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SIMULTANEOUS PHOTOMETRIC AND CONDUCTIVITY DETECTION FOR MICROCOLUMN LIQUID CHROMATOGRAPHY

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SUMMARY

A miniature detector for microcolumn liquid chromatography is described that provides simultaneous conductivity and UV photometric signals. The total volume of the detection space is less than 100 nl and the optical path length is 1 mm. Light is guided by optical fibres. The function of simultaneous detection was verified in the ion chromatography of inorganic and organic anions.

INTRODUCTION

Two recent trends can be noticed in the development of detection techniques for liquid chromatography (LC): miniaturization of detection cells compatible with microcolumns and efforts to increase the versatility of detection. It would be useful to solve both problems simultaneously. A simple and effective solution consists in detectors that provide more independent signals from one detection cell. Compared with the connection of several detectors in series, the advantages of the above-mentioned approach are the smaller contribution to extra-column zone spreading and the fact that there is no time shift of the signals. Simultaneous detectors have been described in which the effluent is monitored on the basis of different detection modes such as conductivity and permittivity detectors[1], UV photometric, fluorimetric and conductivity detectors[2] and amperometric and UV photometric detectors[3]. These detectors, however, are not compatible with microcolumn LC with respect to the volume of the measuring cells. An electrochemical detector[4] has been described recently with a measuring cell of volume 20 nl, providing at the same time independent conductivity and amperometric signals separated electronically.

For UV photometric detection, which is the most widely used detection technique in LC, a miniature flow cell with optical fibres[5] has been developed and tested successfully. Its improved version[6] was used as a basis for the detector described below.

This paper presents a description of the design and verification of the properties of a combined detector with the measuring cell with a volume of less than 100 nl providing the UV photometric and conductivity signals. As the combination of these two detection modes is suitable especially for monitoring ionized substances, the detector was used in ion-pair chromatography for simultaneous conductivity and indirect photometric detection.

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Conductivity detection is a frequently used technique in ion chromatography[7–10]. This method is universal for monitoring the ions, but solutes having a similar conductivity to the ion of the elution agent are detected with lower sensitivity.

Photometry has found wide application in ion chromatography in connection with indirect photometric detection[11]. In contrast to direct photometric detection[12–14] when the ions of the solutes absorb radiation more than the eluent, the indirect method needs an elution agent that absorbs at a suitable wavelength (usually 254 nm). In this region the absorption of inorganic ions is negligible and the detector provides negative peaks. The sensitivity of this method depends on several factors. The molar absorptivity of the eluent and its concentration should be selected in such a way that the absorbance is in the range 0.2–0.8[11]. If the background absorbance is not in this range, the signal-to-noise ratio deteriorates. From the practical point of view, however, the apparatus used also seems to play an important role because it has been found experimentally that a significant increase in noise arises even if the values of the background absorbance are, *e.g.*, 0.5[15] or 1.0[16].

EXPERIMENTAL

Measuring cell design

The design of the combined microcell is based on the design of the UV photometric cell[6]. It is made of stainless steel (Fig. 1a). Optical fibres (4), the inlet capillary from the column (1) and the outlet metal capillary (5) serving as an electrode for conductivity detection are connected to the cell by plastic screws (3). The metal cell

