Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1051 (2004) 43–51
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Adaptation of an evaporative light-scattering detector to micro and capillary liquid chromatography and response assessment

Karen Gaudin, Arlette Baillet, Pierre Chaminade*

Groupe de Chimie Analytique de Paris-Sud: EA 3343, Laboratoire de Chimie Analytique, Faculté de Pharmacie, 5 Rue Jean-Baptiste Clément, 92290 Châtenay-Malabry, France

Available online 15 June 2004

Abstract

A commercially available evaporative light-scattering detection (ELSD) system was adapted for micro and capillary LC. Therefore the various parameters involved in the droplet formation during the nebulization step in the ELSD system were studied. It was shown that the velocity term in the Nukiyama Tanasawa equation remains constant, leading to droplets of the same order of magnitude for narrow bore and capillary columns. Consequently, the ELSD modification was performed by decreasing the internal diameter of the effluent capillary tube in the nebulizer nozzle and by keeping its external diameter constant. Next, response curves for a conventional and the developed micro and capillary LC were compared as to investigate why a linear ELSD response is often obtained when used in micro or capillary LC. By splitting the flow rate post column, we showed that the nebulization process was not at the origin of the phenomenon. For ceramide III and tripalmitin, the response curves were found to be non-linear. However the curvature was less significant when the columns internal diameter decreased. Calculated particle size profiles for micro or capillary LC suggest that the particle entering the detection chamber are bigger than under conventional LC conditions. Last, triethylamine and formic acid were used to increase the response of the detector. The response enhancement, expected from previous studies, was established for the two lipids involved in this study.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Evaporative light-scattering detection; Detection, LC; Calibration; Instrumentation; Lipids

1. Introduction

Adapting the evaporative light-scattering detection (ELSD) for micro-liquid chromatographic use is a growing field of interest. Since the detector's contribution to band broadening is very small, it is very suitable for micro-scale chromatography.

The mechanism of ELSD is complex. Three steps take place in order to obtain detection namely nebulization, evaporation and light scattering. Moreover, in the last step, three different phenomena can occur depending on the particle diameter (D) and wavelength (λ) of the light source, i.e. Rayleigh scattering (when $D/\lambda < 0.1$), Mie scattering (when $0.1 < D/\lambda < 10$) and reflection–refraction (when $D/\lambda > 10$) [11]. Thus, with ELSD several mechanisms of light scattering can occur simultaneously.

According to Stolyhwo et al. [1] the residence time of solutes in the detector was only a few milliseconds which corresponded mainly to the travel time of the solutes across the drift tube (where) propelled by the nebulizing gas. Thus, though a fully miniaturized detector for micro LC has already been reported [2], several adaptations of commercially existing detectors for micro LC were realized by modifying the nebulizer. Various authors [3–7] introduced a laboratory-made nebulizer in a Varex detector. Both Héron and Tchapla [8], using a Sedere detector, and Cobb et al. [9], using a Polymer Labs. detector, modified the detector by sliding a silica tubing of appropriate inner dimension into the standard nebulizer nozzle.

The non-linearity of the ELSD response is often considered as a disadvantage for quantitative analysis with this detector. However, several authors described a linear ELSD response when used with micro and capillary columns (Table 1). It is suspected that the linearity of the response with ELSD in micro or capillary LC might be due to the reduction of the flow rate [9] resulting in a better homogeneity of droplet size distribution.

* Corresponding author. Tel.: +33 1 46835790; fax: +33 1 46835458.
E-mail address: pierre.chaminade@cep.u-psud.fr (P. Chaminade).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.05.021

Table 1
Summary of the literature about micro- and capillary-ELSD

Reference	Detector	Solutes	Calibration range (ng)	*b*-value range[a]	LOD (ng)	Mobile phase composition	Column (mm), particle size (μm)	Flow rate (μL min^{-1})
Micro LC								
2	Laboratory made μELSD	TAG	30–300	1.51–1.65	8	Acetonitrile:acetone (2:8)	Kromasil C_{18} (5 μm) 120 mm × 0.7 mm	15
7	Sedere	TAG	14–80	0.85–1.31		Acetonitrile:acetone (30:70)	Brownlee Spheri 5 RP18 250 mm × 1 mm	40
8	Polymer Labs.	Amino acids	0.5–500	Linear fit	30–50	Acetonitrile-8 mm perfluororinated carboxylic acid in water gradient	Hyperyl BDS C_{18} (5 μm) 1 mm	55
Capillary LC								
3	Varex	Irgafos 168	18–110	1.81	6	Acetonitrile:acetone (80:20)	Nova-pak (4 μm) 190 mm × 0.32 mm	3
7	Sedere	TAG	2–50	1.06–1.31		Acetonitrile:acetone (30:70)	Hypersil C_{18} (3 μm) 150 mm × 0.3 mm	3
6	Varex	CIII			0.5	Acetonitrile with TEA/FA temperature program	Kromasil C_{18} (3.5 μm) 500 mm × 0.32 mm	15
4	Varex	Irganox 3114 and 1076	50–500	Linear fit	<3	90% Acetonitrile (temperature program)	Hyperyl BDS C_{18} (5 μm) 70 mm × 0.32 mm	15
5	Varex	Irganox 3114 and 1077	1–20	Linear fit	<1	Ethylacetate:acetonitrile (10:90)	Open capillary 1.5 m × 20 μm	
8	Polymer Labs.	Amino acids	0.5–500	Linear fit	0.5–1.5	Acetonitrile-8 mm perfluororinated carboxylic acid in water gradient	Hyperyl BDS C_{18} (5 μm) 0.3 mm	5
6	Varex	Glucose	1–25 and 25–461	Linear fit	0.05	Methanol:water (50:50)	Flow injection analysis	3

[a] *b*-term related to Eq. (4).

