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ELECTROKINETIC DETECTION IN LIQUID CHROMATOGRAPHY

MEASUREMENT OF THE STREAMING CURRENT GENERATED ON ANALYTICAL AND CAPILLARY COLUMNS

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SUMMARY

The streaming current that originates due to transport of the diffusion part of the electrical double layer generated in an adsorption column packing has been measured. A technique for the direct measurement of the streaming current from stainless-steel columns and glass capillary columns with an electrometer is described.

INTRODUCTION

An exact representation of the concentration profile on the output from a chromatographic column is a major requirement in detection in chromatography. As a consequence, heavy demands are often made of the fundamental parameters of the detectors in high-performance liquid chromatography (HPLC), such as a small volume of the detection compartment, a suitable time constant of the transfer and recorder and small, appropriately designed couplings between the column and the detector. The column can be prepared in such a way that it also creates the detection element at the same time. This procedure allows high-efficiency packed columns to be used without the occurrence of extra-column distortion of the concentration profiles, and also allows capillary columns to be employed when the detection volume of conventional detectors decreases the separation efficiency substantially.

Direct pick-up of the signal from the chromatographic column is possible if the electrokinetic principle of detection[1,2] is used, which, in addition to the above advantages, also gives a sufficiently high sensitivity for the systems that were selected. It is interesting that the use of electrokinetic phenomena in chromatography with aqueous mobile phases had been suggested theoretically previously[3]. Only in the 1960s was attention drawn to electrokinetic phenomena in low-conductivity liquids[4,5], particularly in connection with the production of electric charge in crude oil and petroleum products, *e.g.*, in pipelines. A two-electrode detection system for liquid chromatography was patented about 10 years ago[6]. In an indirect context, even detectors based on the principle of droplets electrically charged by means of spraying[7] and detectors with a generator of uniform droplets[8] can be mentioned. From the viewpoint of the detection and simplicity of the instrumentation, and particularly

with regard to the possibility of picking up the signal directly from the column, the measurement of electrokinetic streaming would seem to be advantageous.

Proceeding from the theoretical principles[2] of the origin of the streaming current and from the technique for its measurement, the adsorption bed can be replaced with a packed or a capillary column. Particularly with the latter alternative, making the column and the detector identical is useful as it does not necessitate any additional modifications of the detector[9] for picking up the concentration profiles in very small volumes of the mobile phase.

CURRENT GENERATION DURING THE CHROMATOGRAPHIC PROCESS

As shown earlier[2], the magnitude of the charge formed by the liquid stream around the adsorbent is a function of both the nature of the liquid, which is characterized by the relaxation constant, τ, *i.e.*, by the ratio of permittivity to conductivity, τ/κ, and the phase equilibrium, the kinetics of which are described by the product of the mass transfer, h, and the specific surface area of the adsorbent per unit volume, s. The equilibrium state of the ionized component in the mobile and the stationary phases is denoted by the concentrations C_{-0} and C_{-s}, respectively. Introducing the expression for the mean value of the charge, q_{av}:

$$q_{av} = 2F(C_{-0} - C_{-s}) \tag{1}$$

where F is the Faraday constant, an expression is obtained for the instantaneous value of the electrical charge, q, with respect to the length of the streaming, y, at a linear velocity of the liquid, u:

$$q = \frac{s\,h}{1 + s\,h} \cdot q_{av} \left[1 - \exp - (1 + s\,h\,\tau) \cdot \frac{y}{u\,\tau} \right] \tag{2}$$

This expression is equivalent to eqn. 8 in an earlier paper[2]. The kinetics of the establishment of the phase equilibrium according to eqn. 1 can be considered to be identical with those of the establishment of phase equilibrium of the solute in the chromatographic process. The diffusion coefficient, D, of the effluent is assumed to be approximately identical with that of the ionic component of the effluent ($D_+ = D_-$). At the same time, it is necessary to take into consideration that the liquid does not pass the entire specific surface area of the adsorbent[10], so that the diffusion path will always be longer for the chromatographic process than the diffusion path of the ions the equilibrium state of which is disturbed by the streaming liquid. The kinetics of the phase equilibrium, expressed by eqn. 2 as $s\,h$, can therefore be characterized by the relaxation time, τ_c, of the chromatographic process from Einstein's equation[11-13]:

