Picoliter Cell Volume Potentiometric Detector for Open-Tubular Column LC*

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Abstract

A detector for open-tubular column liquid chromatography based on an ion-selective microelectrode cell assembly is described. The microelectrode with tip diameter of approximately 1 μm is directly inserted into the downstream column end. Estimated detector cell volumes are about 10^{-14} l (25-μm i.d. column). Experimental evidence is presented that proves the cell volume to be smaller than 5×10^{-10} l. The detection system should be adequate for open-tubular columns with an inner diameter ≥ 5 μm.

Introduction

There is growing interest in the use of columns of small inner diameter for liquid chromatography (LC) (1). In particular, theoretical and experimental aspects of open-tubular columns are under study by a number of groups (2-17). For a recent review see reference 2.

A severe limitation is imposed by the detector cell volume V_{det} which, according to Knox and Gilbert (3), should be:

$$2V_{det} \leq \sigma_v^0 = \frac{\pi d_c^2 L}{4\sqrt{N}} = \tfrac{1}{4}\sqrt{N}\pi d_c^3 h \qquad \text{Eq. 1}$$

where σ_v^0 = standard deviation of unretained solute peak, d_c = inner diameter of open tubular column, L = column length, and N = number of theoretical plates, if the early-eluting peaks are to be resolved.

Equation 1 leads to a V_{det} of 67 pl for an open-tubular column of 1-m length and 10-μm i.d. ($k' = 0$, $\nu = 13.8$, $h = 0.29$). Maximum detector cell volumes that contribute less than 1% loss of efficiency for columns of ≤ 30-μm i.d. would have to be smaller than 1 nl ($h = 1$, $N = 10^5$, capacity ratio $k' = 10$, diffusion coefficient $D = 10^{-9} m^2 s^{-1}$) (2). For a column of 30-μm i.d., Equation 1 leads to a V_{det} of 6.7 nl ($h = 1$, $N = 10^5$, $k' = 10$, $\nu = 7.2$, length of capillary:3m).

Some interesting approaches to low dead-volume detecto have been reported. These include UV detectors (18,19), lase fluorimetry (20), sheath flow fluorimetric detection (21), fre falling drop detectors (22), on-column optical detecto (12,13,23), and miniaturized electrochemical detectors (24). Ce volumes as small as 0.06 nl have been obtained (2). Althoug ion-selective electrodes (ISE) have been used for the detectio in LC (25-27), the dead-volumes are too large for use with sma bore open-tubular columns.

In this paper, a report is presented on the use of ion-selectiv microelectrodes as detectors of extremely small dead-volume i open-tubular column LC.

Theoretical

The response of an ISE cell assembly can be described by (26

$$E = E_o + \frac{s}{z_i} \cdot \log\left(a_i + \Sigma K_{ij}^{Pot} \cdot a_j^{z_i/z_j}\right) \qquad \text{Eq.}$$

where E = electromotive force of the cell assembly; z_i,a_i = th charge number of the primary I ion and its activity in mol per liter (*M*) in the sample solution; z_j,a_j = the charge numb of any interfering J ion and its activity in moles per liter (*M* in the sample solution; K_i^{Pot} = selectivity factor or a measure the preference by the sensor of the interfering J ion relative t the I ion to be detected (an ideally selective membrane electro would show all $K_{ij}^{Pot} = 0$); E_o = potential difference comprisin a constant (for a given temperature) plus the liquid junctio potential; and $s = 2.303RT/z_iF = 59.16mV/z_i$ (25°C). For a su ficiently large ionic background concentration, variations in t liquid junction potential induced by changes in the activity the primary I ion can be kept negligably small.

