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## Note

# Use of a simultaneous conductivity and amperometric detector with a manganin electrode in the microcolumn liquid chromatography of dicarboxylic acids

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The determination of aliphatic dicarboxylic acids by liquid chromatography is an important method for the analysis of biological samples. Owing to the complexity of the samples and the lack of a strong chromophore in the most common aliphatic dicarboxylic acids, the use of UV detection[1–5] suffers from interference problems and poor detection limits. Conductivity detection with post-column suppression[6–8] takes advantage of the ionic nature of acids. Selectivity for complexing acids can be achieved by amperometric[9] and potentiometric[10] detection on a copper working electrode. However, the use of the copper electrode has the disadvantage of a lower variability of pH and nature of the co-ion in the mobile phase, which decreases the possibility of optimizing acid separations. Further, the non-complexing dicarboxylic acids remain undetected. Simultaneous conductivity and amperometric detection can bring improvements, *e.g.*, the simultaneous detection of complexing and non-complexing inorganic anions was achieved by connecting two detectors in series[11].

Recently, the sensitive amperometric detection of amino acids on a manganin working electrode was reported[12]. On the basis of the described amperometric[9] and potentiometric[10] detection of dicarboxylic acids on a copper electrode, a manganin electrode was tried in this work for the amperometric detection of dicarboxylic acids. Further, the advantage of the use of a simultaneous conductivity and amperometric detector with a single electrochemical cell[13] was applied to the simultaneous detection of both complexing and non-complexing organic acids. A complexing cation, $Zn^{2+}$, was added to the mobile phase to control the retention and separation of acids. This approach is similar to the regulation of the retention of amino acids by adjusting the content of $Cu^{2+}$ ion in the mobile phase[14]. In order to keep the number of system peaks small, the number of ionic components in the mobile phase was minimized. The previously developed instrumentation[12,13] allowed us to carry out experiments using microcolumn liquid chromatography.

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## EXPERIMENTAL

### *Apparatus*

The microcolumn reversed-phase ion-pair chromatography of dicarboxylic acids was performed on a laboratory-built chromatograph. The mobile phase was pumped by an HPP 5001 syringe pump (Laboratory Instruments, Prague, Czechoslovakia) modified for microcolumn liquid chromatography. A 1-$\mu$l volume of an acid solution was introduced on to the microcolumn by a laboratory-made four-port injection valve described previously[15]. A CGC glass microcolumn (150 × 1 mm I.D.) (Tessek, Prague, Czechoslovakia) was packed with 7.5-$\mu$m Silasorb SPH $C_{18}$ (Lachema, Brno, Czechoslovakia) by the viscosity packing technique[16]. A simultaneous conductivity and amperometric detector[13] was equipped with a manganin working electrode[12]. The detector had a flow cell of volume 20 nl and cell constant 1.5 $cm^{-1}$. The d.c. polarizing voltage of the working electrode was +0.3 V. The chromatograms were recorded with an A-25 two pen recorder (Varian, Walnut Creek, CA, U.S.A.).

### *Chemicals*

The mobile phase was prepared from 10 m*M* stock solutions of its components. They were prepared by dissolution of tetrabutylammonium ($TBA^+$) hydroxide (Aldrich, Milwaukee, WI, U.S.A.) phthalic ($P^{2-}$) acid (Lachema), sodium hydroxide (Lachema) and zinc hydrogen phthalate. The solution of zinc hydrogen phthalate was obtained by dissolution of zinc oxide (Lachema) and 2 equiv. of phthalic acid in a small volume of warm water and by subsequent dilution to a 10 m*M* concentration of $Zn^{2+}$.

Stock solutions of $TBA^+$ hydroxide, phthalic acid, zinc phthalate and sodium hydroxide were mixed and diluted with distilled water to obtain the required mobile phase composition. Its final pH was adjusted with sodium hydroxide solution using an OP-208/1 pH meter (Radelkis, Budapest, Hungary). The conductivity of the mobile phase was measured with an OK 102/1 batch conductimeter (Radelkis). Oxalic (Ox), malonic (Mo), malic (Mi), maleic (Me), fumaric (Fu), tartaric (Ta), succinic (Su), glutaric (Gl), adipic (Ad) and citric acids (Ci) were supplied by Lachema.

