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ELECTROKINETIC DETECTION AT DIFFERENT POINTS IN A NARROW-BORE GLASS COLUMN IN LIQUID CHROMATOGRAPHY

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SUMMARY

The measurement of the electrokinetic current at different points in a narrow-bore glass column (0.4–0.5 mm I.D.) packed with sorbent is described. The origin of a "derivative" signal and the dependence of its shape on the capacity ratio of the solute and the flow-rate of the mobile phase are explained and verified experimentally. The values of the height equivalent to a theoretical plate, were determined at two places in the column, and a value of $H_{\min} = 0.11$ mm (*i.e.*, 3.1 d_p) was calculated.

INTRODUCTION

The electrokinetic principle of detection in liquid chromatography (LC)[1,2] makes it possible to pick up a signal directly from the chromatographic column[3]. Extra-column contributions to peak broadening, originating in the detection cell and in the connecting tubes between the column and the detector, are thus eliminated; this is particularly important when working with narrow-bore and capillary columns. A technique has been described[3] for the direct measurement of the streaming current which results from the transport of the diffusion part of the electric double-layer generated in columns packed with sorbent and in capillary columns.

In the present work, the signal was monitored at different places in the glass column, which allows one to observe the profile of a chromatographic peak during its passage through the column. An advantage is that it is possible to detect solutes having high capacity ratios in the first sections of the column, and which might otherwise be difficult to detect at the column end.

Fig. 1 shows schematically the change in concentration of a solute at the peak maximum, $c_{\max}$, along a column of total length L, and which is expressed by

$$c^i_{\max} = \frac{Q_i}{A\,\varepsilon\,\sqrt{2\,\pi\,H_i}\cdot(1 + k_i)}\cdot\frac{1}{\sqrt{L}} \qquad (1)$$

where Q_i is the total amount of solute i introduced into the column, A is the column cross-section, ε is the total column porosity, H_i is the height equivalent to a theoretical

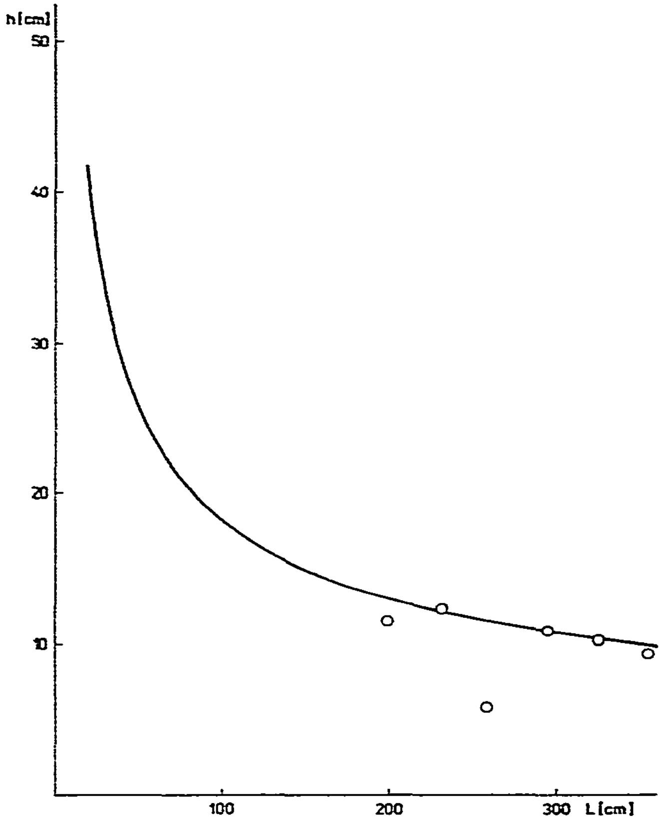

Fig. 1. Change in the concentration of the solute at the peak maximum (expressed in terms of the peak height, h) along the column calculated according to relationship 1 for h = 10.7 cm at L = 295 cm.

plate of solute i and k_i is the capacity ratio of solute i. For a given column and a given experiment, the first fraction at the right-hand side of eqn. 1 is a constant. It is assumed that the column packing is homogeneous enough so that neither $A\varepsilon$ nor H or k changes along the column length. The nearer to the column inlet the detection element is placed, *i.e.*, the shorter is L, the greater will be the concentration of the analysed component at the peak maximum.