The measurement of the potential difference ΔE, which is due to the addition of I ion to a background of the J ions, allows a determination of the activity a_i of the I ion:

$$a_i = \Sigma K_{ij}^{Pot} \cdot a_j^{z_i/z_j} \left\{ 10^{\left(\frac{\Delta E \cdot z_i}{s}\right)} - 1 \right\} \qquad \text{Eq. 3}$$

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For the maximum activity $a_{i,max}$ in an elution profile (see Figure 1) a potential difference ΔE_{max} is obtained; the width of the signal is defined for $a_i = a_{i,max}/2$, where $\Delta E = \Delta E_{1/2}$:

$$\Delta E_{1/2} = \frac{s}{z_i} \cdot \log \left\{ \frac{10^{\left(\frac{\Delta E_{max} \cdot z_i}{s}\right)} + 1}{2} \right\} \qquad \text{Eq. 4}$$

Table I and Equation 4 show that large values of ΔE_{max} lead to potentiometric signals where the width of the elution profile $\Delta E_{1/2}$ (for $a_{i,max}/2$) must be read off at heights above 50% of the potentiometric signal ΔE_{max}. Therefore, elution profiles recorded in ΔE values often look much broader and show more overlap than the more conventional profiles presented linearly in the concentration of the eluted species.

Although ISEs respond to single ion activities, it is permissible to use the corresponding ion concentrations provided that constant ionic background and low activities of the primary ion are maintained and appropriate calibration measures are taken.

Experimental

The experimental set-up shown in Figure 2 was used with the following components: a pump (Altex model 110A); a pressure gauge (Wika Manometer 232.50.100, range 0-10 bar); injection loops (Rheodyne type 70-10, 10 μl and Valco UHP series, 0.2 μl, with two-position electric value actuator); a 25-μm i.d. vitreous silica capillary column of length 0.6 m and 0.3 m, untreated or treated with trichloro-octadecyl-silane (ODS) (Scientific Glass Engineering); and a valve (Whitey SS-22 RS4).

The ion-selective microelectrode detector had a tip diameter of ≅ 1 μm and was inserted directly into the end of the open-

Table I. Height $\Delta E_{1/2}$ in an Elution Profile Corresponding to One Half of the Maximum Activity of the Eluted Ion

	$\Delta E_{1/2}$ (mV)		$\frac{\Delta E_{1/2}}{\Delta E_{max}} \times 100$	
ΔE_{max} (mV)	monovalent ion	divalent ion	monovalent ion	divalent ion
0.1	0.05	0.05	50.04	50.10
0.2	0.10	0.10	50.10	50.20
0.5	0.25	0.25	50.24	50.49
1	0.50	0.51	50.49	50.98
2	1.02	1.04	50.98	51.95
5	2.62	2.74	52.44	54.85
10	5.48	5.95	54.85	59.52
20	11.90	13.56	59.52	67.80
50	35.64	41.38	71.29	82.75
100	82.75	91.12	82.75	91.12
200	182.25	191.12	91.12	95.56
500	482.24	491.12	96.45	98.22