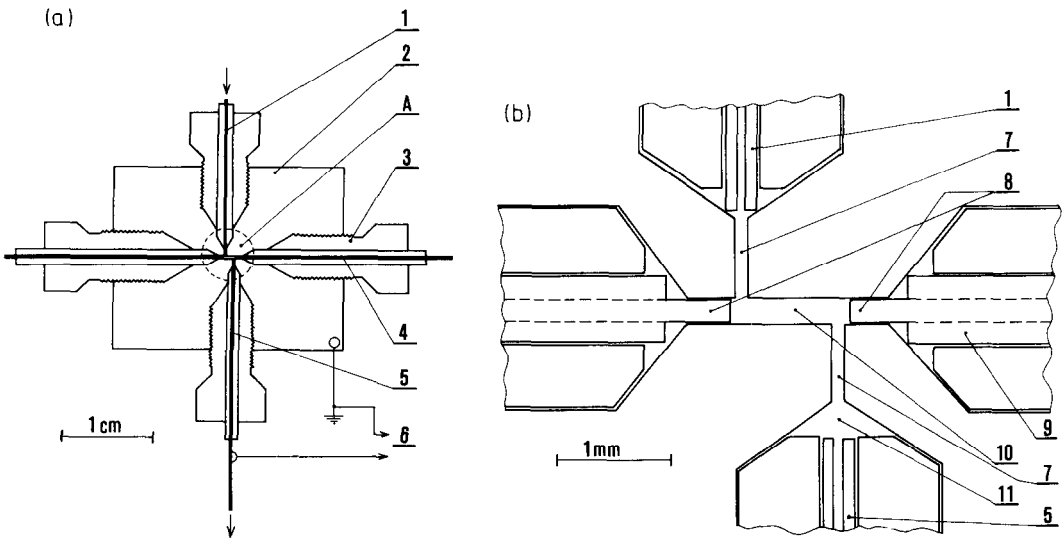

Fig. 1. Measuring flow-through microcell. (a) Schematic representation: 1 = fused-silica inlet capillary (0.1 mm I.D.); 2 = detector body; 3 = plastic sealing screw; 4 = optical fibre; 5 = metal outlet capillary; 6 = contacts of conductivity detection. (b) Detail A: 7 = outlet opening (0.12 mm I.D.); 8 = quartz core of the optical fibre; 9 = cladding of the optical fibre; 10 = detection space of the optical cell (0.25 mm I.D., length 1 mm); 11 = detection space of the conductivity cell.

body (2) is used as the other conductivity electrode. Fig. 1b shows that there are two detection spaces in the cell: one for photometric detection (10) of volume *ca.* 50 nl and optical path length 1 mm and the other for conductivity detection (11) of volume *ca.* 30 nl. The cell constant (K) was measured with a calibration solution and the value $K = 40$ cm^{-1} was verified by calculation. It is evident from Fig. 1b that the total space can be considered to be less than 0.1 μl, including connections (7). However, the difference in volume between the centres of the two detection spaces is decisive for the volume and time shift of both of the signals obtained. Fig. 1b shows that this volume difference is about 0.05 μl.

The UV light from a mercury discharge lamp is guided to the optical part of the detector by the optical fibre and then the other optical fibre guides the radiation to a photomultiplier. The advantages of using optical fibres in miniaturized detection systems have been described earlier[5,6,17,18]. The operating alternating voltage on the electrodes of the conductivity detector was 0.6 V.

Chromatography

The described cell was tested in a chromatograph assembled from modular units. The mobile phase was supplied by an SP 8700 pump (Spectra-Physics, Santa Clara, CA, U.S.A.). The sample of volume 1 μl was injected by a laboratory-made four-port injection valve. The glass cartridge microcolumn (Tessek, Prague, Czechoslovakia) of dimensions 30 $\times$ 0.7 mm I.D. was packed with the sorbent SGX C_{18} Separon, 5 μm (Lachema, Brno, Czechoslovakia). The cell was connected directly to the column outlet by a fused-silica capillary of 0.1 mm I.D. The optical part of the detection cell was connected by optical fibres with a FS 950 detector (Kratos, Ramsey, NJ, U.S.A.) modified to a single-beam photometer at the wavelength 254 nm. The electric outlet of the conductivity cell was connected by a coaxial cable with the electronic part of the electrochemical detector[4]. Both the signals were monitored with a TZ 4200 double-pen recorder (Laboratory Instruments, Prague, Czechoslovakia).

The chromatographic separation of anions was carried out on the sorbent dynamically coated with quaternary ammonium salt[19]. The tetrabutylammonium ion (TBA^+)[8] was used as the ion-pair agent. It was present in the mobile phase at a concentration of 0.1 mmol l^{-1}. The anions can be eluted from the column with organic acids[20], *e.g.*, phthalic acid, salicylic acid[21], nitrophthalic acid[22] or nicotinic acid[20]; we used 3 mmol l^{-1} nicotinic acid ($pK_a = 4.8$). The mobile phase conductivity was 400 $\mu\Omega^{-1}$ cm^{-1} at pH 5.2, adjusted by with potassium hydroxide.