EXPERIMENTAL

Apparatus and columns

Glass columns (2–4 m × 0.4–0.5 mm I.D. × 0.8–1.2 mm O.D.), with thread diameter 100 mm, were used. The columns were packed with Silpearl spherical silica gel (Kavalier, Votice, Czechoslovakia), particle size 30–40 μm. Constantan wire was wound round the column and served as a screening; about 1 cm of the glass surface was left free at the half-length of the column and on the last but one thread. Additionally, a glass column (0.44 mm I.D.) was used which consisted of three segments connected to each other, with two pieces of a stainless-steel tube (length *ca.* 1.5 cm) into which a stainless-steel capillary (0.25 mm I.D.) was inserted in order to decrease the free volume of the connections. The first two segments of the column consisted of four threads, the third segment of one thread. This column was used for measurements without the wire screening.

The mobile phase contained 7% *n*-butanol in *n*-hexane with the addition of 10 ppm of perchloric acid. A mixture of aliphatic alcohols, containing methanol, ethanol, *n*-propanol and *n*-butanol, was injected.

The measurements were performed in the arrangement shown in Fig. 2. The column was placed in an earthed metallic box (1) and was connected to a stainless-steel injection port (2) into which the mobile phase was introduced via an inlet (3). A needle valve connected via inlet 4 served to adjust the splitting ratio between the sample entering the column and the sample which was vented. Unscreened segments of the column were connected to electrometers (Vibron 33B; Electronic Instruments, Richmond, Great Britain) (5, 5′); the segments of the column wound with wire were earthed. When the glass column with metallic connections was used, the stainless-steel segments were connected to the electrometers. The mobile phase was pumped by a VCM 300 pump (Development Workshops, Czechoslovak Academy of Sciences, Prague, Czechoslovakia) after which a pressure pulse damper according to Locke[4] was inserted. The sample was injected through a septum by means of a Hamilton 5-μl high-pressure syringe.

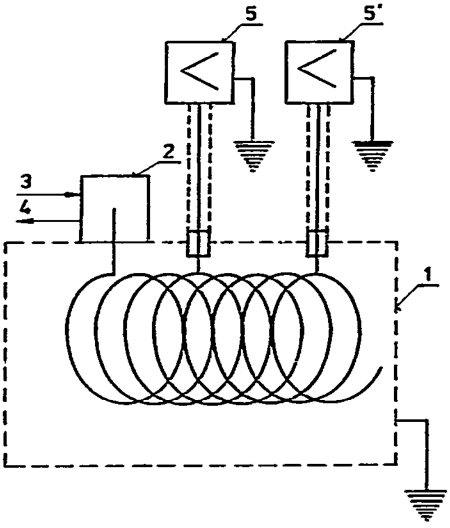

Fig. 2. Diagram of the apparatus for the measurement of the streaming current at two places in the glass column.

RESULTS

Character of the response

An example of a chromatogram obtained by sensing at two places in the glass capillary column with screening is shown in Fig. 3. It is obvious that each solute exhibits a peak that consists of two branches of opposite polarities. This phenomenon is more marked for solutes with lower capacity ratios *k*. The ratio of the heights of the

