measured: 1400, independent: 920, of which 789 had $I > 2\sigma(I)$, 56 parameters, $R1 = 0.0342$, $wR2 = 0.1076$, residual electron density $+0.33/-0.15$ e Å^{-3} (CCDC-103189). e) *n*-Heptane: growing temperature $-110\,^{\circ}$C, space group $P\bar{1}$ (no. 2), $2\Theta_{max} = 60^{\circ}$, reflections measured: 1750, independent: 1674, of which 1388 had $I > 2\sigma(I)$, 128 parameters, $R1 = 0.0499$, $wR2 = 0.3756$, residual electron density $+0.39/-0.19$ e Å^{-3} (CCDC-103190). f) *n*-Octane: growing temperature $-78\,^{\circ}$C, space group $P\bar{1}$ (no. 2), $2\Theta_{max} = 60^{\circ}$, reflections measured: 1356, independent: 1187, of which 991 had $I > 2\sigma(I)$ 73 parameters, $R1 = 0.0386$, $wR2 = 0.1285$, residual electron density $+0.48/-0.15$ e Å^{-3} (CCDC-103191). g) *n*-Nonane: growing temperature $-98\,^{\circ}$C, space group $P\bar{1}$ (no. 2), $2\Theta_{max} = 55^{\circ}$, reflections measured: 3288, independent: 2089, of which 1396 had $I > 2\sigma(I)$, 162 parameters, $R1 = 0.0468$, $wR2 = 0.1549$, residual electron density $+0.31/-0.15$ e Å^{-3} (CCDC-103192). Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publicationnos CCDC-103186 – CCDC-103192. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CD2 1EZ, UK (fax: (+44) 1223-336 – 033; e-mail: deposit@ccdc.cam.uk).

[17] The calculated lattice energies, expressed in kcal mol^{-1} per molecule, show a similar alternation as the melting points: propane: -7.504, *n*-butane: -8.814, *n*-pentane: -11.922, *n*-hexane: -14.783, *n*-heptane: -16.690, *n*-octane: -19.657, *n*-nonane: -21.260. The Cerius^2 program (Version 3.5) was used for these calculations. The experimental coordinates serve as the starting points and the electrostatic potentials were assigned from the AM1-Hamiltonian in MOPAC6. The "Crystal Packer" module with a built in Dreiding II force field was used to minimize crystal structures and the lattice energies were obtained from the final minimized crystal structure.

Coordination-Ionspray-MS (CIS-MS), a Universal Detection and Characterization Method for Direct Coupling with Separation Techniques**

Ernst Bayer,* Petra Gfrörer, and Claus Rentel

Spray techniques such as atmospheric pressure chemical ionisation (APCI)[1] or electrospray ionization (ESI)[2–5] have become important techniques in mass spectrometry. In particular their simple coupling with separation techniques such as HPLC,[6] capillary electrophoresis (CE),[7] and capillary electrochromatography (CEC)[8] is the method of choice for characterization, analysis, and structural elucidation. A characteristic common to these techniques is the formation of droplets from which ions are subsequently liberated. In the case of APCI the ionization occurs by charge transfer from a reagent gas plasma formed by corona discharge at the tip of a metal needle. With ESI, the sample solution is nebulized by application of a strong electric field. Spray formation is usually assisted pneumatically by means of a coaxial gas stream. Ionization is achieved by either protonation of basic groups or deprotonation of acidic groups of the analyte molecules depending upon the polarity of the field employed. While electrospray mass spectrometry has found widespread application, for example in the analysis of peptides and proteins, with their readily protonated amino groups, and of the easily deprotonated oligonucleotides, it fails for a large number of nonpolar substances and is of limited utility for substances with only weakly basic or acidic groups. Numerous classes of natural compounds such as terpenes, sugars, alcohols, aromatic compounds, and vitamins in addition to a large number of synthetic organic compounds such as produced in combinatorial chemistry are either inaccessible to analysis by spray techniques or can be detected only with poor sensitivity.