RESULTS AND DISCUSSION

The described combined microcell was used for the indirect UV photometric detection of anions in microcolumn ion chromatography. Its volume (<0.1 μl) is approximately 100 times smaller than that of the photometric cells (*ca.* 10 μl) used in commercial LC, although the optical path length (1 mm) was only ten times shorter (10 mm for commercial cells). To maintain the sensitivity of indirect photometric detection, *i.e.*, the value of the background absorbance in the range 0.2–0.8, it is necessary to select a suitable elution agent with a higher molar absorptivity (ε). Therefore, nicotinic acid was selected, having $\log \varepsilon = 3.4$ at 262 nm[23]. With a concentration of 3 mmol l^{-1} of nicotinic acid in the mobile phase the value of

background absorbance is 0.82 AU. From the chromatographic point of view nicotinic acid is a substance with a low elution strength, and therefore a short (30 mm) microcolumn can be used.

Fig. 2 shows calibration graphs for the photometric and conductivity channels of the detector. The calibration was carried out with Cl^-, NO_2^- and NO_3^- ions. The peak height (h) represents the magnitude of the detector response corresponding to the concentration of the substance at the peak maximum. Individual points represent the averages of 2–3 values and the regression line results from points corresponding to the NO_3^- ion. The values of twice the peak-to-peak noise ($2n$) are shown and are as follows, with the resulting minimum detectable concentrations (c_{min}): for the optical detector, $2n = 0.002$ AU and $c_{min} = 0.02$ mmol l^{-1}, corresponding to the minimum value of the fluctuation of the nicotinic acid concentration in the mobile phase; for the conductivity detector, $2n = 0.16$ $\mu\Omega^{-1}$ cm^{-1} and $c_{min} = 0.004$ mmol l^{-1} for the Cl^- ion.

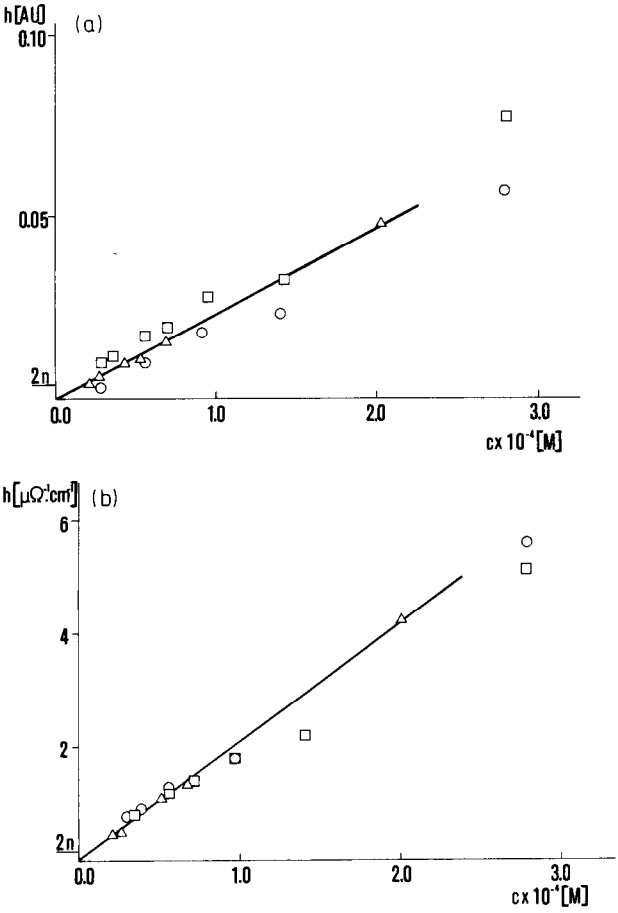

Fig. 2. Calibration graphs for (a) optical and (b) conductivity detectors. Injection volume, 1 μl. Solutes: □, Cl^-; ○, NO_2^-; △, NO_3^-. Column, CGC (30 × 0.7 mm I.D.), packed with SGX C_{18} Separon, 5 μm. Mobile phase: 3 mmol l^{-1} nicotinic acid–0.1 mmol l^{-1} TBA^+; pH = 5.2 adjusted with KOH; conductivity, 400 $\mu\Omega^{-1}$ cm^{-1}. h = Peak height; n = noise; regression line resulting from points corresponding to NO_3^- ion.

