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Improvement of on-line detection in high-speed counter-current chromatography: UV absorptiometry and evaporative light-scattering detection

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ABSTRACT

Improvements in analytical high-speed counter-current chromatography involve reduction of the column volume and a high revolution speed in order to decrease the time of analysis. Continuous detection should be performed instead of laborious fraction collection before reading the UV absorbance of the fractions. In this work on-line UV absorptiometry was performed by adding a "make-up" solvent to the column effluent in order to obtain a homogeneous medium before the detection is carried out. The first attempt to use an evaporative light-scattering detector is discussed.

INTRODUCTION

Recently, counter-current chromatography has been radically improved because high-speed centrifugal apparatus has been developed [1–10] in order to increase efficiency and to reduce the separation time. This involved reduction of the column volume by using 0.85 mm I.D. tubing and increasing the revolution speed to 2000–4000 rpm to sustain the stationary phase in such a small diameter column [9,10]. Thus, high-speed countercurrent chromatography (HSCCC) can perform analytical-scale separations with speeds and resolutions approaching those achieved by high-performance liquid chromatography (HPLC) [3].

In order to become truly efficient, continuous detection has to be achieved on-line with HSCCC. Thermospray mass spectrometry [11,12], Fourier transform IR [13] and UV detection have already been used to monitor the column effluent. In a general way, UV detection is performed after laborious collection of fractions because problems can occur in direct UV detection, as has already been described and classified into four categories by Oka and Ito [14]: (1) carryover of the stationary phase due to improper choice of operating conditions or (2) to overloading of the sample, vibrations or fluctuations of the revolution speed; (3) turbidity of the mobile phase due to the difference in temperature between the column and the detection cell; and (4) gas bubbling after reduction of the effluent pressure. Some of these problems can be solved by optimization of the operating conditions, control of the temperature of the mobile

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phase [9] and addition of some length of capillary tubing after the detector to prevent bubble formation. Unfortunately, in case of stationary phase carryover (2% per hour in centrifugal partition chromatography (CPC) [15]), detection problems may still occur; these can be solved by adding to the column effluent prior detection a solvent which is miscible with both the stationary and mobile phases.

This paper also describes an attempt to use evaporative light-scattering detection (ELSD) on-line with HSCCC because this detector is known to permit evaporation of the mobile phase prior to detection in HPLC [16–18] or in supercritical fluid chromatography [16–20]. After a suitable evaporation step, in the worst case of segmented or emulsified mobile phase the column effluent should always be an aerosol of the solutes before reaching the detection cell.

PRINCIPLE OF THE ELSD

A thorough evaluation of the theory has been given in several papers [21–23]. ELSD involves atomization of the column effluent into a gas stream via a Venturi nebulizer, evaporation of the solvents by passing it through a heated tube to yield an aerosol of non-volatile solutes and finally measurement of the intensity of light scattered by the aerosol. The processes by which the path of electromagnetic radiation can change direction when passing through a medium containing a suspended particulate phase are related to the size of the particles, which can be varied in HPLC by altering parameters such as the density, viscosity, surface tension and velocity of the mobile phase. The radius of the particles also depends on solute concentration and temperature during evaporation.

EXPERIMENTAL

Apparatus

The HSCCC apparatus (Fig. 1) consisted of two Shimadzu Model LC 5 A reciprocating HPLC pumps (Touzart et Matignon, Vitry sur Seine, France) and a Constametric II G (Milton-Roy, Villepinte, France) for pumping the organic and the aqueous phases, respectively.

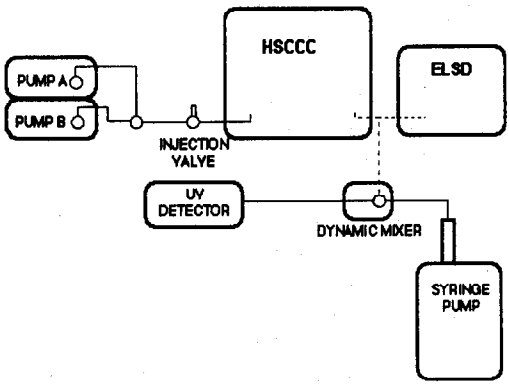

Fig. 1. Schematic diagram of the HSCCC apparatus.

