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Potentiometric detection using a metallic copper electrode in reversed-phase and ion-exclusion chromatography with eluents containing ion-interaction reagents

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Abstract

The response characteristics of a metallic copper wire electrode used as a sensor electrode in a potentiometric detector after ion-interaction separations are determined. Volatile carboxylic acids have been selected as model compounds to test the sensor electrode. It is found that concentrations of an ion-interaction reagent (tetraethylammonium perchlorate) as small as 0.01 mM in an unbuffered mobile phase are optimal for the potentiometric detection and separation in two chromatographic systems, namely reversed-phase and ion-exclusion. The ion-interaction reagent gives improved solute peak shape and results in retention times becoming independent of solute concentration, without any adverse effect on detection sensitivity. An increase in the mobile phase concentration of the reagent results in increased solute retention in the ion-exclusion chromatography mode and variation of this concentration is a new parameter that can be used to control solute retention. Typical detection limits are 250, 510 and 360 pmol for acetic, propionic and butyric acids, respectively.

1. Introduction

Potentiometry has been used frequently for selective detection in flow-injection analysis through the use of membrane electrodes. More recent investigations have shown that metallic wire electrodes can also be utilised successfully as the indicator electrode for potentiometric detection in ion-exchange [1,2], ion-exclusion [3] and reversed-phase [4] chromatography. The application of metallic electrodes has not been previously reported in ion-interaction chroma-

tography, for which other detectors are commonly used (spectrophotometric [5], indirect spectrophotometric [6], conductometric [7], amperometric [8] and inductively coupled plasma [9]).

Volatile carboxylic acids have been selected as model compounds for evaluation of the detection method reported in this paper. Although their separation in high-performance liquid chromatography (HPLC) can be achieved using many chromatographic techniques [10] (normal-phase, reversed-phase, ion-exchange and ion-exclusion chromatography), serious problems have been encountered with their detection in complex sample matrices. UV absorption at 210 nm is the

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most frequently used detection method but due to its sensitivity to even trace amounts of contaminants [11], it cannot be used for complex samples without suitable sample pretreatment because of interference problems.

In a previous paper [12], the electrokinetic detection of carboxylic acids in a reversed-phase system has been reported. When pure water was used as mobile phase, peaks with serious fronting were observed and retention times were dependent on solute concentration, which is disadvantageous from the analytical point of view. Buffering of the mobile phase improved the separation of the acids, but also increased the mobile phase conductivity and therefore the detection limit became poorer. Subsequent work [13] showed that addition of a small concentration of tetraalkylammonium ions without buffer improved the separation without deleterious effects on the sensitivity. We expect a similar effect to occur for potentiometric detection. In this paper we report a study of the use of a metallic copper electrode as a potentiometric detector in reversed-phase and ion-exclusion chromatography using mobile phases containing ion-interaction reagents (IIRs). To our knowledge, IIRs have not been used previously in ion-exclusion chromatography and it is a particular aim of this paper to test the applicability of this technique with potentiometric detection.

2. Experimental

2.1. Instrumentation

The chromatographic measurements were performed using a Millipore–Waters (Milford, MA, USA) Model 510 chromatographic pump, a Model U6K universal injection valve and a Model 484 tunable absorbance detector. Chromatograms were recorded using a Goerz-Metrawatt (Vienna, Austria) SE-120 chart recorder. The columns used were a Brownlee Labs. (Santa Clara, CA, USA) C_{18} Spheri-5 cartridge column (100×4.6 mm I.D.), a Waters Nova-Pak C_{18} column (100×4.6 mm I.D.), and a Bio-Rad

(Richmond, CA, USA) Aminex HPX-87 organic acid analysis column (300×7.8 mm I.D.).

2.2. Potentiometric detection

The electrochemical cell used for potentiometric detection in this study has been described previously [14]. The electrodes were copper and silver wires, both 0.5 mm in diameter. The silver electrode was coated with silver chloride as described elsewhere [15]. The wire electrodes were placed in a Perspex cell of dimensions $4 \times 3 \times 1$ cm with a 1-mm flow-through channel and were mounted with Omnifit connectors (supplied by Activon, Sydney, Australia) and rubber septa. The voltage difference between the two electrodes was measured continuously after each chromatographic injection using a millivolt meter supplied by Activon Australian (Activon pH/mV meter, type 101).