In order to study the causes of this linear tendency, the ELSD response shape was compared for conventional, micro and capillary LC. Therefore, we first adapted a commercial detector to micro and capillary LC. This miniaturization was based on the study of the predicted size of the nebulized droplets. The ELSD system was adapted in such way that we could evaluate the changes in the response profiles rather than achieving a maximum sensitivity or a lower limit of detection.

2. Experimental

Two ELSD systems, Eurosep DDL 31 (Eurosep Instruments, Cergy St. Christophe, France), were employed. Experiments were executed at 1 bar air pressure and the nebulizer was set at 35 °C, the drift tube at 45 °C. The photomultiplier was set at 600 V, which was the recommended voltage with the 50 W halogen lamp. One ELSD system, referred to as the micro-ELSD system or capillary-ELSD system, was modified for micro and capillary use as described in the results and discussion section. The chromatographic systems consisted of a Thermo Separation Products P1000 XR gradient pump equipped with a TSP SCM1000 vacuum membrane degasser (Thermo Separation Products, San Jose, CA, USA). The chromatograms were recorded with a personal computer integrator KromaSystem 2000 1.60 (Bio-Tek Kontron Instruments, Milan, Italy). For the micro- and capillary-HPLC system an IC-400-VAR (LC Packings, Amsterdam, The Netherlands) flow-rate splitter was set prior to a valco INJ-P4 manual injection valve (Valco, Houston, TX, USA) equipped with a 5 and 1 μL external loop (LC Packings).

Five analytical columns were tested in this study. A conventional (4.6 mm i.d. × 250 mm) and a narrow bore (2.0 mm i.d. × 250 mm) column, both packed with 5 μm C_{18}-Kromasil from Interchim (Montluçon, France). A micro column (1.0 mm i.d. × 150 mm) packed with 3 μm Inertsil ODS-3-C_{18}, obtained from LC Packings. Two capillary columns, one (0.3 mm i.d. × 250 mm) packed with 3 μm Inertsil ODS-3-C_{18}, obtained from LC Packings and one (0.32 mm i.d. × 150 mm) packed with 5 μm Zorbax C_3SB from Micro-Tech Scientific (Saratoga, CA, USA), were used.

Tripalmitin (ppp) and triethylamine (TEA) were purchased from Sigma (Saint Quentin Fallavier, France). Ceramide III (CIII) (stearoyl-phytosphingosine) was a gift of Cosmoferm (Delft, The Netherlands). All solvents and formic acid (HCOOH) were HPLC grade from Fisher Scientific (Elancourt, France) and used without further purification.

3. Results and discussion

When comparing ELSD systems for micro and capillary LC described in literature (Table 1), several differences can be remarked. The Varex detector for example uses a relatively short straight drift tube. This direct transfer of the whole aerosol to the drift tube and thus the detection cell, may be considered as an advantage for coupling to microseparation techniques. However, the drift tube is usually set at a temperature higher than 50 °C to allow an appropriate drying of solvent droplets issued from the nebulizer [11]. The complete transfer of the solutes can thus be diminished by these high temperatures resulting in an unfavorable effect on the detector response. By increasing the length of the drift tube from 6 to 25 cm, Alexander [7] showed a tremendous enhancement in signal-to-noise ratio (S/N) with flow rates in the μL min^{-1} range.

Sedere detectors as well as the Eurosep detector selected in this study, are equipped with a nebulization chamber fitted with a drain tube. The role of this drain tube is to eliminate the solvent originating from the collection of big droplets in the nebulization chamber [11]. It allows using high flow rates with various solvents [12] by selecting the smallest droplets before they enter the drift tube. In our experience, the drain tube can be shut when the detector is used at flow rates below 0.4 mL min^{-1}, no solvent appears to flow through. The nebulization also seems to be more efficient at reduced liquid flow rate. The drift tube of the Eurosep detectors is markedly longer than the Varex one and thus allows to operate at lower temperatures (30–50 °C). Still, the DDL 31 ELSD system utilized here had to be modified before being connected to the micro or capillary columns, due to the inner diameter of the capillary entering the nebulizer.

3.1. Adapting the nebulizer to micro-flow

The first step in our micro-flow adaptation was to understand which parameters in the ESLD nebulization process were relevant for the miniaturization.

