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EXPERIMENTAL COMPARISON OF SOME DETECTORS USED IN HIGH-EFFICIENCY LIQUID CHROMATOGRAPHY

MILOŠ KREJČÍ AND NADĚŽDA POSPÍŠILOVÁ

Institute of Instrumental Analytical Chemistry, Czechoslovak Academy of Sciences, Brno (Czechoslovakia)

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SUMMARY

The properties of the detectors that are most often used in high-efficiency liquid chromatography were studied under identical conditions. Sensitivities and contributions to the height equivalent to a theoretical plate were determined for a differential refractometer, a capacitance detector, a UV spectrophotometer and two types of detector with effluent transporter.

INTRODUCTION

Efforts aimed at attaining maximum separation efficiencies have predominated in recent developments in liquid chromatography. The selection of sorbents, the preparation of columns and decreasing the concentration of the sample feed help to achieve this aim. The detection of the actual profile of the concentration of the solute in the mobile phase in the column outlet remains an ideal. The use of a detector with the maximum sensitivity and the minimum effective volume in close proximity to the outlet of the effluent from the column would be approaching this ideal. In liquid chromatography, in contrast to gas chromatography, the detector contributes considerably to the broadening of the concentration profile of the solute at the column outlet[1-3].

The volume variance, σ_V^2, is 10^{-1} to 10^{-3} cm^3 for efficient columns (height equivalent to a theoretical plate, $h < 0.05$ cm), according to our experience. For columns with $n/\text{sec} > 6$ (number of theoretical plates per second), the volume variance is usually 1 to 10^{-2} cm^6. An acceptable variance, σ^2, of the extra-column volume (sampler, connections, detector) should therefore not exceed *ca.* 10^{-3} to 10^{-5} cm^6. Otherwise, the broadening of the chromatographic curve is controlled by the contribution generated in the extra-column volume.

The sensitivity of the detector is also a significant guide to the usability of the detector in liquid chromatography. The detectors must respond quantitatively to such amounts of the separated compounds that do not exceed the adsorption capacity of the column. These amounts lie in the range *ca.* 10^{-6} to 10^{-3} g according to the chromatographic system applied (liquid–solid, liquid–liquid, gel permeation chromatography) and according to the volumes of the columns. If the broadening of the inlet concentration pulse in the column is considered in relation to the partition coefficient,

the maximum concentration of the substance being separated, in the column outlet, can be expected to be 10 to 100 times less. The lowest sensitivity of the detector required for liquid chromatography is *ca.* 10^{-4} to 10^{-8} g.

Comparative studies on detectors in the literature suffer from the disadvantage that standard conditions are not maintained with the use of different detectors and, in some instances, these conditions are even not described reliably. Therefore, we have evaluated a series of commercial detectors for liquid chromatography and also of detectors developed or manufactured in this Institute.

Several groups of detectors were used in our investigation. We used the following non-selective detectors: a differential refractometer of the Fresnel type, a deflection differential refractometer and a capacitance detector connected in a resonance circuit. A UV photometer at a wavelength of 254 nm was used as being representative of selective detectors. The properties of two detectors, a wire detector and a disc detector, with effluent transporters, were measured.

EXPERIMENTAL

A liquid chromatograph of our own construction[4], a Siemens S-200-P chromatograph and a Waters ALC-501 chromatograph were used for the evaluation of the detectors. A column 100 mm long, I.D. 2 mm, packed with glass beads of 140–160 mesh, was used for all the measurements. The weight of the packing was 0.5367 g. A sampler with a guide[5] was used with this column, which enabled a reproducible introduction of the sample to be achieved by means of Hamilton 7001 and 7005 injection syringes. Analytical grade hexane or benzene (Lachema, N.E., Brno, Czechoslovakia) was used as the mobile phase.

The standard column was connected to the detectors directly without any further connecting elements. Only with the capacitance detector, constructed in this Institute, were the inlet connectors changed so as to be able to evaluate their effect on the broadening of the chromatographic curve.