2.3. Reagents

Triply distilled water was passed through a Millipore (Bedford, MA, USA) Milli-Q water purification apparatus. Mobile phases were filtered through a $0.45\text{-}\mu\text{m}$ membrane filter and were degassed in an ultrasonic bath prior to use. Tetraethylammonium perchlorate (TEAP) was used as the ion-interaction reagent and was obtained from G. Frederick Smith Chemical Co. (Columbus, OH, USA). The following acids: formic, acetic, propionic, butyric, valeric, caproic, oxalic and succinic (analytical grade, Sigma, St. Louis, MO, USA), were used without further purification.

2.4. Procedure

Dilute solutions of TEAP in a water–methanol mixed solvent were used as mobile phases. The experiments were performed with flow-rates of 0.4 or 0.6 ml/min. The column was stabilised by passage of mobile phase for 30 min before chromatographic measurements were recorded and its temperature was maintained at 25°C . Stock solutions of the solutes were prepared as 10 mM solutions in Milli-Q water and diluted to

the required concentration before use. Aliquots (generally 10 μl) were injected into the chromatographic system with a 100- μl syringe (Hamilton, Reno, NV, USA).

3. Results and discussion

3.1. Reversed-phase chromatographic separation

From the equation derived in our previous paper [3], it is known that optimum potentiometric detection limits can be expected under conditions of low buffer concentrations in the mobile phase, in mobile phases containing components characterised by low copper complexing strength, and for solutes existing in their dissociated forms. These conditions are quite similar to those suitable for use with the electrokinetic detector [12,13]. As already mentioned, it can be expected that a small concentration of an IIR in an unbuffered mobile phase should improve chromatographic separation and also provide conditions suitable for potentiometric detection at a copper electrode.

TEAP is a suitable IIR because it does not form complexes with copper ions. Chromatograms of some volatile carboxylic acids, separated by reversed-phase chromatography using mobile phases without and with IIR, are presented in Fig. 1. The acids were eluted in the order: acetic, propionic, butyric, valeric and caproic acid. Peaks showing severe fronting and retention times dependent on the solute concentration were obtained in the chromatogram recorded with a methanol–water (20:80, v/v) mobile phase (Fig. 1a). Both of these effects result from decreased retention due to increased dissociation of the solute acids at low concentration and were eliminated after the addition of the IIR to the mobile phase (Fig. 1b). It is worth emphasising that concentrations as small as 0.01 mM made the retention time independent of solute concentration and resulted in symmetrical peaks, without any adverse effect on the detector performance. Other IIRs, such as tetrapropyl- and tetrabutylammonium perchlorate produced similar effects.

