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Modified laser light-scattering detector for use in high temperature micro liquid chromatography[1]

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Abstract

Non-aqueous high temperature micro liquid chromatography with packed capillary columns has been utilized for the investigation of a modified laser light scattering detector. The original nebulizer in a laser light scattering detector, which was not capable of operating at volumetric flow-rates of less than 10 μl/min, was replaced with a capillary nebulizer that was placed at a drift tube distance of 7 cm, measured from the end of the drift tube. This distance combined with an external gas heating device, gave a 'clean' signal without fluctuations and with acceptable baseline noise. The limit of detection was less than 3 ng ($S/N=3$), and a linear direct calibration curve ($R^2=0.9999$) was obtained between 50 and 500 ng for two antioxidants injected on a packed capillary column ($n=3$). Retention control by temperature programming was allowed at a ramp rate of less than 5–6°C/min. Fatty amides and acyl glycerol were chromatographed by temperature programming.
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1. Introduction

The combination of elevated temperature and nonaqueous mobile phases in liquid chromatography is an analytical technique that was developed for the purpose of separating high molecular mass compounds with low water solubility [1].

Non-aqueous mobile phases with high UV-cutoffs, which often are needed for separation of high molecular mass compounds, unfortunately impose limitations on UV detection. Furthermore, many polymers lack UV-absorbing chromophores. To be able to further explore the capabilities of high temperature liquid chromatography with nonaqueous mobile phases, the evaporative laser light-scattering detector (ELSD) is the natural choice. ELSD is a relatively inexpensive alternative method for the detection of heavy compounds that do not absorb UV light compared to mass spectrometry. In contrast to UV detection, which is highly dependent of the presence of UV absorbing chromophores, an ELSD is anticipated to detect all compounds that are less volatile than the mobile phase, with a response that

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is not independent of structure, but is fairly similar. UV detectors are known to have comparatively low limits of detection and linear calibration curves, in contrast to the ELSDs, which are known for their non-linear response and rather poor detection limits.

The use of μl/min flow-rates has some favorable features compared to conventional mobile-phase flows, including lowered consumption of organic solvents, improved signal-to-noise ratios and improved compatibility with some detectors, like the mass spectrometer [2]. Unfortunately, the nebulizer in the commercially available evaporative light scattering detectors is constructed mainly for use with conventional columns with a mobile phase flow-rate of 1–2 ml/min, and not for handling mobile phase flow-rates lower than 10 μl/min.

Hoffmann et al. [3] modified a commercially available ELSD for μl/min flow-rates by replacing the original nebulizer with a laboratory-made nebulizer, which had the ability to adjust the position of the liquid-fed capillary relative to the nebulizer gas capillary. It was found that this parameter was highly critical. Their work showed that the response of the modified detector was non-linear in the nanogram region, and the limit of detection was found to be 5 ng.

Alexander IV [4] has recently modified the same version of an ELS detector for use in micro liquid chromatography (μ-LC) as the one presented in this paper. This modification too was initiated as a result of the inability of the original nebulizer to handle μl/min flow-rates. Alexander used an integral restrictor for feeding the mobile phase to the nebulizer gas. In principle, this is a more or less miniaturized copy of the original nebulizer. A linear response curve and a limit of detection of 50 pg was achieved with a direct injection system, without a chromatographic column.

We have developed a nebulizer that has been employed both in a laser light-scattering detector, as well as an interface for the coupling of μ-LC to an inductively coupled plasma mass spectrometer (ICP–MS) [5]. This was necessary due to the inability of the original instrumental set-ups in both detectors to handle a micro-flow-rate of less than 10 μl/min. The modified ELSD has been evaluated with regard to the limit of detection, linearity and response to temperature programming.

2. Experimental

2.1. HTLC–evaporative laser light-scattering detection

The experimental set-up consisted of a Merck-Hitachi LaChrom L-7100 pump (Merck, Darmstadt, Germany) or a Waters model 590 pump (Waters, Milford, MA, USA) with modified inlet and outlet check-valves, a Valco model CI4W manually operated injection valve equipped with a 60-nl internal loop volume (Valco Instruments, Houston, TX, USA), a Hewlett Packard 5700A gas chromatograph as the column oven (Hewlett Packard, Cupertino, CA, USA), and a modified version of a laser light-scattering detector (Varex Mark III, Alltech, Deerfield, IL, USA). The capillary column was connected to the injector by a fused-silica capillary (30 μm I.D., 375 μm O.D., 25 cm long). The fused-silica capillary transfer line (20 μm I.D., 375 μm O.D., 40 cm long) from the column to the nebulizer in the laser light scattering detector also acted as a restrictor, preventing the mobile phase from boiling at higher temperatures.

