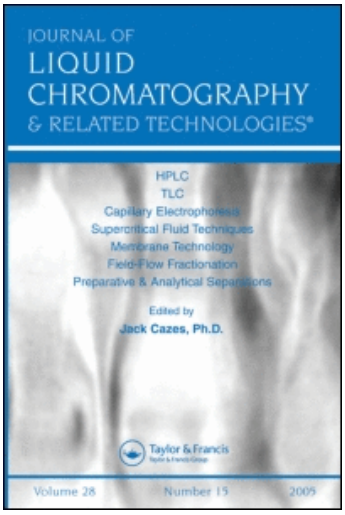

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INFLUENCE OF VARIOUS PARAMETERS ON THE RESPONSE FACTORS OF THE EVAPORATIVE LIGHT SCATTERING DETECTOR FOR A NUMBER OF NON-VOLATILE COMPOUNDS

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ABSTRACT

We have investigated the influence of the most important operation parameters of the evaporative light scattering detector (ELSD) on its response factors. When the detector is operated under proper experimental conditions, the repeatability of the response factor is around 1%. The response factors and the signal to noise ratio are maximum for a certain flow rate of scavenger gas, which depends much on the exact dimensions of the nebulizer. The temperature of the drift tube has no effect, as long as the vapor pressure of the solvent is larger than ca 200 torrs. The nature of the mobile phase has some effect, related to the surface tension and viscosity of the solvent used. The most important factor, however, is the nebulizer itself. Replacing the nebulizer by another one, made with the same tubes, or changing slightly the position of the liquid nozzle in the gas nozzle may change markedly the response as well as its relationship with sample size. The peak area increases exponentially with increasing sample size. but the exponent depends very much on the exact design of the nebulizer. Similar response factors are obtained for fatty acid methyl esters, triglycerides, sugars, polynuclear aromatic hydrocarbons and polystyrene.

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INTRODUCTION

The evaporative light scattering detector (ELSD) derives from early work by Charlesworth [1] and McRae [2]. The principle of the detector is that of a transport detector [3], using a scavenger gas stream rather than a material conveyor. The effluent is nebulized immediately at the exit of the chromatographic column, in a concentric nebulizer, in a stream of warm gas. The solvent vaporizes and leaves a cloud of particles made by the non-volatile content of the eluate. This contains the solutes as well as the non-volatile residue of the solvent. These particles are carried by the gas stream across an intense light beam. The amount of scattered light collected on a photocell or a photomultiplier is a measure of the amount of non-volatile solute in the effluent stream.

Further theoretical studies have been published on the detector mechanism by Mourey and Oppenheimer [4,5,6,7] while Stolyhwo et al. designed an improved version with a much lower detection limit and the capability to work with narrow bore diameter columns [8,9,10]. A number of papers have described applications of this detector to the analysis of triglycerides and other lipids [9-10,11,12,13].

At first, it would seem that the response of the detector is predictable, since the work of Mourey and Oppenheimer has resulted in good agreement between the experimental response factors and the values calculated on the basis of the Atkinson equation (see equation 2) and the Mie theory of light scattering [4-7]. Their work has also predicted that the detector response cannot be truly linear in a significant mass range, even on a log-log plot, but that it is rather sigmoidal, that the response factors also depend on the nature of the mobile phase (namely, on its density, viscosity and surface tension), and to some extent on the

refraction index of the solute. However, there is little experimental data available to confirm or infirm these predictions, beyond the limited data presented ([4-7]). For almost all chromatographic detectors known, the response does not vary exactly as predicted by theory. Sometimes the discrepancy is negligible, but in most cases it is very important. Work recently published has shown that the droplet size distribution of the aerosol reaching the light scattering cell does not always correspond to the prediction of the simple theory and that the variations of the response factors resulting from a change in the mobile phase do not seem to be predictable ([14]).