When using pneumatic nebulizers, as is the case here, the droplet size can be estimated via the Nukiyama and Tanasawa empirical equation [13]. The surface volume mean droplet diameter D_{sv}, also known as the Sauter mean diameter, of the wet aerosol can be calculated from:

$$D_{sv} = \left[\frac{585\sqrt{\sigma}}{(\nu_g - \nu_l)\sqrt{\rho}}\right]_\alpha + \left[597\left(\frac{\mu}{\sqrt{\sigma\rho}}\right)^{0.45}\left(1000 \times \frac{Q_l}{Q_g}\right)^{1.5}\right]_\beta \quad (1)$$

where σ is the mobile phase surface tension, ρ its density, μ its viscosity, $(\nu_g - \nu_l)$ the difference between the nebulizer gas and liquid velocities, Q_l/Q_g the ratio of liquid and gas volumetric flow rates. Eq. (1) can be split into a liquid and gas velocity term (subscript α) and a liquid and gas flow ratio term (subscript β).

The aim of our modification was to keep the gas velocities the same as in the original ELSD system by maintaining the annular space between the effluent capillary and the

Table 2
Calculation of D_{sv} for acetone ($\rho = 0.79\,g\,mL^{-1}$, $\sigma = 23.32$ dyn/cm, $\mu = 0.36$ cP)

Nebulizer setting	Capillary i.d. (μm)	Liquid flow rate (μL min^{-1})	Gas flow rate (L min^{-1})	Gas velocity (m s^{-1})	Liquid velocity (m s^{-1})	α[a]: (velocity term, μm)	β[a] (flow ratio term, μm)	D_{sv} (μm)
Conventional	100	1000	2	388	2.12	8.2	69.2	77.4
	100	300			0.64	8.2	11.4	19.6
Micro	50	50			0.42	8.2	0.8	9.0
Capillary	20	5			0.27	8.2	0	8.2
Conventional	100	1000	3	582	2.12	5.5	37.7	43.1
	100	300			0.64	5.5	6.2	11.7
Micro	50	50			0.42	5.5	0.4	5.9
Capillary	20	5			0.27	5.5	0	5.5

[a] Refers to Eq. (1).

inside diameter of the nebulization gas tube (i.e. by keeping constant the outer diameter of the effluent capillary tube). Therefore, experiments were executed at a gas flow rate of 2 or 3 L min^{-1} through the nebulizer outlet (typical settings according to the manufacturer) for acetone with various nebulizer settings. Results shown in Table 2 reveal that according to Eq. (1) the droplet size (D_{sv}) with the conventional nozzle is about four times bigger at 1 mL min^{-1} than at 0.3 mL min^{-1}. Calculation of the two terms α and β from Eq. (1) shows that the differences in D_{sv} values are mainly governed by the flow ratio term (β). An almost equal contribution of these two terms exists at 0.3 mL min^{-1} but the flow ratio term (β) is mainly responsible of the droplet size increase at higher flow rates. The velocity term is constant for the two liquid flow rates but like the flow ratio term it is influenced by the gas velocity.

These calculations constituted the basis of our modification of the ELSD nebulizer. The two settings for micro and capillary LC shown in Table 2 use 50 and 20 μm i.d. silica tubes. These tubes are connected directly from the column to the nebulizer. In addition to minimising the solute dispersion these internal diameters allowed to ensure an equal liquid flow velocity at the nebulizer outlet for our different settings. The silica tube's outer diameter was 375 μm, matching the outer diameter of the standard nozzle. In the schematic representation of the nebulizer shown in Fig. 1, the silica tube (4) was tightened in a 1/16 in. (1 in. = 2.54 cm) polyther ether ketone (PEEK) sleeve (1) in order to fit the connector at the top of the nebulizer (2).

By keeping the outer diameter of the tube constant, the velocity of the gas was not modified. In Table 2 the D_{sv} values are similar for micro and capillary settings but they are only half of those obtained with the conventional settings when operated at 0.3 mL min^{-1} (narrow bore columns). These settings were also expected to maintain the nebulization efficiency and no evidence of solvent flowing from the drain was found.

In a next step the capillary tube that introduces the effluent in the nebulizer had to be adjusted. The height of the tip of the capillary tube (4 in Fig. 1) had to be adjusted relatively to the nebulizer outlet (5 in Fig. 1). The position of the tip is important for the nebulization process because it affects the stability of the spray and therefore affects the background noise and the substance's response. Using a nebulizer constructed from a modified bellow valve, Alexander [7] showed the need to realize a nebulizer with an adjustable capillary tip position. When the tip emerged from the nebulizer *S*/*N* was higher when using a hydro-organic mobile phase compared to pure water.

The design of our DDL 31 nebulizer shown in Fig. 1, allowed to make this modification since one revolution of the upper part corresponds to a 1 mm translation of the capillary into the nebulizer. The evolution *S*/*N* when adjusting the position of the capillary tip (50 μm i.d. × 375 μm o.d.) relative to the nebulizer outlet for a flow rate of 50 μL min^{-1} was studied (Fig. 2). Considering the factory preset position (with the standard capillary) as the zero position, an optimum was reached for a position where the tip is 1.5 mm further into the nebulizer. In this work we will refer to this setting as micro-ELSD. Using the 20 μm i.d. capillary at

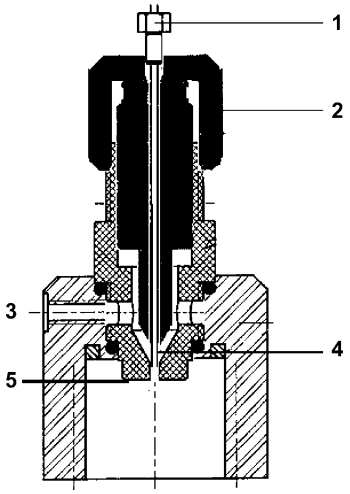

Fig. 1. Schematic representation of the nebulizer: (1) locking nut with peak sleeve, (2) upper part of the nebulizer with capillary guide, (3) gas inlet, (4) effluent capillary tube, (5) bottom part of the nebulizer.