$$\tau_c = d^2/D \geqslant 1/(s\,h) \tag{3}$$

where D is the diffusion coefficient and d^2 is the mean square distance diffused. The diffusion path is usually expressed as a function of the mean particle size, d_p:

$$\tau_c = \omega\,d_p^2/D \tag{4a}$$

where ω is a constant, or, for adsorption liquid chromatography[14]:

$$\tau_c = d_p^n/D \tag{4b}$$

where n is a constant characterizing the chromatographic system. The relaxation time can be expressed by the following relationship for capillary columns[11] of radius r_c:

$$\tau_c = r_c^2/D \tag{4c}$$

As the height equivalent to a theoretical plate, H, in liquid chromatography is mostly directly proportional to the mass transfer coefficient, C, (prevalently in the mobile phase) and to the flow-rate, it holds that

$$H = C u = E(k) \tau_c u \tag{5}$$

where $E(k)$ is a value dependent on the capacity ratio, k. If the process that controls the mass transfer takes place in the mobile phase, the dependence of E on k can be neglected for $k \gg 1$ and eqn. 5 can be simplified to

$$H = E \tau_c u \tag{6}$$

where E is a constant.

By substituting $s\,h$ from eqn. 3 into eqn. 2, an expression is obtained for the charge created on the column as a function of the relaxation time of the mobile phase, τ, and the relaxation time of the chromatographic process, τ_c, where the former value is characteristic of the mobile phase while the latter value reflects the kinetics of the chromatographic process:

$$q = \frac{\tau}{\tau - \tau_c} \cdot q_{av} \left[1 - \exp - \left(\frac{1}{\tau} + \frac{1}{\tau_c} \right) \frac{y}{u} \right] \tag{7}$$

The values of τ_c, varying ca. from 10^{-2} to 10^{-3} sec, are characteristic of the present state of the art of HPLC. Low-conductivity mobile phases have relaxation times of ca. $1 - 10^{-1}$ sec. As $\tau \gg \tau_c$, eqn. 7 can thus be simplified to

$$q = q_{av} \left[1 - \exp - \frac{y}{\tau_c u} \right] \tag{8}$$

With the aid of eqn. 6, q can further be expressed as a function of H:

$$q = q_{av}[1 - \exp - Ey/H] \tag{9}$$

It is often stated[11-13] that $E = 2$; under these conditions, $q \approx q_{av}$ if $y > 2H$.

An equilibrium charge is thus established on the chromatographic column on a section, y, that is approximately equal to twice the height equivalent to a theoretical plate. The change of this charge with time (ref. 2), the streaming current, is measured at the instant when the solute leaves the chromatographic column. The dependence of the streaming current on time thus represents faithfully the concentration profile of the

solute in the column at all points from the injection point to a distance greater than two theoretical plates. A linear dependence of the response on the concentration is obviously also assumed, which must be verified experimentally for a given range of concentrations.

Analytical columns

The streaming current was measured directly from an electrically insulated analytical column, a schematic diagram of which is shown in Fig. 1. The column (1), of length 180 mm and I.D. 2 mm, was made of stainless steel and was insulated electrically from a metallic shielding jacket (2) with a PTFE insulation (3). It was packed with Silpearl spherical silica gel (Kavalier, Sklárny, Czechslovakia) of particle size 30–45 μm. The column was coupled electrically via a connector (4) to an electro-meter (5) with an input resistance of $10^{10} \Omega$. The mobile phase, consisting of n-butanol–n-hexane (15:85, v/v), entered the column via an injection block (6). The column input and output were sealed with PTFE (7) according to Huber[15]. The effluent was led from the column into a withdrawing tube (8), which was either earthed or connected with another electrometer (9). The liquid was led to waste via a PTFE tube (10).
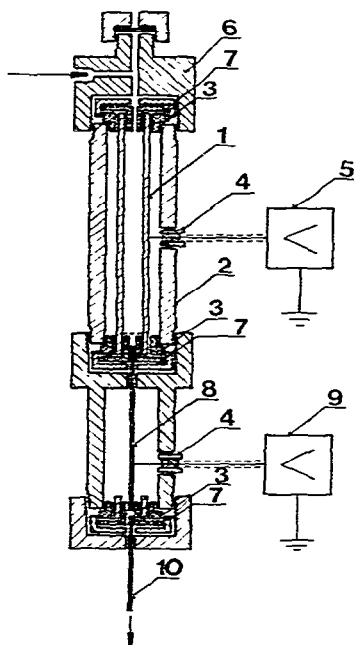

Fig. 1. Arrangement for measurement of streaming current generated on analytical column.