Thus, we decided that it was necessary to undergo a systematic study of the influence of the detector parameters on its response, with emphasis on the nebulizer design and operation, before studying other transport detectors of the same class. This is of special importance in the case of the ELSD, because of some of its particular properties and because of contradictions found in the literature. Experimentalists have reported that the response factors do not depend on the nature of the analytes ([2,3,8-13]). On the other hand, theory predicts a dependence on the refraction index ([4-7]). In practice, this is not necessarily contradictory, because the effect may be small and lower than the precision of the measurements made so far, especially since most determinations have been done for closely related compounds which may have very similar refractive indices. Theory predicts a significant influence of the nature of the solvent used on the response (see Theoretical section below), whereas it has been suggested that the detector could be used for quantitative analysis in gradient elution ([10]). Clearly, the response factor depends on the characteristics of the nebulizer.

We report here experimental results obtained with one detector using several nebulizers, with compounds of different chemical groups, different solvents, and under different experimental conditions. Two nebulizers of very different design have been used in this work.

THEORETICAL

At the exit of the column, the eluent stream is nebulized, and the cloud of droplets formed is carried by a scavenger gas stream through a drift tube where the solvent vaporizes. The droplets shrink to the volume of non-volatile material contained in the eluent. The particle diameter is related to the diameter of the original droplet by:

$$D = d_p \, (C/d)^{1/3} \qquad (1)$$

where:

- D is the particle diameter,
- d_p is the diameter of the original droplet,
- C is the concentration of the non-volatile material in the droplet,
- d is the density of this material.

Accordingly, the average particle size in the cloud at a given time and the particle size distribution can be derived from the elution profile of the analyte and the droplet size distribution given by the nebulizer.

The average diameter, D_o, of the particles formed in a concentric nebulizer is given by the Atkinson equation ([15]):

$$D_o = \frac{A \, \sigma_l^{1/2}}{u \, \rho_l^{1/2}} + B \left[\frac{\eta_l}{(\sigma_l \, \rho_l)^{1/2}}\right]^{0.45} \left[\frac{1000 \, Q_l}{Q_g}\right]^{1.5} \qquad (2)$$

where:

- A and B are constants. With D_o in um, these constants are 585 and 597, respectively ([5]),

- σ_l is the surface tension of the mobile phase,

- ρ_l is the density of the mobile phase,

- η_l is the viscosity of the mobile phase,

- u is the relative velocity of the gas and liquid streams in the nebulizer (i.e., the cross-section average velocity of the gas stream between the gas and the liquid nozzles, minus the cross-section average velocity of the solvent in the liquid tube),

- Q_l is the volume flow rate of the mobile phase,

- Q_g is the volume flow rate of the scavenger gas.

Equation 2 predicts that the average droplet size decreases with increasing gas flow rate, since then both terms of equation 2 decrease, and with decreasing solvent flow rate, since in both cases the relative velocity increases and both terms of equation 2 decrease. It also predicts that the average droplet size will depend on the nature of the solvent, for given values of the liquid and gas flow rates, because of the dependency of the two terms on the density, surface tension and viscosity of the nebulized liquid.

Whereas equation 2 seems to predict reasonably well the average droplet size ([4-7]), there is no model in the literature predicting the droplet size distribution, which, from empirical, but limited evidence, appears to be a log - normal distribution with a standard deviation comparable to half the logarithm of the average droplet diameter ([4-7]).

As explained above, the droplets formed in the nebulizer shrink while the solvent vaporizes in the drift tube. When all the solvent is vaporized, the diameter of each particle is related to that of the

original droplet by equation 1. The average droplet size being between 5 and 15 um in the nebulizer, the average particle size for a solute at a 1 ppm concentration will be between 0.05 and 0.15 um. This is just at the limit between the Mie and the Rayleigh regions of light scattering, located approximately at a ratio of the particle diameter to the light wavelength of 0.2. Particles corresponding to larger concentrations will scatter light as predicted by the Mie theory.