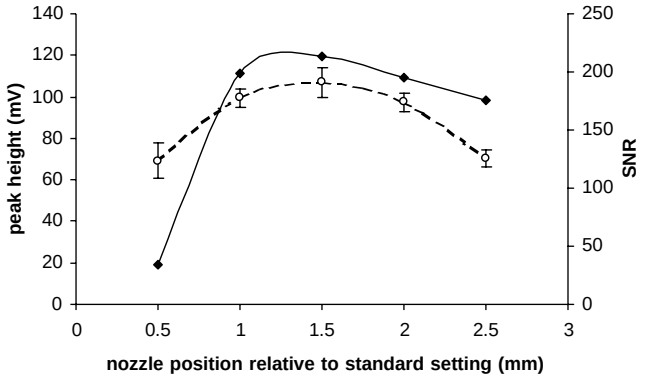

Fig. 2. Influence on the signal of the capillary position relative to the nebulizer outlet. Column 1 mm i.d., ceramide III. Solid line (◆): *S/N*; dashed line (○): peak height.

5 μL min^{-1}, a 3 mm translation was needed to reach optimal *S/N* conditions. This is called the capillary-ELSD setting.

3.2. Study of the ELSD response in micro and capillary LC

Since the ELSD response also depends on the nebulizing solvents, ELSD parameters and solutes, it can be difficult to compare performances between the systems described in different publications. Still, the detection limit in micro LC is generally around 10 ng while for capillary LC the 1 ng range is reached. Those results were obtained by either using non-aqueous mobiles phases for lipids [2,6,8], Irganox [4,5] or amino acids with an acetonitrile:water mobile phase [9]. A 50 pg detection limit was reported for glucose [7] but it was measured in a flow injection analysis system. Thus, this result may be due to the extremely low peak dispersion found with this technique.

The nanogram barrier can be considered as a theoretical limit of ELSD [11]. It arises from the solute concentration as it leaves the column and the droplet size decrease during the transfer of the wet aerosol in the drift tube. For injected amounts in the nanogram range, particles around the 100 nm diameter are expected, which is the lowest size for an efficient light scattering. According to Charlesworth [10] the size of a particle after solvent evaporation (D) is related to the droplet size at the nebulizer outlet (D_o), the solute concentration in the mobile phase (C) and its density (ρ):

$$D = D_o \sqrt[3]{\frac{C}{\rho}} \tag{2}$$

The ELSD response is expressed using the classical relationship:

$$y = am^b \tag{3}$$

where y is the solute peak area, m the injected amount and a and b are numerical coefficients.

Typical values for b are between 0.7 and 1.8–2 depending on the light scattering phenomenon. If large particles interact with the light beam the reflection refraction phenomenon is mainly responsible for the response and it leads to low b values. If little particles are illuminated the light is diffused according to the Rayleigh theory and b values are close to 1.8–2. For intermediate size particles, the Mie diffusion occurs and leads to intermediate values of b.

The ELSD response is thus related to the particle size, that is associated to the concentration of the solute entering the detector. The solute concentration at the peak maximum (C_{max}) is classically described by Eq. (4) [14]:

$$C_{max} = \frac{m\sqrt{N}}{\pi/4\sqrt{2\pi}d_c{}^2\varepsilon L(k+1)} \tag{4}$$

where m is the amount of solute injected, N the column plate number, d_c and L the column internal diameter and length, ε the column porosity and k the solute capacity factor.

This equation shows that the sensitivity achieved with two columns of different inner diameter should be related to their squared diameter ratio if all other parameters are kept unchanged. It is valid for low injected amounts, where no chromatographic overload occurs.

ELSD response is known to be sensitive to the peak shape. As already reported, for comparable amount injected, band broadening induces an increase in the b term of Eq. (3) [15]. This is the consequence of the lower concentration entering the detector that conducts to smaller particles in the detection chamber. For the same reason, increasing the retention reduces the C_{max} value and modify the ELSD response [15]. Comparison of ELSD response profiles should thus pay attention to chromatographic dispersion. Unfortunately, unlike UV detection, ELSD does not allow an accurate measurement of the plate count. The detector non-linearity (the value of parameter b) influence the recorded peak width. The consequence is that the peak is thinner than the concentration profile [16] and; therefore, only an apparent plate count can be reported. Therefore, we will not compare our different settings in term of efficiency or peak shape since response influence peak geometry and vice versa.