The following detectors were used for the study.

(1) A differential refractometer of the Fresnel type (Laboratory Data Control, Riviera Beach, Fla., U.S.A.).

(2) A deflection differential refractometer (Waters Associates, Framingham, Mass., U.S.A.).

(3) A capacitance detector in the resonance circuit[6] (this Institute).

(4) A UV photometer (Development Workshops of the Czechoslovak Academy of Sciences, Prague, Czechoslovakia) with a cell of cylindrical shape with an optical path of 1 mm, and with a slit shaped cell with an optical path of 7 mm (both cells were constructed in this Institute).

(5) Detectors with effluent transporters: a wire[7,8] and a disc detector[9,10] (both manufactured in this Institute).

All the measurements were carried out under identical experimental conditions. Therefore, the contributions to the peak broadening that originated in the column and sampler were considered to be constant. The inlet connecting tubes must be considered to be integral parts of the detector volume with respect to the construction of the majority of the detectors, as their construction is mostly not touched by the operator.

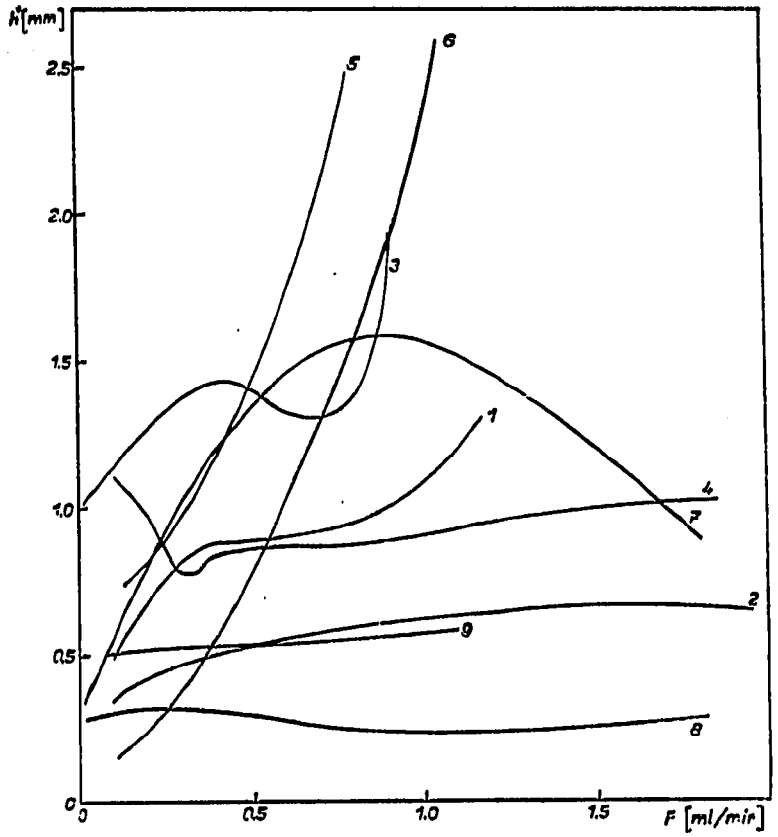

Fig. 1. Dependence of the height equivalent to a theoretical plate, h^+, on the mobile phase flow-rate, F. 1 = Refractometer (Laboratory Data Control); 2 = refractometer (Waters Associates); 3 = UV detector, l = 7 mm; 4 = UV detector, l = 1 mm; 5 = wire detector; 6 = disc detector; 7 = capacitance detector, shaped capillaries of I.D. 1 mm; 8 = capacitance detector, capillaries of I.D. 1 mm, packed with glass beads; 9 = capacitance detector, capillaries of I.D. 25 mm.