In the Rayleigh region the amount of light scattered is proportional to the square of the particle volume and to the reverse of the fourth power of the light wavelength. It is also function of the complex refractive index of the solute, which depends on the normal refractive index and the absorptivity at the wavelength used. It is maximum in the direction of the incident light and minimum in the perpendicular direction. The amount of light scattered in the Mie region is a more complex function of the same parameters. Detailed calculations have been carried out by Mourey and Oppenheimer ([4-7]). They predict a response curve with a sigmoidal shape.

Theory permits a few conclusions regarding the design and operation of the nebulizer. In order to maximize the response factors, we need to produce as large as possible solvent droplets. The upper limit will be set by the requirement of properly operating the nebulizer under steady conditions. For example, we want to operate the nebulizer at the lowest gas flow rate compatible with its steady state behavior, since both terms of equation 2 increase with decreasing gas flow rate. If the scavenger gas flow rate is too low, however, some huge droplets are occasionally formed, resulting in spikes on the detector response. Although these spikes can be eliminated by software, their number should be minimized to avoid errors in quantitative analysis. This concern should guide in the

determination of the minimum gas flow rate used. It seems that proper operation of a concentric nebulizer requires a gas velocity of the order of the sound speed. In order to achieve that velocity with as small as possible a volume flow rate, we need a small nebulizer. Then equation 2 will be reduced in practice to the first term. The response should not depend on the solvent viscosity but on the ratio of the surface tension to the density.

Data regarding the solvents most commonly used in liquid chromatography are reported Table I, together with the values of the two coefficients of equation 2 corresponding to the optimum set of experimental conditions adopted in this work. The range of variation is not large and the possibilities of adjustment are limited.

T A B L E I

Coefficients of Equation 2 for different Solvents

Solvent	Viscosity (cP)	Density (g/mL)	Surface Tension dyne/cm	Coef 1	Coef 2	Droplet Diameter (um)
Acetone	0.31	0.787	23	3163	184	4.76
Chloroform	0.53	1.498	27	2484	195	4.03
Ethyl Acetate	0.45	0.924	23.6	2956	208	4.93
Acetonitrile	0.32	0.783	29	3560	177	5.14
Methanol	0.52	0.791	22.6	3127	232	4.97
Benzene	0.65	0.879	28.5	3331	238	5.17
n-Heptane	0.38	0.684	19	3083	217	4.78

**G.W.C. Kaye and T.H. Laby, Tables of Physical and Chemical Constants, Longmans, Green and Co, London, UK, 1956.

EXPERIMENTAL

One of the detectors used has been described in detail previously ([3,8,9]). The only important differences are in the data acquisition system, which uses a microcomputer and in the nebulizer. Experiments have also been made with a Varex (Rockville, MD) LSD.

In some of the experiments, the column effluent is split between the nebulizer and a conventional UV detector. The axial connecting tube to the ELSD, is 0.1 mm i.d.. The perpendicular connecting tube, to a Nupro fine metering valve and the UV detector, is 0.3 mm i.d.. The flow rate to the nebulizer is kept at 0.3 mL/min. The excess solvent (ca 0.7-0.8 mL/min) is sent to the UV detector or to waste. In experiments where the detection limits are determined, the entire column effluent is sent to the detector.

The gas stream is carefully filtered before admission to the nebulizer. Its flow rate is controlled and measured. It is heated at a controlled temperature, adjustable between 25 and 100C.

The light beam used is produced by a 1 mw He-Ne laser (Hughes Aircraft Co, Carlsbad, CA; wavelength: 632 nm). The scattered light is collected by an optical fiber and its power measured by a photocell.

The signal is acquired with an IBM PC microcomputer (IBM, Boca Raton, FA), using Labtech Notebook (Laboratory Technology, Cambridge, MA) software. The acquisition frequency is 5 Hz. The base line is determined from two sets of points acquired before and after the peak, by drawing a straight line between the mass center of each set. The peak area is calculated by summing up the differences between the signal and the

interpolated base line for each data point acquired during the elution of a peak. Determinations of detection limits have been made using a Hewlett-Packard 3392A integrator, directly connected to the photocell and to the computer, for data storage.