Our comparison only focuses on the shape of calibration curves obtained with the different settings. The reason of our interest is that in six of the eight studies listed in Table 1, the response was found to be linear. The linearity was assessed by a linear fit or by a statistical comparison of the b term of Eq. (3) with 1.0. These results are in apparent contradiction with Eq. (1) that showed that in micro-flow nebulization smaller droplets are obtained and thus the response should shift to the Rayleigh region of response, increasing the b term of Eq. (4).

To investigate the effect of the micro-flow rate in the nebulization process and the response, an experiment was performed with both the modified and unmodified ELSD systems. A post column flow splitter was installed at the outlet of a 2 mm column: the main flow was entering the unmodified ELSD system and 1/100th of the flow rate was directed towards the capillary-ELSD system. Ceramide III was chromatographed using an acetonitrile:acetone (10:90) mobile phase. It is assumed that the peak dispersion

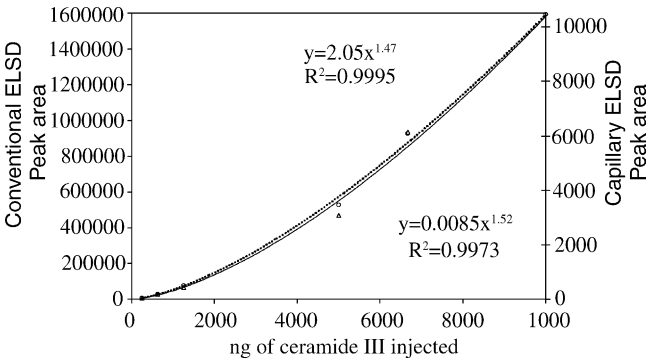

Fig. 3. Response curve obtained for ceramide III chromatographed with propanol-1:acetonitrile (50:50). Narrow bore column (2 mm i.d.) coupled to a post column splitter connected to a conventional ELSD system (dotted line and circle points, upper equation) and a capillary-ELSD system (plain line and triangle points, lower equation). Each point represents the mean of a duplicate injection.

introduced by the flow splitter is of the same order of magnitude in the two exiting channels and, that the concentration profile entering the two detectors is identical. Fig. 3 shows the calibration curves recorded simultaneously with this setting: the *b* term of Eq. (4) is about 1.5 in both cases. The response curve from the capillary-ELSD system was thus almost identical to the narrow bore one. This result is consistent with the concentration dependence of the ELSD response. It can also be assumed that the reduction of the droplet size introduced by the 20 μm i.d. tube used in the capillary-ELSD system was not important enough to noticeably influence the response shape. At least the ratio between the *a* term of the two equations (1/250) is found lower than the actual split ratio. This suggest that our capillary nebulizer is two- or three-fold less efficient than the standard one.

Next, different calibration curves were recorded using either the conventional and the narrow bore column or the micro and capillary columns. The results are shown in Table 3 for ceramide III and triplamitin chromatographed with propanol-1:acetonitrile (50:50) and acetonitrile:acetone (10:90), respectively. Examples chromatograms are presented in Figs. 4 and 5. As in the preceding experiment, the calibration curve for ceramide III shows a strong curvature with *b* values at about 1.5 for the narrow bore column. The *b* value of calibration curve with the capillary detector is slightly lower and is around 1.4. A further comparison of these curves is difficult since the calibration range is reduced to 40–400 ng in capillary LC compared to 250–10,000 ng for the narrow bore system. In the case of triplamitin, *b* increased when the column's inner diameter decreased in the case of the conventional and narrow bore columns. These two columns were employed with the same conventional ELSD system and the increase in *b* was consistent with the decrease of the droplet size shown in Table 2. An important difference was also noticed between the apparent plate number of the two columns that may also explain the higher *b* value of the narrow bore column. The *b* value for

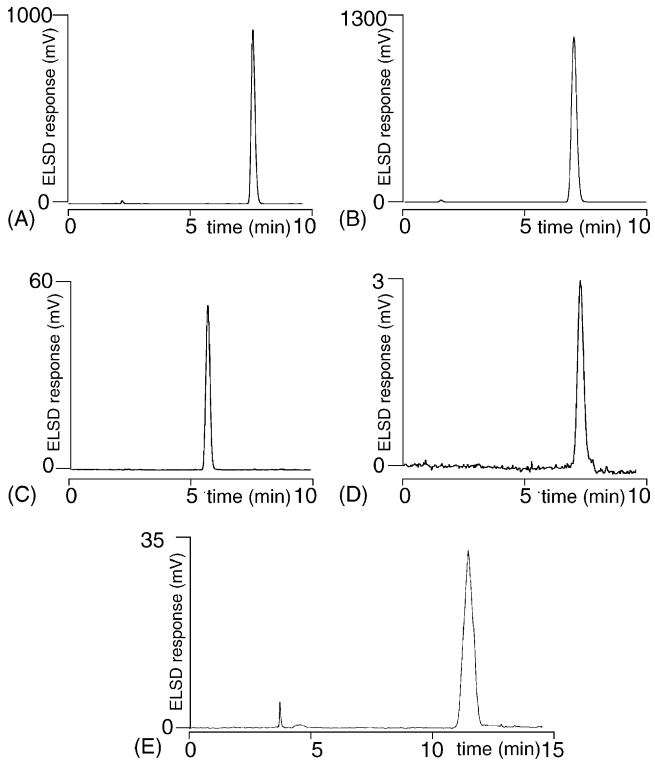

Fig. 4. Chromatograms of tripalmitin obtained with the different settings: (A) and (B) conventional 4.6 and 2 mm columns (2200 ng injected), (C) micro-ELSD with the 1 mm column (176 ng injected) and (D) and (E) capillary-ELSD with 300 μm columns kromasil and zorbax.