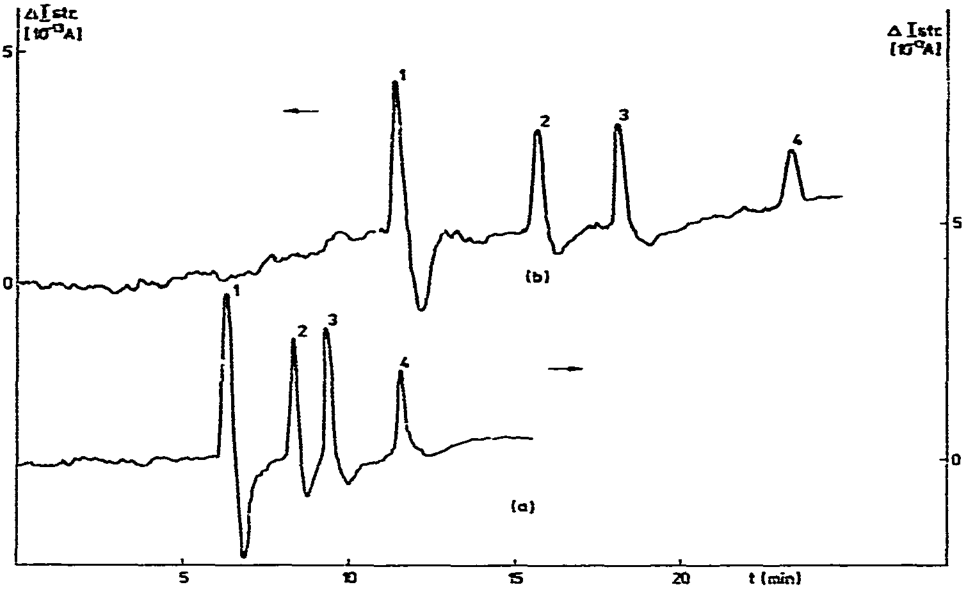

Fig. 3. Chromatogram of a mixture of *n*-butanol (1), *n*-propanol (2), ethanol (3) and methanol (4) obtained at two places in the glass column. L = 185 cm (a) or 310 cm (b).

branches of each peak (h^+/h^-, the plus sign denoting the first branch) does not depend on the mobile phase flow-rate for a given solute (Table I). However, it does depend on the capacity ratio of the solute, and on the position of sensing, increasing with increasing capacity ratio and for sensing at the column end. For methanol, which has a capacity ratio k = 1.4, the height of the second branch of the peak is

TABLE I

DEPENDENCE OF THE RATIO OF HEIGHTS, h^+/h^-, FOR *n*-BUTANOL (k_1 = 0), *n*-PROPANOL (k_2 = 0.35) AND ETHANOL (k_3 = 0.67) ON THE LINEAR VELOCITY OF THE MOBILE PHASE

u (*cm/sec*)	*L* = *1850 mm*			*L* = *3100 mm*		
	h_1^+/h_1^-	h_2^+/h_2^-	h_3^+/h_3^-	h_1^+/h_1^-	h_2^+/h_2^-	h_3^+/h_3^-
0.20	1.7	2.0	3.4	1.7	2.4	4.4
0.27	2.2	2.7	3.8	1.8	3.0	4.0
0.39	1.7	2.6	3.6	1.7	2.8	4.3
0.43	1.9	2.0	4.3	1.8	2.3	4.4
0.47	1.8	3.0	3.6	1.4	3.1	5.5
0.53	2.0	2.8	3.9	1.5	5.3	5.4
0.90	1.8	2.5	3.8	1.6	2.7	5.3
1.0	1.7	2.4	3.4	1.3	3.1	5.7
1.1	1.8	2.8	3.6	1.5	3.2	4.0
1.6	1.9	2.6	3.5	1.4	3.5	4.8
2.5	1.9	2.5	3.1	1.8	2.8	4.2
Average	1.8	2.5	3.6	1.6	3.1	4.7

usually too small to be measured with sufficient precision, and the ratio h^+/h^- is therefore not given for this solute.

When a solute enters the unscreened segment of the column, a change occurs in the density of the electric charge of the entering liquid, corresponding to the concentration profile of the solute. The similar change but of opposite polarity takes place when the solute leaves the unscreened segment. Both of the processes can be identified, as shown previously[3]. In the present case, the resulting signal is the sum of the inlet and the outlet signals at the given column segment. Chromatographic peaks with even more complicated shapes for the negative branch were obtained by sensing from the metallic connecting segments of the column. In this case, the situation is made more complicated by the presence of another solid phase, the stainless steel of the connecting capillaries.

The dependence of the concentration of the analysed component at the peak maximum on the distance of the detection element from the column inlet was verified by measurements on the glass column with eleven threads (L = 330 cm). The first five threads were earthed and the signal was picked up from the remaining six threads. *n*-Butanol was injected into the column and the height of the first branch of the chromatographic peak was measured at various sensing points. The measured values of h were plotted in Fig. 1, which also shows th. dependence of the chromatographic peak height on the distance of the place of sensing from the column inlet, calculated according to relationship 1. The value h = 10.7 cm for L = 295 cm, as measured on the last but one thread of the column, was taken as the basis for the calculation.