If an electrically neutral mobile phase enters the adsorbent in the chromato-graphic column, an equilibrium electric charge density is established along the whole column. If a constant flow velocity is maintained, a change in the electric charge density occurs only on the column outlet where the charged liquid flows into the

withdrawing tube. Consequently, a basic streaming current, corresponding to the composition and flow-rate (and hence to the mass) of the mobile phase is produced. The existing phase equilibrium is disturbed by the solute concentration pulse entering the column. The values of C_{-0} and C_{-s} in eqn. 1 are time dependent and therefore the streaming current, $dq/dt = I_{str}$, is measured in the form of an input pulse. However, over a distance equal to several heights equivalent to a theoretical plate according to eqn. 9, the equilibrium charge density is re-established on the column and the original basic streaming current is measured again. The stainless-steel column, which conducts perfectly, does not allow a change in the equilibrium electric charge density that reflects the peak travelling along the column. At the moment when the solute is leaving the column, the electric charge density on the column is changed with time and the response (streaming current of the solute) corresponding to the solute peak is recorded. A current of the opposite sense with respect to the response can be measured from the withdrawing tube of the column, because of similar electrical insulating and shielding to those of the column. The currents measured directly from the column and from the withdrawing tube have the same absolute value but are of opposite sense, assuming that the radius of the withdrawing tube is large enough[2] not to generate its own streaming current. The identity of the response measured on the column and on the withdrawing tube is obvious from Table I.

TABLE I

RATIOS OF PEAK HEIGHTS FROM CAPILLARY TO THOSE FROM COLUMN

Parameter	Solute						
	Ethanol	Methanol	Water	Nitro-benzene	o-Nitro-aniline	m-Nitro-aniline	p-Nitro-aniline
Capacity ratio (k)	1.0	2.0	12.1	0.28	1.21	2.9	5.0
Amount of solute (mg)	0.4	0.4	0.2	0.9	0.09	0.06	0.03
Ratio of peak heights at flow-rates:							
0.46 ml/min	0.95	1.02	1.10	0.97	0.95	0.99	0.95
0.22 ml/min	0.90	0.92	1.01	0.93	0.95	1.00	1.10
Peak areas in pA·sec at flow-rates:							
0.46 ml/min	103	203	281	133	83	160	210
0.22 ml/min	100	204	289	125	85	155	200

Capillary columns

The possibility of picking up the response directly from the column was utilized in the detection of the solute from capillary columns in liquid chromatography. Capillary columns were prepared by drawing soft alkaline glass tubes[16]. The tubes used for drawing capillaries were first purified with chromic–sulphuric acid and then packed loosely with spherical Silpearl adsorbent of bead size 25–35 μm. The adsorbent was thermally activated prior to being packed in the tubes. For column preparation we used modified procedures[17–19] that had proved satisfactory in gas chromatography. The adsorbent beads (melted into the surfaces of the capillary columns) were not washed out by the mobile phase at the given experimental condi-
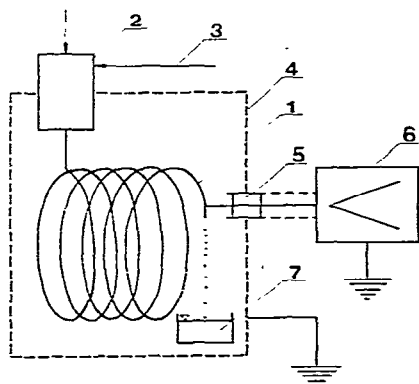
Fig. 2. Arrangement for measurement of streaming current generated on capillary column.

tions. The inner diameter of the columns varied from 0.10 to 0.20 mm and the diameter of the coil was 10 cm. Adaptation of the column ends was similar to that used in capillary gas chromatography.