The dependence of the height equivalent to a theoretical plate on the flow-rate (F) of the mobile phase was measured in the range F = 0.1–2 ml/min. The results of the measurements for the individual detectors are shown in Fig. 1. The following relation[11] exists between the measured values h^+ and h on the column, which takes into account the effect of the instrument, for an inert sample and a column packed with non-porous material:

$$h^+ = L\frac{\sigma_V'^2 + hq^2L}{(V' + qL)^2} \qquad (1)$$

where h^+ is the HETP measured; h is the HETP on the column; L is the column length; q is the free column section; V' is the free volume of the detector equipment; and $\sigma_V'^2$ is the volume variance characterising the detector contribution.

Considering the minimum contribution of the column to the peak broadening, the measured h^+ can be taken as a detector contribution, h', to the peak broadening:

$$h' = L\frac{\sigma_V'^2}{(V' + qL)^2} \qquad (2)$$

The measured h^+ values were compared with the values calculated according to eqn. 2. The average deviation was $|\Delta h| = 0.055$ mm. The volume variance, $\sigma_V'^2 = F^2 \cdot \sigma_t'^2$, which characterises the peak broadening due to the detector effect, was measured at the same time. The results are summarised in Fig. 2.

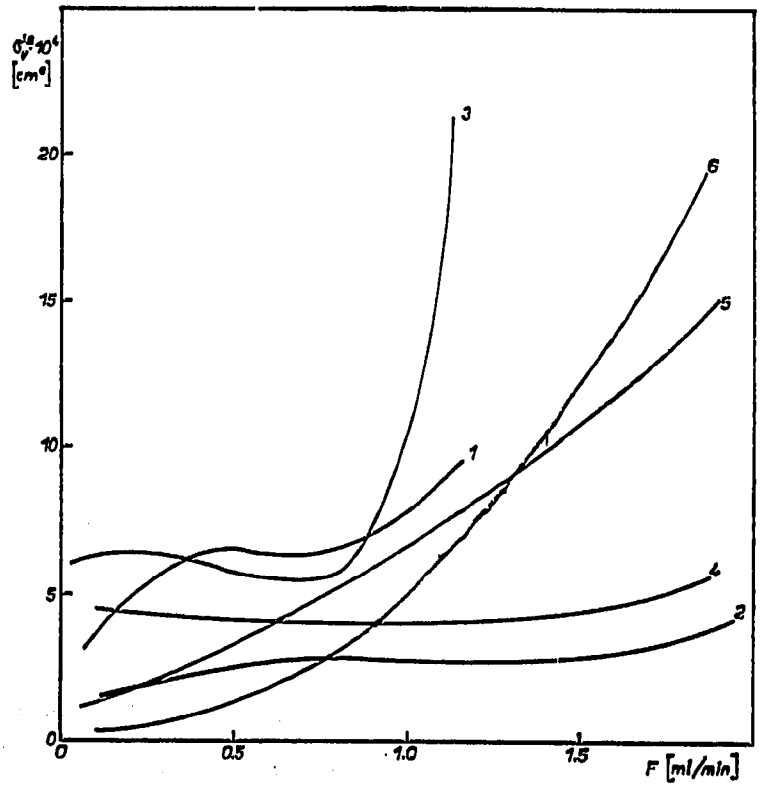

Fig. 2. Dependence of the variance, $\sigma_V'^2$, on the mobile phase flow-rate, F. Numbers on curves as in Fig. 1.

The minimum detectable concentrations and sensitivities of the detectors were determined for the compounds measured. Characteristic values are given in Table I.

RESULTS AND DISCUSSION

The detector response is proportional to the concentration or the weight of the compound under analysis. The dependence of the response on the concentration of the solute in the mobile phase is a function of the difference between the analytical properties of the effluent, a_{t_0}, and those of the mobile phase, a_0, so that it can be written as:

$$R_i = K[a_{t0}(c) - a_0] \quad (3)$$

where K is the constant of proportionality, R_i is the detector response to the substance i and c is the concentration of the solute in the mobile phase.