Verification of the mechanism of the response

In order to verify the mechanism of the response picked up from a segment of a narrow-bore glass column, a system described earlier[2,3] was employed. The electrokinetic signal was generated in a packed column (200 × 4 mm I.D.). Metallic capillaries of various dimensions, connected after the column, were applied. The lengths (minimum 120 mm) stainless-steel capillaries (0.3–0.8 mm I.D.) were selected so that the sensing of electrokinetic streaming currents might be performed under the conditions (*e.g.*, flow-rate, conductivity of the mobile phase) providing mass character of the response[2]. The 3200-mm capillary with large volume (2.50 ml) and 1 mm I.D. was made of copper.

The column was packed with Silpearl silica gel. Hexane, purified and dried over activated alumina, with the addition of 5% (v/v) isopropanol was used as the mobile phase. Model solutions of nitroanilines in nitrobenzene, *o*-nitroaniline in nitrobenzene and benzyl cyanide were injected.

The experiments confirmed that measurable electrokinetic currents are generated when solute in the mobile phase flows through empty metallic capillaries. In accord with the theory of the generation of streaming currents[1–3], the magnitudes of these currents depend on the linear velocity of the mobile phase in the capillary. That is why they are greater for a capillary with a given diameter at a higher volumetric flow-rate; at a given flow-rate they increase with decreasing diameter of the capillary (Tables II and III, values with a minus sign). An exception was observed in the case of exclusion response. The "derivative" character of the responses was observed not only with the injected solutes but sometimes also with vacancy of the isopropanol and with the exclusion response[5].

TABLE II

DEPENDENCE OF THE MAGNITUDES OF THE RESPONSES GENERATED IN THE COLUMN AND IN THE STAINLESS-STEEL CAPILLARY (0.8 mm I.D.) ON THE MOBILE PHASE FLOW-RATE

The magnitudes of responses are expressed in terms of 10^{-14} A; similarly in Tables III and IV. Responses generated in the sensing capillary possess a negative sign.

Flow-rate (ml/min)	*Exclusion response*		*Vacancy*		*Nitrobenzene*		*o-Nitroaniline*		*m-Nitroaniline*	
	+	−	+	−	+	−	+	−	+	−
3.6	84	20	156	0	144	40	52	8	3	0
0.95	36	12	112	0	112	10	44	0	0	0
0.47	8	0	71	0	78	0	32	0	0	0
0.22	0	0	45	0	47	0	24	0	0	0

The experiments further suggested that mutual shift of the maximum of the signal generated by the solute in the column and of the signal generated in the sensing capillary is associated with the volume of the sensing capillary. The volumes of the applied stainless-steel capillaries were too small to enable a differentiation between the signals. A detection capillary was therefore used with a volume of 2.50 ml, selected so that it might hold not only the zone of the solute (benzyl cyanide) but also the isopropanol vacancy eluted before it. Under these conditions, the responses generated in the column (zones 1–3 in Fig. 4) were perfectly separated from the benzyl cyanide response generated in the sensing capillary (zone 4). The flow-rate dependence of the responses generated in this case in the column and in the sensing capillary is summarised in Table IV. The last column of this table contains the distances between the maxima of the electrokinetic responses generated in the column and in the sensing capillary, expressed in terms of volume. The values measured are in a good accord with the volume of the sensing capillary calculated from its dimensions.

It was thus confirmed that measurable electrokinetic signals are generated not only by the flow of the mobile phase through the chromatographic column but also by its flow along the surface of tubes with diameters sufficiently small that retention can be considered to be negligible. The magnitude of the electrokinetic streaming current generated on the surface of a tube depends (for a given tube material, com-

TABLE III

DEPENDENCE OF THE MAGNITUDES OF THE RESPONSES GENERATED IN THE COLUMN AND IN THE STAINLESS-STEEL CAPILLARY (0.3mm I.D.) ON THE MOBILE PHASE FLOW-RATE

Responses generated in the sensing capillary possess a negative sign.

