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A POTENTIOMETRIC MEMBRANE CELL AS A DETECTOR IN LIQUID CHROMATOGRAPHY

R. S. DEELDER*, H. A. J. LINSSEN, J. G. KOEN and A. J. B. BEEREN
DSM Research, Geleen (The Netherlands)

SUMMARY

The design and operating characteristics of a micro-type membrane cell as a differential detector for ionized compounds are described. The detector consists of two compartments separated by an ion-exchange membrane. The column effluent is passed through one compartment of the detector, and a reference electrolyte solution of the same composition as the eluent through the other. Changes in the composition of the solution in the measuring compartment bring about changes in the membrane potential. The performance of the detector was investigated under conditions prevailing in "classical" as well as dynamic solvent-generated ion-exchange chromatography. Experimental data on basic characteristics such as sensitivity, linearity, detection limits and dynamic behaviour are presented. The device has a detection limit in the nanomole range, provided that sufficiently diluted buffers are used as eluents. The linear working range is about 2–3 decades.

INTRODUCTION

The development of suitable column-packing materials for reversed-phase liquid chromatography marks an important breakthrough. Nowadays, reversed-phase chromatography is the method of choice for separating mixtures of polar and ionized substances.

Modern high-performance liquid chromatographic techniques, however, have not been widely used for the separation and quantitative determination of common inorganic anions in mixtures. Probably, a major reason for this is the lack of suitable detectors.

Small *et al.*[1] developed a technique called ion chromatography, which combines separation by ion-exchange chromatography and detection by conductance monitoring; the background conductance of the eluent is eliminated by the use of a so-called suppressor column. Although the detection limits are extremely low, the over-all separation efficiency leaves much to be desired. Recently, Gjerde *et al.*[2,3] showed that suppressor columns can be omitted if low-conductivity buffer solutions are used as eluents. Again, the column performance seemed rather poor. Reeve[4] described the use of reversed-phase ion-pair chromatography for separating inorganic anions. Since low-wavelength UV detection was used in this study, only a limited number of ions was detectable.

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The use of an ion-exchange membrane cell as a non-selective detector for ionic compounds was proposed as early as 1962 (refs. 5–7), but this idea has received only limited attention. More recently, the application of ion-selective membrane electrodes as detectors for liquid chromatography was described[8,9]. In the present study, we investigated the operation and characteristics of a miniaturized membrane cell equipped with a non-selective ion-exchange membrane as a detector in ion-exchange as well as in reversed-phase ion-pair chromatography.

THEORY

Two electrolyte solutions differing in composition, which are separated by an ion-exchange membrane, differ in their electrical potentials. The so-called membrane potential, E_m, can be measured in a membrane cell of the type:

reference electrode	solution 1	membrane	solution 2	reference electrode

When appropriate electrodes are used, the e.m.f. of this cell is equal to the membrane potential. Several theoretical approaches have been devised for calculating membrane potentials[10,11]. The most simple situation occurs when two solutions contain the same electrolyte in different concentrations. For an ideally perm-selective cation-exchange membrane with no water transference, the membrane potential is related to the monovalent-cation activities $(a_+)_1$ and $(a_+)_2$ through the Nernst equation:

$$E_m = \frac{RT}{F} \ln \frac{(a_+)_1}{(a_+)_2} \tag{1}$$

where R, T and F are the gas constant, the temperature and the Faraday constant, respectively. When the membrane cell is used as a detector in ion-exchange chromatography, solution 1 corresponds to a reference solution and solution 2 to the column effluent. Suppose that both solutions contain equal concentrations of an electrolyte CX, where C^+ and X^- represent a monovalent cation and anion, respectively. Normally $(a_{C^-})_1 = (a_{C^-})_2$. When a monovalent sample cation, S^+, is eluted from the column, solution 2 will contain the cations C^+ and S^+. Now, the e.m.f. of the cell is given by the equation[10]:

$$E_m = \frac{RT}{F} \ln \frac{\bar{D}_{C^+}(a_{C^+})_1/\bar{f}_{C^+}}{\bar{D}_{C^+}(a_{C^+})_2/\bar{f}_{C^+} + \bar{D}_{S^+}(a_{S^+})_2/\bar{f}_{S^+}} \tag{2}$$

