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Note

Off-line combination of isotachophoresis and mass spectrometry

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Isotachophoresis (ITP) is an excellent separation method for permanent ions or for substances able to form ions in solution. On the other hand, identification can be performed only by comparing the detected characteristics, *e.g.*, UV absorbance or electrical conductivity, of the sample with those of a standard. So, if no standard is available or some substances exhibit identical behavior with respect to the characteristics mentioned, spectroscopic methods, *e.g.*, mass spectrometry (MS), can be advantageously combined with ITP in order to get a greater degree of certainty concerning the identity of the sample. For ITP, the sample components to be separated must exist, at least partially, as ionic species under working conditions, a property not required for MS with electron-impact (EI) ionization, so that the combination of the two methods was not previously proposed in the literature. Nevertheless, combination of ITP and MS for separation and identification is possible, as will be shown in this paper. Detailed conditions for a more general use of ITP–MS, *e.g.*, the choice of buffering counter ions for several pH values, are the subject of a current investigation and will be published later[1].

In this paper, an example of the potential of ITP–MS is given by the use of MS in the identification of the pesticides paraquat and diquat after separation from cations, typical of plant extracts, by ITP. Sample pretreatment itself, using displacement chromatography, is of minor interest for demonstrating the combination of ITP with MS and is the subject of a future paper[2]. Identification cannot be achieved by ITP alone because of the identical ionic mobilities of the two quarternary ammonium compounds under the given conditions. However, after trapping the pesticide zones, the substances can easily be identified by MS.

EXPERIMENTAL

Reagents

Chemicals (p.a. grade) were obtained from E. Merck (Darmstadt, G.F.R.). The pesticides paraquat (1,1′-dimethyl-4,4′-dipyridinium-dichloride) and diquat (9,10-dihydro-8a,10a-diazaphenanthrene-dibromide), both technical grade, were obtained from S.u.I. Ehrendorfer (Augsburg, G.F.R.). Double-distilled water from a quartz apparatus was used as solvent.

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Electrolyte system for separation

As unbuffered leading electrolyte (LE), 0.01 *M* hydrochloric acid (pH 2) was used. Terminating electrolyte (TE) was a 0.01 *M* solution of tris(hydroxymethyl)-aminomethane (TRIS; adjusted to pH 6 with HCl). For preliminary experiments, but not for the trapping procedure, 0.05% (w/w) poly(vinyl alcohol) (Mowiol 8-88; Hoechst, Frankfurt, G.F.R.) was added to the leading electrolyte.

Procedure

After separation by ITP, the sample zones of interest were trapped by the trapping device described below. The solutions, 3 μl containing 3 μg of pesticide, were transferred into a crucible (aluminum alloy) normally used for MS and the solvent was evaporated. Then the crucible was brought into the ion source of the mass spectrometer via the solid sample inlet.

Apparatus

ITP separations were carried out by the use of an instrument similar to that described by Everaerts *et al.*[3] and made at Komensky University, Bratislava, provided with an electrical conductivity detection cell designed by Stankoviansky *et al.*[4]. The separation capillary made from PTFE, was 0.3 mm I.D. for preliminary experiments (50 μA current) and 0.8 mm I.D. for trapping experiments (250 μA current). The detector signal was recorded by the use of a two-channel recorder (Model 42.00; H. Knauer, Berlin, G.F.R.).

Trapping experiments were performed using the PTFE three-way valve[5] shown in Fig. 1. Position 1 shows the operational mode for trapping the sample zone of interest in the valve capillary. The time of migration from the detector to the valve can either be calculated or measured by the aid of a colored marker. After the valve capillary is filled with the sample zone, the migration of the electrolytes is stopped and the valve switched to position 2. The valve capillary, containing the sample solution is then depleted using a syringe, and the solution is transferred into a crucible as used for MS. The valve capillary is switched into position 3, filled with leading electrolyte

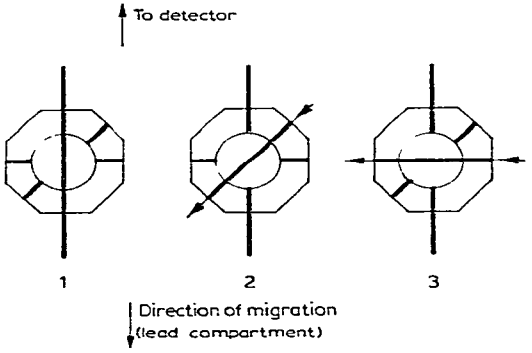

Fig. 1. Trapping device consisting of a three-way valve for collecting isotachophoretically separated sample components. 1 = Filling position (the zone of interest is migrating from the detector into the three-way valve). 2 = Transferring position (the sample solution is transferred into the MS crucible). 3 = Refilling position (the capillary is filled with leading electrolyte).

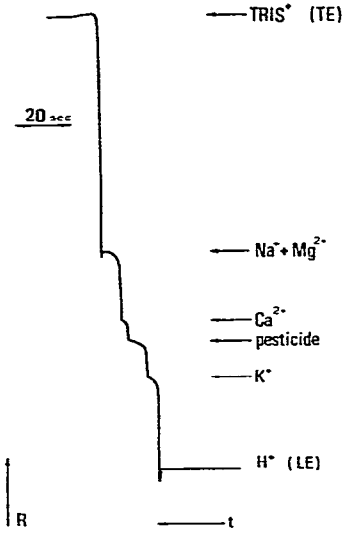

Fig. 2. Isotachopherogram for trapping experiments of a model mixture of cations typical for plant extracts with paraquat and diquat added. No additive was used with the LE. Current: 250 μA, capillary I.D. 0.8 mm, t = time, R = increasing resistance.

and ITP can be continued after switching back to position 1. The valve enables a complete fractionation of the separated constituents into a discrete number of fractions which can be evaluated by other techniques.

