ELSEVIER

Journal of Chromatography A, 712 (1995) 269–284

JOURNAL OF CHROMATOGRAPHY A

Industrial applications of capillary zone electrophoresis–mass spectrometry

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Abstract

Capillary zone electrophoresis was coupled on-line via a tricoaxial sheath flow interface with a benchtop quadrupole mass spectrometer (CZE–MS) equipped with a (pneumatically assisted) electrospray ionization source. Experimental parameters were optimized for operation in the negative-ion scan mode. The system was applied to the fingerprinting of an alkylsulphate detergent, to the identification of an impurity in a chloramine-T disinfectant, to the characterization of a complex polyethoxylated alkylphosphate emulgator, to the analysis of phenoxy acid herbicides and to the impurity profiling of MCPP. The optimized system was found to fully maintain the CZE separation performance, showing up to 1 million theoretical plates for the low-molecular-mass analytes studied. The sensitivity in the full-scan mode was typically in the picogram range and sufficient for the separation and identification of minor impurities in these industrial chemicals, down to the 0.1% level. In addition, the feasibility of on-column transient isotachophoresis (ITP–CZE–MS) under high electroosmotic flow conditions has been demonstrated for sample preconcentration and subsequent identification of trace impurities.

1. Introduction

Initially, capillary zone electrophoresis (CZE) [1] was mainly applied to biochemical analyses, but since 1989 its separation power has been demonstrated in the other fields of chemical analysis as well [2,3]. The ability of CZE to resolve mixtures which are hard to separate by other (chromatographic) methods puts a strong demand on the coupling of CZE with identification methods such as mass spectrometry (MS). As a rule CZE–MS coupling must be performed on-line because of the small elution volumes (nanolitres) of the zones which migrate through the capillary.

The on-line coupling of CZE with electrospray (ESI)-MS was demonstrated for the first time by Olivares et al. [4], followed by Lee et al. [5] for the coupling with pneumatically-assisted electrospray (ionspray, ISP). The developments in on-line CZE–MS, including the coupling with continuous-flow fast atom bombardment (FAB)-MS, have been described in two recent reviews [6,7]. From our literature survey of on-line CZE–MS, covering 100 publications in the period 1987–1994, we concluded that (a)

0021-9673/95/$09.50
SSDI 0021-9673(95)00289-8

(pneumatically-assisted) electrospray and (b) coaxial interface designs are being preferred, (c) about 85% of the CZE–MS work described so far is about positive-ion CZE–MS and, (d) most of the applications are in the biochemistry field: the other applications deal with (a.o.) drugs, metabolites, toxins, dyes and pesticides. Negative-ion electrospray CZE–MS is obviously more demanding, especially when the stability of the ESI is concerned. The onset of corona discharge has been reported to occur at significantly lower field strengths in negative-ion ESI of aqueous solutions versus positive-ion operation because of the fact that electrons can easily emanate from the edges of the stainless-steel electrospray capillary held at high negative potentials [8,9]. Especially in CZE–MS with coaxial sheath flow interfaces, the electrical contact at the CZE capillary outlet with the negative ESI potential might aggravate an unstable situation [9].

Other major concerns in on-line CZE–MS coupling are the maintenance of the separation integrity and the poor (concentration) sensitivity. The former can be realized by proper interface designs and good operating conditions (see below), and the latter covers the most important current research topics in CZE–MS: improvement of the concentration sensitivity via increased sample loadability of the CZE capillary using isotachophoretic principles [10], improvement of the ionization efficiency using sheathless electrospray interfaces and small I.D. (5–10 μm) capillaries [11,12] and the use of highly sensitive ion-trap mass spectrometers [13].

In this work, a commercial benchtop CZE–MS system was used based on a quadrupole MS equipped with an atmospheric pressure source, pneumatically-assisted electrospray ionization, and a tricoaxial CZE–MS interface. This system was applied to the analysis of a variety of industrial chemicals: the fingerprinting of a sodium alkylsulphate detergent, the analysis of the disinfectant chloramine-T, the characterization of a complex polyethoxylated alkylphosphate emulgator and the analysis of phenoxy acid herbicides and related impurities. In all cases the MS was operated in the negative-ion scan mode.

The results obtained show good separation performance, up to 1 million theoretical plates, and adequate stability and sensitivity for the identification of impurities (down to the 0.1% level) in these products.

2. Experimental

2.1. Apparatus

A Lauerlabs (Emmen, Netherlands) Model PRINCE capillary electrophoresis system was used, equipped with an F.u.G. (Rosenheim, Germany) Model HCN 35-35000 power supply, operated in the constant voltage mode. CZE was performed in 80–125 cm × 50–75 μm I.D., 375 μm O.D. fused-silica capillaries from Polymicro Technologies (Phoenix, AZ, USA), from which the first 30 cm were thermostated in the oven at 30°C. Samples were introduced into the capillary via the controlled pressure system, typically at 40 mbar during 0.1 min, unless stated otherwise. The CZE system was interfaced with a VG-Platform (Fisons Instruments VG–Biotech, Altrincham, UK) mass spectrometer equipped with an API source and a tricoaxial CZE–MS interface with pneumatically-assisted electrospray ionization. The drying and the nebulizing gas were both nitrogen. Depending on the application, the flows of the drying and the nebulizing gas varied between 40 and 150 and 30 and 45 l/h, respectively, the source temperature between 40 and 60°C, the electrospray voltage was −3 to −4 kV, the HV lens −0.2 kV and the cone voltage varied between −15 and −90 V; the multiplier was set at 650 V. The mass spectrometer was calibrated in the positive-ion mode using a mixture of PEG–ammonium adducts.

The make-up liquid, iso-propanol–water (4:1), was provided by an Applied Biosystems (San Jose, CA, USA) Model 140B syringe pump at 5–10 μl/min. The pump was equipped with a capillary fused-silica restrictor yielding a back-pressure of approx. 80 bar in order to guarantee a stable microflow.

2.2. Chemicals

Water was purified in an Alpha-Q (Millipore, Bedford, MA, USA) apparatus. The detergent

Teepol HB7 was obtained from Sigma (St. Louis, MO, USA). Mesityloxide, chloramine-T, the phenoxy acid herbicides, MCPP and the polyethoxylated alkylphosphate were from laboratory stock. All other chemicals were analytical grade and obtained from J.T. Baker (Deventer, Netherlands).

2.3. Methods

The electrophoretic mobilities, the coefficient of electroosmotic flow, and the plate numbers were calculated using the equations in Ref. [14]. New CZE capillaries were flushed with 1 *M* NaOH, water and finally with the buffer under investigation. In between subsequent analyses the CZE capillary was flushed with buffer only.

3. Results and discussion

3.1. General considerations

For a successful operation of a CZE–MS system with a tricoaxial interface at least the following critical parameters must be taken into account: (a) the stability of the CZE current via the sheath electrode; (b) the composition and flow-rate of the sheath liquid; (c) the compatibility of the CZE buffer; (d) the relative position of the three capillaries at the probe tip; (e) the flow-rates of the drying and the nebulizing gas; and (f) sample discrimination. The impact of some of these parameters on stability and sensitivity might be rather different from one CZE–MS design to the other and will be discussed here for the particular instrument as described in section 2.

Following the suggestions of Straub and Voyksner [15] we obtained an improved electrospray stability for isopropanol–water versus a methanol sheath flow and a better sensitivity (up to two-fold) at 5 μl/min as compared with 10 μl/min. From a compatibility point of view, the best CZE buffer would be volatile and contain a significant percentage of organic solvent (ideally the buffer would be the same as the sheath liquid; in particular situations, e.g. in on-line ITP–CZE–MS [10], they even must have the same composition) in order to assure efficient mixing with the sheath flow at the probe tip, and a low conductivity, the latter providing a more efficient electrospray ionization [16,17] and might be an alternative to the use of very small I.D. (5–10 μm) CZE capillaries [11,12]. As in LC–MS, however, such an ideal CZE–MS buffer often differs significantly from the ideal CZE buffer, in practice a good compromise has thus to be found for each application.