where $\bar{D}_{C^+}$ and $\bar{D}_{S^+}$ are diffusion coefficients and $\bar{f}_{C^+}$ and $\bar{f}_{S^+}$ are molar activity coefficients in the membrane for the ions C^+ and S^+, respectively. Eqns. 1 and 2 are valid only for the limiting case of complete membrane diffusion, so that concentration gradients in the solution can be neglected. Further, it is assumed that $\bar{D}_{C^+}/\bar{D}_{S^+}$ and $\bar{f}_{C^+}/\bar{f}_{S^+}$ are constant throughout the membrane. Further simplifications were proposed by Spencer and Lindstrom[5]. They replaced solution activities, a, by concentrations, and with $[C^+]_1 = [C^+]_2 + [S^+]_2$ and $[C^+]_1 \approx [C^+]_2 \gg [S^+]_2$ they found

$$E_m = \frac{RT}{F}\left(1 - \frac{\bar{D}_{S^+}\bar{f}_{C^+}}{\bar{D}_{C^+}\bar{f}_{S^+}}\right) \frac{[S^+]_2}{[C^+]_1} \tag{3}$$

which gives the e.m.f. of the cell as a linear function of the sample-ion concentration $[S^+]_2$. It should be noted that eqn. 3 is slightly different from the relation proposed by Spencer and Lindstrom[5], who used intramembrane mobilities instead of diffusivities and activity coefficients[10,12]. It is evident that the same theoretical approach holds for the cell response to anions; in this instance, the solutions in the cell are separated by an anion-exchange membrane.

A condition of the use of membrane cells as detectors in flow systems is that their response faithfully reflects, at all times, the concentration of the ions of interest in the solution. Changes in the concentration of these ions bring about concentration gradients in the boundary layer between the flowing sample solution and the membrane surface, as well as in the ion-exchange membrane itself[10]. Transient potentials, *i.e.*, the potential responses following stepwise changes in the concentration of the ion of interest, have been the subject of many studies[13–18]. A relatively simple situation has been described theoretically by Conti and Eisenman[16], who concluded that, for a fixed-site ion-exchange membrane, the membrane potential is time-independent once the membrane–solution interface has reached equilibrium, although the concentration and potential profiles in the interior of the membrane are still changing with time. This conclusion is valid provided that the values of the diffusion coefficient, $\bar{D}$, and the activity coefficient, $\bar{f}$, are concentration-independent. If this is so, the transient phenomena are due only to the limited rate of mass transfer from the bulk solution to the membrane surface and vice versa. This particular problem has been investigated for ion-selective membranes, and equations for the potential–time response have been reported[13,14,18]. These equations predict an exponential behaviour of potential *vs.* time, *i.e.*,

$$\frac{E_t}{E_\infty} = 1 - e^{-t/\tau} \tag{4}$$

where E_t is the cell e.m.f. at time t, E_∞ is the final e.m.f. of the cell and τ a time constant. The first-order detector response will modify the chromatographic peaks exponentially. The influence of time constants on peak shape in liquid chromatography was recently discussed by Kirkland *et al.*[19]. The conditions for which eqn. 4 has been derived do not differ from those prevailing in the present detector when only one electrolyte is involved (see eqn. 1).

EXPERIMENTAL

The detector cell is shown in Fig. 1, which is a side view. The cell is composed of two identical 4 × 4 × 2 cm Lucite blocks bolted together and holding between them the ion-exchange membrane. Two types of membrane were used, the Selemion CMV cation-exchange membrane and the AMV anion-exchange membrane (Mitsubishi). The column effluent and the reference solution flow through inlet channels of I.D. 0.4 mm to the measuring compartments, which are formed by PTFE spacers of thickness 200 μm; the length of the duct is 10 mm, and the width is 0.4 mm. From the measuring compartments, the fluid flows to vertical cylindrical holes drilled in each of the blocks. PTFE sleeves provide a tight fit of the KCl–agar salt bridges into these cavities. The salt bridges establish connection between the liquid in the detectors and the $Hg–Hg_2Cl_2$ reference electrodes (Electrofact R 111). The reference electrodes are