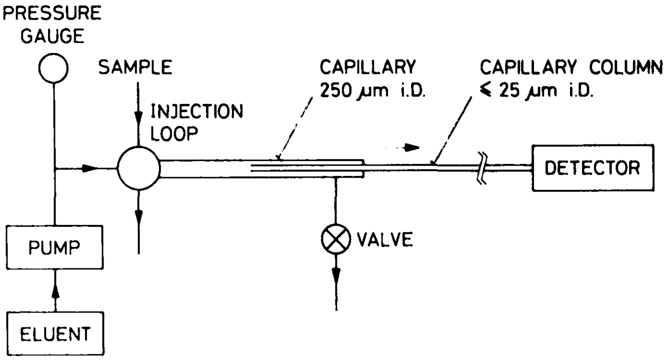

Figure 2. Schematic diagram of the open-tubular column liquid chromatograph

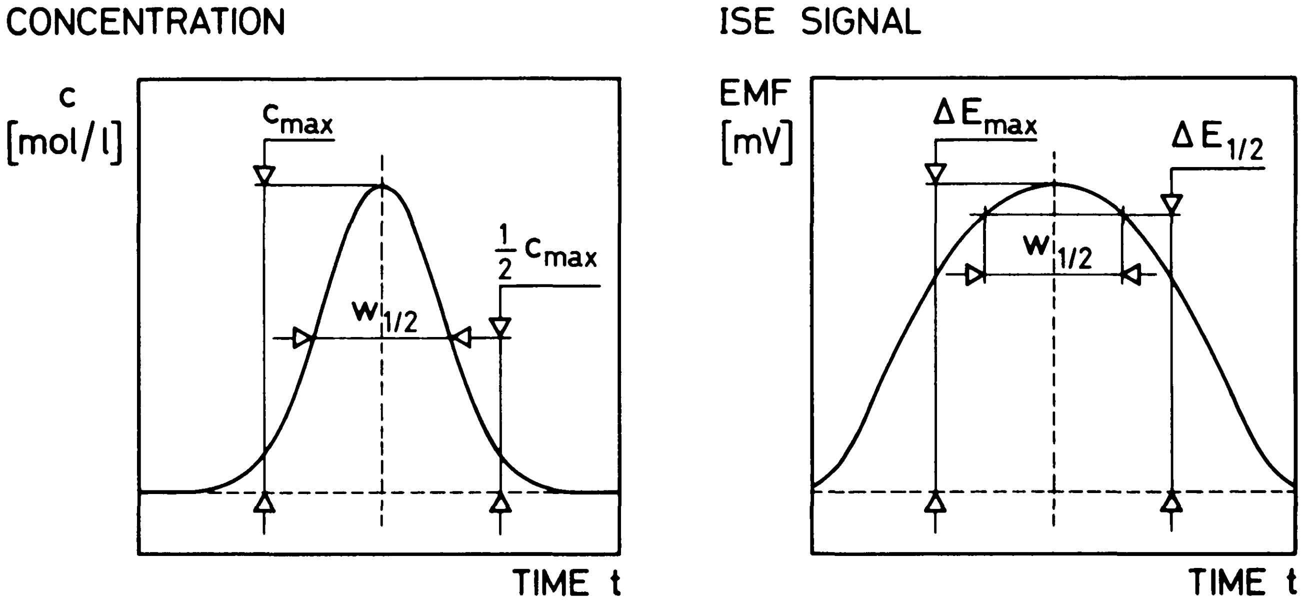

Figure 1. Elution profiles for a detector with linear (left) and logarithmic (right) response

tubular column (see Figure 3). Its membrane was a classical cation-exchanger solution (3% w/w potassium tetra-*p*-chlorophenylborate in 2,3-dimethylnitrobenzene) (29,30). The reference electrode was a chlorinated silver wire inserted into a micro pipet (filled with $3M$ KCl). The potential difference between ion-selective microelectrode and reference electrode was measured as described earlier (30) and recorded (Kontron, W + W Recorder Model 314). For further details see reference 31.

Because of the relatively low electrical resistance [$2 \times 10^9\ \Omega$ for a tip diameter of 1.5 μm (29)] of the microelectrode based on a classical cation-exchanger, the response time of the electrochemical cell assembly is less than 10 msec (32). Even with neutral carrier based systems (29,33,34) showing inherently higher membrane resistances, microelectrodes with response times of about 7 msec have been prepared (35).

Results and Discussion

The detection volume of the detector described here is determined by the surface area ($\cong 8 \times 10^{-11} m^2$) of the ion-selective electrode and the stagnant diffusion layer extending from the membrane into the sample solution. Assuming a thickness of this layer of approximately one column inner diameter (here 25 μm), an effective detector volume of 20×10^{-15} l results. An experimental verification of the influence of the volume of the detector on the elution profile was performed by measuring the reduced plate height *h* for an unretained component as a function of the reduced linear velocity ν of the mobile phase. Figure 4 indicates perfect agreement between experimental values (points) and those predicted by the Golay equation (curve). The calculations were based on a column diameter $d_c = 25$ μm, a diffusion coefficient $D = 10^{-9} m^2 s^{-1}$, and $k' = 0$. Equation 13 in reference 3 was used to calculate the curve plotted in Figure 4. The minimum in Figure 4 corresponds to a value of $h = 0.29$ (HETP = 7.2 μm, N = 83.000) for $\upsilon = 13.8$ (16.3 nl/min). Together with Equation 1, the detection volume is therefore estimated to be smaller than 500 pl. Unfortunately, the separation system used leads to a drastic increase in HETP with increasing capacity ratio (see Figure 5) so that it is unsuitable for analytically relevant separations of cations (see Figure 6).