Flow-rate (ml/min)	*Exclusion response*		*Vacancy*		*Nitrobenzene*		*o-Nitroaniline*		*m-Nitroaniline*	
	+	−	+	−	+	−	+	−	+	−
3.6	22	5	4	49	0	80	0	36	2	0
2.2	18	4	4	44	0	70	0	33	2	0
0.96	13	5	8	26	0	33	0	18	0	0
0.47	2	0	7	11	0	13	0	6	0	0

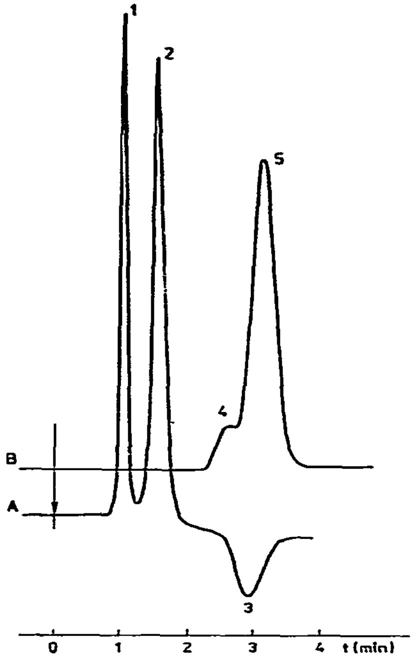

Fig. 4. Chromatogram of benzyl cyanide with sensing of the electrokinetic streaming current by use of a large-volume capillary. A, Record of the electrokinetic response: 1 = response to butanol vacancy generated in the column; 2 = response to the benzyl cyanide zone generated in the column; 3 = response to the benzyl cyanide zone generated in the sensing capillary. B, Comparative record by a refractometric detector connected after the sensing capillary: 4 = response to butanol vacancy; 5 = response to the benzyl cyanide zone. The time of injection is denoted by the arrow.

position and flow-rate of the mobile phase) on the tube diameter. If this tube is applied as a sensing element, its own signal is combined with the signal generated in the column and transported by the mobile phase (*cf.*, Tables II and III with Table IV). At the same time, the mutual shift in time of the maxima of the signals measured in the column and those measured in the sensing tube is determined at a given volumetric flow-rate by the volume of the sensing tube. Composite signals can therefore be expected for sensing elements with sufficiently small volumes (*e.g.*, unscreened seg-

TABLE IV

FLOW-RATE DEPENDENCE OF ELECTROKINETIC RESPONSES OF BENZYL CYANIDE, GENERATED IN THE COLUMN (h) AND IN THE LARGE-VOLUME SENSING CAPILLARY (h')

ΔV is the difference in the elution volumes of the maxima of the responses generated in the column and in the sensing capillary, calculated from the chromatograms.

Flow-rate (ml/min)	h	h'	$\lvert h'/h \rvert$	ΔV *(ml)*
4.56	560	−40	$7.1 \cdot 10^{-2}$	2.50
1.86	220	−16	$7.3 \cdot 10^{-2}$	2.60
0.94	126	9	$7.1 \cdot 10^{-2}$	2.64

ments of a sufficiently non-conducting packed capillary column, a metallic sensing capillary inserted between the two segments of the glass column, a sensing capillary after the packed column). If the polarities of component signals are of opposite sign, the resulting signal can, when the magnitudes of the individual components are comparable, imitate the course of the derivative of the elution peak. If tubes with larger volumes are used for sensing, the parasitic signal of narrow zones can be separated entirely from the column signal, differentiated and quantified. With broader zones, however, it coincides with the column response, in such a way that it distorts the shape of the desorption branch (Fig. 5). Distortion of the time course of the signal sensed from the column, caused by the parasitic signal generated in the sensing capillary, cannot be observed if the secondary signal is small or if it is generated by the zone which, with respect to the volume of the sensing element, can be considered as relatively broad.