We have developed a new method of chemical ionization in which positively or negatively charged complexes are formed by the addition of a suitable central atom to the analytes, and these complexes can be detected by mass spectrometry. Since both polar and nonpolar organic compounds can form coordination compounds with an appropriate central atom, this form of ionization is highly versatile. The wealth of experience available in the area of coordination chemistry can be drawn upon in this technique and thus new classes of compounds can be analyzed with the spray technique. We therefore refer to this new technique as coordination-ionspray mass spectrometry (CIS-MS). Neither an electric field nor the formation of a reagent gas plasma by corona discharge is necessary for ionization. Efficient nebulization in the ion source is however mandatory. Since pneumatic nebulization in the absence of an electric field is often insufficient to obtain a suitable spray, a supporting voltage was applied in some cases to stabilize the nebulization process.

In the case of poorly ionized compounds, such as fully protected peptides, the formation of clusters leads to complex spectra that are difficult to interpret and to a reduction in sensitivity. In , The ESI mass spectrum of the fully protected 28-mer peptide of the potassium channel of the transmembrane sequence of *Drosophila melanogaster*[9, 10] is shown in Figure 1 a. With the exception of the terminal amino group all the basic groups are protected and are thus not available for protonation, and only the clusters with sodium and potassium ions are observed within the mass range of the quadrupole mass spectrometer. The ion yield is only poor and the interpretation of the spectra is problematic.

It is known that lithium salts form complexes with peptides and that these prevent the association of peptide chains. Seebach et al.[11] have employed lithium salts to increase the solubility and to improve the yield in the solid-phase synthesis of peptides that have a high propensity for aggregation by the formation of β structures. The addition of lithium iodide to the transmembrane peptide leads to specific stable metal complexes that can be detected in high ion yield and which allow simple determination of the molecular mass (Figure 1 b).

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[**] This work was financially supported by the Deutsche Forschungsgemeinschaft within the framework of the Graduiertenkolleg "Quantitative analysis and characterization of pharmaceutically and biochemically relevant substances".

  1433-7851/99/3807-0992 $ 17.50+.50/0

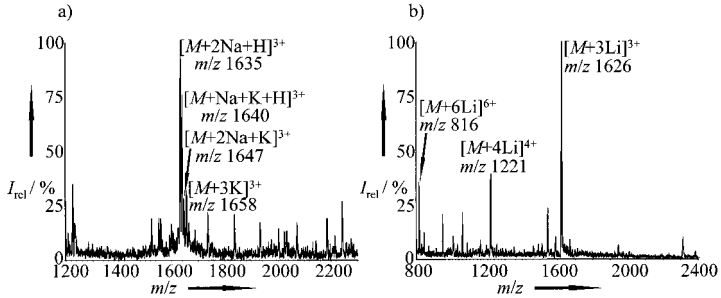


Figure 1. a) ESI mass spectrum of the fully protected transmembrane peptide $[NH_2]$-Lys(BOC)$_{28}$-Ile-Val-Gly(Tmob)-Ser(Trt)-Leu-Cys(Trt)-Val-Val-Ala-Gly(Tmob)-Val-Leu-Thr(Trt)-Ile-Ala-Leu-Pro-Val-Pro-Val-Ile-Val-Ser(Trt)-Asn(Trt)-Phe-Asn(Trt)-Tyr(tBu)$_1$-[COOH] (*m/z* 4857) from *Drosophila melanogaster* under normal electrospray conditions; 4.5 kV, flow rate 3.5 μl min^{-1}. b) CIS mass spectrum after addition of 20 μl mL^{-1} lithium iodide and with reduced field strength of 1.5 kV; flow rate 10 μL min^{-1}.

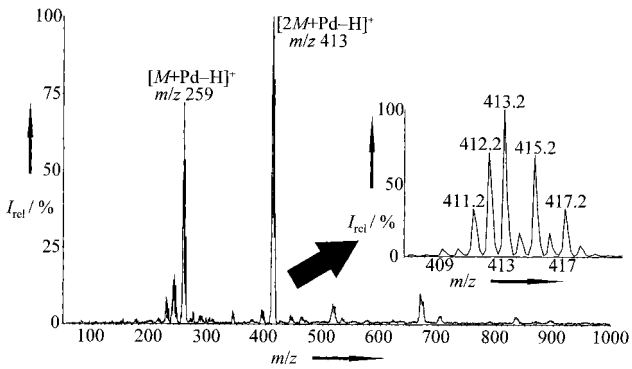


Figure 2. CIS mass spectrum of isopulegol complexed with 100 μg mL^{-1} Pd^{II} acetate; no voltage applied, flow rate 10 μl min^{-1}.