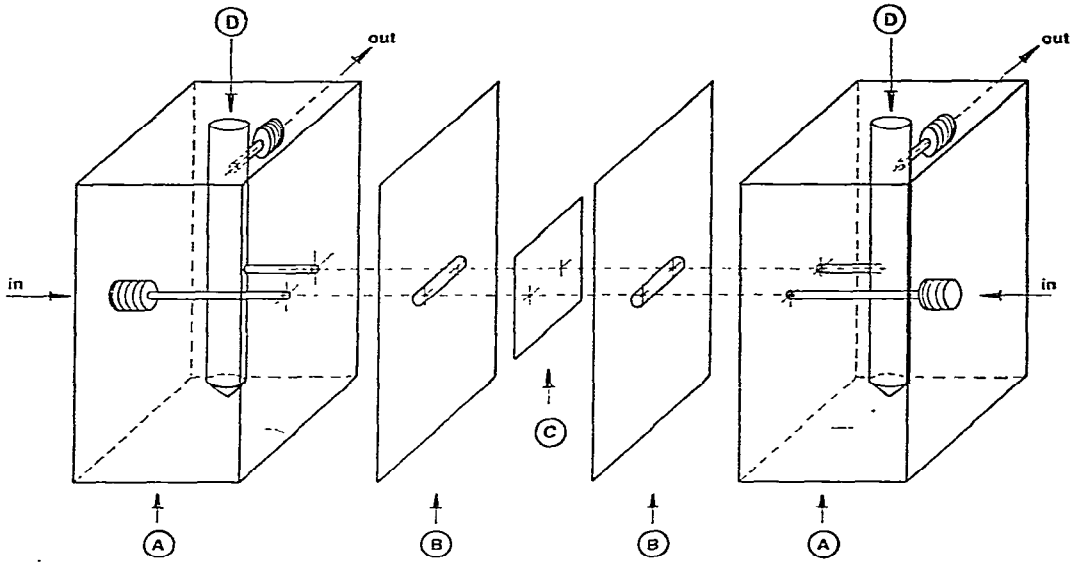

Fig. 1. Detector construction. A, Lucite blocks; B, PTFE spacers; C, ion-exchange membrane; D, cavities for salt bridges to reference electrodes. Screws for pressing the blocks together are not shown.

connected through an impedance transformer (Knick 72 W) to a microvoltmeter (Philips PM 2435). Both the cell assembly and the reference electrodes are thermostatically controlled at 25°C. The reference flow is maintained at 10 cm^3/h by means of a constant-flow metering pump (Kontron, Labotron LDP 13 A). The recorder used for registration of the chromatograms (Kipp BD 9) is equipped with a 0.5-sec RC-filter to eliminate high-frequency noise from the microvoltmeter.

Transient responses to stepwise changes in concentration were recorded in digitized form by means of a digital voltmeter (Hewlett-Packard 3437 A) and a cassette recorder (Tektronix 4924), which permitted further processing of the data on a desk-computer (Tektronix 4051).

Various liquid chromatographs were used. Ion-exchange chromatography with the corrosive chloride-containing eluents was carried out with laboratory-built equipment. A detailed description of this type of equipment has been given by Huber and Van Urk-Schoen[20]; the only modifications now made concern the pumping system.

In the present study a corrosion-resistant Hastalloy-C membrane pump (Orlita DMP 1515) was used. Pump pulses were damped out by means of a restriction and bourdon-spring manometers. These springs were filled with oil and protected from the corrosive eluent by a flexible PTFE sealing element. Injections were carried out with a PTFE–Kel-F injection valve (Chromatronix SVA-31 K), which was leak-proof at pressures up to 35 bar. Columns were of thick-walled glass tubing, 15 cm long and 4 mm I.D. Separations were carried out on the cation exchanger Aminex A-4 (particle size 10–15 μm) and the anion exchanger Aminex A-27 (particle size 8–12 μm) (Bio-Rad). Reversed-phase ion-pair chromatography was carried out on a Spectra-Physics Type 3500 liquid chromatograph. Columns were 15 cm long and

4.6 mm I.D. (316 ss), and LiChrosorb RP-18 (mean particle size 10 μm) (Merck) was used as column packing. A high-pressure sampling valve (Rheodyne 70-10) was used for sample injection. All columns were thermostatically controlled at 25°C by means of a circulating water bath. All chemicals used were of analytical grade.