## RESULTS AND DISCUSSION

The dependence of the capacity factors, $k$, of the acids studied on the content of $Zn^{2+}$ ions in the mobile phase are summarized in Fig. 1. In agreement with previous results[9], a mobile phase containing only $TBA^+$ phthalate (*i.e.*, without $Zn^{2+}$ ions) (see Fig. 1) cannot separate dicarboxylic acids effectively. Neither the substitution of phthalate ions by other co-ions, *e.g.*, benzoate or sulphate, nor the variation of the $TBA^+$ ion concentration in the mobile phase improved the separation of dicarboxylic acids. Addition of $Zn^{2+}$ ion to the mobile phase led to a decrease in solute retention, depending on its ability to complex with the metal cation. For instance, the retentions of oxalate and citrate anions are the most susceptible to variations in $Zn^{2+}$ concentration (see Fig. 1). The decrease in retention is due to the formation of uncharged complexes of acids with $Zn^{2+}$ ion, which decreases the interaction of acids with $TBA^+$ ion adsorbed on the hydrophobic stationary phase.

An example of the separation of some dicarboxylic acids is shown in Fig. 2. In

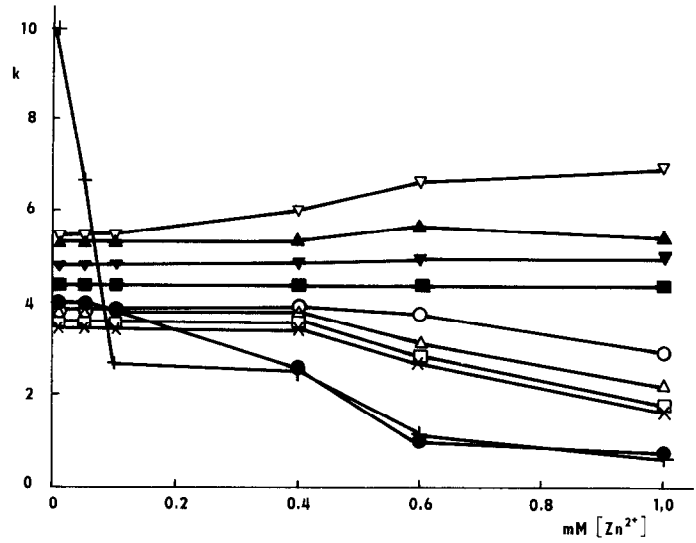


Fig. 1. Dependence of acid retention on $Zn^{2+}$ concentration. Mobile phase, 1 m*M* $TBA^{+}$–2m*M* $P^{2-}$ with the indicated concentration of $Zn^{2+}$: pH = 6.0, adjusted with sodium hydroxide. Flow-rate, 40 $\mu$l/min. Column: glass CGC (150 × 1 mm I.D.), packed with Separon SPH $C_{18}$ (7.5 $\mu$m). Solutes: ● = oxalate; × = malate; □ = malonate; △ = tartrate; + = citrate; ○ = succinate; ■ = glutarate; ▼ = fumarate; ▽ = adipate; ▲ = maleate.

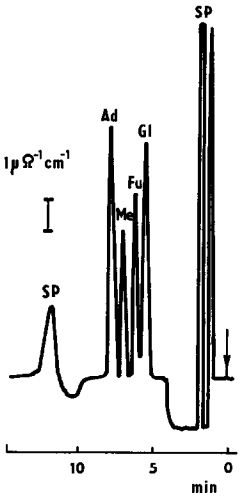


Fig. 2. Chromatogram of a mixture of glutarate (Gl), fumarate (Fu), maleate (Me) and adipate (Ad), each 3 nmol. SP = system peaks. Mobile phase: 1 m*M* $TBA^{+}$–2 m*M* $P^{2-}$–0.6 m*M* $Zn^{2+}$–1.8 m*M* $Na^{+}$; pH = 6.1. Conductivity, 460 $\mu ohm^{-1}$ $cm^{-1}$. Column as in Fig. 1. Detection: conductivity output of the detector described under Experimental.

spite of their low complexing ability, their separation can be improved by the presence of $Zn^{2+}$ ion in the mobile phase. On the other hand, the low complexing ability suggests the use of conductivity detection.

Further optimization of acid separations can be achieved by the simultaneous reduction of the concentration of all the mobile phase components. Similarly to the retention of inorganic anions eluted by tetrabutylammonium phthalate mobile phase[17], the retentions of organic anions increase with increasing dilution of the mobile phase. A lower ionic concentration in the mobile phase is advantageous also for the improvement of conductivity detection.

The application of a mobile phase with decreased concentrations of ionic components to the simultaneous conductivity and amperometric detection of dicarboxylic acids is shown in Fig. 3. The chromatograms indicate that complexing acids, *e.g.*, tartaric and malic acid, can be detected amperometrically by the proposed device with sufficient sensitivity. In spite of giving a high amperometric response, citrate and oxalate are not shown in this example, as they are co-eluted with system peaks close to the dead volume. Tartrate and malate are almost undetected conductrimetrically (see Fig. 3), which indicates that they are present in the mobile phase mainly in the uncharged form of the $Zn^{2+}$ complex. On the other hand, Fig. 3 demonstrates that weakly complexing anions, *e.g.*, adipate, glutarate and fumarate, are better detected conductimetrically than amperometrically.