Those detectors which have a response R_i independent of the flow-rate of the mobile phase are included in the category of concentration detectors; those detectors for which R_i is a function of the flow-rate of the mobile phase are considered to be mass detectors. For $a_{t_0}(c) =$ constant for different solutes, the detectors are non-

selective[12]. Function $a_{i_0}(c)$ varies considerably for individual compounds or groups in the case of selective detectors. Destructive and non-destructive detectors are further differentiated according to whether or not the compound changes while passing through the detector.

Differential refractometers

Two differential refractometers based on different principles were compared. Refraction is used by both of them as an analytical property. The detector response is proportional to the difference between the refractive index of the pure mobile phase, n_0, and that of the mixture of the mobile phase with the solute i, n_{i_0}, so that the following relation can be written:

$$R_i = K(n_{i0} - n_0) \tag{4}$$

where K is the constant of proportionality characterising the instrumental arrangement. The concentration dependence of the analytical property of the detector, $a(c)$, is expressed as

$$n_{i0} = n_0 v_0 + n_i v_i \tag{5}$$

where v is the volume fraction of the solute (subscript i) and of the mobile phase (subscript o).

A differential refractometer using the Fresnel principle[13] (manufactured by Laboratory Data Control) measures the intensity of the reflected light, which is dependent on the refractive index of the liquid in the measuring cell and on the angle of the incident light beam. The latter condition is the reason why several prisms are used, the selection of which is influenced by the refractive index of the mobile phase. The volume of the cell was 5 μl.

A deflection differential refractometer (Waters Associates) is based on the deflection of the light beam by the liquid in the measuring cell. The deflection is scanned by a photoelectric cell that is sensitive to the position of the impinging beam. The volume of the measuring cell was 10 μl.

The sensitivities of both detectors (Table I) are approximately the same, 10^{-7} n. The contribution of the detector to the broadening of the concentration curve, expressed by the variance (Fig. 2), does not show any substantial differences as for the absolute values of $\sigma_V'^2$ at flow-rates up to *ca.* 1 ml/min. Greater broadening of the curve occurs above this flow-rate with the Fresnel-type detector. These differences are more evident when the broadening of the concentration curve is expressed in terms of the height equivalent to a theoretical plate, h^+ (Fig. 1), where less curve broadening is found with the deflection-type detector, even at higher flow-rates.

Differential refractometers are concentration-dependent, non-destructive detectors. They belong to the class of non-selective detectors whose response is dependent on the difference between the refractive index of the mobile phase and that of the solute. Both detector sensitivity and the contribution to the height equivalent to a theoretical plate are satisfactory for high-efficiency liquid chromatography. The differential refractometer of Waters Associates, tested by the authors, is more suitable for columns with larger diameters in which the flow-rates exceed 1 ml/min.

TABLE I

CHARACTERISTIC PROPERTIES OF THE DETECTORS TESTED

Detector	*Cell Volume* (μl)	*Analytical property*	*Noise*	*Sensitivity*
Refractometer (Laboratory Data Control)	5.0	Difference in refractive index, Δn	$1.43 \cdot 10^{-7}$	$2.87 \cdot 10^{-7}$
Refractometer (Waters Associates)	10.0	Difference in refractive index, Δn	$2.03 \cdot 10^{-7}$	$4.06 \cdot 10^{-7}$
Capacitance detector	11.7	Difference in dielectrical constants, $\Delta\varepsilon$	$5.90 \cdot 10^{-6}$	$11.8 \cdot 10^{-6}$
UV detector, $l = 1$ mm	27.2	Absorbance, A	$4.34 \cdot 10^{-3}$	$8.68 \cdot 10^{-3}$
UV detector, $l = 7$ mm	49.0	Absorbance, A	$0.78 \cdot 10^{-3}$	$1.56 \cdot 10^{-3}$
Wire detector	—	Ionisation current, I	$1.95 \cdot 10^{-13}$	$3.9 \cdot 10^{-13}$
Disc detector	—	Ionisation current, I	$7.43 \cdot 10^{-11}$	$14.86 \cdot 10^{-11}$

