combined approach with LC–MS and LC–NMR, the tocotrienol isomers could be separated and identified in crude palm oil extract [99]. As in the previous work, coordination ionspray was carried out using Ag(I). Bayer et al. demonstrated that the coordination ionspray method can also be applied in combination with capillary electrochromatography (CEC) [100]. In this work, unsaturated fatty acid methyl esters, vitamins of the D group and estrogenic compounds were investigated.

Porter et al. expanded the coordination ionspray technique to peroxidation products of cholesterol linoleate and cholesterol arachidonate [101]. Complex peroxide mixtures were successfully investigated using this method. As in the original method of Bayer et al., Ag(I) was added after liquid chromatographic separation of the peroxides. The same group subsequently determined diacyl peroxides by coordination ionspray with tandem mass spectrometry [102]. The site-directed fragmentation of neutral and polar lipids by Ag^+ in coordination ionspray was the subject of a more recent report by Porter and coworkers [103]. Rudzinski and Zhang recently reported the analysis of organosulfur compounds in petroleum products by the addition of Pd(II) [104]. Dibenzothiophene and related compounds could be determined by coordination ionspray-tandem mass spectrometry. Takino et al. [105] described the determination of PAHs with the post-column addition of Ag(I).

Very recently, coordination ionspray was applied to triglycerides in vegetable oils after separation by supercritical fluid chromatography. Sandra et al. [106] obtained an increase in sensitivity by a factor of 100 compared with UV detection. For saturated triglycerides, only the $[M+Ag]^+$ peaks were ob-

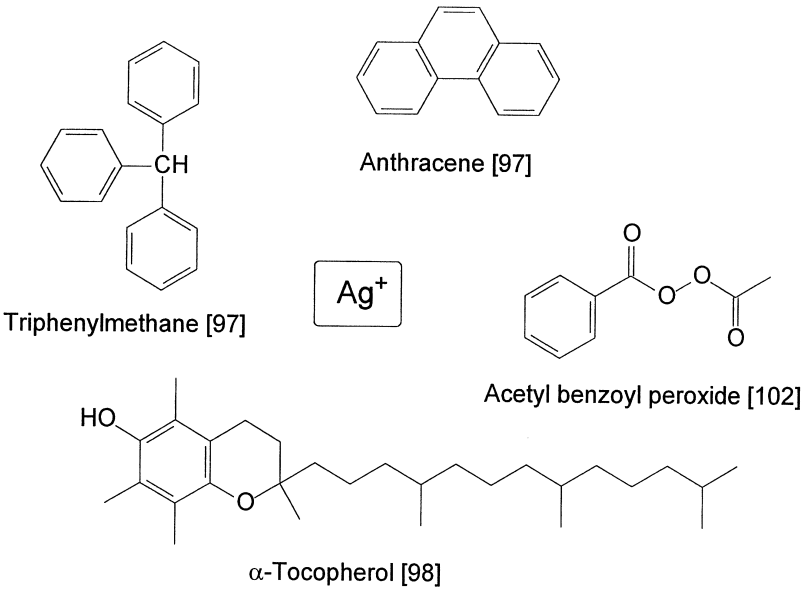

Fig. 10. Selection of substances which can be analyzed by coordination ionspray-MS with addition of silver(I) ions.

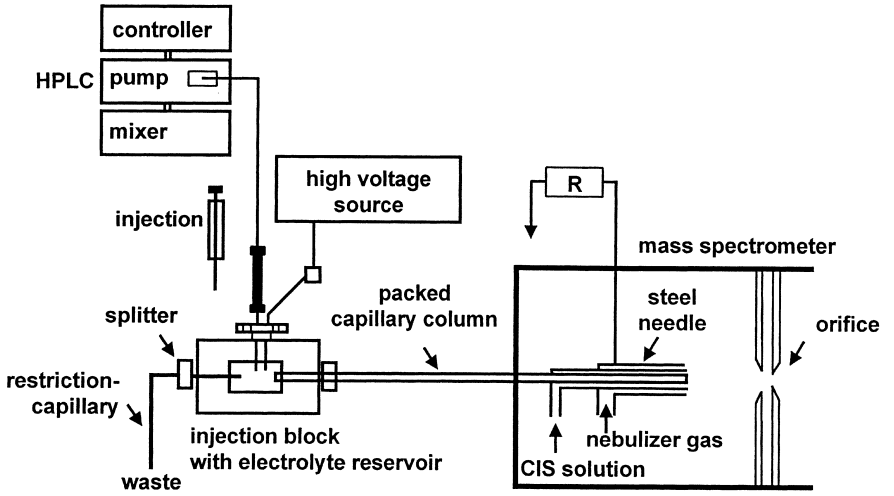

Fig. 11. Instrumental setup for direct coupling of capillary separation techniques with CIS-MS by adding a solution of the coordinating ion in a sheath flow stream. Reprinted, with modifications, from Ref. [97], with permission.

served, while unsaturated triglycerides were detected as $[M+H]^+$ and $[M+Ag]^+$.

Coordination ionspray is therefore comparable to the previously discussed techniques. Because the method has been introduced very recently, significant new applications are likely to be presented by an increasing number of scientific groups in the near future. These may occur, for example, in the field of the analysis of non-polar compounds of biological relevance or in environmental analysis of heteroaromatic substances. Although no significant effects have yet been reported in the literature, the addition of Ag(I) salts may lead to the deposition of significant amounts of silver in the instrument. This will depend on the characteristics of the individual interface, but should be considered prior to the application of high silver salt concentrations. A possible strategy to overcome this problem would be the use of nanospray interfaces with flow-rates as low as possible, which would drastically reduce the total amount of silver introduced into the system.

6. Conclusions

A series of four new techniques for the LC–MS analysis of non-polar compounds has been presented. None of these will achieve the status of a ‘‘universal’’ ionization technique for non-polar compounds, but all show good potential for use in particular applications. The coupling of electrochemistry and MS will be limited to electroactive analytes, but the exploitation of this technique may deliver exciting new insights into biological redox reactions, and extremely low limits of detection may be achieved in combination with dedicated derivatizing agents. The APPI approach is probably the most generally applicable of those presented in this paper. APPI is already on the way to becoming a complementary technique to APCI even for less polar compounds. Especially for the analysis of aromatic compounds with few polar functionalities, APPI is likely to be superior to APCI. Electron capture ionization may be carried out on an existing LC–APCI-MS instrument, but its application is limited to compounds with strongly electron-withdrawing substituents, e.g. polyhalogenated and nitroaromatic compounds. In the bioanalytical field, the increasing development of dedicated derivatizing agents for electron capture APCI-MS can be expected. For coordination ionspray-MS, the application of Pearson’s classification is likely to lead to new and currently unexpected applications for the analysis of non-polar compounds.

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