RESULTS AND DISCUSSION

Fig. 2 shows the chromatogram of a mixture of lithium chloride and sodium chloride; the detector was equipped with the Selemion CMV cation-exchange membrane, and separation was effected by ion-exchange chromatography. A large negative peak is observed in the beginning of the chromatogram at the time corresponding with the column liquid hold-up. This peak is due to the counter-ion H^+ being displaced by the sample ions Li^+ and Na^+. The detector response for H^+ follows eqn. 1.

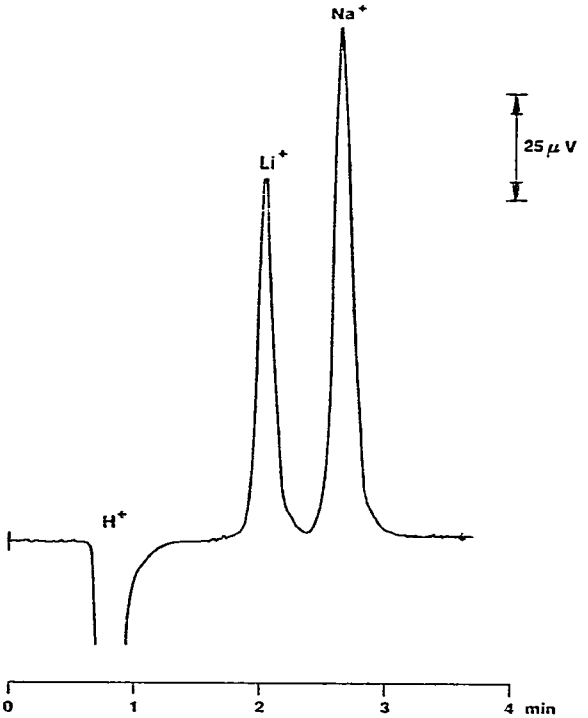

Fig. 2. Separation of LiCl (1 μmole) and NaCl (1.5 μmoles). Column, 15 cm × 4 mm (glass); packing material, Aminex A-4; eluent, 2 *M* HCl; flow-rate, 50 cm³/h; pressure drop, 25 bar; injection volume, 50 μl.

When the sample bands for Li^+ and Na^+ pass through the detector, the signal follows eqn. 3; it is positive, since $\bar{D}_{H^+}\bar{f}_{H^+}/(\bar{D}_{H^+}\bar{f}_{Li^+}) < 1$ (ref. 21). Fig. 3 gives an example of the use of the detector for measuring anions; here, the Selemion AMV membrane was used. Two different procedures were followed for testing the linearity of the membrane cell as a detector for anions.