For the study on the detector response no column was used, but the sampling valve (Rheodyne, Berkeley, CA, model 7125) was connected to the ELSD through a 30 cm long, 0.25 mm i.d. tube. The sample volume was 10 or 20 μL. The peak obtained was somewhat unsymmetrical. Its elution lasted approximately 15 seconds.

Three different nebulizers were studied. The two concentric nebulizers were both built after the same design as those previously used ([3,8,9]). A 0.007" i.d., 1/32" o.d. capillary tube carries the liquid stream to the nozzle. The gas stream arrives through a concentric tube 0.02" i.d., 1/16" o.d.. The tip of the liquid feeder is placed inside the gas tubing, at an adjustable distance from its end, between 1/16 and 1/2". Both concentric nebulizers were very similar, although the length of the liquid tube was slightly longer for the second one.

The solvents used where chromatography grade, from J.T. Baker (Phillipsburg, NJ). The solutes were from Sigma (St Louis, MO).

RESULTS and DISCUSSION.

I - Background Signal and Base Line Noise.

The dark current observed without light beam is 5pA. When the laser is on, the current is 0.2 nA and the background noise 10 pA. It results probably from diffraction of the laser light beam on the window of the

cell. There is no increase of the background signal or noise when the solvent flow is switched on, as long as filtered, freshly distilled or pure HPLC grade solvent is used.

II - Influence of the carrier gas flow rate.

In this study, the mobile phase flow rate has been kept constant at 0.3 mL/min. A plot of the response for a constant sample amount versus the scavenger gas flow rate exhibits a maximum at some intermediate flow rate, and so does the plot of the signal to noise ratio versus flow rate. The two maxima are achieved for different flow rates, however. At large flow rates the decrease in response due to an increase in the gas flow rate is easy to understand. The average particle size of the solute cloud decreases with increasing gas flow rate (see equation 2). The response decreases accordingly. At low flow rates the nebulizer does not work properly. The response decreases rapidly with decreasing flow rate, the noise increases and spikes appear. This is related to the fact that the flow velocity of the scavenger gas in the concentric nebulizer should be in the sonic range in order for the nebulizer to function properly. A low gas flow rate results in very large droplets which vaporize too slowly, hence the spikes ([3,4]).

The maximum response is observed for a flow rate of approximately 2.7 L/min, the maximum signal to noise ratio for a flow rate of 3.6 L/min. At this flow rate, however, a change in the gas flow rate of 1% results in a change of the response of about 1%. A good flow rate control is thus necessary for good quantitative performance of the detector.

Table II gives statistical results on a systematic study of the repeatability of the detector response. A standard deviation of 1.1% is achieved for 13 measurements made at 4.5 L/min, a value for which the

T A B L E I I

Repeatability of Peak Area Determination

Analysis #	Peak Area
1	3.12
2	3.14
3	3.16
4	3.15
5	3.11
6	3.11
7	3.16
8	3.16
9	3.15
10	3.11
11	3.16
12	3.19
13	3.23
Average	3.15
RSD (%)	1.05

Sample: 59.3 μg of olive oil.
Solvent flow rate: 0.3 mL/min
Gas flow rate: 4.5 L/min.
Drift tube temperature: 55C.

response varies rapidly with the flow rate. The error propagation coefficient is now about 3.5. (The change in peak area is 3.5% for a 1% change in the scavenger flow rate). This demonstrates, however, that it is possible to achieve extremely good control of the detector parameters. The repeatability of the sampling valve and the flow rate fluctuations taking place during injection are the most important error contributions under these experimental conditions.

III - Temperature of the Drift Tube.