In agreement with Varghese and Cole [9] we found protruding capillaries to provide the best sensitivity, but in contrast with their work we obtained adequate stability even with protruding capillaries. Probably a tapered end of the CZE capillary [7] would give better mixing characteristics at the probe tip, but we still did not use that option since the tip would be more fragile.

Many authors [e.g. 13,18] report about the aspirating effect of the coaxial nebulizing gas which might cause the introduction of air during the injection in the CZE system and which introduces a laminar flow inside the CZE capillary during the actual electrophoretic separation. We observed such an aspirating effect at very high nebulizing gas flows (above 50 l/h) only. Actually the reverse situation is true in the design of our API source: a small restriction in the drying gas exhaust tube causes a small overpressure which generates a laminar flow in the direction of the CZE inlet vial and hinders the injection process. In order to study the impact on the CZE performance, the following experiment was carried out. In a first series of experiments the tricoaxial probe was outside the API source while the ESI voltage was off and the nebulizing gas was on. In the second series of experiments the probe was inside the API source with the ESI voltage still off, but with both the drying and the nebulizing gas on. The electroosmotic flows and the plate numbers were determined using a neutral marker, mesityloxide, which was detected half-way the CZE capillary by UV absorbance. Care was taken to assure that the liquid level in the inlet vial was at the same height as the probe tip in both cases. From the results of the second series of experiments relative to the first series, i.e. relative to the situation outside the API source, it was concluded

that the impact of the pressure-induced flow on the plate number and the electroosmotic flow was significant: with 50 μm and 75 μm I.D. capillaries the electroosmotic flows were reduced to 95 and 85%, respectively, and the plate numbers were reduced to 70% and 5%, respectively. Fortunately, our CZE apparatus is capable of programming either a positive or a negative pressure on the inlet vial during the electrophoretic separation, thus we can compensate for a deviation from atmospheric pressure in the source. Nevertheless 50 μm I.D. (or even smaller I.D.) CZE capillaries are to be preferred, also because of the lower conductivity. The negative impact of the pressure-induced flow on the injection can be simply overcome by switching off the gases during the injection [13]. In addition one should switch off the ESI voltage during pressure injection in order to avoid sample discrimination.

To summarize, for this particular CZE–MS system we recommend the parameters and settings for negative-ion CZE–MS as shown in Table 1. Using these settings it is possible to fully maintain the CZE separation performance, even up to 1 million theoretical plates as shown in the following application.

3.2. Fingerprinting of linear alkylsulphates

Linear alkylsulphates are widely used in detergent formulations. Usually they are complex mixtures of components with the anionic group in common but with different lengths of the alkylchain. Fingerprinting of these detergents is necessary in order to assess the particular application area. Liquid chromatography (LC) has been used for this purpose for many years. Apart from difficulties in obtaining baseline resolution, another problem is the absence of a suitable chromophoric group for UV-absorbance detection.

However, the use of on-line LC–MS with an ion spray interface has shown to be a good alternative for detection problems in this particular field [19,20]. We used the separation power of CZE for the analysis of a commercial detergent, Teepol HB7 [21]. Using a UV-absorbing background electrolyte, we were able to detect the negative peaks as a characteristic fingerprint (actually five fingers indeed) of this detergent featuring baseline resolution, plate numbers of up to 440 000 and an analysis time of 3 min only [21]. The peaks were identified by standard addition and found to represent C_9–C_{13} alkylsulphates. These results were confirmed later by others who also showed the effect of the buffer cation in these separations [22]. It was demonstrated by Smith et al. [23] that sodium dodecylsulphate is very well amenable to CZE–MS. In this work we have studied the use of CZE–MS for the fingerprinting and the identification of the commercial C_9–C_{13} alkylsulphate sample. We used a 50 μm I.D. CZE capillary with a 10 m*M* ammonium acetate buffer of pH 8.9 and a voltage of 30 kV. The actual voltage difference was 34 kV (400 V/cm) because of the ESI voltage of −4 kV.

A 1:1000 dilution of the detergent with the electrophoresis buffer was made and injected at 40 mbar during 0.1 min, followed by the injection of a similar plug of buffer. Next, the CZE voltage, the ESI voltage and the gases were switched on and the MS started scanning from

Table 1
Guidelines for negative-ion CZE–MS using the system as described in section 2

1. Monitor your CZE current during CZE–MS.
2. Use 50 μm (or smaller) I.D. CZE capillaries.
3. Use a nebulizing gas flow of 30 l/h and a drying gas flow lower than 50 l/h.
4. Avoid a height difference between the liquid level in the CZE inlet vial and the probe tip.
5. Use a stable sheath flow of iso-propanol–water (4:1) at 5 μl/min.
6. Remove the polyimide outerlayer of the CZE capillary at the probe tip and protrude the CZE capillary 0.2–0.3 mm relative to the sheath flow capillary, and protrude the sheath flow capillary 0.8–1 mm relative to the nebulizing gas capillary.
7. Switch off the drying and the nebulizing gas and the ESI voltage during injection.

100 to 300 amu in 0.5 s. Fig. 1 shows both the total ion current (TIC) and the reconstructed ion electropherograms (RIE) of the [M − H] ions indicated. The fingerprint can be easily recognized from the TIC, i.e. even without any prior knowledge of the composition. The separation of the individual components is quite satisfactory. The analysis time has almost doubled as compared with our previous work [21] because of the longer CZE capillary and the lower electroosmotic flow in the ammonium acetate buffer versus the sodium veronal buffer. However, the most striking result here is the identification of the C_8 ([M − H] at m/z 209) and C_{14} ([M − H] at m/z 293) homologues in this detergent sample which were not found before. In order to support the identification, the same sample was analyzed under different cone voltage settings thus promoting in-source CID fragmentation patterns. All components showed the $[HSO_4]^-$ ion (m/z 97) upon in-source CID, in addition to their characteristic $[M - H]^-$ ions. The overall performance of the CZE–MS system was investigated with the same detergent but at a lower concentration and a smaller injection volume (20 mbar, 0.1 min of a 2000 × dilution). The MS was scanned over a smaller range at high speed, 215–300 amu in 0.2 s. The total ion current thus obtained (Fig. 2) is really amazing: ultra-high resolution and plate numbers ranging from 700 000 to 1 000 000 (except for C_{10} alkylsulphate: 330 000). Two additional peaks were observed in between the homologues, which represent, possibly, branched alkylsulphates. When dispersion in CZE is diffusion-limited only, the plate number equation is according to Ref. [1]: $N = mV/2D$, in which N is the plate number, m the mobility, V the voltage difference over the capillary and D the diffusion coefficient. The plate numbers and mobilities obtained here correspond with calculated diffusion coefficients in the order of $0.8 \cdot 10^{-5}$–$1.2 \cdot 10^{-5}$ $cm^2\ s^{-1}$, which are very realistic for low-molecular-mass analytes like these. Thus it can be concluded that the theoretically maximum attainable plate number in CZE can be realized in this CZE–MS system provided that the instrument is optimized using the guidelines in Table 1 and overloading of the CZE capillary is being avoided. This linear alkylsulphate application provides very reproducible results and is currently used as an overall performance standard for negative-ion CZE–MS in our laboratory.

3.3. Analysis of chloramine-T

Chloramine-T is widely used as a disinfectant in e.g. the food industry. It can be analyzed by CZE in its anionic form. CZE–MS was carried out using a 75 μm I.D. fused-silica capillary and a sheath flow-rate of 10 μl/min, i.e. the CZE–MS system was not optimized yet with regard to the guidelines in Table 1. A solution of 0.1 mg/ml chloramine-T in buffer was prepared and injected by a pressure of 40 mbar, 0.1 min, which corresponds to 2 ng. The CZE voltage was switched on and the MS was scanned from 70 to 500 amu in 0.5 s. The total ion current (TIC) and the reconstructed ion electropherogram (RIE) are shown in Fig. 3 (left). Despite the non-optimized conditions, a sharp symmetrical peak, showing 120 000 theoretical plates, could be observed in the TIC and is very obvious from the reconstructed ion electropherogram. The detection limit using the reconstructed ion electropherogram would be 100 pg (scan mode). In an attempt to detect the impurities in chloramine-T, an excess (10 mg/ml) was injected and analyzed by CZE–MS under the same conditions. The total ion current (Fig. 3, right) clearly shows an impurity around 8 min. This result is in good agreement with preliminary CZE–UV studies which also showed one impurity in the electropherogram estimated at the 0.1% level relative to chloramine-T. In the present CZE–MS experiment this corresponds to an injected amount of 200 pg. The impurity was identified using the background subtracted mass spectra shown in Fig. 4. The spectrum of chloramine-T (Fig. 4, lower left) shows the negative ion at m/z 204 and the isotope cluster of one chlorine atom. The impurity (Fig. 4, upper left) has a negative ion at m/z 170 and contains no chlorine at all. The analysis was repeated at an (absolutely) higher cone voltage (−60 vs. −16 V) in order to generate in-source CID fragmentation patterns.