First, solutions of potassium acetate (KAc) and KCl (subscript 2) and of KCl

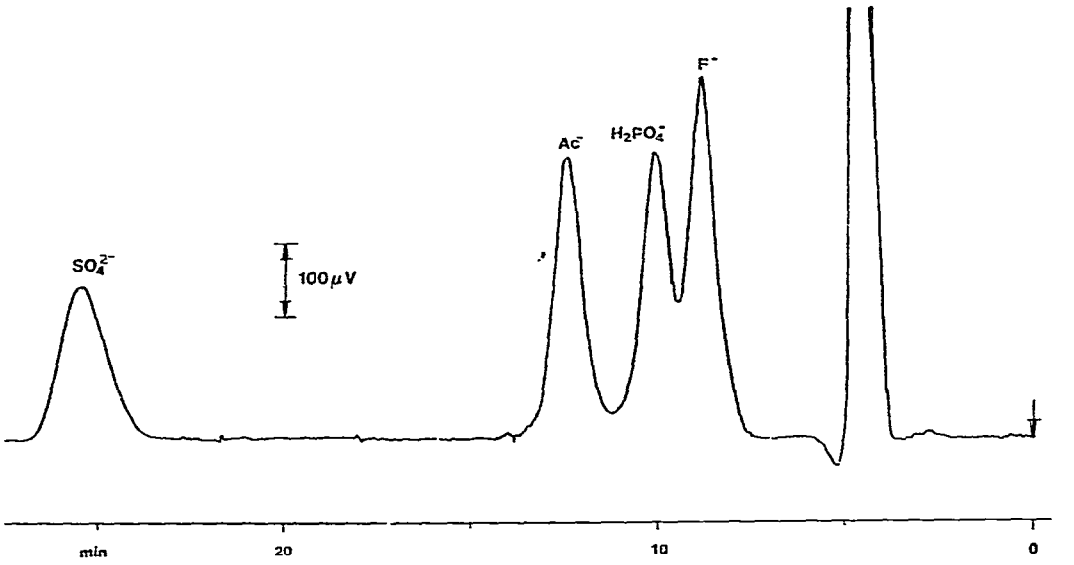

Fig. 3. Separation of F^- (1.1 μmoles), $H_2PO_4^-$ (0.7 μmole), Ac^- (1 μmole), and SO_4^{2-} (0.5 μmole). Column, 10 cm × 4 mm (glass); packing material, Aminex A-27; eluent, 0.25 *M* KCl + 0.005 *M* KH_2PO_4 (pH 4.5); flow-rate, 10 cm^3/h; pressure drop, 15 bar; injection volume, 50 μl.

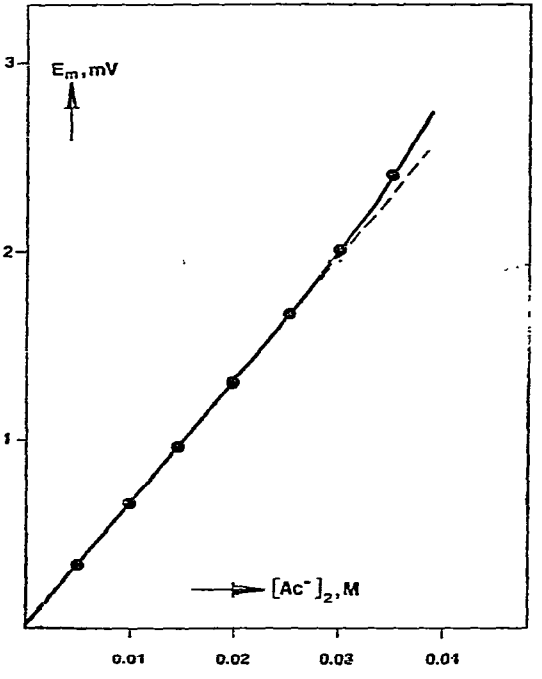

Fig. 4. Graph of cell e.m.f. (E_m) *vs.* concentration for potassium acetate (KAc) in KCl, with $[Ac^-] + [Cl^-] = 0.25$ *M*. Reference, 0.25 *M* KCl.

(subscript 1) with $[Ac^-]_2 + [Cl^-]_2 = [Cl^-]_1 = 0.25\ M$ were fed into the measuring and the reference compartment of the detector, respectively, and the cell e.m.f. was measured for a series of values of $[Ac^-]_2$. The results given in Fig. 4 indicate that the detector response was linear for $[Ac^-]_2$ up to *ca.* 0.025 *M*, *i.e.*, $[Ac^-]_2/[Cl^-]_1 = 0.1$. The detector signal was flow-independent, at least if changes in flow-rate did not bring about temperature changes of the fluid in the cell. Secondly, solutions with increasing concentrations of KAc were injected into the ion-exchange column (for conditions, see Fig. 3). It can be seen from Fig. 5 that the response of the detector was linear for amounts up to *ca.* 4 μmoles, which corresponds to a concentration of 0.025 *M* in the detector at the peak maximum. The standard deviation of the peak area, σ, increases with decreasing sample size, and the broken lines in Fig. 5 indicate the confidence region at $\pm 3\sigma$. An arbitrary detection limit may be defined as the amount of the component that can be detected at a given confidence level, *e.g.*, average peak area/σ = 3 (refs. 22 and 23). For Ac^-, 26 nmoles could be detected.