2.2. Nebulizer modification to the ELSD

The laboratory-made nebulizer (Fig. 1) consisted of stainless (316) steel tubing, 5 cm long, 1/16″ outer diameter and with a 0.02″ inner diameter, and a capillary restrictor (described above) placed inside the steel tubing. Four holes (0.4 mm diameter) were drilled through the nebulizer steel tubing to allow the nebulizer gas to enter the tubing. This part of the nebulizer tubing was placed inside a ‘cavity’ in the nebulizer housing. The nebulizer gas was introduced into the nebulizer steel tubing in this cavity. Two pieces of 1/16″ through-bore Valco unions were welded on both sides of the housing to make gas-tight connections.

The original nebulizer with its mounting plate was removed from the drift tube, and a new plate with an inner diameter that was slightly larger than that of the nebulizer housing was installed. The nebulizer housing was supplied with two O-rings (heat resistant) for adjusting the length of the drift tube by sliding the nebulizer body up and down the original drift tube and for securing gas tightness. The restric-

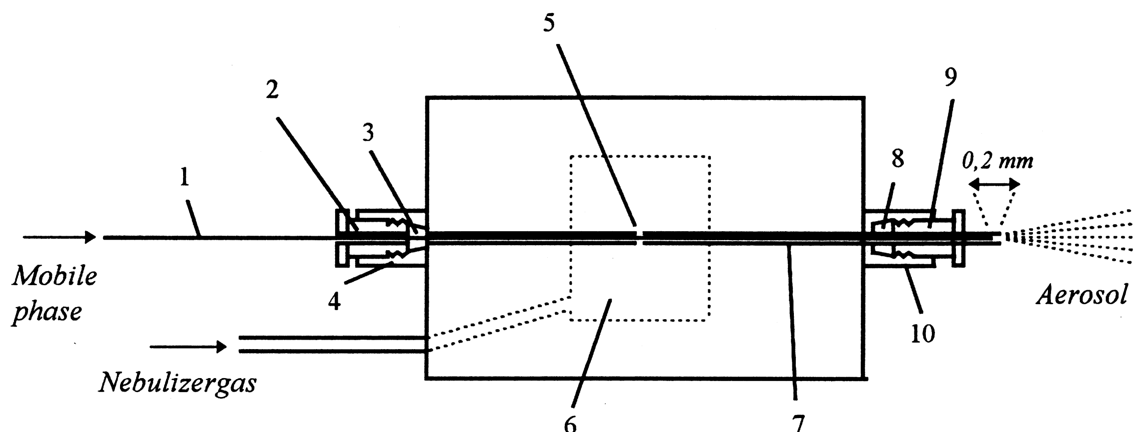

Fig. 1. Detailed (not to scale) drawing of the nebulizer; (1) restrictor capillary, (2 and 7) outer tubing, (3 and 8) ferrule, (4 and 10) $\frac{1}{2}$ Valco union, (5) holes for the nebulizer gas in the outer tubing and (6) nebulizer gas cave.

tor capillary was placed 0.2 mm inside the nebulizer steel tubing in accordance with previous results [3]. Preheating of the nebulizer gas was performed using a laboratory-made gas heating device. This heating device consisted of a coil of copper tubing (1/8″) and two heating cartridges placed inside the coil.

2.3. Columns and mobile phases

The capillary columns were packed with 3 or 5 μm porous Hypersil ODS or BDS (Hypersil, Shandon, UK) or 5 μm Kromasil C_{18} particles (Shandon Southern Products, Cheshire, UK), with I.D.s of 0.32 mm and different lengths. All columns were packed according to the procedure described in Ref. [1]. Experiments performed with the original nebulizer were performed using a 63-cm long column packed with 5 μm porous Hypersil ODS (Hypersil, Shandon UK) operated at a minimum of 100°C. The mobile phases used for all HTLC–ELSD experiments consisted of 10% dimethylformamide (DMF), 10% ethylacetate or 5% pyridine in acetonitrile.

2.4. Materials

The fused-silica capillaries came from Composite Metal Services, unions and ferrules were from Valco (Valco Europe, Schenkon, Switzerland) and carbon dioxide (99.99%) was from AGA (Oslo, Norway). HPLC-grade acetonitrile, ethylacetate and DMF were from Rathburn and pyridine, puriss, was from Fluka (Buchs, Switzerland).