The detection limits can be derived with the help of the peak-to-peak noise

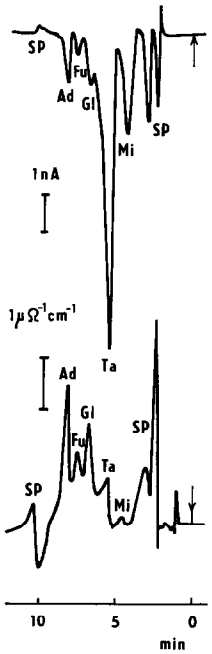


Fig. 3. Chromatogram of dicarboxylic acids detected with simultaneous conductivity (lower trace) and amperometric (upper trace) detector. Sample: malic (Mi), tartaric (Ta), glutaric (Gl), fumaric (Fu) and adipic (Ad) acids, each 1 nmol. Mobile phase: 0.9 m*M* $TBA^+$–0.45 m*M* $Zn^{2+}$–0.9 m*M* $P^{2-}$; pH = 6.6. Flow-rate, 40 μl/min. Column as in Fig. 1.

measured under the chromatographic conditions for both detection modes. Based on the amount of the acid injected and the experimental conditions, the calculated amperometric response to tartrate is 0.2 mA l mol$^{-1}$. Together with the noise of 40 pA this gives a detection limit of 0.4 $\mu M$ in the detector or 1 ng or 1 mg/l in the sample for a peak height of twice the peak-to-peak noise. For comparison, detection limits of 5 ng for oxalate and 30 ng for malonate were found[9] for amperometric detection on the copper electrode and a 100 × 4.6 mm I.D. separation column. Simultaneously, a conductimetric noise equivalent to 0.03 $\mu$ohm$^{-1}$ cm$^{-1}$ was found with a mobile phase of conductivity 460 $\mu$ohm$^{-1}$ cm$^{-1}$. The conductimetric noise then corresponds to 1/15 000 of the mobile phase conductivity. Based on the parameters of the adipate peak, its detection limit in the sample can be calculated to be about 2 mg/l.

## CONCLUSION

The simultaneous conductivity and amperometric detector with a single electrochemical micro-flow cell can work efficiently with a manganin working electrode. Such a device detects sensitively both complexing and non-complexing dicarboxylic acids separated by reversed-phase ion-pair microcolumn liquid chromatography. The control of solute retention by the content of $Zn^{2+}$ cation in the mobile phase is compatible with both detection modes. The retention and selectivity control together with simultaneous conductivity and amperometric detection can help to identify sample components.

## REFERENCES

1 V. T. Turkelson and M. Richards, *Anal. Chem.*, 50 (1978) 1420.
2 E. Rajakylä, *J. Chromatogr.*, 218 (1981) 695.
3 P. Vrátný, O. Mikeš, P. Štrop, J. Čoupek, L. Rexová-Benková and D. Chadimová, *J. Chromatogr.*, 257 (1983) 23.
4 J. F. Keefer and S. M. Schuster, *J. Chromatogr.*, 383 (1986) 297.
5 D. Tusseau and C. Benoit, *J. Chromatogr.*, 395 (1987) 323.
6 R. D. Rocklin, R. W. Slingsby and C. A. Pohl, *J. Liq. Chromatogr.*, 9 (1986) 757.
7 R. D. Rocklin, C. A. Pohl and J. A. Schibler, *J. Chromatogr.*, 411 (1987) 107.
8 A. A. Ivanov, O. A. Shpigun and Yu. A. Zolotov, *Zh. Anal. Khim.*, 42 (1987) 694.
9 W. Th. Kok, G. Groenendijk, U. A. Th. Brinkman and R. W. Frei, *J. Chromatogr.*, 315 (1984) 271.
10 P. R. Haddad, P. W. Alexander and M. Trojanowicz, *J. Chromatogr.*, 315 (1984) 261.
11 R. D. Rocklin and E. L. Johnson, *Anal. Chem.*, 55 (1982) 4.
12 K. Šlais, *J. Chromatogr.*, 442 (1988) 111.
13 K. Šlais, *J. Chromatogr.*, 436 (1988) 413.
14 S. Levin and E. Grushka, *J. Chromatogr.*, 384 (1987) 249.
15 K. Šlais and D. Kouřilová, *J. Chromatogr.*, 258 (1983) 57.
16 D. Kouřilová, K. Šlais and M. Krejčí, *Collect. Czech. Chem. Commun.*, 49 (1984) 764.
17 M. Cooke, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 515.