The solvent contained in the droplets formed in the nebulizer must be completely vaporized during the migration of these droplets down the

T A B L E I I I

Influence of the Drift Tube Temperature on the Response Factor

Temperature (C)	Peak Area
25	18.6
30	18.8
35	18.6
37	18.9
40	18.9
45	18.9
50	18.8
60	18.7
70	18.9
80	19.0
90	18.9
100	19.0
Average	18.8
RSD (%)	0.70

Sample: 37.1 μg of olive oil.
Solvent flow rate: 0.3 mL/min
Gas flow rate: 3.5 L/min.

drift tube. Thus a compromise between the flow velocity of the scavenger gas and the temperature of the drift tube has to be chosen. The temperature must be low enough that the analytes are not vaporized, which would result either in a systematic error (small extent of analyte vaporization) or in a total loss of signal (total vaporization of analyte). We have limited our investigations to organic solvents used for the analysis of oils and fats.

Table III gives the response factors for trioleine measured at increasing temperature from 25 to 100 C. There is no trend in the series of measurements. For the 12 data points the relative standard deviation

is only 0.7%, which is even better than the repeatability measured at 35C for 13 measurements (see previous section and Table II). This better repeatability may be explained by the use of a lower gas flow rate, at which the error propagation coefficient is only 1.

The results in Table III demonstrate that the residence time of the droplets of solution in the drift tube is large enough and the kinetics of heat transfer in this tube fast enough to ensure complete vaporization of the solvents used in this work (chloroform and acetonitrile). Data regarding the vapor pressure and the latent heat of vaporization of solvents used in HPLC are reported in Table IV. These data explain why the vaporization of the solvent is easy with acetone, acetonitrile or chloroform but difficult with water. In this case, a compromise between a low residence time (small nebulizer, narrow drift tube), giving a small detector time constant, and a rather low scavenger gas velocity (large nebulizer, wide drift tube), permitting a complete vaporization of the solvent, has to be chosen. With a gas flow rate of 4.5 L/min, the average residence time of the droplets in the drift tube is less than 10 msec, which is short to permit the vaporization of water. Indeed, a response time below 0.01 sec is rarely necessary.

IV - Response Factor.

As firmly established on theoretical and experimental ground by Mourey and Oppenheimer ([4-7]), the response of the evaporative light scattering detector is not linear. Although this work was performed on a different ELSD, using a much wider and longer drift tube and a larger scavenger gas flow rate, these conclusions apply to all light scattering detectors. They merely result from the fact that droplets scatter light with an intensity which increases much faster than the third power of their diameter.

T A B L E I V

Physical Properties of the Solvents Used in HPLC

Solvent (C)	Vap. Pr. (40C)	Vap. Pr. (80C)	Vap. Pr. (100C)	Heat of Vaporiz	Visc. (cP)	Dens. (g/mL)	Surf. Tens.
Acetone	400.0	Eb @ 56.5C		119.08	0.31	0.787	23.7
Chloroform	3.4	27.2	63.1	59.26	0.53	1.498	27.1
Carbon Tetrachloride	214.9	843.4	1475.4	43.82	0.97	1.632	17.3
Toluene	59.1	291.2	556.3	86.80	0.58	0.867	28.4
n-pentane	867.1	2753.5	4420.2	85.40	0.21	0.626	16
Heptane	92.5	427.7	795.7	75.60	0.38	0.684	19.3
Methanol	400 @50C	Eb @ 64.7C		290.50	0.52	0.791	22.6
Acetonitrile	169.1	733.9	1334.6	183.50	0.32	0.783	29.6
Water	55.2	355.2	159.9	540.00	1	0.996	72.7
Ethyl acetate	200 @42C	Eb @ 77.1C		94.20	0.45	0.924	23.6

Vap. Pr.: Vapor Pressures in mm Hg.
Heat: Latent heat of Vaporization, in cal/g.
Visc.: Viscosity, centiPoise.
Dens.: Density, g/mL
Surf. Tens.: Surface Tension, Dyne/cm.