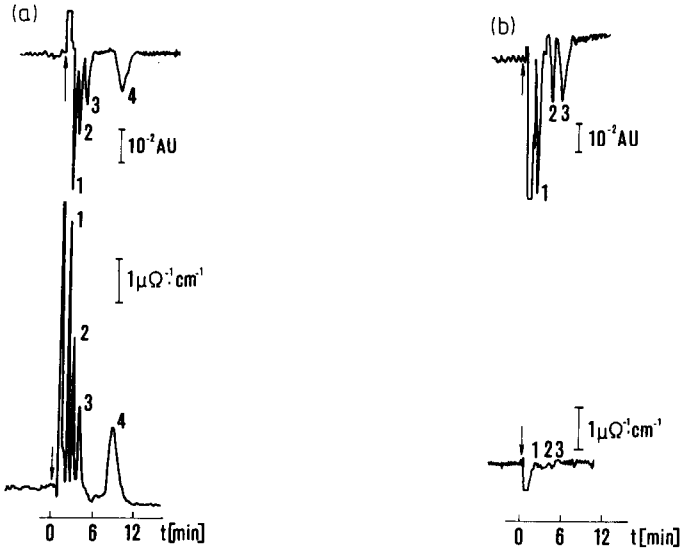

Fig. 3. Simultaneous UV photometric and conductivity detection of anions. (a) Chromatogram of inorganic anions. Mobile phase flow-rate, 26 μl min^{-1}; sample volume, 1 μl. Solutes: 1 = Cl^- (1 mmol l^{-1}); 2 = NO_2^- (1 mmol l^{-1}); 3 = NO_3^- (1 mmol l^{-1}); 4 = I^- (3 mmol l^{-1}). Detection: simultaneous indirect UV photometric detection at 254 nm (upper line) and conductivity detection (lower line) with the described detector. Other conditions as in Fig. 2. (b) Chromatogram of carboxylic acids. Solutes: 1 = butyric acid (0.4 mmol l^{-1}); 2 = isovaleric acid (0.8 mmol l^{-1}); 3 = valeric acid (1.2 mmol l^{-1}). Other conditions as in (a).

An example of the chromatographic separation of inorganic anions is shown in Fig. 3a. These substances are optically transparent at 254 nm and, considering the value of their equivalent ion conductivities (see Table I), the combined detector provides a simultaneous record of both chromatograms. The time shift (Δt) of individual signals obviously depends on the flow-rate (F) of the mobile phase. Taking into account the actual value of the flow-rate (F = 26 μl min^{-1}), the time delay (Δt) = 0.11 s) caused by the cell arrangement can be neglected.

TABLE I

EQUIVALENT ION CONDUCTIVITIES (λ) OF THE IONS USED AT 25°C

Ion	$\lambda \times 10^4$ ($m^2\ \Omega^{-1}\ mol^{-1}$)			
	Ref. 24	*Ref. 25*	*Ref. 26*	*Ref. 27*
Nicotinate			29.5	33.4
Cl^-	76.5	76.3		
NO_2^-	71.8	71.8		
NO_3^-	71.4	71.4		
I^-	76.6	76.8		
Butyrate	32.6	32.6		32.6
Isovalerate		32.7		31.0
Valerate	28.8		30.5	

Similarly to other simultaneous detection methods, the independent conductivity and photometric signals enhance the identification possibilities of solutes. Moreover, this approach increases the versatility of the detector when one of the detection modes has a low sensitivity; see Fig. 3b, showing the chromatogram of a mixture of carboxylic acids. These compounds cannot be detected sensitively in the system used with the conductivity method, as can be seen from the chromatogram and from the equivalent ion conductivities of the ions used in Table I. Organic solutes and nicotinic acid have similar ion conductivities and, therefore, the conductivity detector response is very low. The photometric channel, on the other hand, permits sensitive detection as for inorganic ions.

CONCLUSION

The detector for liquid flow analyses was designed and tested to provide simultaneous photometric and conductivity signals. With respect to the small volume of the measuring cell (<0.1 μl) it can be connected to a microcolumn chromatograph. The detector design is simplified substantially by using optical fibres in the optical section. The design of the flow cell permits its easy dismantling. The time shift of the photometric and conductivity responses is negligible with respect to the values of the mobile phase flow-rate in microcolumn LC. In practical applications the combined detector increases the possibilities of solute identification due to selective detection and improves the detector versatility. It could also be used in chromatography with varying compositions of the mobile phase for simultaneous recording of the course of the mobile phase gradient and the chromatogram.

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