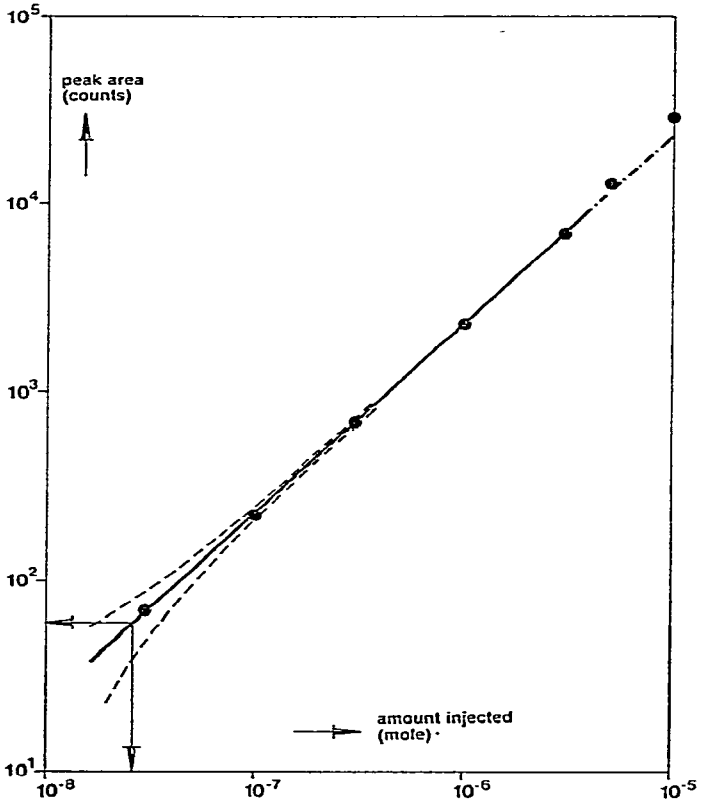

Fig. 5. Calibration curve for potassium acetate. Conditions as in Fig. 3.

Further, the influence of the counter-ion concentration, $[Cl^-]_1$, on the detector response was measured at a fixed concentration of the sample ion, $[Ac^-]_2$; the results are shown in Fig. 6 and are in accordance with eqn. 3. These findings suggest that detection limits may be considerably improved by decreasing the concentration of

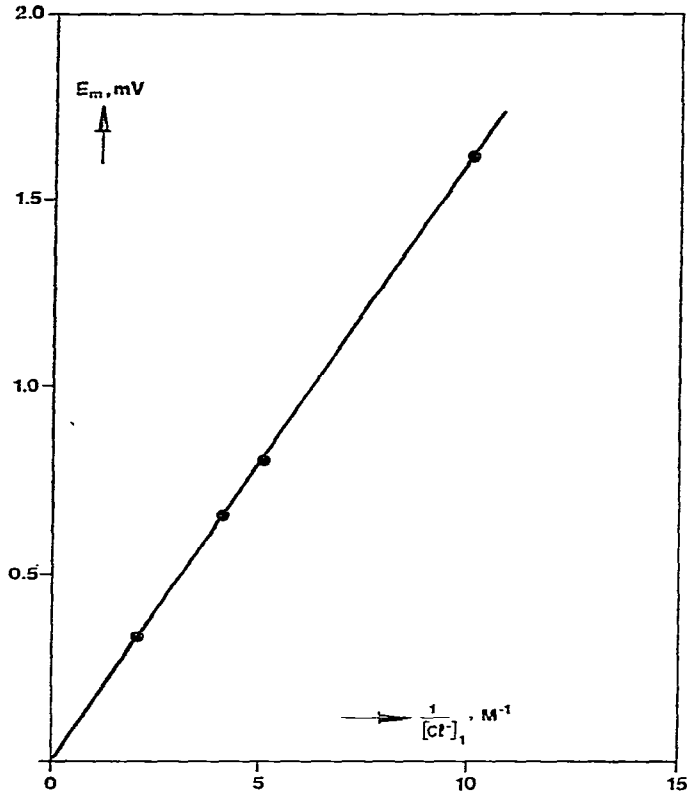

Fig. 6. Graph of cell e.m.f. (E_m) for 0.01 M acetate in KCl as a function of Cl^- concentration.