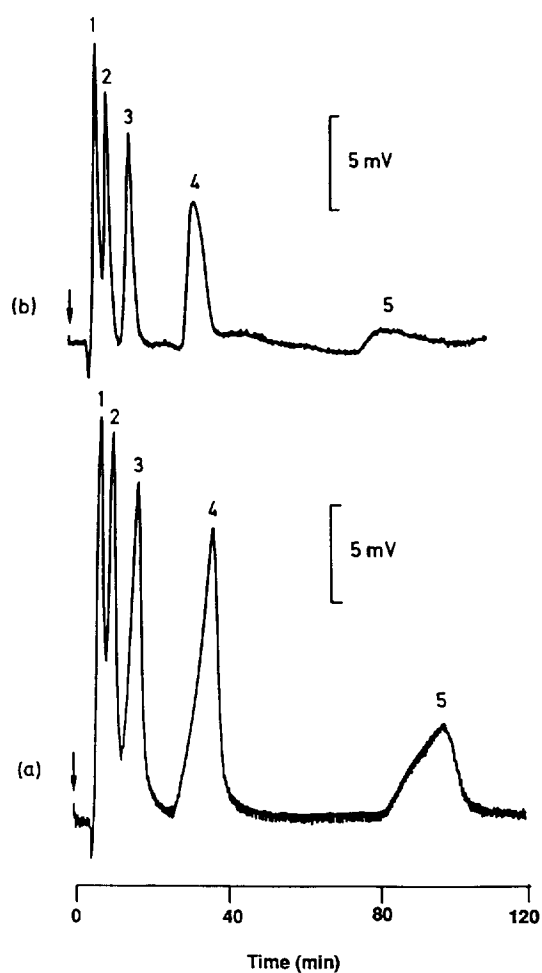


Fig. 1. Reversed-phase chromatograms obtained using (a) water–methanol (80:20, v/v), and (b) 0.01 mM TEAP in water–methanol (80:20, v/v) as mobile phase. Conditions: Brownlee Spheri-5 column, flow-rate 0.4 ml/min, copper wire sensor electrode, silver–silver chloride reference electrode. Peaks: 1 = acetic; 2 = propionic; 3 = butyric; 4 = valeric; 5 = caproic acids. Samples were injected at 1 mM concentration using an injection volume of 10 μl .

3.2. Ion-exclusion chromatographic separation

Ion-exclusion chromatography involves the use of a strong anion- or cation-exchange resin for the separation of ionic solutes from weakly ionised or neutral solutes. In this mode of chromatography, ionic solutes tend to be repelled (or excluded) from the resin, whilst neu-

tral solutes are able to penetrate the resin and are hence retained. Besides the charge on the solute, several other factors are known to play a part in the retention process. These include the size and especially the hydrophobicity of the solute. The mechanism by which acidic solutes may be separated by ion-exclusion chromatography using a cation-exchange resin has been described previously [16]. We now introduce another parameter that exerts an influence on retention in this form of chromatography, namely, the concentration of IIR in the eluent. This technique resembles the use of inclusion compounds [17] and that of micellar ion-exclusion chromatography [18].

The ion-exclusion system studied here consists of water used as mobile phase in conjunction with a sulfonated cation-exchange resin and mono- and dicarboxylic acids were used as solutes. Chromatograms of solute acids (oxalic, succinic, formic, acetic, propionic and butyric) were recorded using mobile phases both with and without IIR. As was observed for reversed-phase chromatography, severely fronted peaks having retention times dependent on solute concentration were obtained when water was used as mobile phase. Chromatograms of the same acids obtained in the 0.01 mM TEAP mobile phase are shown in Fig. 2, from which it can be seen that even a small IIR concentration in an unbuffered mobile phase yielded better shaped peaks. The solute retention times were independent of solute concentration, and were much lower than observed for the reversed-phase separations. The addition of IIR to the mobile phase resulted in increased solute retention times (Fig. 3), which can be explained by the decrease of resin charge caused by the interaction of the cationic TEAP ions with the anionic resin functional groups, enabling the negatively charged solute species to penetrate the resin network.

The characteristic s-shape dependence in ion-exclusion chromatography of the solute retention time on the pK_{a1} was observed for 0.01 M TEAP in the mobile phase for the short-chain organic acids employed in this study. This confirms that the IIR does not significantly alter the ion-exclusion mechanism of the solute retention process