The attachment of the capillary column to the chromatograph is illustrated schematically in Fig. 2. The glass capillary column (1) is connected to a mobile phase inlet (3) via a stainless-steel injection block (2). The column is placed in an earthed metallic cylinder (4), which serves as a shield. The capillary column is wound along the whole length of the coil with copper wire of diameter 0.1 mm, which is connected to an electrometer (6) via a connector (5). The capillary end is drawn into a jet in such a way that the leaving effluent will create a continuous jet stream and not individual droplets, which could change the electrical capacity of the column and could be recorded by the electrometer as noise. The effluent is collected in a beaker (7). In some instances the effluent was led by a PTFE capillary outside the space shielded by the cylinder, in which case the withdrawing capillary was sufficiently long and carefully earthed. As this work was not concerned with optimization of the capillary columns but rather the exploitation of the detection principle, neither a sampler with a splitter was used nor was an optimal design from the viewpoint of the column efficiency sought.

An example of a chromatogram is shown in Fig. 3. At the moment of injection of the sample into the column, a peak is recorded that corresponds to the change in electric charge density of the entering liquid on the one hand and to the instantaneous change in the mobile phase velocity on the other. n-Butanol (peak 1 in Fig. 3) is eluted in the dead volume of the column, $V_M = 40\ \mu l$, which corresponds to the dead time, $t_M = 7.5$ sec, at a given linear velocity of the mobile phase. The capacity ratio for phenacetin (peak 2) was $k = 14$, with a theoretical plate number of $n = 300$, which corresponds to 2.5 effective plates per second. The response was $2.0 \cdot 10^{-12}$ A for 1 μg of phenacetin at a noise level of $4 \cdot 10^{-14}$ A.

The chromatographic relaxation time according to eqn. 6 with constant $E = 2$ for phenacetin was $\tau_c = 12.5$ msec under the experimental conditions used. The relaxation constant of the mobile phase was measured as $\tau = 250$ msec. Eqn. 9 holds under these conditions and the changes in the streaming current measured directly from the capillary column follow the concentration profile of the solute at the column
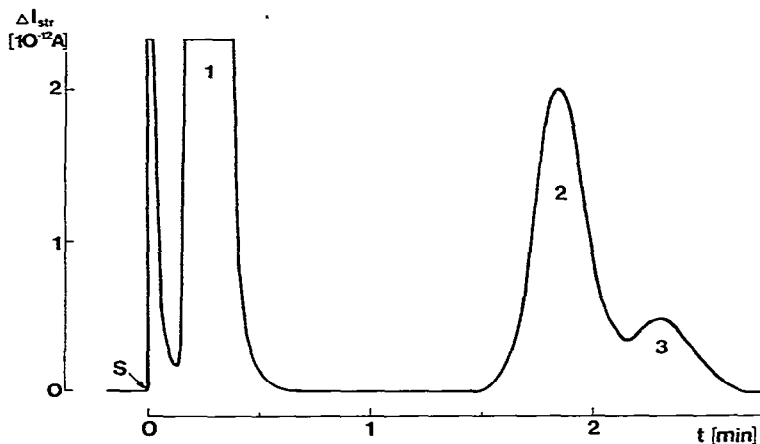
Fig. 3. Example of chromatogram obtained with capillary column (150 cm × 0.16 mm I.D.). Mobile phase, *n*-butanol–*n*-hexane (95:5, v/v); pressure drop, 7.5 MPa: flow-rate, 20 cm/sec. Peaks: 1 = *n*-butanol (solvent); 2 = phenacetin (1 μg in 1 μl of solvent); 3 = unknown.

outlet. The latter conclusion also holds for packed columns, where the chromatographic relaxation time[14] varies from several to tens of milliseconds.

CONCLUSION

Electrokinetic detection can be used by measuring the streaming current generated on an analytical or capillary chromatographic column. The changes in the streaming current reflect the concentration profile of the solutes with time constants of several to tens of milliseconds. A small time constant and the adequate sensitivity permit the detection of solutes in capillary columns with no problems with detection devices of sufficiently small volume.

In the systems described, the column creates an equipotential unit. With glass columns the streaming current can be measured even in individual column segments. The course of the chromatographic process can thus be investigated. This arrangement allows strongly adsorbed components to be investigated in the initial segments of the columns with greater sensitivity than at the end of the columns. A column with a number of detection segments that do not disturb the separation can be created in capillary liquid chromatography. The above systems are being further investigated.

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