the counter-ion. However, as far as detection limits are concerned, there is little point in decreasing the counter-ion concentration, at least in "classical" ion-exchange chromatography. For example, in the separation of anions (Fig. 3) the capacity factor, k', of the sample ions is inversely proportional to the concentration of the counter-ion, $[Cl^-]_1$ (ref. 20). On the other hand, the dilution of the sample zones during the chromatographic separation is inversely proportional to $k' + 1$ (ref. 24). Therefore, at decreasing counter-ion concentration, the influence of increasing detector sensitivity will be almost completely offset by an increasing dilution of the sample zones. In reversed-phase ion-pair chromatography on chemically bonded alkyl phases, the retention of ionized compounds is strongly enhanced by addition of suitable amphiphilic substances, such as long-chain quaternary ammonium compounds or alkylsulphonates, to the aqueous eluents[25,26]. The amphiphilic ions are strongly adsorbed on the hydrophobic surface of the bonded phase, and the resulting surface layer shows ion-exchange properties[27–30]. In these phase systems, one can influence k' values both through the concentration of the amphiphilic ion in the eluent and through the counter-ion concentration; at low counter-ion concentrations, reasonable k' values are readily obtained. A practical illustration of the principle of reducing detection limits by using low concentrations of counter-ions is shown in Fig. 7, which illustrates the separation of quaternary ammonium ions in reversed-phase ion-pair

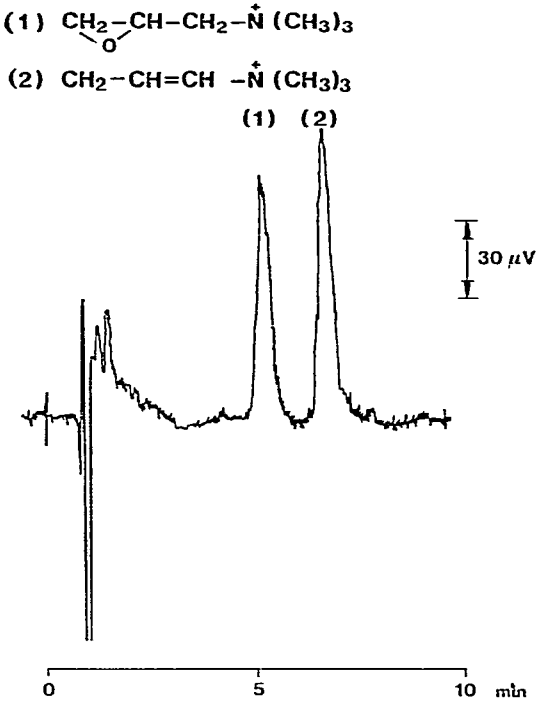

Fig. 7. Separation of N,N,N-trimethyl-3-hydroxy-1-prop-1-enaminium chloride (1) and N,N,N-trimethyloxiranylmethylaminium chloride (2). Column, 15 cm × 4.6 mm (316 ss); packing material, LiChrosorb RP-18 (10 μm); injection volume, 20 μl; eluent, phosphate buffer (0.05 *M* Na^+, pH 7.00) + 5% 2-propanol and 0.5 m*M* sodium 1-dodecanesulphonate.

systems. The last peak in the chromatogram corresponds to 81 nmoles, and the coefficient of variation for the peak area is 3.2%. The detection limit is *ca.* 7 nmoles.

The dynamic behaviour of the detector was investigated by making stepwise changes in the concentrations of the ions of interest in the solution flowing through the measuring compartment of the detector. For this purpose, a sampling valve equipped with a 2.5-ml sample loop was mounted immediately in front of the detector. On injection, the sample front brought about a nearly perfectly stepwise change in concentration, and axial dispersion could be neglected. Typical response curves are given in Fig. 8. An initial delay in the response curves is due to the connection tubing between the injection valve and the measuring compartment. Apart from the initial part, the response curves for Cl^- (a) and SO_4^{2-} (b) are correctly described by eqn. 4; τ values of 2.1 and 2.6 sec were found. The apparent thickness (d) of the stagnant boundary layer at the membrane surface was estimated from the relation $\tau = d^2/(2D)$, where D is the diffusion coefficient of the ions in the boundary layer[14]. For $\tau = 2.1$ sec and $D = 10^{-5}$ cm^2 sec^{-1}, we find $d = 60$ μm, which is a reasonable value compared with the depth of the flow-channel along the membrane. Further, this value is similar to the values for the diffusion layer at membranes in stirred vessels[31]. Low values for τ were also found for F^-, acetate and NO_2^-. However, for NO_3^- (c), the response is much slower and the response curve no longer agrees with eqn. 4. Apparently the kinetics