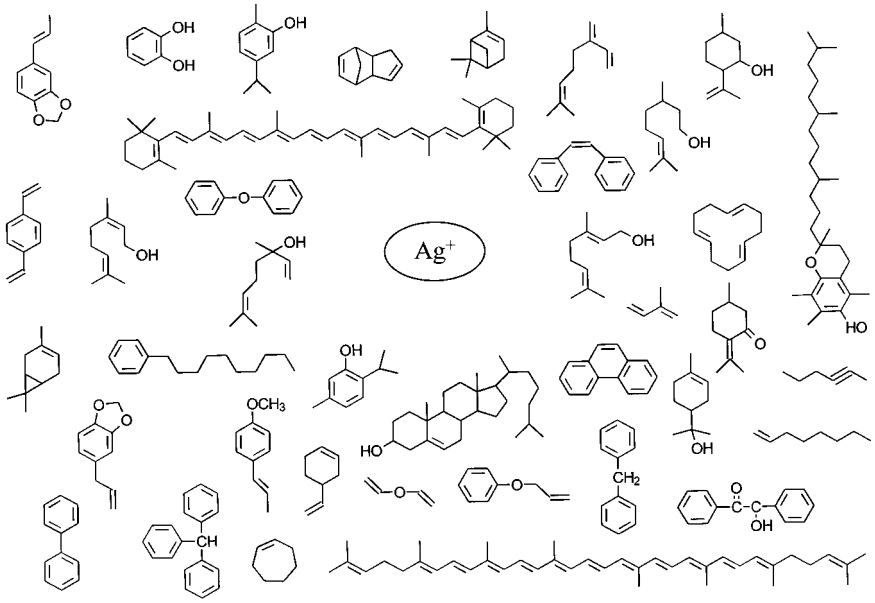
Reduction of the electric field from 4.5 to 1.5 kV leads to an improvement in the signal-to-noise ratio, since other competing mechanisms of ionization and the formation of clusters are suppressed.

Although metal complexes such as metal proteins[12–16] and alkali and alkaline earth ionic adducts[17–19] of polar analytes have been measured with electrospray-MS, it has hitherto not been recognized that conversion into charged complexes represents a general method of ionization in solution for mass spectrometric purposes, that it requires neither activation nor conditions of ESI-MS, and that it is therefore applicable to nonpolar analytes. The sample is subjected to less thermal stress than APCI and the sensitivity is significantly improved. Lower detection limits in the range of 300 – 500 fmol are attained for carotinoids.[20] The detection limit of palmitoleic acid methyl ester was 370 fmol in the "single ion mode" and 37 fmol with "multiple reaction monitoring". These substances were detected as their silver complexes.

Since olefins and polyolefins such as terpenes, sesquiterpenes, carotinoids, vitamins, and steroids either cannot be detected at all or only with poor sensitivity with ESI-MS they would appear particularly attractive for CIS-MS. With metals of the first and eighth transition groups of the periodic table, for example, Cu^{I}, Ni^{II}, Pd^{II}, Pt, and Ag^{I}, these unsaturated compounds form highly stable π or π-allyl complexes. Figure 2 shows the CIS-MS of the palladium – π-allyl complex of the terpene alcohol isopulegol without the electrical field that is mandatory for ESI-MS. The $[M+Pd-H]^+$ ions with the isotopic distribution characteristic of palladium are the prominent species. If silver(I) is used as central atom the π complexes rather than the π-allyl complexes are detected. Here also, avoidance of other mechanisms of ionization leads to clear-cut spectra with low noise levels.

We have investigated a large number of olefins, polyolefins, and aromatic compounds by CIS-MS, including steroids, vitamins of the D and E families, carotinoids, polystyrenes, terpenes, and unsaturated fatty acids. Scheme 1 shows a selection of the analytes measured in the form of their Ag^{I} complexes. On-line formation of the charged species is carried out after the chromatographic separation since in most cases the chromatographic resolution of even relatively stable complexes formed prior to separation was unsatisfactory.