the 1 mm i.d. column was also comparable to the 2 mm i.d. column. Our choice to keep the same liquid velocity in the nebulizer capillary created droplets of similar size and led to similar response curves for narrow and micro chromatography. When using our capillary-ELSD system with

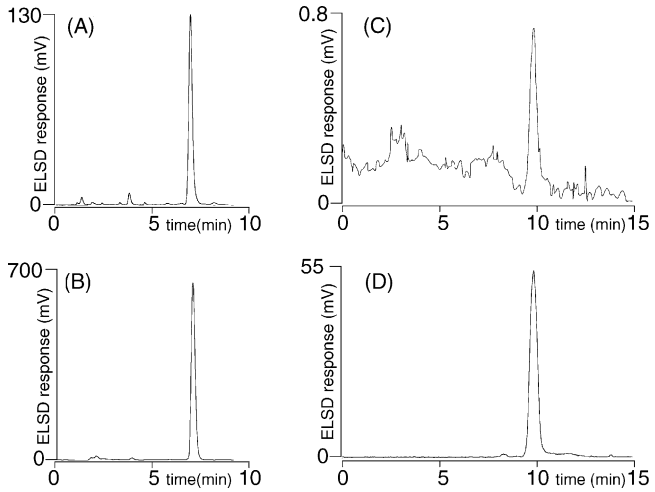

Fig. 5. Effect of triethylamine and formic acid (TEA:HCOOH) on ELSD response in conventional and capillary LC. Ceramide III 625 ng injected on the 2 mm i.d. column without (A) and with (B) TEA:HCOOH in mobile phase. Same solute, 40 ng injected on 300 μm i.d. column without (C) and with (D) TEA:HCOOH.

Table 3
Response of ELSD with different chromatographic systems and triethylamine and formic acid

Stationary phase, i.d. × length (mm) and particle size (μm)	Flow rate (mL min^{-1})	Retention time (min)	Apparent number of theoretical plates	Calibration range (ng)	Levels	a	Log (a) ± S.D.	b ± S.D.	r^2	F[a]	d.f.
ppp[b]											
Kromasil 4.6 × 250 (5)	1	8.7	10,000	44–6,600	7	2.57	0.41 ± 0.08	1.45 ± 0.03	0.996	3,056	12
Kromasil 2 × 250 (5)	0.3	7.0	4,300	44–4,400	5	1.42	0.15 ± 0.10	1.60 ± 0.03	0.994	2,441	14
Inertsil 1 × 150 (3)	0.05	5.6	3,300	15–250	5	2.50	0.40 ± 0.05	1.72 ± 0.06	0.997	5,092	15
Zorbax 0.32 × 150 (5)	0.005	11.3	3,300	12.5–250	5	4	0.6 ± 0.12	1.01 ± 0.02	0.967	266	5
Inertsil 0.3 × 250 (3)	0.008	7.3	4,700	30–1,000	7	1.34	0.13 ± 0.08	1.25 ± 0.03	0.996	1,272	12
CIII[c]											
Kromasil 2 × 250 (5)	0.3	9.9	2,800	250–10,000	6	1.95	0.29 ± 0.08	1.47 ± 0.03	0.997	3,464	10
Inertsil 0.3 × 250 (3)	0.005	7.0	6,200	40–400	4	1.8	0.25 ± 0.08	1.37 ± 0.04	0.999	1,225	6
Results with triethylamine and formic acid											
CIII[c]											
Kromasil 2 × 250 (5)	0.3	9.9	3,400	250–2,500	4	7.62	0.88 ± 0.07	1.54 ± 0.02	0.999	4,313	6
Inertsil 0.3 × 250 (3)	0.008	7.1	6,000	1–80	5	209.89	2.32 ± 0.02	1.26 ± 0.01	0.999	12,133	8
ppp[b]											
Inertsil 0.3 × 250 (3)	0.008	7.3	3,300	8–950	4	31.55	1.50 ± 0.17	1.06 ± 0.08	0.966	169	6

[a] F-test between regression-residual variance with associated degrees of freedom (d.f.).
[b] Mobile phase condition; acetonitrile:acetone (10:90, v/v).
[c] Mobile phase condition; propanol-1 and acetonitrile (50:50, v/v).

two different 300 μm i.d. columns, the b value decreased significantly to 1.01 or 1.25 despite differences in apparent plate numbers and retention time. Those values are in the range observed by Héron and Tchapla [8] using the same compounds with a similar mobile phase composition. Moreover, values shown in Table 2 indicate that the droplet size at the nebulizer outlet should be comparable or even smaller than for the 1 and 2 mm i.d. columns.