The pumps were connected to a Model CPHV 2000 HSCCC system (SFCC, Neuilly-Plaisance, France) equipped with three identical and independent columns symmetrically arranged around the central axis of the centrifuge. Each column was prepared from 93.5 m × 0.80 mm I.D. PTFE tubing wound onto a holder to give a capacity of 47 ml. The columns underwent synchronous planetary motion and revolved around their own axis at the same angular velocity as the revolution around the central axis, avoiding twisting of the column flow tubes. The maximum speed attainable was 2000 rpm. The β value (ratio of the rotational radius to the revolution radius) ranged from 0.32 at the internal terminal to 0.7 at the external terminal. The separation utilized only one column connected to the pump using 0.8 mm I.D. PTFE tubing. The other two columns were only used to counterbalance the centrifuge after filling with an appropriate mixture of stationary and mobile phases. Samples were injected into the column via a Rheodyne Model 7125 injection valve equipped with a 315-μl loop. Injections of the samples dissolved in the mobile phase were carried out into the mobile phase after filling the column with the stationary phase.

Prior to the ELSD, a Model 2550 UV detector (Varian, Orsay, France) was used to monitor the absorbance of the column effluent. When only UV detection had to be used, isopropanol was continuously added to the column effluent using a Model 8500 syringe pump (Varian) before detection via a Model 811 dynamic mixing chamber (Gilson, Villiers-le-Bel, France) in order to reduce the background level in case of carryover of the stationary phase.

The Sedex 45 ELSD system (Sédéré, Vitry sur Seine, France) manufactured for HPLC was used without modification.

Solvent system

A two-phase solvent system composed of chloroform–methanol–water (3:1:3, v/v/v) was used to separate a test mixture of phenols. The solvent mixture was equilibrated at room temperature and the phases were separated shortly before use.

All organic solvents were of HPLC grade. Alcohols were purchased from Prolabo (Paris, France), other organic solvents from Rathburn (Chromoptic, Montpellier, France). Solvents were filtered before use. Water was doubly distilled. Nitrogen (L'Air Liquide, Paris, France) supplied the nebulizer of the ELSD system.

RESULTS AND DISCUSSION

Investigation of HSCCC coupled with UV detection

In order to study the potential of our HSCCC apparatus, the separation of a test mixture of phenols was done with UV detection (Fig. 2). Using the organic phase as the mobile phase, the retention of the aqueous phase was 80% at 1850 rpm and flow-rate 0.5 ml/min. The partition efficiency was calculated according to the HPLC equation for Gaussian peaks, $N = 5.54\,(t_r/\delta)^2$, where N is the number of theoretical plates (TP), t_r the retention time of the solute used to determine N and δ the width at half-height of the considered peak. The efficiency ranged from 650 TP for the first-eluting peak to 1830 for the third peak and increased with retention. The extra-column dispersion of the chromatographic system was measured by using the linear extrapolation method [24]. The total variance, σ^2, was plotted against the square of the retention volume, V_r, for a 0.5 ml/min flow-rate of the mobile phase (Fig. 3). Line a was obtained by

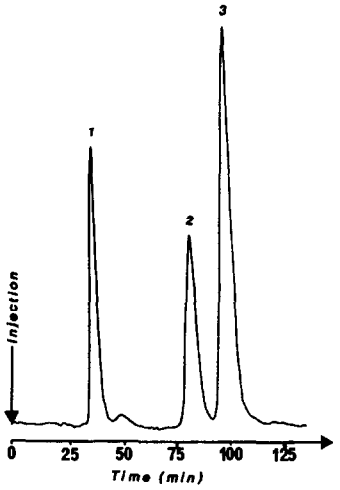

Fig. 2. On-line HSCCC–UV detection of a test mixture of phenols. Solvent, $CHCl_3$–CH_3OH–H_2O (3:1:3, v/v/v); retention of the stationary phase (aqueous phase), 80%; rotation speed, 1860 rpm; mobile phase flow-rate, 0.5 ml/min; isopropanol flow-rate, 0.5 ml/min (added to the column effluent); UV detection at 270 nm, 1 a.u.f.s. Solutes, 1 = *o*-nitrophenol; 2 = *p*-nitrophenol; 3 = phenol.