* R.R. Dreisbach, Physical Properties of Chemical Compounds American Chemical Society, Washington, DC, 1955, 1959, 1951
**G.W.C. Kaye and T.H. Laby, Tables of Physical and Chemical Constants, Longmans, Green and Co, London, UK, 1956.
*** Handbook of Chemistry and Physics, CRC Press, Cleveland, OH, 1977.

When the solution is nebulized at constant mobile phase and scavenger gas flow rates, the size distribution of the eluent droplets remains constant. It does so also during the elution of the analyte, provided the surface tension of the solution does not change significantly (see equation 2), which is probable with most organic solution, but may not be always true for the aqueous solutions used in

reversed phase analysis. During the vaporization of the solvent the droplets shrink and their final volume is proportional to the analyte concentration. Hence, the response of the detector cannot be linear but is given by:

$$y = a \quad m^b \qquad (3)$$

where a and b are numerical coefficients.

As a result, plots of the peak height or peak area versus the sample size in double logarithmic coordinates are linear with a slope b. Also, the column efficiency appears to be multiplied by b ([8]). Experimentally, b is always smaller than 2, the value which would be obtained if light scattering were to follow the Rayleigh law. This is not surprising, since we are using a 632 um wavelength light beam and the average diameter of the particles is larger than 0.05 um for a 1 ppm concentration.

The intensity of the light beam collected by the optical fiber and transmitted to the photomultiplier is the integral over a rather large solid angle of the light scattered by the analyte particles. The intensity of the light scattered in any direction by a particle is a function of the direction, of the particle size and of the wavelength of the incident light. The averaging process involved in the light collection ensures that the signal increases monotonically with increasing sample size, but it cannot be made linear ([6]).

In a large range of sample size, however, the response follows equation 3. A plot of the logarithm of the peak area versus the logarithm of the sample size is a straight line, with a slope b and an ordinate a ([3-11]). Beyond a certain sample size the response increases more slowly than predicted by equation 3. The phenomenon does not result from a saturation of the photocell which remains linear in the range

investigated. It has been described and explained by Oppenheimer and Mourey and is related to the particle size distribution ([4-7]).

Values of the coefficients a and b (see equation 2) are reported in Tables V to VIII, for different series of measurements, corresponding to the calibration of the ELSD for various compounds or to the study of the influence of different experimental conditions on the response factors. The parameters a and b are derived by a linear regression of the data points (logarithm of the peak area versus logarithm of the sample size). A value is also obtained for the confidence interval.

The detection limits result from the combined effects of the response factor for a given compound and of the base line noise. It is related to the peak height, not the peak area. It depends on the band width of the peak obtained, i.e., on the quality of the chromatograph and the column, and on the retention of the analyte considered. Accordingly, it may vary rather largely from one column to another, for a given analyte, and from analyte to analyte, on a given column. With our instrument, for triglycerides analyzed in non-aqueous reversed phase conditions, with a concentric nebulizer, it is of the order of 30 to 100 ng. For pyrene analyzed in normal phase chromatography, with n-hexane as mobile phase and a T-shape nebulizer, Righezza and Guiochon ([14]) found a detection limit of about 3 ng.

V - Influence of the Instrument Parts on the Response.

We have studied the influence of the laser power, of the design of a concentric nebulizer, and of the design of the light scattering cell on the response of the ELSD for olive oil. In the next section we report on the comparison between the performance of a concentric and a T-shape nebulizer.

T A B L E V

Response of different
Light Scattering Detectors

Slopes (b)	Horizontal Cell	Vertical Cell
Nebulizer #1	1.46 +/- 0.04	1.55 +/- 0.04
Nebulizer #2	1.26 +/- 0.04	1.33 +/- 0.03

Intercepts (log a)	Horizontal Cell	Vertical Cell
Nebulizer #1	-1.41 +/- 0.04	1.10 +/- 0.03
Nebulizer #2	-1.75 +/- 0.03	0.73 +/- 0.03

Response for 10 ug	Horizontal Cell	Vertical Cell
Nebulizer #1	1.12	446.
Nebulizer #2	0.32	115.