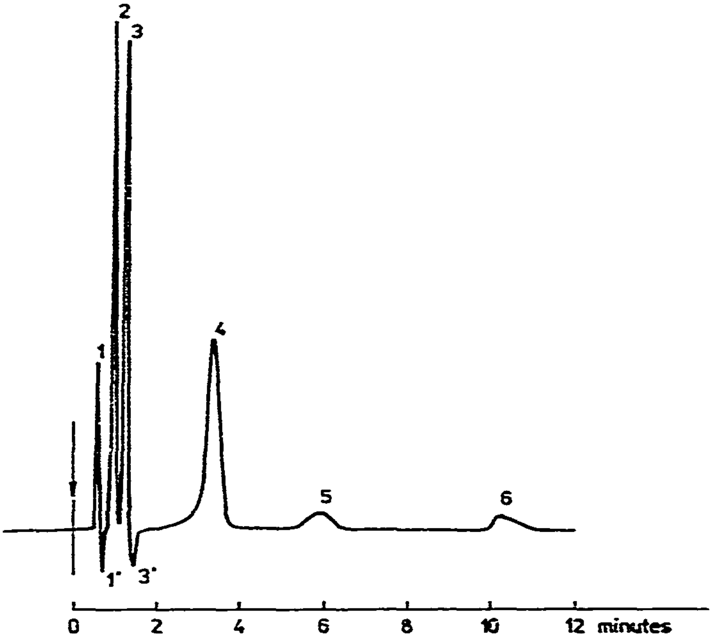

Fig. 5. Chromatogram of a model mixture of nitroanilines. Column: stainless steel, 200 × 4 mm, packed with Silpearl silica gel. Mobile phase: 5% isopropanol in hexane, flow-rate 2.2 ml/min. Sensing capillary: 120 × 0.8 mm. Zones: 1 = exclusion response; 2 = butanol vacancy; 3 = nitrobenzene; 4 = *o*-nitroaniline; 5 = *m*-nitroaniline; 6 = *p*-nitroaniline. The responses generated in the sensing capillary are denoted by primes, the injection time by the arrow.

Measurement of H

In order to calculate *H*, the peak of methanol was selected, for which it is supposed, with respect to the ratio of the heights of the branches of the peak, that the position of the maximum and the shape of the positive branch will be distorted only negligibly by the negative branch. The calculation was performed on the positive branch. The dependence of the height equivalent to a theoretical plate, *H*, calculated for both segments of the column, on the linear velocity of the mobile phase, *u*, is shown in Fig. 6. The values of *H* measured in both segments agree well for various *u*.

It was found that $H_{min} = 0.11$ mm, *i.e.*, 3.1 d_p. For a well packed column it was reported[10] that $H_{min} < 3\ d_p$. It is assumed[6,7] that the column can be well packed providing the ratio of the column diameter and the particle diameter $d_c/d_p > 10$. For the present micropacked column $d_c/d_p = 12$ and in this instance the value of H_{min} is considered acceptable. Furthermore, the constant C of Van Deemter's equation was determined from the graph to be $C \approx 0.04$ and was compared with the value calculated according to[8]

$$C = \frac{1 + 6k + 11k^2}{96\,(1 + k)^2} \cdot \frac{d_p^2}{D_m}$$

where D_m is the diffusion coefficient of the solute in the mobile phase. The value $D_m = 3 \cdot 10^{-5}$ cm^2/sec, taken from ref. 9, was used for the calculation. The calculated value of $C = 0.02$ sec is less than the value taken from the graph ($C = 0.04$ sec).

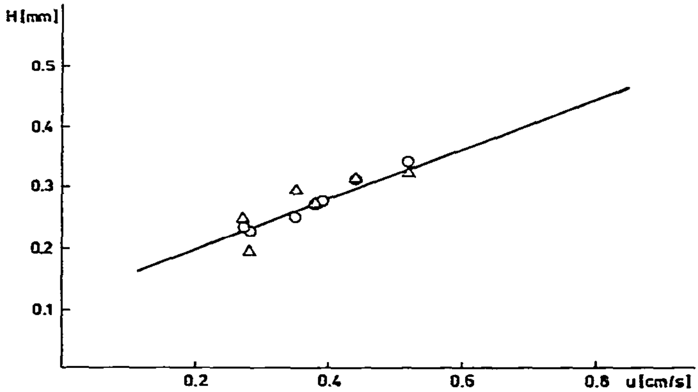

Fig. 6. Dependence of the height equivalent to a theoretical plate, H, on the linear velocity of the mobile phase, u. Column packing: Silpearl (d_p = 30–40 μm). Mobile phase: 7% (v/v) *n*-butanol in hexane, containing 10 ppm perchloric acid. Solute: methanol. L = 185 cm (○) or 310 cm (△).

CONCLUSIONS

The principle of the measurement of electrokinetic streaming currents in chromatographic columns[3] makes it possible to measure the response at different places in the column. The responses obtained for solutes in a single column are comparable with those obtained by measurements in several columns connected in series.

The appearance of a "derivative" signal, the genesis of which was explained and verified experimentally, is a disadvantage of the technique described. However, using this technique, the homogeneity of the packing of narrow-bore columns can be checked by determining the values of the height equivalent to a theoretical plate at different points throughout the column length.

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