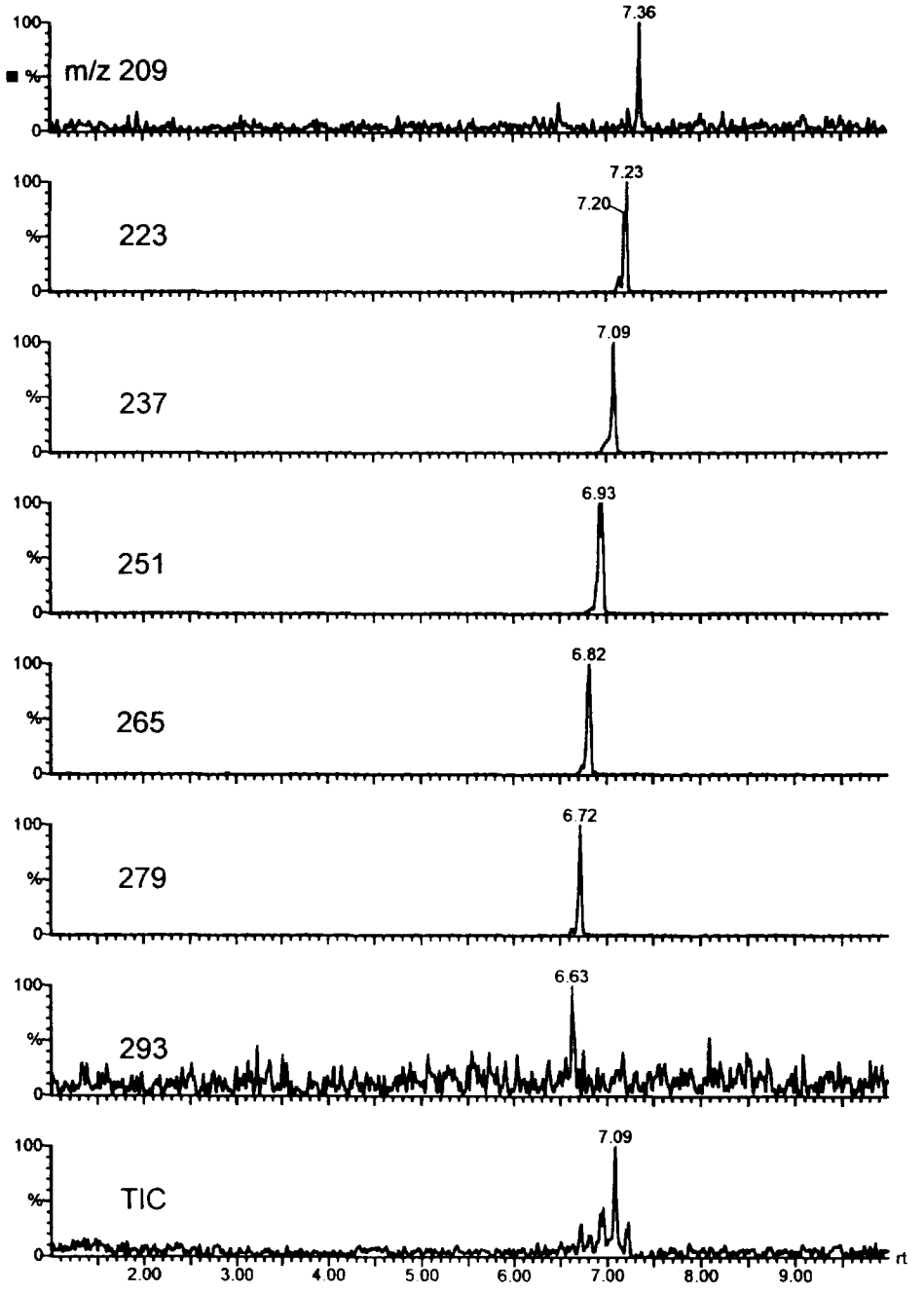

Fig. 1. The analysis of Teepol HB7 by full-scan negative-ion CZE–MS. Total ion current (TIC) and reconstructed ion electropherograms (RIE) of the $[M-H]^-$ ions indicated. Conditions: injection of a 1:1000 dilution with electrophoresis buffer by pressure (40 mbar, 0.1 min); CZE in a 85 cm × 50 μm I.D. fused-silica capillary using a 10 m*M* ammonium acetate buffer pH 8.9, constant voltage 30 kV; sheath flow, 5 μl/min isopropanol–water (4:1); ESI voltage, −4 kV; cone voltage, −30 V; source, 50°C; drying gas, 50 l/h; nebulizing gas, 30 l/h; scan range, 100–300 amu in 0.5 s. Other conditions, see text.

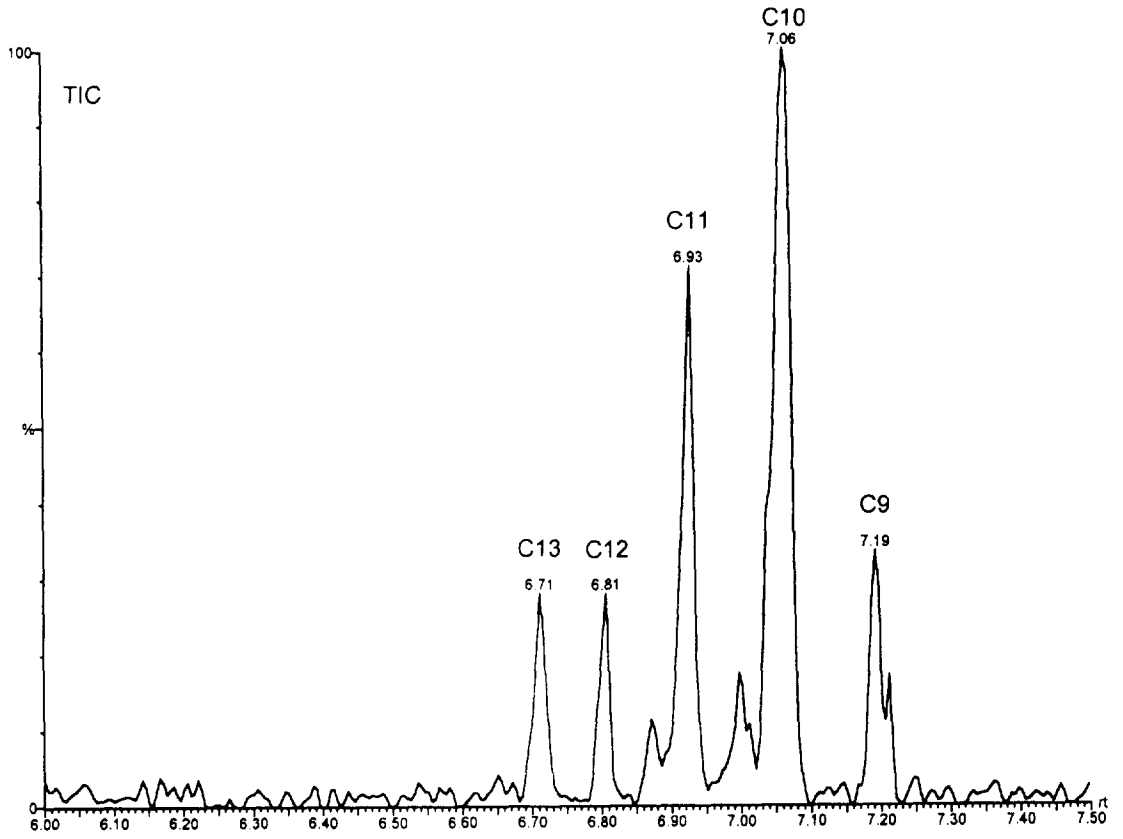

Fig. 2. Total ion current (TIC) showing ultra high-performance CZE–MS of C_9–C_{13} linear alkylsulphates. Injection of a 1:2000 dilution of Teepol HB7 (20 mbar, 0.1 min). Scan range, 215–300 amu in 0.2 s. Other conditions, see Fig. 1.