To estimate the number of moles of the ions to be detected, the following equation was used:

$$\frac{n_i}{n_c} = \frac{V_s}{V_c} \qquad \text{Eq. 5}$$

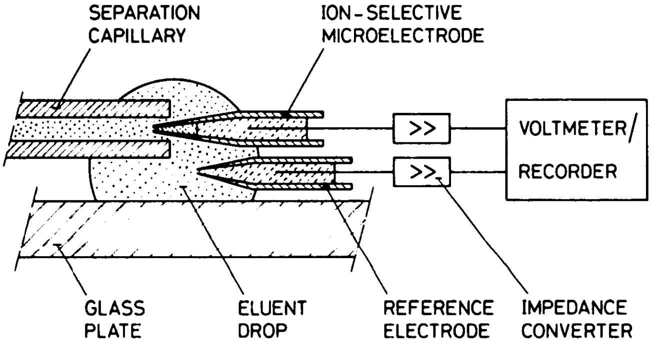

Figure 3. Schematic diagram of the ion-selective detection system

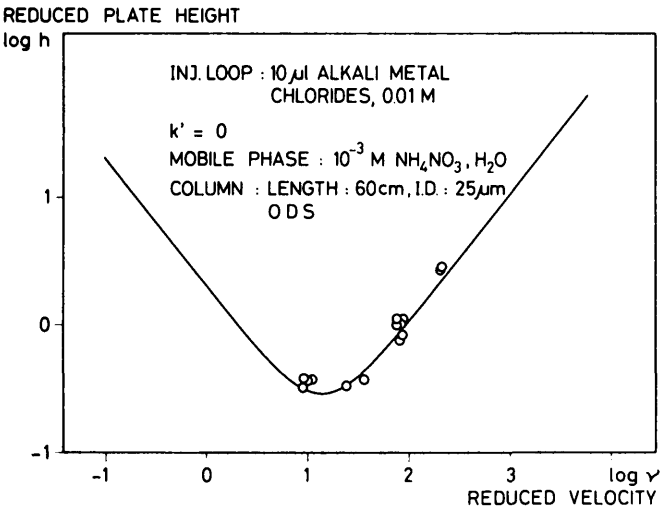

Figure 4. Reduced plate height as a function of the reduced velocity. Curve: Equation 13 in reference 3. Points: Experimental values

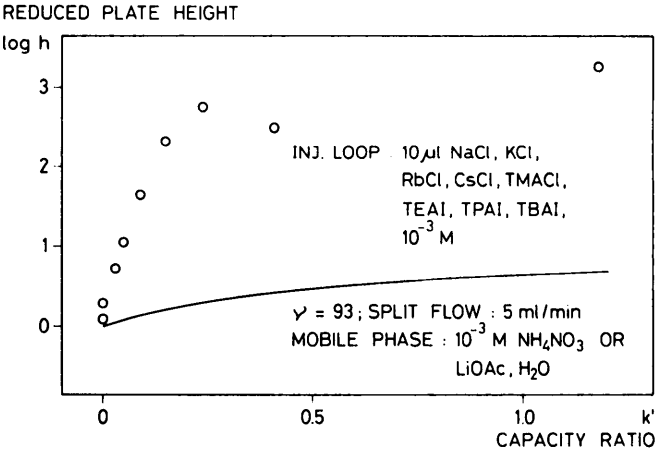

Figure 5. Reduced plate height as a function of the capacity ratio. Curve: Equation 13 in reference 3. Points: Experimental values. Column: 60 cm, 25-μm i.d., ODS.

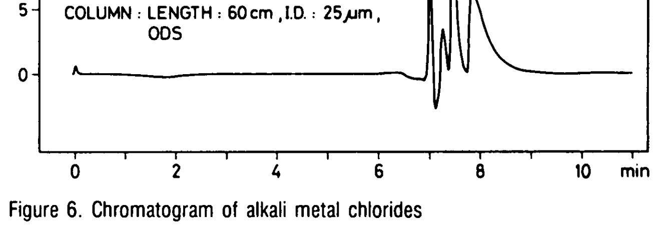

Figure 6. Chromatogram of alkali metal chlorides

where n_i = moles of ions in injection loop, n_c = moles of ions injected into the open tubular column, V_c = volume flow (ml/min) of mobile phase through open tubular column, and V_s = split flow (ml/min).