An explanation for this response shape can be found by calculating the particle size at the peak maximum (i.e. by combining Eqs. (2) and (3)). Fig. 6 shows the increase in particle diameter for a hypothetical solute injected at amounts between 1 and 1000 ng and with the same droplet size at the nebulizer outlet. It is clear that the particle size increases much faster in capillary LC than in micro or conventional LC. Thus, assuming that the same quantity could be injected on both type of columns, capillary chromatography is likely to produce bigger particles than conventional, narrow bore or even micro chromatography. This increase in particle size shifts the response away from the Rayleigh diffusion and the b term of Eq. (3) decreases. Still here, even when used in capillary LC, the ELSD system detector is not linear even if the occurrence of a linear relationship is higher.

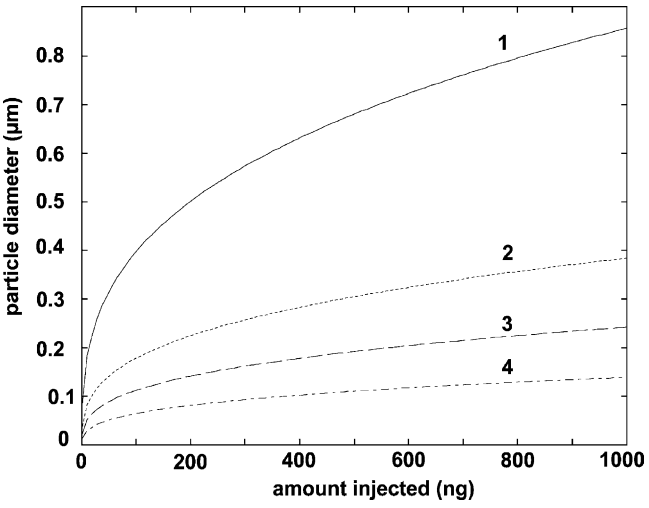

Fig. 6. Calculated particle size after solvent evaporation as a function of the injected amount for capillary (1), micro (2), narrow bore (3) and conventional columns (4).

A theoretical sensitivity increase from 1 to 40 can be calculated from Eq. (4) when comparing narrow bore and capillary LC. However, this expectation is usually not met and sensitivity improvements in the order of 10–20% for the expected value are found with conventional detection methods such as UV [17]. The only study comparing conventional, micro and capillary-ELSD [8] just focuses on the response shape without examining sensitivity. The calibration curves from Cobb et al. [9] only show that the slope of calibration curves is higher in capillary than micro LC. The sensitivity obtained with our different settings can be roughly compared by examining the a term of Eq. (4). Thus, Table 3 shows that the sensitivity does not increase with the decreasing diameter of our columns. The sensitivity appears comparable with our different settings except for the shorter capillary column. In addition to the lowest b value the calibration using the 0.32 mm i.d. × 150 mm capillary column is also the most sensitive with an a term around 4. The possible lowest efficiency of the nebulizer at micro-flow rates was envisaged from the results with the post column flow splitter.

Also, this can be a consequence of the low inner diameter of the capillary tube. Due to the log-normal distribution of the droplets' diameter at the nebulizer outlet, little droplets are more frequent than big ones [15]. It could then be assumed that our nebulizer produces a significant amount of small droplets that remain undetected and reduce the sensitivity. In the case of the 150 mm × 0.32 mm i.d. column used at a lower flow rate than the 0.3 mm i.d. × 250 mm droplets should be bigger but also more abundant. This may also explain the results of Cobb et al. who connected their micro and capillary column to the detector using the same 127 μm i.d. tubing. It can be thought that the lower flow rate they encountered in capillary LC significantly increased the droplet size at their nebulizer outlet compared to micro LC. A compromise between sensitivity and chromatographic efficiency is likely to be found to improve the intrinsic detection performances of a capillary-ELSD system.

3.3. Assessing the sensitivity improvement with triethylamine et formic acid in capillary-ELSD

Our group is interested in the sensitivity improvement of ELSD by the addition of triethylamine (TEA) and formic acid (HCOOH) for lipid analysis. This phenomenon was first reported by Gaudin et al. [18] and further investigated by Deschamps et al. [19,20]. As demonstrated in a later study [20] an equimolar amount of TEA and HCOOH added to the mobile phase increased the ELSD sensitivity, i.e. increased the *a* term of Eq. (4), but little or no effect was noticed on the *b* term of the equation. When applying an experimental setup consisting out of flow injection or size-exclusion chromatography (SEC) an enhancement in ELSD response with TEA/HCOOH was found to be solvent, solute, flow rate and temperature dependant. Tripalmitin was among the solutes with the lowest increase in response by this addition (1.6-fold) whereas ceramide III was one of the most affected (4.7-fold). These values were recorded with SEC with pure chloroform as a mobile phase. Higher values (up to 30×) were obtained with other solvents and ceramide III as test solute. The increase in sensitivity was assumed to be due to a supra-molecular assembly between the ion pair TEA:HCOOH and solutes. This explains the decrease of this effect with increasing drift tube temperatures. The sensitivity improvement with reduced flow rates remained unsolved even if a rearrangement of the particle distribution at low flow rates was suspected. It was thus attractive to evaluate this phenomenon in capillary LC.