considering the mobile phase flow-rate (0.5 ml/min) without taking into account the isopropanol flow-rate (0.5 ml/min). Line b was obtained for the total flow-rate in the detector (mobile phase + isopropanol). The extra-column variance, σ_{ec}^2 was obtained from the intercept of the lines on the ordinate (Table I). For line b, the determined value of σ_{ec} (1.3 ml) was close to that calculated from the chromatogram in Fig. 2 ($\omega = 4\sigma$, where ω is the base width of the peak) and corresponded to the mixing chamber

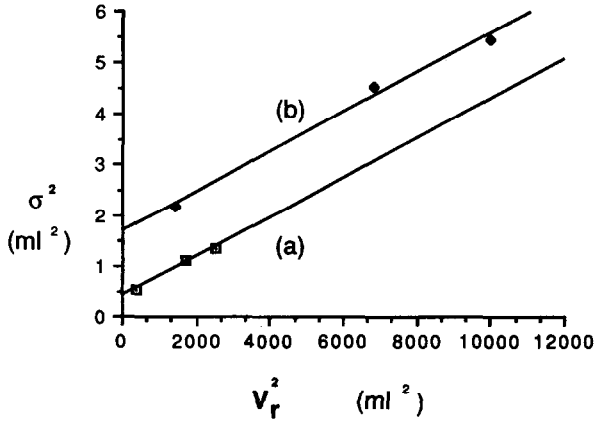

Fig. 3. Plot of total variance (σ^2) of the HSCCC–UV chromatographic system *versus* the square of the retention volume (V_r^2) of the phenolic compounds. Conditions as in Fig. 2. For line a, only the mobile phase flow-rate is considered in calculating V_r; for line b, V_r is calculated for a 1 ml/min flow-rate (mobile phase + isopropanol).

TABLE I

σ^2, σ AND N_c VALUES CALCULATED FROM THE DATA IN FIG. 3

Flow-rate (ml/min)	σ^2_{ec} (ml^2)	σ_{ec} (ml)	N_c (TP)
0.5	0.43	0.66	2600
1	1.7	1.3	2600

volume (1.5 ml). This means that the calculation made to obtain the line b is the correct approach to determine the extra-column variance for our system.

The true efficiency (N_c) of the column, 2600 TP, was obtained from the slope of the graph assuming the same efficiency for the compounds. The apparent and true values of the efficiency were fairly good for a single-column apparatus in comparison with HSCCC with two columns in series [1]. One can also establish that our apparatus permitted efficient counterbalancing of the centrifuge that minimized vibrations.

It must be pointed out that the sensitivity of UV detection was greatly improved by using 0.5 ml/min isopropanol "make-up" before detection; a range of less than 0.1 absorbance unit full scale (a.u.f.s.) was attainable instead of 2–20 a.u.f.s. because the background noise was reduced. Other types of mixing chambers are under investigation in order to reduce band broadening.