The following polymer additives were supplied by Borealis, Stathelle, Norway: Irganox 1076 [octadecyl 3-(3,5-di-*tert*.butyl-4-hydroxyphenyl)-propionate]; Irganox 3114 [tris-(3,5-di-*tert*.butyl-4-hydroxybenzyl)-isocyanurate]; Irgafos 168 [tris-(2,4-di-*tert*.(butylphenyl)phosphite]; Tinuvin 327 [2(2′-hydroxy-3′,5′-di-*tert*.butylphenyl)-5-chloro-benzotriazole]; oleamide [*cis*-9,10-octadecenoamide]; stearamide, [octadecanamide]; erucamide [*cis*-13-docosenamide] and glyceryl monostearate (technical grade).

3. Results and discussion

3.1. The capillary nebulizer

The reason for the flow-rate limitation with the original nebulizer is probably related to the inner diameter of the steel capillary tubing connected to this nebulizer, which is three times larger than the I.D. of the fused-silica transfer line (restrictor). The low flow-rate from the restrictor is probably not sufficient to 'fill' the volume of the nebulizer tubing, resulting in an inhomogeneous liquid flow being exposed to the nebulizer gas and, consequently, a flickering and unstable signal.

The laboratory-made capillary nebulizer (Fig. 1) used the restrictor (fused-silica capillary transfer line

from the column) for solvent delivery directly into the nebulizer gas. The fact that this nebulizer gave a stable performance at flow-rates lower than 10 μl/min supported the assumption that the inner diameter of the original nebulizer tube was too large for mobile-phase flow-rates of lower than 10 μl/min.

However, the laboratory-made nebulizer had a limited capacity for transferring the heat from the heated drift tube to the nebulizer gas, due to the lack of direct contact between the nebulizer and the heated drift tube, and a laboratory-made device to preheat the nebulizer gas improved the signal-to-noise ratio. The current preheating procedure takes place outside the detector, affecting the response to temperature changes and, due to the length of the transfer line, requiring some time to stabilize the aerosol conditions in the detector. On shutting down the detector and taking the nebulizer apart, it took at least 60 min before the baseline was stabilized when the system was restarted, due to the gas heating element being placed outside the detector. The time needed for the system to be equilibrated during system startup could be reduced to approximately 30 min by reducing the nebulizer gas flow-rate to 0.2 l/min at night, keeping the HTLC system running around the clock.

Preheating the nebulizer gas gave a more stable and homogeneous aerosol formation, which led to a smoother baseline at a relatively low drift tube temperature. Not preheating the nebulizer gas made it necessary to use a higher temperature on the drift tube, which, in turn, gave rise to a more unstable and spiked baseline.

Hoffmann et al. [3] investigated the position of the mobile phase transfer capillary versus the outer tubing, and found that the optimum position for the inner capillary was 0.2 mm inside the outer tubing. By placing the inner capillary 0.2 mm inside the end of the outer nebulizer tubing, the nebulizer gas is forced to mix with the mobile phase (cross-flow) at an earlier stage than when the inner capillary is placed outside the nebulizer tubing. Placing the capillary less than 0.1 mm inside or outside this optimal position resulted in a drastic reduction in the signal response. Their experiment was performed using a laboratory-made adjustment screw device that could adjust the position of the inner capillary versus the outer nebulizer tubing. The modification performed on the same detector model by Alexander IV [4] differs from the present one in that the fed capillary tip is placed outside the outer nebulizer tubing.

The use of a small massive brass cylinder that had a 0.2-mm long steel rod implanted simplified the placement of the inner capillary. The outer tubing of the nebulizer was placed upon and around the steel rod on the brass cylinder, then the inner capillary in the nebulizer's outer tubing was pushed forward until it stopped against the steel rod and, finally, the inner capillary was tightened in this position.

3.2. Length of the drift tube

The 'barrel' shape of the nebulizer and the two O-rings positioned on the nebulizer's body made it possible to adjust the length of the drift tube. However, changing the length of the drift tube did not give any significant improvement in the limit of detection. By keeping all parameters constant, except for the length of the drift tube, a worse S/N ratio could be observed at shorter lengths of the drift tube (Table 1), indicating that the distance was too short for the evaporation process. This could be compensated for by increasing the gas flow or the temperature of the preheated nebulizer gas. Approximately the same limit of detection was achieved at all of the lengths investigated by regulating the gas flow and the temperature of the preheated nebulizer gas and the drift tube.