The spectra thus obtained (Fig. 4, lower right) indicate that m/z 170 is also a decomposition product in the chloramine-T spectrum. The decomposition pathways are rather different: the chloramine-T spectrum shows a.o. benzene and toluene, while the impurity spectrum (Fig. 4, upper right) shows a.o. the loss of SO_2. Despite of the dissimilarities in the fragmentation patterns, the most likely identity of the impurity is the decomposition product toluenesulfonamide, formed by the exchange of a chlorine by a hydrogen atom, thus yielding an $[M - H]^-$ ion at m/z 170 without any chlorine atoms.

3.4. Characterization of a polyethoxylated alkylphosphate

Polyethoxylated alkylphosphates are used as emulgators and exhibit, a.o., antistatic properties, which are very useful in specific applications. As in the detergent analysis, these products consist of complex mixtures and detailed knowledge of the constituents is required. In the present case, we knew already the presence of phosphor from XRF analysis and the presence of alkyl and ethylene oxide units from NMR analysis. A first attempt with flow injection negative-ion electrospray MS, i.e. without CZE, confirmed the presence of more than 20 components, representing polyethoxylated decyl- and dodecyl-phosphates. Polyethoxylated alkylphosphates cannot be analyzed directly via GC, and LC analysis suffers from detection problems and requires gradient elution. Mono- and dialkylphosphates are weak acids which can be, respectively, double and single negatively charged, depending on the pH of the CZE buffer. We used a 75 μm I.D. capillary and a

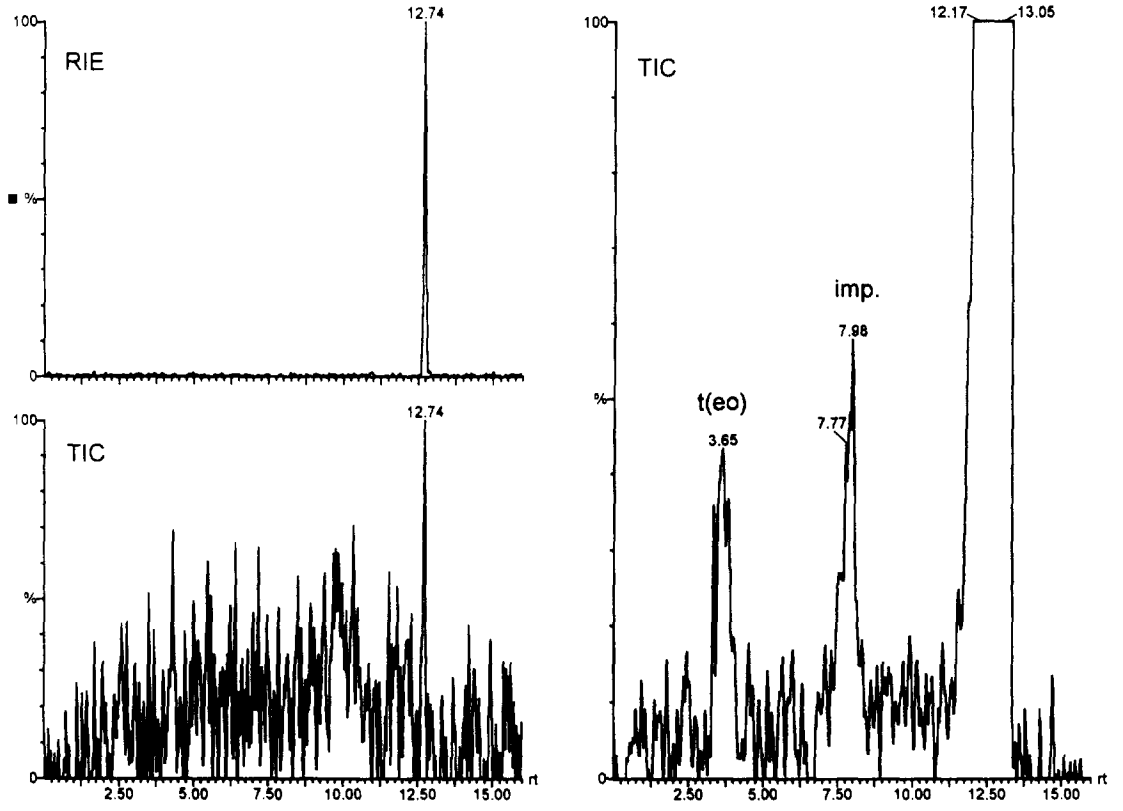

Fig. 3. The analysis of chloramine-T by full-scan negative-ion CZE–MS. Lower left, total ion current (TIC) after injection of a solution of 0.1 mg/ml in buffer (40 mbar, 0.1 min); upper left, reconstructed ion electropherogram (RIE, m/z 204 + 206) of the same analysis. Right, total ion current (TIC) after injection of a 10 mg/ml solution. Conditions: CZE in a 90 cm × 75 μm I.D. fused-silica capillary using a buffer of 10 m*M* ammonium acetate pH 10; constant voltage, 30 kV; sheath flow, isopropanol–water (4:1) at 10 μl/min; ESI voltage, −3.4 kV; cone voltage, −16 V; source, 60°C; drying gas, 150 l/h; nebulizing gas, 45 l/h; scan range, 70–500 amu in 0.5 s. Other conditions, see text.

buffer of 10 m*M* ammonium acetate (pH 9)–methanol (4:1) in order to obtain sufficient solubility and resolution. The presence of methanol and the relatively high potassium content of the sample decreased the electroosmotic flow as expected, but in conjunction with the overpressure in the ion source (cf. above) rather long analysis times were obtained (> 30 min). Therefore, the pressure-induced laminar flow in the direction of the inlet vial was compensated by programming a pressure of 25 mbar on the inlet vial during the actual CZE separation. Note that the CZE–MS system was not optimized yet with regard to the guidelines in Table 1 and was scanned from 220 to 1000 amu in 0.5 s. The total ion current thus obtained is shown in Fig. 5 (bottom). About 12 peaks can be easily distinguished, and in between them a few additional minor peaks can be seen. The mass spectra of these peaks showed all $[M-H]^-$ ions, corresponding with ethoxylated decylphosphates (higher peaks) and ethoxylated dodecylphosphates (smaller peaks). Using the mass spectral data, summed ion electropherograms were reconstructed for both series (Fig. 5, middle and top). The decylphosphates showed 0–15, and the dodecylphosphates 0–12 ethylene oxide units, together 29 components. Looking for even high-