Mass spectra were obtained by the use of a double focusing instrument (Model 112; Varian MAT, Bremen, G.F.R.) with an EI ion source. Ion source conditions: pressure 10^{-6} Torr, temperature 200°C, electron energy 70 eV. The resolution of the mass spectra was 800 (10% valley).

RESULTS AND DISCUSSION

The main problem in the combination of ITP and MS, even when a transfer of the separated components can be carried out with the aid of trapping devices which are commercially available from Shimadzu (Kyoto, Japan)[6] and LKB (Bromma, Sweden)[7], is due to the fact that for ITP, ionic species are required, which seems to be highly undesirable for MS with EI ionization because of the low volatility of the compounds and the presence of the counter ions which interferes with the mass spectra. Nevertheless, conditions can be chosen in such a way that the substances under consideration may give interpretable mass spectra, *e.g.*, by using a low-molecular-weight counter ion which does not interfere in the *m/e* region of interest. For this reason, in order to separate paraquat and diquat from the cations typical of plant material extracts, chloride was chosen as a counter ion. The isotachopherogram of a standard mixture of these cations is shown in Fig. 2. Paraquat and diquat cannot be distinguished using an electric conductivity detector because of the identical ionic mobilities which cause mixed zones. It should be noted that the two pesticides are usually not used together in formulations. In order to identify the two substances, the sample zone was trapped and mass spectra of the sample were recorded. Because of the different MS patterns of the two pesticides as shown in Fig. 3, identification can be

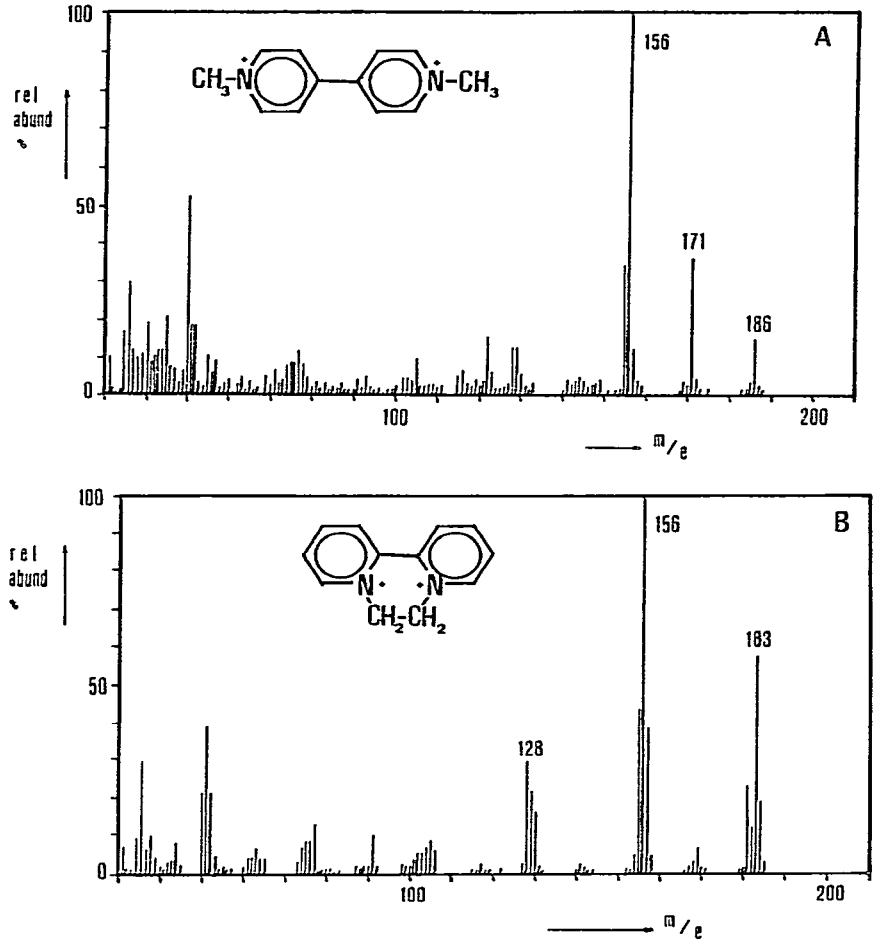

Fig. 3. EI mass spectra of paraquat (A) and diquat (B), after trapping of the ITP zone, where the pesticides exist as chlorides. Solid probe temperature: 300°C.

made even for the permanent ions under consideration with high degree of certainty, *e.g.*, with *m/e* 186 and 171 for paraquat and *m/e* 183 for diquat. In addition, as can be seen from the mass spectra, identification of the two components is possible in mixtures when the substances in the mixed ITP zone are trapped and MS is used.

REFERENCES

1 E. Kenndler and D. Kaniansky, in preparation.
2 J. F. K. Huber, E. Kenndler and P. Pilarski, in preparation.
3 F. M. Everaerts, J. L. Beckers and Th. P. E. M. Verheggen, *Isotachophoresis—Theory, Instrumentation and Applications*, Elsevier, Amsterdam, Oxford, New York, 1976.
4 S. Stankoviansky, D. Kaniansky and M. Koval, *Czech. Pat.*, No. 190, 933, (1978).
5 D. Kaniansky and M. Koval, *Czech. Pat.* applied for.
6 S. Kobayashi, Y. Shiogai and J. Akiyama, in F. M. Everaerts (Editor), *Analytical Isotachophoresis, Proc. 2nd Int. Symp. Isotachophoresis, Eindhoven, September 9–11, 1980*, Elsevier, Amsterdam, Oxford, New York, 1981, p. 47.
7 L. Arlinger, *J. Chromatogr.*, 119 (1976) 9–24.