In the case of polyolefins, $[M]^{\bullet+}$ radicals are often found as by-products in addition to the $[M+Ag]^+$ ions, as is seen in the HPLC separation and CIS-MS detection of carotinoids in Figure 3.[20] It is apparent from Figure 3 that on-line complexation and mass spectrometric detection do not lead to peak broadening relative to UV detection. The kinetics of complexation are rapid under the chosen experimental conditions. In addition to elements of the first and eighth transition groups of the periodic table, many other metals are suitable as central atoms and can be selected according to the principles of complex chemistry. Table 1 gives a selection of potential reagents for various ligands.



Scheme 1. Selection of compounds that can be detected by CIS-MS as their positive π complexes with Ag$^+$.

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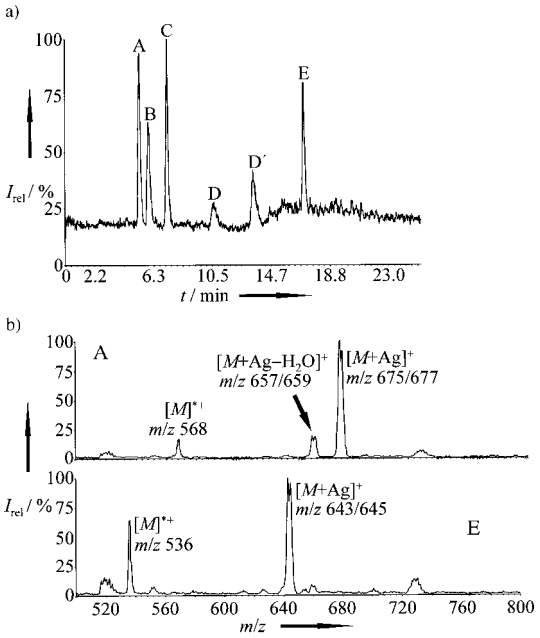

Figure 3. a) Ion chromatogram of the HPLC-MS of lutein (A), zeaxanthin (B), canthaxanthin (C), echinenon (D, D′), and β-carotine (E). b) CIS mass spectra of the separated carotinoids A and E.

Table 1. Reagents for CIS-MS

Central atom	Reagent	Solvent	Analyte
B^{III}	BF_3	organic solvent	alcohols, ethers, esters
B^{III}	H_3BO_3	H_2O, alcohol	sugars, polysaccharides, alcohols
B^{III}	$B(OCH_3)_3$	H_2O, alcohol	ethers, alcohols
Ag^{I}	$AgNO_3$	H_2O, alcohol, acetonitrile	arenes, olefins, polyolefins, carotinoids
Pd^{II}	$Pd(OAc)_2$	H_2O, alcohol acetonitrile	vitamins A, D, and E, estrogens
Li^{I}	Li halogenides	H_2O, alcohol, acetonitrile	peptides, sugars, alcohols

CIS-MS is not restricted to positive ions but can also be used in the negative mode, in particular when the central atom of the complexing reagent has an electron deficiency. Boron(III) is such a central atom. Aliphatic alcohols and sugars are difficult to detect with electrospray MS. However after reaction with boron(III) trifluoride, the negatively charged boron complexes are readily detected in CIS-MS, as demonstrated by the mass spectrum of cholesterol (Figure 4a). Here also, a reduction in the field strength (Figure 4b) causes suppression of other ionization mechanisms, which leads to simpler spectra and lower limits of detection. Boric acid is another reagent that is commonly used for structural investigation of sugars. Adjacent hydroxyl groups form more stable boron complexes than isolated hydroxyl groups. Under the conditions selected for CIS-MS, only the boron complexes with adjacent hydroxyl groups, for example, with fructose (Figure 5), are stable and detectable by MS whereas glucose is not detected. Once again, reduction of the field strength suppresses nonspecific ions and results in more clear cut