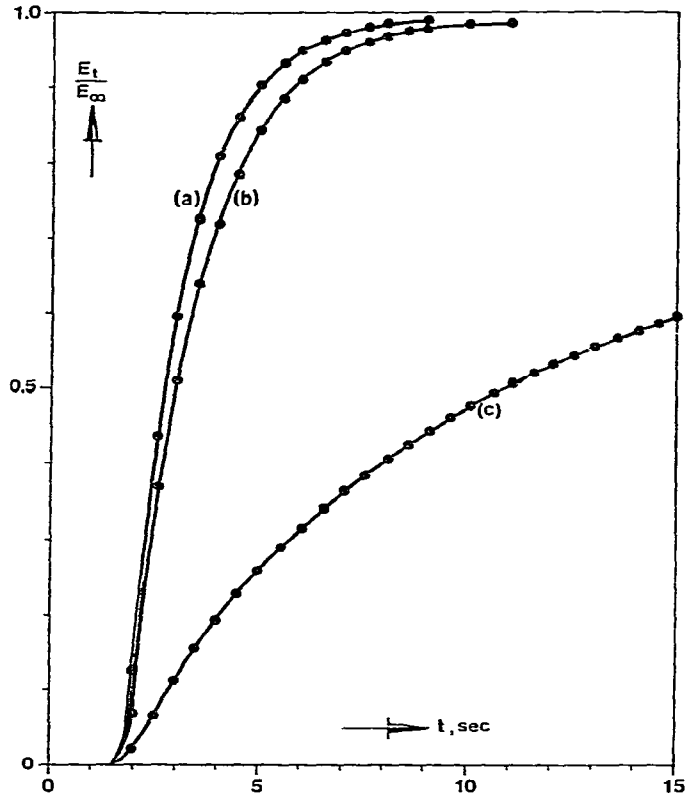

Fig. 8. Plot of response curves (see eqn. 4). (a) Initial concentration 0.02 *M* Cl^-, final concentration 0.022 *M* Cl^-; (b) initial concentration 0.02 *M* Cl^-, final concentration 0.018 *M* Cl^- + 0.001 *M* SO_4^{2-}; (c) initial concentration 0.02 *M* Cl^-, final concentration 0.018 *M* Cl^- + 0.002 *M* NO_3^-.

of the membrane electrode are complicated by other rate-determining processes, *e.g.*, slow ion-exchange processes in the membrane accompanied by changes of intra-membrane diffusivities and activity coefficients. An equally sluggish behaviour was observed for I^-. The influence of eluent-flow velocity on detector response time was investigated for Na^+ in HCl; the data are presented in Table I. In this instance, the speed of response is quite satisfactory[19,32,33].

It should be noted that, even at the upper limit of the linear working range, the detector signal is rather low, as the detector is linear only for sample-ion concen-

TABLE I

SPEED OF RESPONSE FOR Na^+ IN 1 *M* HYDROCHLORIC ACID

Flow-rate, cm³ min⁻¹	*Time constant (τ), sec*
0.15	5.8
0.33	3.1
0.60	2.3
1.20	1.3

trations $[S^+]_2$ that are considerably lower than the concentration of the counter-ion $[C^+]_1$ (see eqn. 3). Fig. 4 indicates that, for Ac^- in 0.25 *M* KCl, the upper limit corresponds to a cell e.m.f. of *ca.* 1.5 mV. The major instrumental problem is working at noise levels as low as possible. It can be seen from Fig. 7 that the detector noise is about 5 μV (peak-to-peak). This noise originates mainly in the electronics (operational amplifier and microvoltmeter). When both compartments of the detector are filled with identical electrolyte solutions, a residual voltage of at least 0.1–0.2 mV is observed; this results from differences in e.m.f. of the reference electrodes. This residual voltage is fairly constant, and the drift of the detector is *ca.* 50 μV/h.

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