The original nebulizer (fixed length of the drift tube) and the capillary nebulizer with the longer drift tube lengths suffered from a fluctuating peak signal, which could be observed by the use of a recorder operated at high speed. The use of a lower recorder speed did not expose this problem. A fluctuating peak signal should be avoided because it could give rise to non-reproducible area measurements. We believe that contact between the aerosol and the stainless steel wall in the drift tube was causing this problem. The use of a 7-cm long drift tube did

Table 1
S/N ratios obtained using different lengths of drift tube

Length of drift tube	20 cm	15 cm	10 cm
S/N	10	6	4

overcome this problem. The initially more noisy baseline (because of the shorter length) could easily be improved by increasing both the nebulizer gas flow and the temperature in the preheating element.

3.3. Response curve

The different light-scattering processes are dependent on particle size [6], and the light-scattering detector is usually considered to be a non-linear detector, with a response according to:

$$A = am^x$$

where A is area, a is the response factor, x is the slope of the response line and m is the mass injected on the column [7]. Although the light scattering detector is considered to be non-linear, it has been shown that linearity can be obtained in a limited concentration range [8].

Direct calibration curves were obtained with a 70 cm×0.32 mm capillary column packed with 5 μm Hypersil BDS particles. The curves showed linearity between 50 and 500 ng for two compounds (R^2= 0.9999) with different retention times (Irganox 3114 and Irganox 1076). The RSD values (n=3) for Irganox 3114 ranged from 0.2% (499.2 ng) up to 1.6% (49.9 ng) in this interval. For Irganox 1076, the RSD values were quite similar, ranging from 0.4% (502.4 ng) up to 1.6% (50.2 ng). Despite their different molecular masses (Irganox 3114; M_r=784, Irganox 1076; M_r=531), the linear response was closely related in this range;

Irganox 3114: $Y = 453.7 \cdot X - 9807$

Irganox 1076: $Y = 454.2 \cdot X - 9903$

The extended calibration curve for Irganox 1076, ranging from 10 to 50.2 ng, indicated that the linear range could possibly be extended. As mentioned earlier, the use of typical packed capillary column dimensions is probably an advantage for the ELS detectors with regards to linearity. The smaller dilution of the chromatographic band is probably one of the main reasons for the linear part of the calibration curve. By reducing the extra-column volumes, it might be possible to further extend the lower end of the linear range of the ELS detector. The linear calibration curve obtained using the other recently modified nebulizer was obtained by a direct injection system [4]. The fact that a direct injection system does not contain any column or couplings

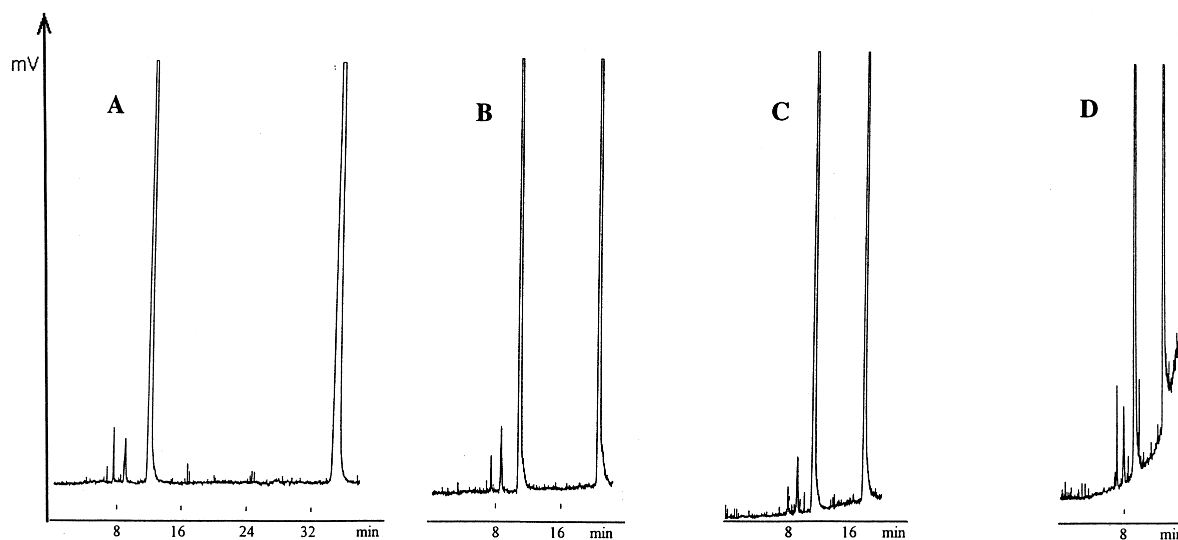

Fig. 2. Response to temperature programming. Flow, 5 μl/min; Column, 5 μm Kromasil C_{18} (70 cm×0.32 mm I.D.); mobile phase, 10% DMF in acetonitrile. Drift tube temperature, 80°C; gas-heater, 80°C; Nebulizer gas, 1.54 standard liter per minute (SLPM) (Nitrogen). Length of drift tube, 7 cm. The initial temperature was 50°C, which was held for 2 min, then the temperature was increased to 150°C; A=Isothermal 50°C, B=2°C/min, C=4°C/min and D=8°C/min.

gives us reason to believe that extra-column dispersion is critical for the length of the linear range of the ELS detector.