Capacitance detector

The dielectric constant, ε, is used as the analytical property for the capacitance detector. The general expression for the detector response is

$$R_i = K(\varepsilon_{i0} - \varepsilon_0) \tag{5}$$

where K is the constant of proportionality, ε_0 is the dielectric constant of the mobile phase and

$$\varepsilon_{i0} = \varepsilon_0 v_0 + \varepsilon_i v_i \tag{6}$$

In our work the detector was in the resonance circuit[6,14] and the useful detector response was thus decreased by additional capacities in the detection system used. The following equation was therefore used:

$$R_i = K' f_1 \left[1 - \frac{[(1 + k)\varepsilon_0]^{\frac{1}{2}}}{(k\varepsilon_0 + \varepsilon_{i0})^{\frac{1}{2}}} \right] \tag{7}$$

where f_1 is the basic frequency of the oscillating circuit and k is the factor characterising the decrease in the sensitivity due to the variable capacities of a constructional character.

For constant experimental conditions, the factor k can be included in the instrument constant K'. In our work, for $f_1 = 1.8 \cdot 10^7$ Hz, $k = 0.444$ and $\varepsilon_{01(\text{hexane})} = 1.890$, then $K' = 0.699 \cdot 1.8 \cdot 10^7 \cdot K''$, where K'' is the transport function ($R_i = K''(\Delta f)$) and (Δf) is the deviation from the basic frequency.

The volume of the measuring cell of the capacitance detector is 11.7 μl. As the capacitance detector is considerably affected by temperature, a suitable inlet capillary must be used, which serves as a heat exchanger so that the temperature

Minimum detection		*Measured compound*	*Mobile phase*	*Note*
mol/sec	*mol/ml*			
$5.67 \cdot 10^{-11}$	$2.67 \cdot 10^{-8}$	Methyl ethyl ketone	Benzene	$n^{20}_{CH_3COC_2H_5} = 1.3814$ (ref. 19) $n^{20}_{C_6H_6} = 1.5011$ (ref. 19)
$9.86 \cdot 10^{-10}$	$9.13 \cdot 10^{-8}$	Chloroform	Benzene	$n^{20}_{CHCl_3} = 1.4456$ (ref. 19)
$4.81 \cdot 10^{-9}$	$3.38 \cdot 10^{-7}$	Benzene	Hexane	$\varepsilon^{20}_{benzene} = 2.284$ (ref. 4) $\varepsilon^{20}_{hexane} = 1.890$ (ref. 4)
$4.73 \cdot 10^{-10}$	$5.69 \cdot 10^{-8}$	Naphthalene	Hexane	at $\lambda = 254$ nm, $\varepsilon = 1.526 \cdot 10^3$ for naphthalene
$4.21 \cdot 10^{-11}$	$1.46 \cdot 10^{-9}$	Naphthalene	Hexane	
$4.93 \cdot 10^{-8}$	$8.89 \cdot 10^{-6}$	Squalane	Hexane	
$4.45 \cdot 10^{-9}$	$3.92 \cdot 10^{-7}$	Squalane	Hexane	

of the entering liquid can be stabilised and maintained at the detector temperature. Different types of the inlet capillary were selected: shaped capillaries[15] with I.D. 1 mm and volume 346.7 μl; unshaped capillaries with I.D. 1 mm, packed with glass beads (free volume 174.6 μl); and unshaped capillaries with I.D. 0.25 mm and volume 40 μl. Their effects on the final broadening of the curve were studied. It is obvious from Fig. 3a that the inlets with larger volumes — the shaped capillaries (curve 1) — classify the detector as being a detector of a type that broadens the concentration pulse the most. If the volume of the inlet capillaries is decreased in such a manner as filling with glass beads or replacing it with the capillaries of I.D. 0.25 mm, the broadening is suppressed remarkably. The values of $\sigma_V'^2$ are then very similar (curves 2 and 3, Fig. 3a) even though the values of h^+, measured for packed capillaries with a volume nearly four times greater than that of capillaries of I.D. 0.25 mm, are lower (Fig. 3b).