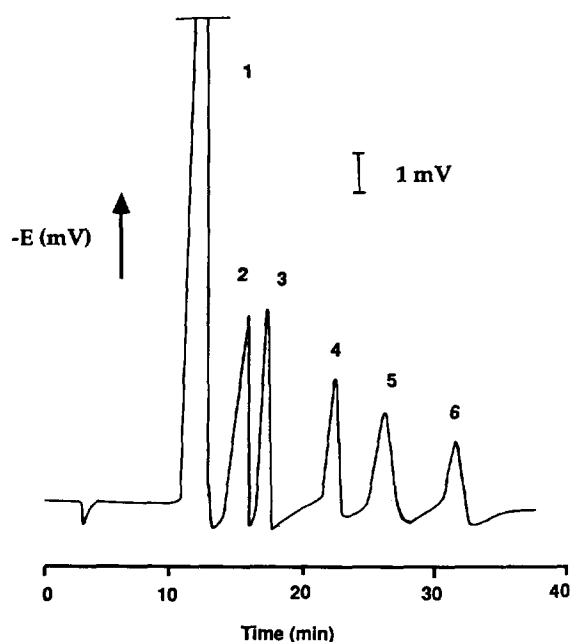


Fig. 2. Ion-exclusion chromatogram obtained using 0.01 mM TEAP as mobile phase. Conditions: Aminex Ion Exclusion HPX-87H column, flow-rate 0.6 ml/min, electrodes as for Fig. 1. Peak: 1 = oxalic; 2 = succinic; 3 = formic; 4 = acetic; 5 = propionic; 6 = butyric acids. Samples were injected at 1 mM concentration using an injection volume of 10 μ l.

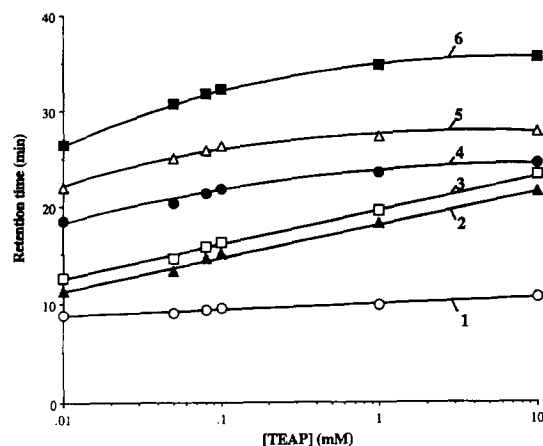


Fig. 3. Influence of the concentration of TEAP in the mobile phase on sample retention in ion-exclusion chromatography. 1 = Oxalic; 2 = succinic; 3 = formic; 4 = acetic; 5 = propionic; 6 = butyric acids. Conditions as for Fig. 2.

although it clearly affects the solute retention. The retention of solute acids having long aliphatic chains is known to be governed predominantly by hydrophobic adsorption onto the neutral areas of the stationary phase resin [19]. Therefore, the presence of an IIR ion having opposite charge to the solute may lead to increased retention of these solutes provided that the IIR becomes adsorbed to the underivatized parts of the resin and thereby provides cationic sites for solute interaction. This adsorption of IIR is unlikely to occur at the very low concentrations of IIR used in this study.

3.3. Detector characteristics

Table 1 summarises the effect of methanol and TEAP on the reversed-phase chromatographic performance of the detector. It was found that the presence of IIR in the mobile phase affects not only the solute retention but also the detector performance. Previous studies [1,3] have indicated that the copper wire electrode responds at varying levels of sensitivity to a wide range of anions. In this study, Fig. 1 and Table 1 show that addition of TEAP to the mobile phase not only changed the retention time for each solute, but also gave lower baseline noise levels as a result of the increased conductivity of the mobile phase. However, Table 1 shows that higher baseline noise was observed when using methanol in the mobile phase. In the case of ion-exclusion chromatography, methanol is not

Table 2

Effect of TEAP and methanol on detection limits (DLs) for carboxylic acids in reversed-phase chromatography

Mobile phase	TEAP (0 mM), methanol 20%	TEAP (0.01 mM), methanol 20%
Sample	DL (pmol)	DL (pmol)
Acetic	480	250
Propionic	500	310
Butyric	580	360

required, and further improvements in noise levels are achieved as shown in Fig. 2.

3.4. Detection limits and calibrations

Detection limits determined in reversed-phase chromatography are shown in Table 2. It is clear that improvements are achieved by use of TEAP when combined with methanol in the mobile phase. The ion-exclusion detection limits are approximately the same as those for reversed-phase with TEAP and 20% methanol, despite the fact that the peaks are much lower in ion exclusion. This is due to the lower baseline noise level in the latter case, as mentioned above. Calibration plots observed for the ion-interaction reversed-phase method are presented in Fig. 4 and those for ion-exclusion chromatography are given in Fig. 5, from which it can be seen that the response follows the anticipated non-linear

Table 1

Influence of the concentrations of TEAP and methanol in the mobile phase on chromatographic parameters for reversed-phase chromatography