Table 3 summarizes the results of calibration curves obtained with and without triethylamine and formic acid using ceramide III as solute and a 2.1 mm i.d. column. The *a* term of Eq. (4) increases with a factor of ∼4, the *b* value is only slightly affected. When using a 300 μm i.d. capillary column, the *a* term is multiplied by a factor of ∼160 while *b* decreases from 1.37 to 1.26. With tripalmitin in capillary LC, the *a* term increase is 23-fold and the *b* term decreases more than for ceramide III, from 1.25 and 1.06.

These results were in agreement with the assumption that TEA and HCOOH form an ion-pair which interacts with the solute in order to increase the particle size in the detection chamber. The use of this response enhancement method in capillary LC offers several advantages. By enhancing the sensitivity of ELSD, TEA and HCOOH allow the analysis of diluted solutions or low injected amounts, which is the natural scope of capillary LC. It can be pointed out that the detection limit of our capillary-ELSD system with ceramide III is of the same order of magnitude (0.5 ng) than the value reported by Molander et al. [6] who also used TEA:HCOOH as response enhancer. Moreover, by lowering the *b* term of Eq. (4), the dynamic range is widened allowing a calibration over two decades. In previous studies, we demonstrated that the response amplification reached a plateau for 0.1% (v/v) of an equimolar amount of TEA and HCOOH added in the mobile phase. A closer examination of the *b* value with TEA:HCOOH would need some additional studies to evaluate the possible dependence of *b* on TEA:HCOOH addition. A recent study, devoted to triacylglycerols shows that the *b* value can be modified in large extend by adding silver nitrate or cholesterol as additives [21].

In conclusion, despite of the predictable effect on droplet size consecutive to the reduction of the inner diameter of the capillary inside the nebulizer, ELSD appears to be simple to adapt to micro and capillary LC. The nebulization at micro flow rates has a low influence on the detector response shape but limits its sensitivity. The higher concentration profile associated with capillary LC influences the detector linearity rather than the diffused light intensity. When used with capillary columns the non-linearity of the detector is less pronounced. In addition, the response enhancement obtained with triethylamine and formic acid was found more pronounced in capillary LC than with narrow bore columns. This solute and solvent dependent phenomenon must be further investigated to ascertain its impact on response linearity. At the present time it allows to reach the nanogram limit of detection for ceramide III.

Acknowledgements

We thank Ann Detroyer for her careful reading of the manuscript and Eurosep Instruments for their technical assistance with the ELSD apparatus.

References

[1] A. Stolyhwo, H. Colin, G. Guiochon, J. Chromatogr. 265 (1983) 1.
[2] M.B. Andersson, L.G. Blomberg, J. Microcol. Sep. 10 (1998) 249.
[3] S. Hoffmann, H.R. Norli, T. Greibrokk, J. High Resolut. Chromatogr. 12 (1989) 260.
[4] R. Trones, T. Andersen, I. Hunnes, T. Greibrokk, J. Chromatogr. A 814 (1998) 55.
[5] R. Trones, T. Andersen, T. Greibrokk, J. High Resolut. Chromatogr. 22 (1999) 283.

[6] P. Molander, A. Holm, E. Lundanes, T. Greibrokk, E. Ommundsen, High Resolut. Chromatogr. 23 (2000) 653.
[7] J.N. Alexander, J. Microcol. Sep. 10 (1998) 491.
[8] S. Héron, A. Tchapla, J. Chromatogr. A 848 (1999) 95.
[9] Z. Cobb, P.N. Shaw, L.L. Lloyd, N. Wrench, D.A. Barret, J. Microcol. Sep. 13 (2001) 169.
[10] J.M. Charlesworth, Anal. Chem. 50 (1978) 1414.
[11] J.A. Koropchak, L.E. Magnusson, M. Heybroek, S. Sadain, X.H. Yang, M.P. Anisimov, Adv. Chromatogr. 40 (2000) 275.
[12] M. Dreux, M. Lafosse, L. Morin-Allory, LC-GC Int., March (1996) 148.
[13] S. Nukiyama, Y. Tanasawa, Trans. Soc. Mech. Eng. 4 (1938) 86.
[14] P.J. Schoenmakers, Optimization of Chromatographic Selectivity, Elsevier, Amsterdam, 1986, p. 308.
[15] P. Van der Meeren, J. Vanderdeelen, L. Beart, Anal. Chem. 64 (1992) 1056.
[16] A. Stolyhwo, H. Colin, M. Martin, G. Guiochon, J. Chromatogr. 288 (1984) 253.
[17] J.P. Chervet, R.E.J. Van Soest, J.P. Salzman, LC-GC Int. 5 (1992) 33.
[18] K. Gaudin K, P. Chaminade, D. Ferrier, A. Baillet, A. Tchapla, Chromatographia 49 (1999) 241.
[19] F.S. Deschamps, K. Gaudin, E. Lesellier, A. Tchapla, D. Ferrier, A. Baillet, P. Chaminade, Chromatographia 54 (2001) 607.
[20] F.S. Deschamps, A. Baillet, P. Chaminade, Analyst 27 (2002) 35.
[21] S. Heron, M. Dreux, A. Tchapla, J. Chromatogr. A 1035 (2004) 221.