It follows from these results that chromatographic zone broadening does not only depend on the value of the dead volume but also on its shape. The connections made from capillaries of I.D. 0.25 mm, having the smallest dead volume, a sufficiently low value of $\sigma_V'^2$ and a low pressure gradient, are the most suitable of the tested possibilities. Packed capillaries are satisfactory as regards the value of $\sigma_V'^2$, and h^+ is better with capillaries packed with glass beads than with those of I.D. 0.25 mm. A high pressure gradient is a disadvantage; for example, it is 16 atm at a flow-rate of 3 ml/min. The shaped capillaries of I.D. 1 mm are not suitable. The measurements also proved the assumption that the broadening caused by the detection cell of the capacitance detector is small[6]. In accordance with expectation, the sensitivity of the capacitance detector used is comparable with the sensitivities of refractometric detectors, and is $10^{-6}\,\varepsilon$ (see Table I). The sensitivity and the volume variance of the detector are satisfactory for its use in high-efficiency liquid chromatography. The

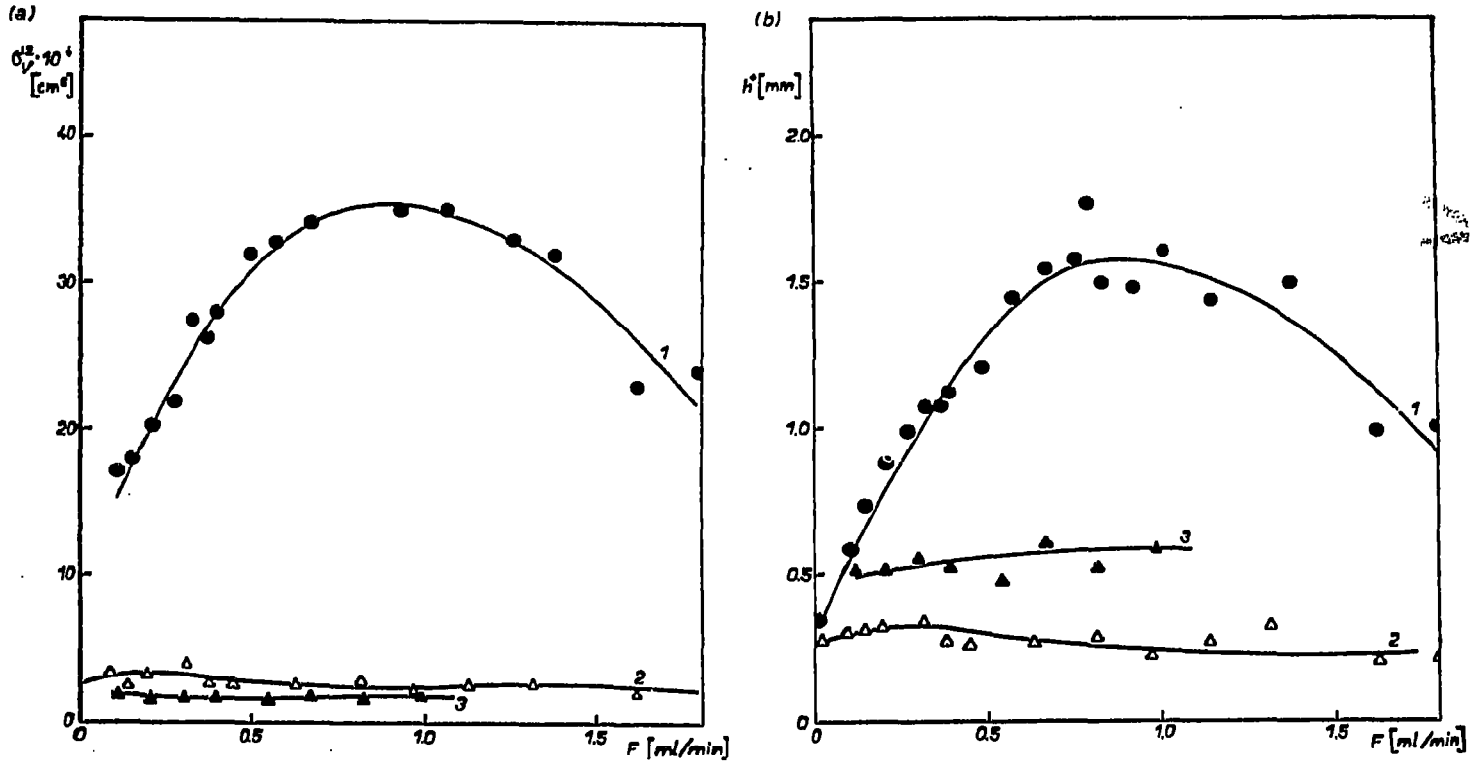

Fig. 3. Dependence of (a) $\sigma_V'^2$ and (b) h^+ for the capacitance detector. 1 = Shaped capillaries of I.D. 1 mm and volume 346.7 μl; 2 = capillaries packed with glass beads of I.D. 1 mm and free volume 174.6 μl; 3 = capillaries of I.D. 0.25 mm and volume 40 μl.

capacitance detector is a non-destructive, concentration-dependent detector with non-specific response to all substances whose dielectric constants are sufficiently different from the dielectric constant of the mobile phase. The usability of the detector differed from that of the refractometric detectors in that the walls of the measuring condenser were metallic in our work, so that it was not possibile to work with conductive compounds.