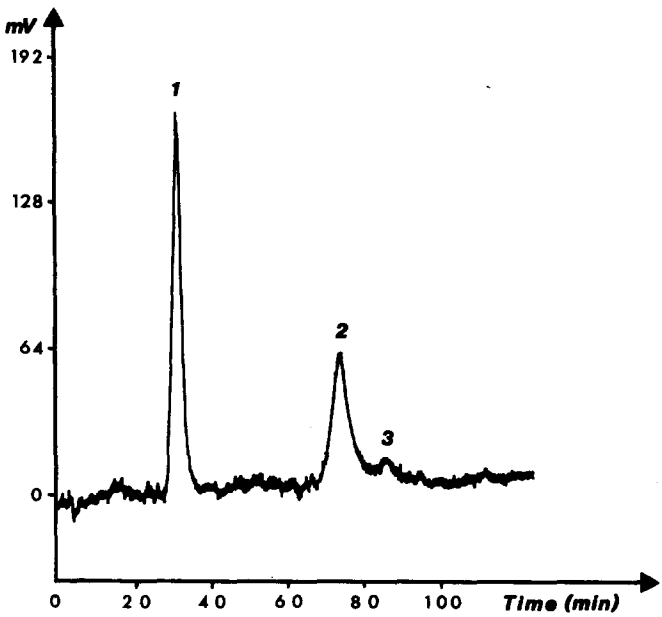

Fig. 4. On-line HSCCC–ELSD of a test mixture of phenols. HSCCC conditions as in Fig. 2. ELSD conditions: evaporation tubing temperature, 82°C; nitrogen pressure, 2 bar.

TABLE II

ELSD RESPONSE FOR VARIOUS COMPOSITIONS OF THE MOBILE PHASE PUMPED AT 1 ml/min

The first column is the mobile phase used after saturation with the stationary phase (in parentheses). The role of the two phases can be reversed.

Solvent system Mobile phase...saturated with...(stationary phase)		Detector signal (mV)
H_2O–CH_3OH	($C_2H_4Cl_2$)	8
$C_2H_4Cl_2$	(H_2O–CH_3OH)	1100
Heptane–ethyl acetate	(H_2O)	11
H_2O	(Heptane–ethyl acetate)	0
$CHCl_3$	(H_2O–CH_3OH)	>1339
H_2O–CH_3OH	($CHCl_3$)	−7
$C_2H_4Cl_2$	(HCOOH–CH_3OH)	1290
HCOOH–CH_3OH	($C_2H_4Cl_2$)	0
CH_3COOH–CH_3OH	(Heptane)	9
Heptane	(CH_3COOH–CH_3OH)	13
Heptane	(H_2O–CH_3OH)	−7
H_2O–CH_3OH	(Heptane)	−13
Acetone–HCOOH	(Methyl isobutyl ketone)	4
Methyl isobutyl ketone	(Acetone–HCOOH)	160
Heptane	(CH_3CN–CH_2Cl_2)	−2
CH_3CN–CH_2Cl_2	(Heptane)	37
n-Butanol	(H_2O)	415
H_2O	(*n*-Butanol)	35

On-line HSCCC–ELSD

This is the first report of on-line coupling of HSCCC and ELSD. Fig. 4 shows the HSCCC–ELSD result for the phenol mixture. Because sublimation of these compounds can occur during the evaporation of the mobile phase (82°C), phenol was difficult to detect. Work is being carried out with non-UV-absorbing molecules to show that ELSD can be used to advantage for detection in HSCCC.

The ELSD response was found to be noisy with the chloroform-containing mobile phase; Table II reports the values (in mV) of the signal of the detector for various CCC mobile phases in comparison with the signal recorded with water taken as a reference [25]. The data reported here are in good agreement with previous results [26], showing that chlorinated solvents give the highest signals. As has already been reported [27], the ELSD response is a function of the mean size of the droplets formed during the nebulization and depends on the properties of the solvents constituting the mobile phase. The response also varies with the refractive index of the scattering centre [27]: the higher the refractive index, the higher is the signal. In the worst case, *i.e.*, systems of solvents with chloroform, monitoring of the signals higher than 1300 mV required a decrease in the range of sensitivity of the photomultiplier. This has to be considered as a drawback because chloroform-containing mobile phases are often

used in CCC. The choice of a high quality of chloroform and solvents in general should be carefully investigated according to Dreux's experience [25].

CONCLUSION

UV detection can be performed on-line with HSCCC by using the experience gained by Oka and co-workers and by adding a make-up solvent in order to obtain a homogeneous phase prior detection. For molecules without chromophore or fluorophore groups or with mobile phases with a high UV cut-off (*e.g.*, toluene, acetone or ethyl acetate), ELSD can be useful but attention has to be paid to the quality of the solvents involved in the separation, especially when chlorinated solvents are used.

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