In Figure 7, these calculated values (curve) are compared with those obtained experimentally using Equation 3 (points). The rather large deviations are mainly a result of an independent and perhaps inappropriate determination of the selectivity factor ($K^{Pot}_{K^+,NH_4^+} \cong 1$), which had been obtained by the separate solution method (28) on $10^{-3}M$ solutions of the chlorides, and also a result from inadequate description of the system by Equations 3 and 5. Small deviations are also due to the substitution of concentrations for single ion activities.

An indication of the detection limit is presented in Figure 8 for the rather unfavorable case of the injection of K^+ into a background of NH_4^+. Since the electrode system does not heavily discriminate between these two ions, the detection limit must be rather high (see Equation 3). Even for this extreme case, ions in the pmol range can be detected. Selectivity factors log K^{Pot}_{KJ} (29) are ca. -2.8 [J: $(CH_3)_4N^+$], 1.3 (J: Cs^+), 0.6 (J: Rb^+), 0 (J: K^+), -1.8 (J: Na^+), and -2.5 (J: Li^+); thus considerably lower detection limits can be expected for certain other ions [e.g., $(CH_3)_4N^+$], even when using the same electrode system and the same ionic background. In extreme cases, detection limits of neutral carrier based liquid membrane electrodes are about $10^{-9}M$ (Ca^{2+} in $0.125M$ K^+) (33) or even $< 10^{-10}M$ (H_3O^+ in $1.0M$ K^+ or Na^+) (34).

The noise of the electrode system including the electronic equipment is < 0.5 mV, corresponding to an uncertainty in the concentration of <2% at high values of the signal measured.

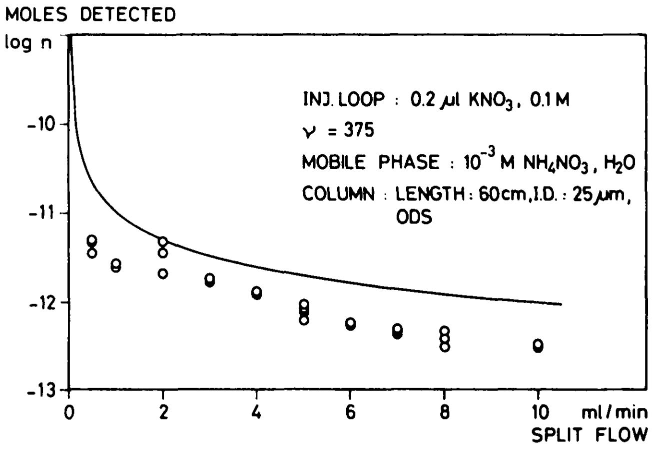

Figure 7. Number of moles detected as a function of the split flow. Curve: Equation 3. Points: Experimental values obtained through Equation 5.

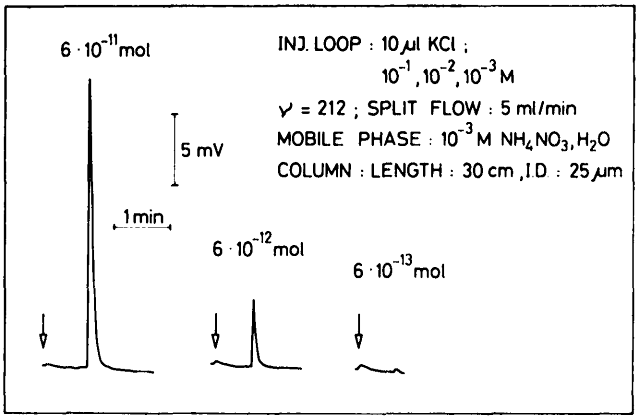

Figure 8. Detection limit of the ISE detector system

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