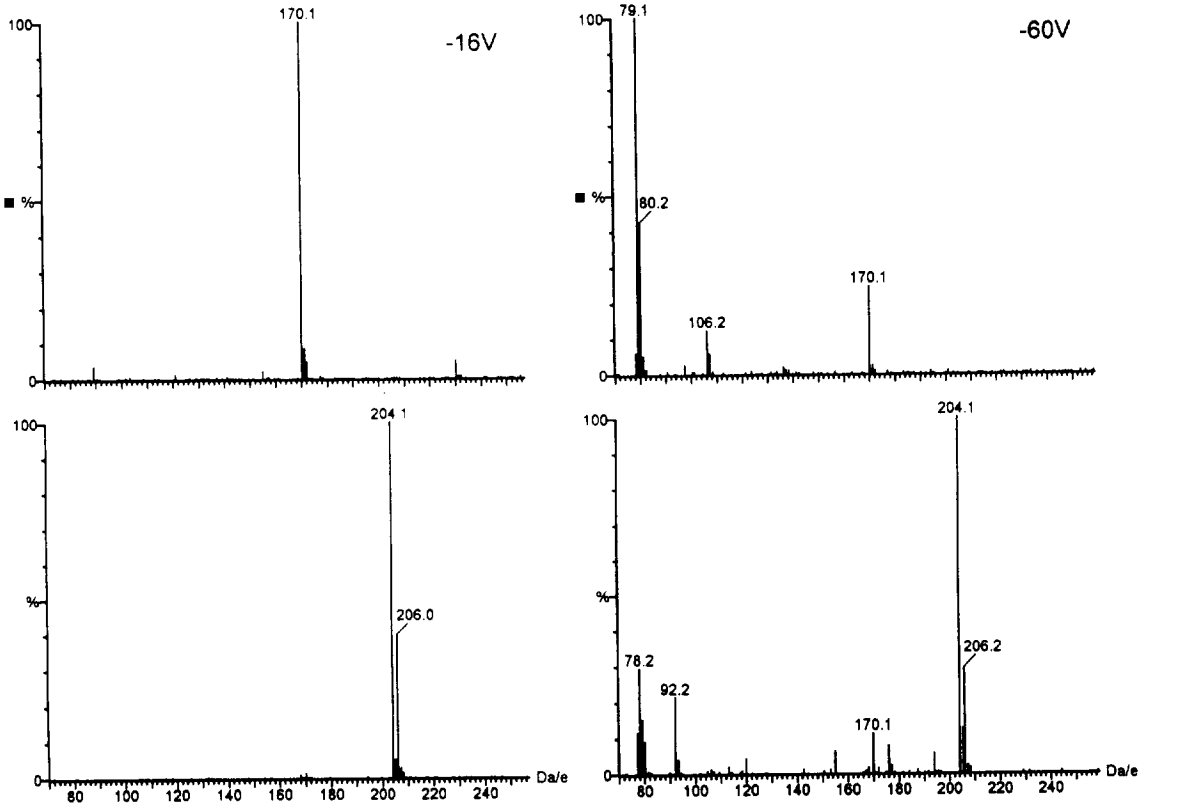

Fig. 4. Background subtracted mass spectra of chloramine-T (lower spectra) and the impurity (upper spectra) using, left, the conditions of Fig. 3 and, right, an (absolutely) higher cone voltage, −60 V vs. −16 V.

er degrees of ethoxylation, the total ion current was further examined at shorter migration times (i.e. at higher apparent mobilities but lower electrophoretic mobilities). Thus all spectra between 8 and 12.5 min were combined and background subtracted, giving the mass spectrum of Fig. 6. Apart from the higher ethoxylated decyl- and dodecylphosphates, a series of didecylphosphates having 0–14 ethylene oxide units showed up in this spectrum. Dialkylphosphates can be single charged only, so it is not surprising that even the lower ethoxylated didecylphosphates appear in the electropherogram before (i.e. at lower electrophoretic mobilities than) the double charged mono-alkylphosphates. To summarize, although a first look at the total ion current showed only about 15 resolved peaks, up to 45 individual components could be identified in this sample using CZE–MS.

3.5. Analysis of phenoxy acid herbicides and impurity profiling of MCPP

MCPP, 2-(2-methyl-4-chlorophenoxy)propionic acid, belongs to the group of phenoxy acids, and is widely used in agriculture as a selective herbicide. Formulations of these herbicides can be analyzed by capillary gas chromatography [24] but derivatization of the carboxyl group is required. Liquid chromatography can be applied directly but might not give sufficient resolution for the analysis of related impurities [24]. CZE, on the other hand, shows baseline resolution for the separation of the structurally

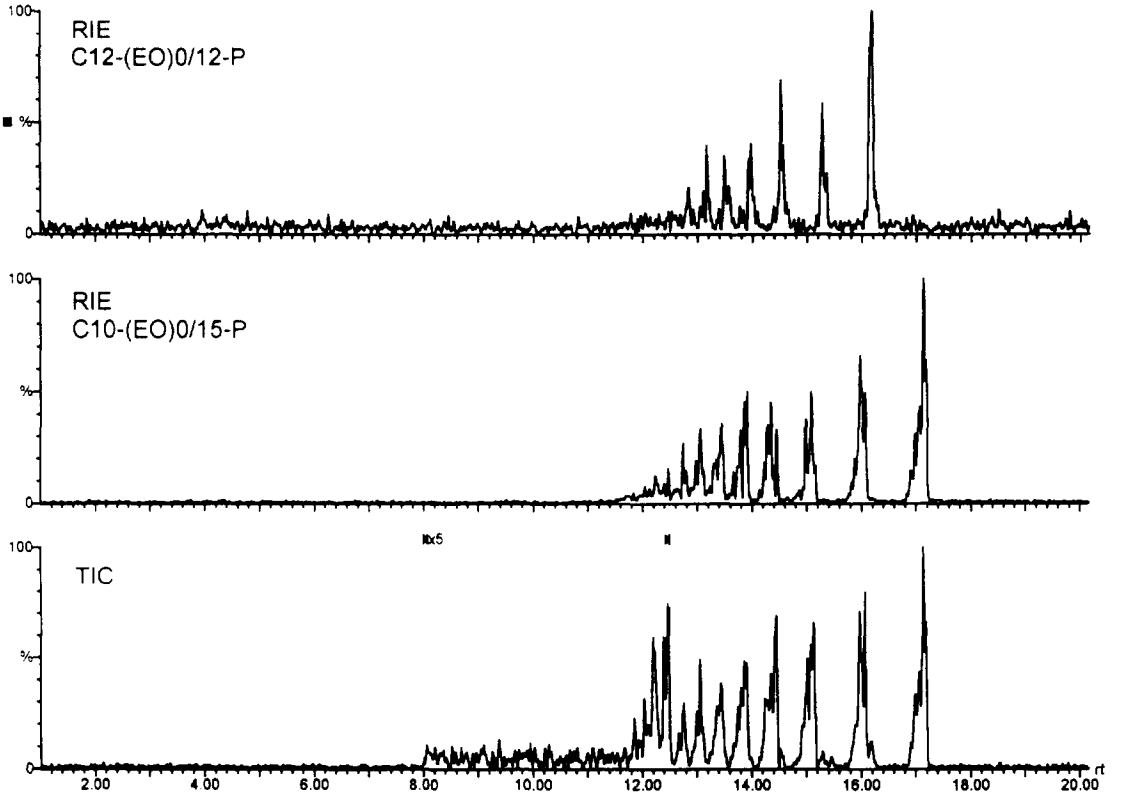

Fig. 5. The characterization of a polyethoxylated alkylphosphate by full-scan negative-ion CZE–MS. Bottom, the total ion current (TIC); middle, the reconstructed ion electropherogram (RIE) of the decyl-polyethoxylated phosphates; and top, the RIE of the dodecyl-polyethoxylated phosphates. Conditions: injection of a 10 mg/ml solution in buffer by a pressure of 40 mbar, 0.1 min; CZE in a 90 cm × 75 μm I.D. fused-silica capillary using a buffer of 10 m*M* ammonium acetate (pH 9)–methanol (4:1); constant voltage, 30 kV and 25 mbar pressure on the inlet vial during the actual electrophoretic separation; sheath flow, isopropanol–water (4:1) at 5 μl/min; ESI voltage, −3 kV; cone voltage, −25 V; source, 60°C; drying gas, 100 l/h; nebulizing gas, 30 l/h; scan range, 220–1000 amu in 0.5 s. Other conditions, see text.

similar phenoxy acid herbicides MCPP, 2,4-DP, MCPA and 2,4-D (for structures, see Fig. 7), provided that the pH of the electrophoresis buffer is chosen correctly [25,26]. In our previous work we have studied the separation of phenoxy acid herbicides and analyzed the impurities in MCPP using the entire different selectivities as obtained after the addition of different types of cyclodextrines to the CZE buffer [25]. Identification was simply done via standard addition of known impurities to the MCPP samples with subsequent analyses using the different CZE buffer systems. The feasibility of CZE coupled with ion spray mass spectrometry for the detection of 2,4-D was demonstrated by Lee et al. [27], who separated some phenoxy acids (including 2,4-D) using a high acetonitrile content in the buffer, and detected them in the single-ion recording (SIR) mode.