MeOH concentration (%)	0	0	20	20				
TEAP concentration (mM)	0	0.1	0	0.1				
Noise level (mV)	0.4	0.15	0.5	0.2				
Sample	<i>h</i> (mV)	<i>t_R</i> (min)	<i>h</i> (mV)	<i>t_R</i> (min)	<i>h</i> (mV)	<i>t_R</i> (min)	<i>h</i> (mV)	<i>t_R</i> (min)
Acetic	12	12.5	3.2	7.8	23	6.3	6	4.3
Propionic	11	20.0	2.0	12.5	20	10.0	5	5.0
Butyric	10	37.5	1.6	26.3	18	16.3	3	7.5

h = Peak height (mV); *t_R* = retention time (min); injection volume 10 μl, sample concentration 1 mM, flow-rate 0.4 ml/min.

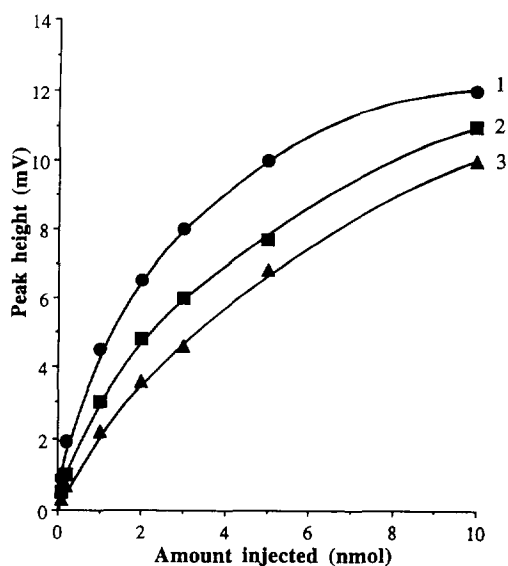


Fig. 4. Calibration curves obtained using reversed-phase chromatography. Conditions as in Fig. 1. 1 = Acetic; 2 = propionic; 3 = butyric acids.

behaviour [3]. The sensitivity is clearly higher in the reversed-phase case, but with the disadvantage of much longer retention times. The higher sensitivity in ion-exclusion to oxalic acid in

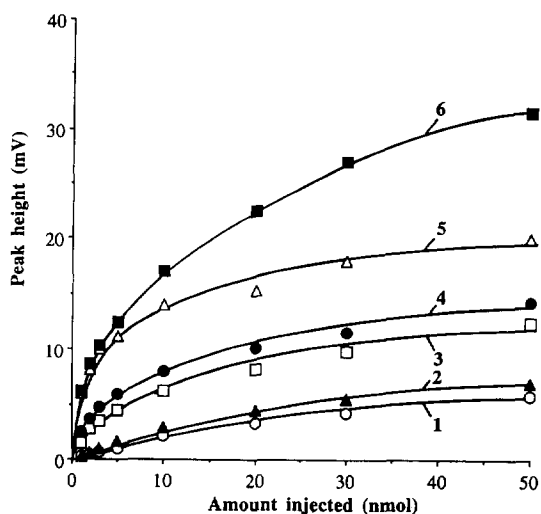


Fig. 5. Calibration curves obtained using ion-exclusion chromatography. Conditions as in Fig. 2. 1 = Butyric; 2 = propionic; 3 = acetic; 4 = formic; 5 = succinic; 6 = oxalic acids.

comparison with the other acids, as shown in Fig. 5, is due to the much higher stability constants for the formation of copper oxalato complexes at the electrode surface.

4. Conclusions

This study has shown that potentiometric detection can be suitable for ion-interaction chromatography (either in reversed-phase or ion-exclusion modes) using an unbuffered mobile phase. Detection limits as small as 0.25 nmol (for acetic acid) can be obtained, comparing favourably with UV detection. The use of an IIR provides an additional parameter for control of the solute retention (especially in ion-exclusion chromatography) and is also valuable for the improvement of separation efficiency. It is clear that the ion-exclusion method gives faster separations for the carboxylic acids of concern here, and therefore offers a major advantage over the reversed-phase separation method.

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