Sample: Olive oil.
Gas Flow Rate: 3.5 L(NTP)/min.
Liquid Flow Rate: 0.3 mL/min.
Drift tube temperature: 55C.

V.a - Influence of the Laser Power.

Replacing the 1 mW He-Ne laser used in our first experiments ([3,8,9]) by a similar one from the same manufacturer, with a 10 mW power results in an improvement of the signal to noise ratio by a factor 4.

V.b - Influence of the Nebulizer.

We have used two different concentric nebulizers (see Section "Experimental"), for which the results regarding glycerol trioleate are

T A B L E V I

Influence of the Nature of the Eluent on the Response of the ELSD

Solvent	Acetone	CHCl3	n-C7H16	CCl4	n-C5H12
Slope (a)	1.32	1.28	1.32	1.36	1.22
Intersection (b)	-0.73	-0.43	-1.01	-0.48	0.76
Solvent Viscosity	0.285	0.514	0.356	0.843	0.215
Solvent Surface Tension	22.01	26.53	19.27	26.15	15.00
Density	0.7899	1.4832	0.6838	1.5940	0.6262

(a) Standard deviation: 0.02 (1.4%).
(b) Standard deviation: 0.02, except for pentane 0.03

Viscosity in cP, Surface tension in dyne/cm,
Density in g/mL.

Sample: Pure Olive Oil.
Solvent flow rate: 0.3 mL/min.
Gas flow rate: 3.5 L(NTP)/min.
Temperature of the drift tube: 55C.

reported in Table V. The noise observed was the same in all cases, but the characteristics of the response were significantly different, although the nebulizers were made successively, using the same tubes for the gas and liquid lines. The difference was essentially in the position of the liquid nozzle inside the scavenger gas tube. The detection limits are about three times lower with the first nebulizer than with the second.

T A B L E V I I

Response of the First Detector

Compounds	Slope	Intersection
Methyl Oleate	1.49	13.1
Tricaprine	1.57	12.2
Trioleine	1.59	12.5
Olive Oil	1.53	12.3
Linseed Oil	1.55	12.5

Solvent: Acetone. Flow rate: 0.3 mL/min.
Gas flow rate: 4.5 L/min.
Drift tube temperature: 55C.

Standard deviation: 0.02 (1.4%).
Standard deviation: 0.1 (0.8%).

The response of any one of these nebulizers was very repeatable (see Tables II and III), even on a long term basis, but recalibration was necessary each time they were taken apart for cleaning and reassembled.

V.c - Influence of the Cell Design.

Two cells have been used. One of them has been described previously ([3,8]). In this cross-path design, the laser beam travels perpendicularly to the scavenger gas stream which carries the analyte particles vertically. The scattered light is collected by an optical fiber in the third perpendicular direction. In this cell the path length of interaction between the light beam and the particulate stream is short, a few mm at most.

T A B L E V I I I

Response of the Second Detector

Compounds	Solvent	Slope (a)	Intersection (b)
Trioleine	Acetone	1.32	- 0.73
Fructose	Methanol	1.31	- 2.63
Maltose	Methanol	1.34	- 2.73
Glucose	Methanol	1.31	- 2.75
Fructose	CH3OH-H2O*	1.31	- 2.71
Sorbose	CH3OH-H2O*	1.32	- 2.68
Saccharose	CH3OH-H2O*	1.34	- 3.25
Xylose	CH3OH-H2O*	1.45	- 3.02
PS Mw=4250	Toluene	1.42	- 1.72
PS Mw=39,000	Toluene	1.44	- 1.90
PS Mw=505,000	Toluene	1.40	- 1.94
Pyrene	Ethanol	1.30	- 0.70

* Methanol: 80%, Water: 20% (v/v).

Solvent flow rate: 0.3 mL/min.
Gas flow rate: 4.5 L/min.
Drift tube temperature: 55C.

(a) Standard deviation: 0.02 (1.4%).
(b) Standard deviation: 0.1 (0.8%).