In the present study, a mixture containing 20 ppm (corresponding to 80 pg) of MCPP, 2,4-DP, MCPA and 2,4-D was pressure-injected and analyzed using the CZE–MS system in full-scan negative-ion mode, optimized according to the guidelines in Table 1. The reconstructed ion electropherograms are shown in Fig. 8. Baseline separation and high plate numbers were obtained (300 000–500 000 theoretical plates), versus 240 000 plates in the CZE–UV system (cf. Ref. [25]). The higher plate numbers in CZE–

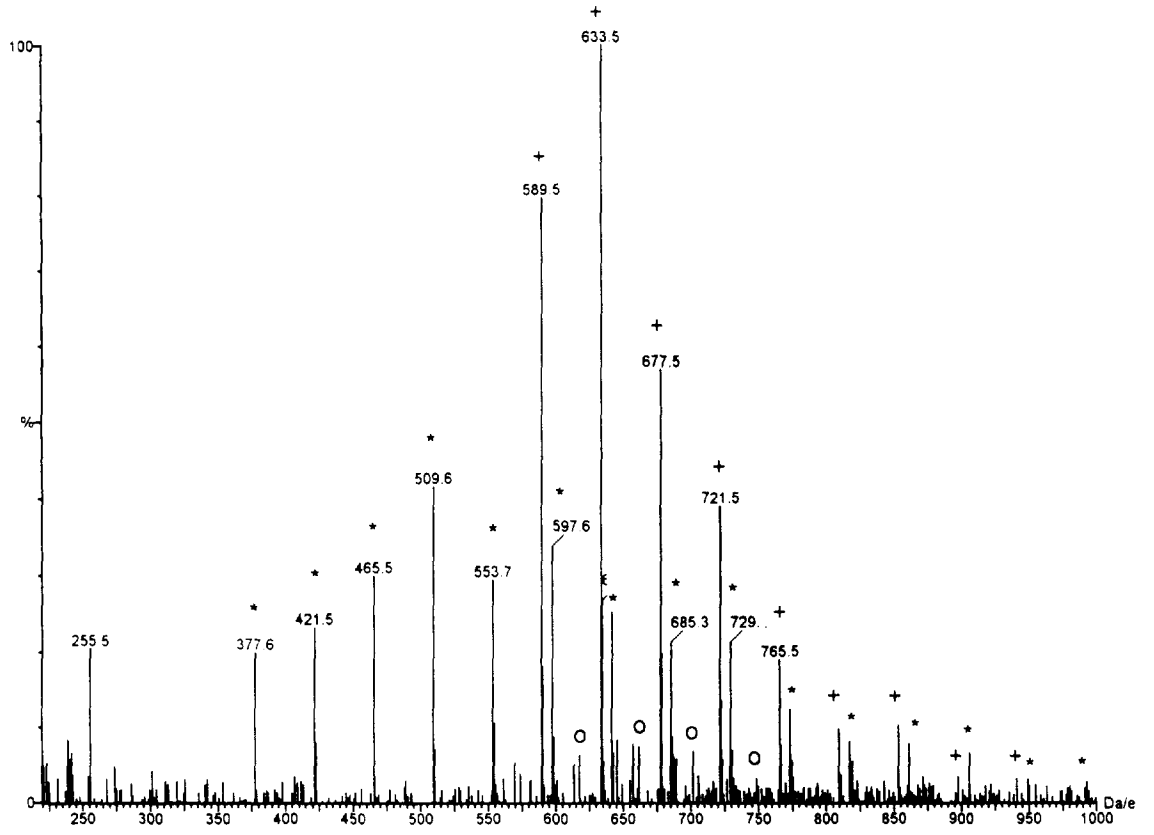

Fig. 6. Background subtracted mass spectrum combined from the data between 8 and 12.5 min in the total ion current of Fig. 5. Symbols: + = $C_{10}(EO)_{8-16}$phosphates; ○ = $C_{12}(EO)_{8-11}$phosphates; * = C_{10}/$C_{10}(EO)_{0-14}$phosphates.

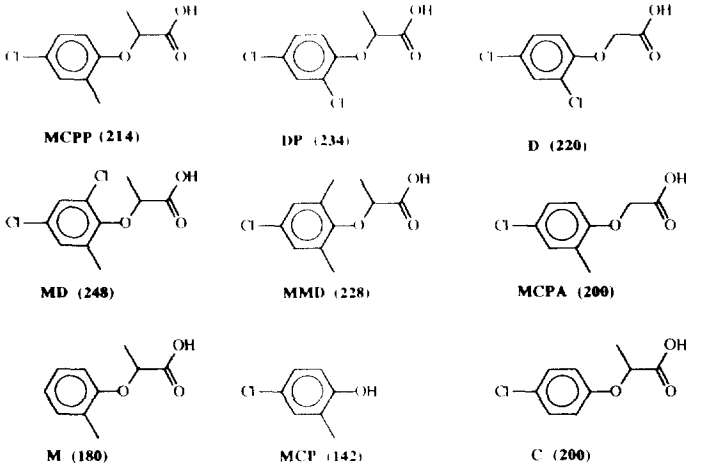

Fig. 7. Structures of MCPP and related compounds (molecular mass in brackets).