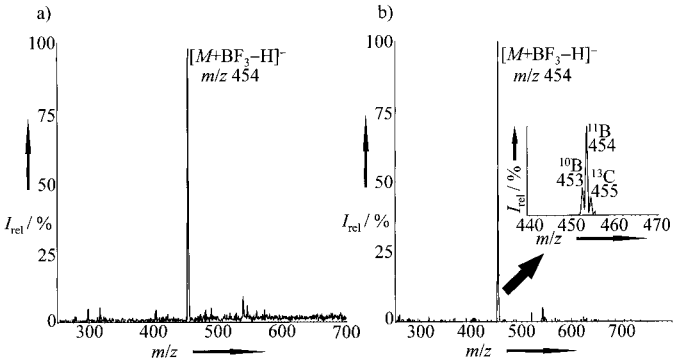

Figure 4. CIS mass spectrum of cholesterol after complexation with BF_3 in diethyl ether a) at -4.5 kV with a flow rate of 3 μL min^{-1} and b) at -1.2 kV and a flow rate of 10 μL min^{-1}.

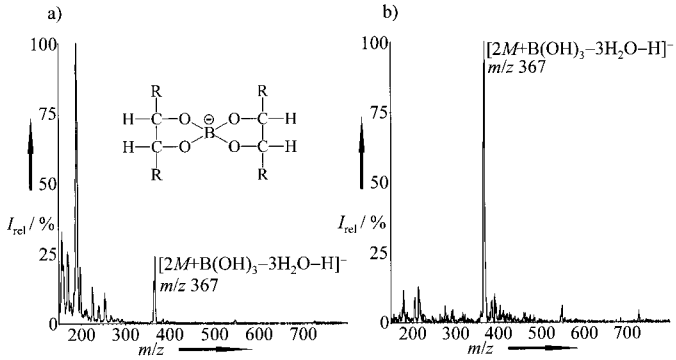

Figure 5. CIS mass spectrum of fructose a) at -3.7 kV, and b) at -1.7 kV. Flow rates are as in Figure 4.

spectra (Figure 5b), since the nonspecific formation of solvent clusters in the lower mass range is hindered. CIS-MS of oligosaccharides represents a relatively simple method for the characterization and structural elucidation of oligosaccharides, especially in complex mixtures in combination with previous separation by HPLC, CEC, or CZE.

The fragmentation of complexes and the analysis of the fragment ions is interesting on account of the fragmentation patterns encountered, as has been demonstrated for conjugates with alkali ions.[18]

Direct coupling of all common separation methods with CIS-MS is possible, with increasing interest being focussed in recent times upon the miniaturized techniques such as capillary HPLC (CHPLC), CZE, and CEC.

Figure 6 shows the separation of unsaturated fatty acid methyl esters by pressure-assisted CEC, which we developed for gradient elution,[21–23] with CIS-MS detection of the silver complexes. The silver complexes were formed after the separation and before entry into the ion source. The CIS mass spectra of the fatty acid methyl esters obtained on-line from the CEC separation are shown in Figure 6b.

The combination of separation in the liquid phase and CIS-MS is a new and widely applicable analytical method, the diverse possibilities of which will become fully apparent with the rigorous application of the known principles of coordination chemistry.

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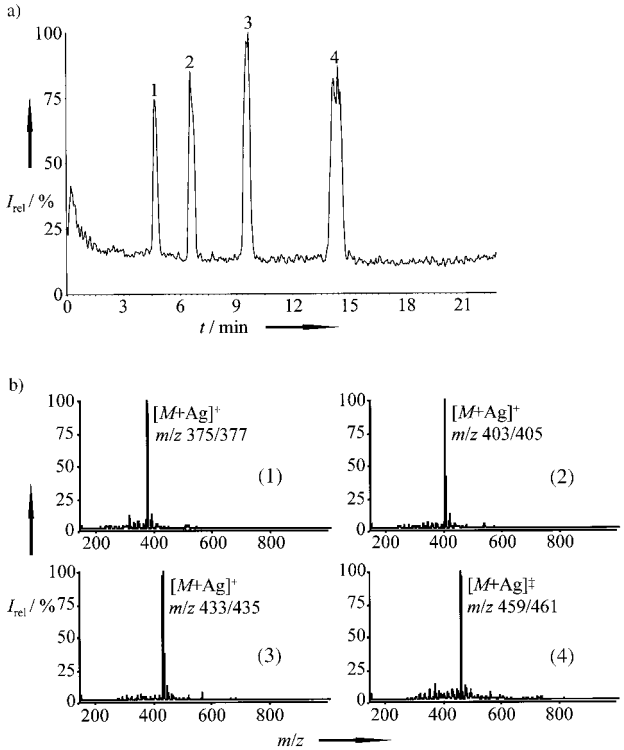