UV detector

The detector that is most used in liquid chromatography at present is that based on absorption of UV light by the solution under measurement. As liquids with $a_0 = 0$, *i.e.*, with the molar absorption coefficient $e_0 = 0$, are generally used as mobile phases, the following relation can be written for the response:

$$R_i = K \cdot l \cdot \frac{e_i}{V_{M_i}} \cdot v_i \qquad (8)$$

where l is the optical path, e_i is the molar absorption coefficient and V_{Mi} is the molar volume of substance i.

The detector based on the absorption of light of wavelength 254 nm was used. The absorption cells were of our own design and are shown in Fig. 4. The slit cell had an optical path of 7 mm and the total volume of the flow cell was 49 μl. As with a Laboratory Data Control refractometer, whose measuring cell is of the slit type, a dependence of the broadening of the concentration pulse on the flow-rate of the

mobile phase was found for the slit cell of the UV detector. Both the value of h^+ and the value of the variance increase rapidly at a flow-rate of 1 ml/min.

A capillary quartz tube with an optical path, l, of 1 mm was used for the comparison of the flow characteristics. The cell of this design did not show any of the hydrodynamic dependences found with the slit cells (see Figs. 1 and 2). The sensitivity of the detector (absorbance, A) was proportional to the optical path, *i.e.*, $9 \cdot 10^{-3}$ for $l = 1$ or $2 \cdot 10^{-3}$ for $l = 7$.

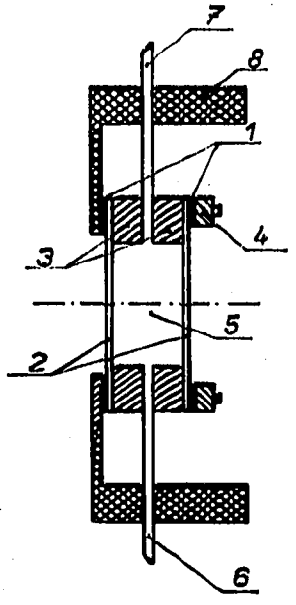

Fig. 4. Schematic diagram of the cell with volume $V = 49\ \mu l$ for the UV detector. 1 = PTFE seal; 2 = quartz glasses; 3 = brass block; 4 = brass lid; 5 = space for the optical cell; 6 = inlet capillary; 7 = outlet capillary; 8 = cell holder.