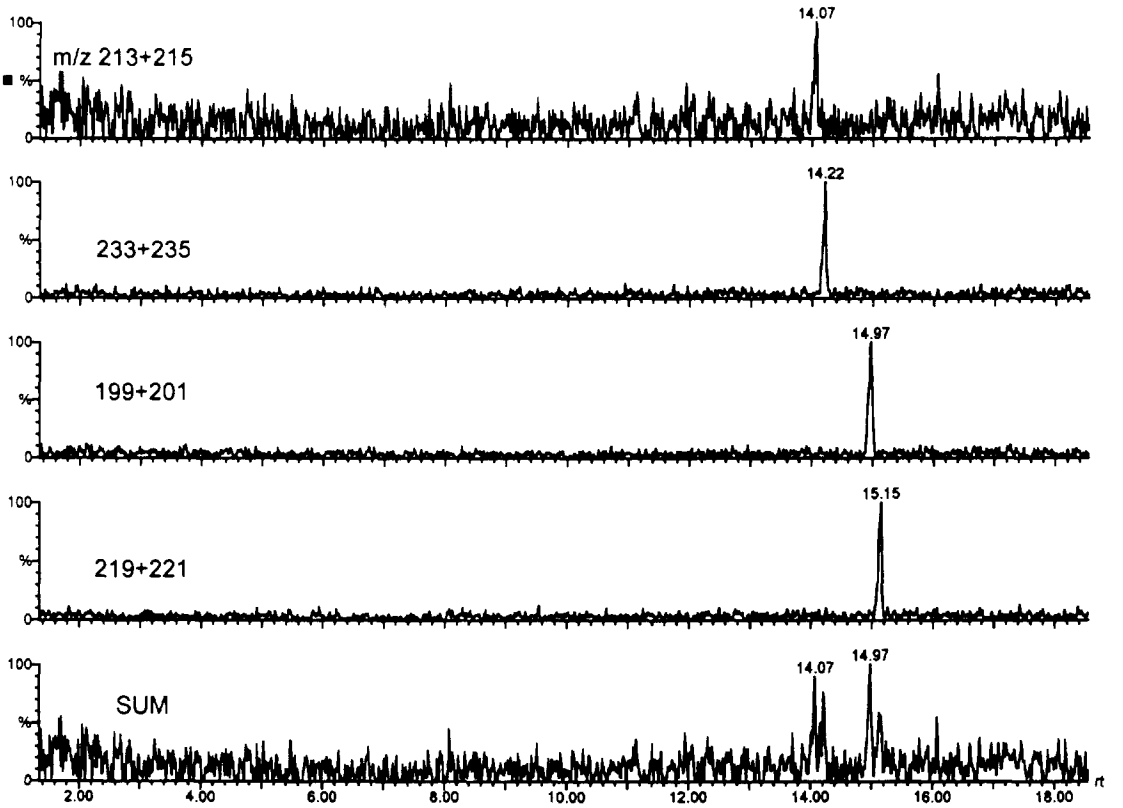

Fig. 8. The analysis of phenoxy acid herbicides by full-scan negative-ion CZE–MS. Reconstructed ion electropherograms (RIE) of the $[M-H]^-$ ions indicated. Conditions: injection of 20 μg/ml (80 pg) of each herbicide (in buffer) by pressure of 40 mbar, 0.1 min, followed by injection of a similar plug of buffer; CZE in a 87 cm × 50 μm I.D. fused-silica capillary using a buffer of 10 m*M* ammonium acetate pH 4.8; constant voltage, 30 kV; sheath flow, isopropanol–water (4:1) at 5 μl/min; ESI voltage, −4 kV; cone voltage, −25 V; source, 50°C; drying gas, 50 l/h; nebulizing gas, 31 l/h; scan range, 100–300 amu in 0.5 s. Other conditions, see text.

MS versus CZE–UV might be attributed to the lower buffer concentration (less Joule heating) used in the present work. The detection limits as calculated from the reconstructed ion electropherograms are typically in the order of 20 pg, except for MCPP whose [M-H]-ion, 213, is very close to a persistent background ion at *m/z* 212. It might be estimated that approximately 120 pg of each herbicide will be required in order to distinguish these four peaks in the total ion current (TIC). The background subtracted mass spectra are shown in Fig. 9. The number of chlorine atoms can be readily seen from the isotope distributions around both the $[M-H]^-$ ions and the main in-source CID decomposition products, the corresponding phenoxy ions.

Next, an excess of an MCPP sample (10 mg/ml) was injected and analyzed by CZE–MS in order to identify the impurities. In the past, MD, MCPA and M, were found to be present in the range of 0.1–1.3% relative to MCPP by CZE–UV [25]. The total ion current and reconstructed ion electropherograms are shown in Fig. 10. A huge peak of MCPP can be seen preceded by a peak at 7.7 min, which corresponds to neutral analytes migrating under the influence of the electroosmotic flow only, a peak at 13.1 min, and a small peak at 15.6 min. The background

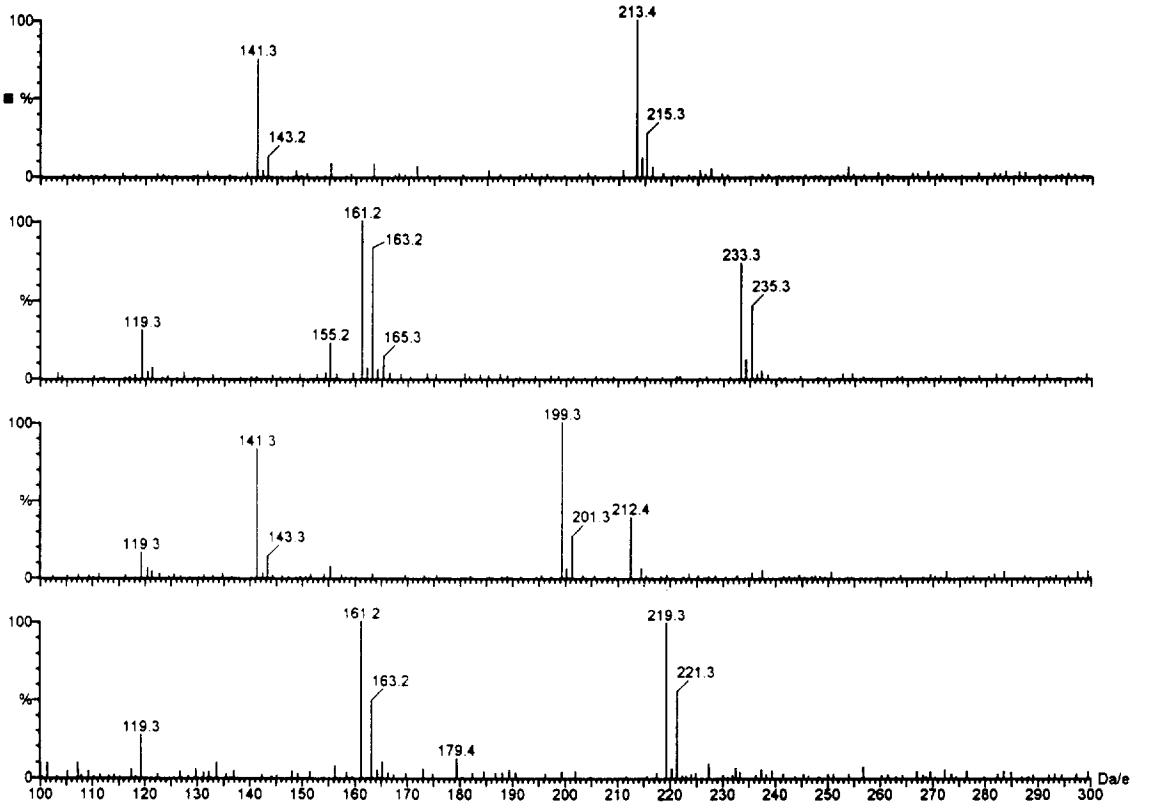

Fig. 9. Background subtracted mass spectra of the four phenoxy acid herbicides in Fig. 8. Top, MCPP; upper middle, DP; lower middle, MCPA; below, D.

subtracted mass spectra of these peaks are shown in Fig. 11. The neutral impurity (Fig. 11, top) shows an $[M-H]^-$ ion at m/z 141 and one chlorine atom (note that m/z 119 and 212 are background ions). MCP is a good candidate which will behave as a neutral analyte indeed at pH 4.8. The next mass spectrum (Fig. 11, middle) shows at least four compounds which co-migrate at the rear of the MCPP peak and which show extremely narrow peaks (2 s width) in the reconstructed ion electropherograms of Fig. 10. The spectrum contains ions which correspond to a mixture of the MCPA and DP spectra (cf. Fig. 9), extended with m/z 127, 179 and 107. The latter two do not contain any chlorine, making M a good candidate. The ion at 127 does contain one chlorine and must be an acid because of its position in the electropherogram. The impurity C (M_r 200) will be a good candidate thus providing a nice demonstration of the value of the CID decomposition product at 127, since the parent compound has the same $[M-H]^-$ ion at m/z 199 and the same chlorine cluster as one of the other impurities, MCPA. The very high plate numbers (2–4 million theoretical plates!) of these compounds are due to a transient isotachophoretic effect [28], as follows. In short, when a sample contains a high concentration of a high mobility co-ion and the CZE buffer ion has a lower mobility than the sample ion, transient isotachophoresis takes place in which the sample co-ion acts as the leading ion and the CZE buffer co-ion as the terminating ion. The sample ion, having a mobility in between, will be focussed in a narrow band as long as the isotachophoretic conditions are maintained. The present situation