Figure 6. Direct coupling of the separation of fatty acid esters with pressure-assisted electrochromatography (pCEC) with CIS-MS detection. 1) Hexadecenoic acid methyl ester, 2) oleic acid methyl ester, 3) *cis*-eicos(9)enoic acid methyl ester, 4) eruca acid methyl ester. a) Total ion current and b) CIS mass spectra of the silver complexes.

Experimental Section

At present no commercial ion source exists that is optimized for CIS-MS. Since all electrospray ion sources require strong electric fields not only for the ionization but also for the generation of as monodisperse a spray as possible, only ion sources with vigorous mechanical nebulization are able to sustain a satisfactory spray under conditions of reduced electric field. In addition, an increase in the temperature of the curtain gas to up to 80 °C is recommended. These basic requirements are not fulfilled by all commercial electrospray ion sources. The ion source of the API III and API 3000 mass spectrometers from PE SCIEX (Toronto, Canada) were used in our investigations but were not optimized for CIS-MS. Nebulization occurs pneumatically by means of a gas stream.

The experimental setup for coupling a separation technique to the interface for the addition of the coordinating compound is shown schematically in Figure 7. We used the previously described apparatus for capillary HPLC,

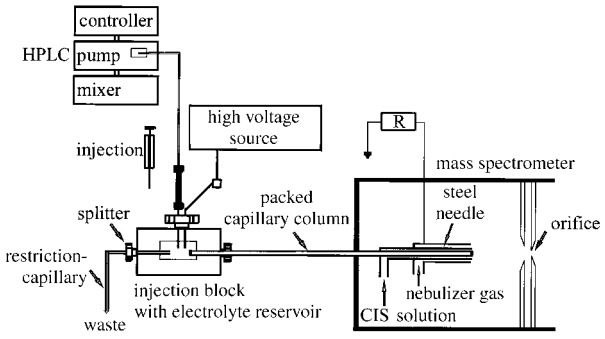

Figure 7. Instrument setup for the direct coupling of CHPLC, CZE, or pCEC with CIS-MS with addition of the coordinating central atom by means of a sheath flow stream.

capillary electrophoresis, and gradient capillary electrochromatography, which can be applied alternatively to all three techniques.[21–23] Addition of the complexing reagent by the "sheath-flow" method described by Smith et al.[7] for coupling capillary electrophoresis with ESI-MS (Figure 7) has proved satisfactory. The end of the capillary column is enclosed in a steel capillary (internal diameter 210 μm) through which the reagent solution is pumped by means of a syringe pump. These capillaries are held within a second steel capillary through which the nebulizing gas flows. Alternatively, the complexing reagent may be added through a T-junction. The following coordinating reagent solutions were used: 20 μg mL^{-1} LiI, 100 μg mL^{-1} Pd^{II} acetate, 50 μg mL^{-1} $AgNO_3$, and 100 μg mL^{-1} boric acid. The flow rate before entry into the ion source was between 3 and 40 μL min^{-1}. Voltages of between 80 and 150 V are applied to the orifice. Further experimental details are given in the legends to the figures.

The HPLC separation of carotinoids (Figure 3 a) was performed on a C3D-RP phase according to the conditions described in reference [24].

A Gromsil ODS-O AB (particle size = 3 μm) packed, fused silica capillary of 100 μm internal diameter and 25 cm long was used for the CIS-MS coupling. The eluent consisted of 40 mmol ammonium acetate (pH 9) and acetonitrile (5/95). An aqueous solution of 100 μg mL^{-1} silver nitrate was added at a flow rate of 3 μl min^{-1} by means of a syringe pump for the sheath flow. The ratio of sheath flow to CEC flow was approximately 3:1

Received: September 17, 1998 [Z 12423 IE]
German version: *Angew. Chem.* **1999**, *111*, 1046 – 1049

Keywords: alkenes · analytical methods · mass spectrometry · olefins · vitamins

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