The UV detector is a selective, concentration-dependent, non-destructive detector. The sensitivity of the detector that was used is satisfactory for work in high-efficiency liquid chromatography.

Detectors with effluent transporters

The analytical property of the sensing element is the ionization of the solute, which is proportional to the number of the effective carbon atoms. The following equation can be written for the response:

$$R_i = K\alpha(\Sigma C_{eff})_{ip}(dN_{i\,eff}/dt) \qquad (9)$$

where K is the instrument constant, α is the ionization efficiency of the sensing element, C_{eff} is the number of effective carbon atoms of the substance entering the sensing element, (ip indicates that the solute was changed during the pyrolysis process into a mixture of chemically different compounds), t is the time,

$$N_{i\,eff} = \beta(N_{id} - N_{is}) \qquad (10)$$

where β is the efficiency of the pyrolysis process, N_{id} is the number of the moles carried by the transporter into the detection system, and N_{is} is the number of moles carried from the wire by the nitrogen from the drying furnace.

Two types of detector with effluent transporters were studied, a wire detector[8] of our own construction and a disc detector[9,10]. The fundamental differences between both systems consist in the different values of N_{td}. While only a negligible portion of the column effluent is carried by the transporter into the drying and pyrolysis furnaces with the wire detector, in the disc detector almost all of the effluent is carried into the sensing element. Regarding variable values of β, N_{td} and N_{ts}, the wire detector can be considered to be only semi-quantitative, and calibration under identical conditions as those used for analysis is necessary. The use of the disc detector is more advantageous, as conditions can be adjusted that necessitate a smaller number of variable quantities.

The transporters used cannot be perfectly purified, and as a consequence use of detectors with effluent transporters is always associated with a large amount of noise. Electric filters[16] are therefore used, which decrease the noise level but at the same time decrease the time constant of the detectors. As the detectors used have no connecting capillaries, the contribution of the detectors to the broadening of the chromatographic curve is small. Owing to the large time constant of the detector, however, h^+ and $\sigma_V'^2$ (Figs. 1 and 2) increase proportionally to the increasing flow-rate of the mobile phase. When calculating the values of h^+ from the chromatogram, the values of the elution time must be corrected for the time needed for the transport of the effluent from the column outlet into the sensing element of the detector.

The detectors with effluent transporters are destructive detectors. Their sensitivity and dynamic characteristics are satisfactory for work in high-efficiency liquid chromatography at low flow-rates of the mobile phase. The detectors are sensitive to those compounds that evoke the response of the flame ionization sensing element, with the restriction given by the required differences between the volatility of the mobile phase and that of the solute.

CONCLUSIONS

The UV photometric detector is the most used detector in high-speed, high-efficiency liquid chromatography at the present time. The main advantages of this detector are its simple construction, high sensitivity, selectivity and relatively lower dependence on the thermal stability of the effluent. The limited usability of the UV detector (with respect to its selectivity) led to a considerable extension in the use of non-selective detectors. Differential refractometers are the detectors that are most readily available commercially at present. Capacitance detectors have been shown to be usable both theoretically[17] and practically[6]. Our experiments proved that differential refractometers are as suitable as the capacitance detector in terms of both sensitivity and dynamic characteristics. The possible use of commercial bridges[3,18] for the measurement of the capacity of the measuring condenser promises a further extension in the use of such detectors. The detectors with effluent transporters are suitable for the special purpose of the analysis of low-volatility compounds. As regards quantitiveness of the response, these detectors can hardly compete with the other non-destructive detectors that were studied in our work.

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