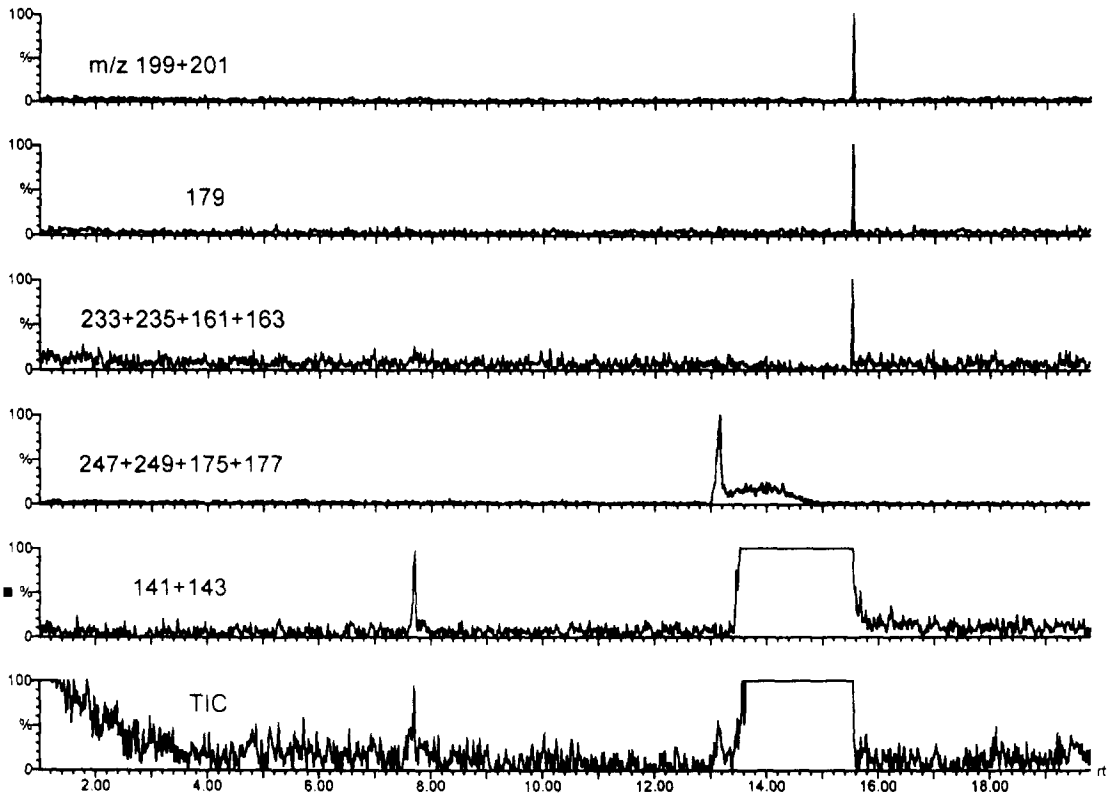

Fig. 10. Impurity profiling of MCPP by full-scan negative-ion CZE–MS. Reconstructed ion electropherograms (RIE) of the $[M-H]^-$ ions indicated and total ion current (TIC). Injection of 10 mg/ml (in acetonitrile–methanol–CZE buffer, 1:2:2) by pressure of 40 mbar, 0.1 min, followed by injection of a plug of buffer. Other conditions, see Fig. 8 and text.

is different from Ref. [28] because of the presence of a strong electroosmotic flow in a direction opposite to the electrophoretic migration. Nevertheless similar principles apply in such a situation [29], the only difference being that the excess co-ion in the sample should be a low mobility ion and the CZE buffer should contain the high mobility ion. In our case, the excess of MCPP represents the excess (0.05 *M*) sample co-ion having a low electrophoretic mobility, while the acetate CZE buffer ion (0.01 *M*) represents the high electrophoretic mobility ion. The impurities M, C, MCPA and DP have electrophoretic mobilities in between MCPP and acetate and will be focussed during the transient isotachophoretic stage. On the other hand, impurities migrating close to the front of MCPP might be broadened, as can be seen in the reconstructed ion electropherograms of Fig. 10. The spectrum of the broadened peak (Fig. 11, below) shows a mixture of $[M-H]^-$ ions at *m/z* 247 and 227, with corresponding fragment ions at *m/z* 175 and 155, the former having two chlorine and the latter only one chlorine atom. MD and MMD will be good candidates for these impurities. The spectrum of the main component, MCPP, was already shown in Fig. 9. It is interesting to note that apart from the MD, MCPA and M impurities found in the past [25] after spiking of the sample, additional impurities (C, DP, MCP and MMD) were identified now by CZE–MS. In-source CID was essential for their identification and transient isotachophoretic focussing provided the signal-to-noise ratio required for the identification of the trace impurities C and DP, which were not detectable in

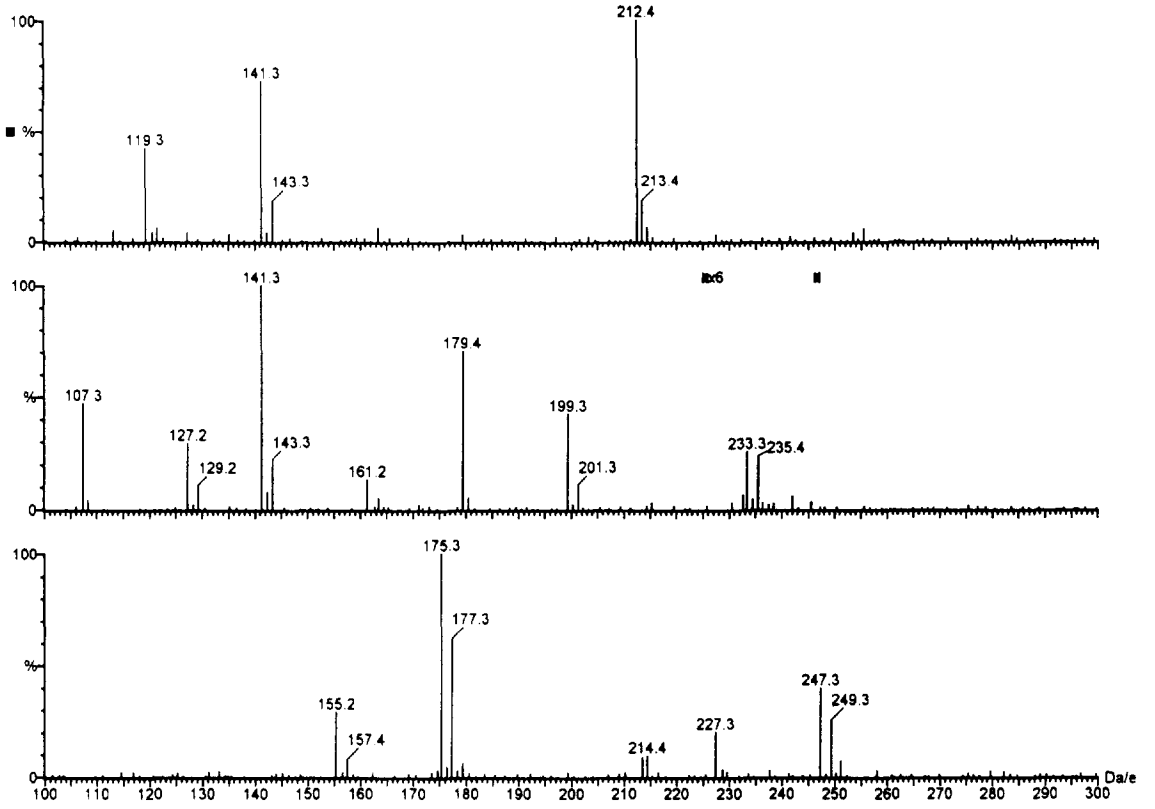

Fig. 11. Background subtracted mass spectra of the impurities in MCPP. Top, spectrum of neutral impurity (MCP) migrating at 7.7 min; middle, spectrum of impurities (MCPA, DP, M, C) migrating at 15.6 min; and bottom, spectrum of impurities (MD, MMD) migrating at 13.1 min in the total ion current (TIC) of Fig. 10.

the CZE–UV approach, i.e. which were present at levels significantly lower than 0.1% relative to MCPP.

4. Conclusions

CZE–MS can be successfully applied to realistic chemical analyses in an industrial research environment. The benchtop system used shows good stability and sensitivity in the negative-ion scan mode, particularly when critical operating parameters are taken into account and optimized. In that case the CZE separation performance is not compromised at all: the reconstructed electropherograms showed plate numbers up to 1 million, even higher than those in comparable CZE–UV studies.

The sensitivity, typically in the picogram range under full-scan conditions, enabled the identification of unknown minor impurities down to the 0.1% level in the examples shown. In addition, transient ITP–CZE–MS under high electroosmotic flow conditions, yielding extremely narrow peaks (up to 4 million theoretical plates), was found to be very useful for on-column preconcentration and subsequent identification of trace impurities ($\leqslant$0.1%).

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