3.4. Response to temperature programming

Fig. 2 shows the response of the modified light-scattering detector to temperature programming. The initial temperature was 50°C for 2 min, then different temperature ramps up to 150°C were performed. The steepest temperature ramp in this experiment, 8°C/min, gave rise to a steeply increasing baseline. This is probably related to the momentary expansion of the mobile phase, which gives a briefly increased flow-rate and a subsequently rising baseline. However, a temperature gradient of 4–6°C/min or less is sufficient for most separations.

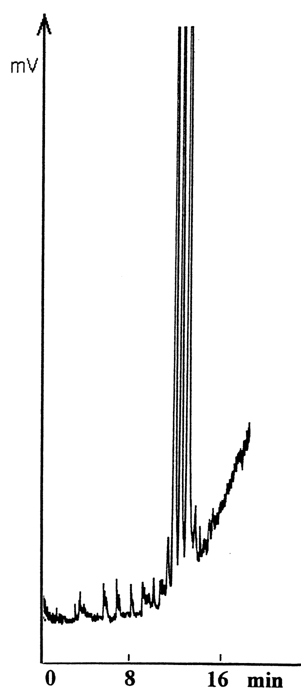

Fig. 3. Temperature-programmed HTLC separation. The starting temperature was 80°C, then this was increased by 4°C/min to 150°C. Flow-rate, 3 μl/min; column, 3 μm Hypersil ODS (70.5 cm×0.32 mm I.D.); mobile phase, 5% pyridine in acetonitrile. Detector temperature, 85°C; gas-heater, 110°C; nebulizer gas, 1.71 SLPM (Nitrogen). Length of drift tube=7 cm. Oleamide (1), stearamide (2) and erucamide (3).

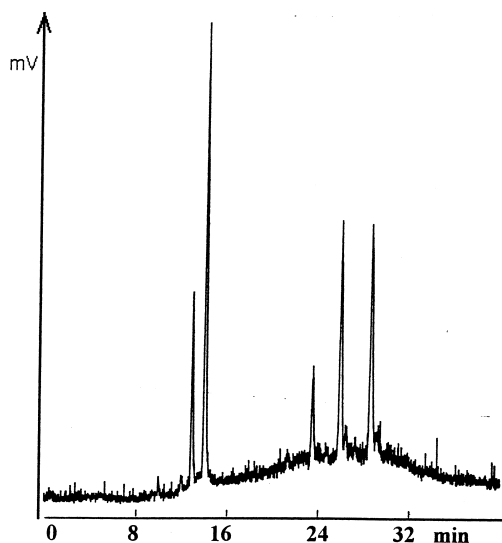

Fig. 4. Temperature-programmed HTLC separation of technical-grade glycerylmonostearate: The initial temperature was 70°C, which was held for 8 min, then the temperature was increased by 2°C/min to 100°C. Flow-rate, 3 μl/min; column, 3 μm Hypersil ODS (67 cm×0.32 mm I.D.), mobile phase, 5% pyridine in acetonitrile. Detector temperature, 85°C; gas-heater, 100°C; nebulizer gas, 1.84 SLPM (Nitrogen). Length of drift tube, 7 cm.

Fig. 3 shows a temperature-programmed HTLC separation of a mixture containing three fatty amides (polymer additives); oleamide, stearamide and erucamide. Peak number 1 also contains stearic acid, which is the main impurity in stearamide [9]. This was exposed by performing purity tests on the fatty amides. The steeply rising baseline is believed to be caused by the long temperature ramp (4°C/min) from 80 to 150°C. By stopping the temperature ramp at 120°C, the same separation with a slowly rising baseline was achieved. Fig. 4 shows a temperature-programmed HTLC separation of technical-grade glyceryl monostearate containing impurities of mono- and di-triglycerides. The temperature ramp was 2°C/min and, by stopping the ramp at 100°C, the influence from the rapidly evaporating mobile phase could be minimized. These examples show that temperature programming can be performed with the present HTLC–ELSD system, but awareness of the limitations caused by the expanding mobile phase have to be considered.

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