The other cell is a parallel-path designed so that the laser beam and the gas stream follow the same path. The stream of analyte particles may interact with the photons in the light beam over a much longer distance (10 to 20 times longer), and we expected a higher signal to noise ratio from this design.

The noise level recorded was the same with the two cells. The response observed was much smaller with the new horizontal cell, however, and this design was abandoned. We observed that, because the gas stream

is curved at the cell inlet, a large fraction of the droplets hit the wall and adhere to it. They are lost for detection. Probably the proportion of lost droplets increases with increasing diameter, which explains the large decrease of the response.

All determinations have been made on the cross path cell, already described ([3]).

VI - Response Functions of Various Compounds.

We have determined the response curves for the following series of compounds: (i) different triglycerides, in solution in acetone (Table VII), (ii) different sugars, in solution in methanol or water/methanol mixtures (Table VIII), (iii) various samples of polystyrene with different molecular weights, in solution in toluene (Table VIII) and, finally, pyrene, in solution in ethanol (Table VIII).

We have had to use methanol/water mixtures (80/20) for the determination of the response curves of sugars (Table VIII), because our present detector cannot operate with water. The maximum temperature at which we may operate the drift tube and the gas velocity in this tube prevent complete vaporization of the water. The gas must be heated at 90C to achieve good results with the methanol/water mixture. Because of the fast flow velocity of the gas, however, and because of the finite rate of heat transfer across the gas stream, it is most probable that the temperature in the particle cloud is not so high, which explains the difficulties with water, which has a very high latent heat of vaporization.

The same response was obtained for fructose in pure methanol and in the methanol/water mixture used. The responses are very similar for all sugars studied, except xylose.

The variation of the refractive index from one compound to another in each series of compounds studied are too small to permit an investigation of the correlation between this index and the response. The difference between the response obtained for different series may be ascribed, at least in part, to the influence of the solvent, through their surface tension, viscosity and density (see Tables I and IV).

The comparison between the responses obtained for different compounds in different conditions is easier when one keeps in mind that the ordinate, a, in the Tables is in fact the response for a 1 μg sample. The larger the response, the larger the sensitivity of the detection and the lower the detection limits.

The response for the different samples of polystyrene are very close. There is no discernible effect of the molecular weight. This shows that the ELSD can be used in size exclusion chromatography as well as with field flow fractionation ([7]). It will be noted that for polystyrene samples the slope, b, is larger than for most other compounds studied, while the ordinate is lower.

There is no clear correlation between the melting point of solutes and their response. Some compounds such as methyl stearate, methyl arachidonate, phospholipids, give a response which is much larger than the one obtained with most other compounds. This could be accounted for by assuming (i) that their presence changes the surface tension of the eluate, hence the average droplet size, or (ii) that they have time to solidify during their migration down the drift tube, or (iii) that their refractive index is different. Assumption ii and iii may be related, solids may have a refractive index significantly different from the one of liquids. In order for a compound to crystallize in the drift tube, two

conditions must be met. First, its melting point must be above the drift tube temperature. Secondly, a crystal germ must appear in almost each droplet during the short time spent by the particle in the drift tube, and it must grow to involve all the matter in the particle. Most organic compounds which meet the first requirement are not going to satisfy the second one.

Assumption i may be ruled out by the parallelism of the response lines. An effect due to a change in the surface tension of the solvent by the analyte should appear and increase progressively, resulting in a change of the coefficient b as well as a. The refractive index of these compounds in their liquid state is very similar to the one the other compounds of the series. This leaves assumption ii or a combination of ii and iii. We note that pyrene gives the same response as triglycerides, although its melting point is 156C and it condenses solid readily.

CONCLUSION

The evaporative light scattering detector is not a truly mass detector as it has sometimes been called. The response depends to some extent on the nature of the solvent, and the response per unit weight varies quite significantly from analyte to analyte, albeit to a much lesser degree than with other non-selective detectors, and especially than with the refractive index detector.

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