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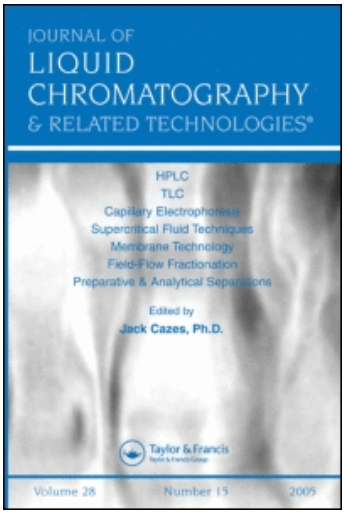

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EFFECTS OF THE NATURE OF THE SOLVENT AND SOLUTES ON THE RESPONSE OF A LIGHT-SCATTERING DETECTOR

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ABSTRACT

The effects of the nature of the solvent and solutes on the response of an evaporative light scattering detector (ELSD) are discussed. Data have been measured for six solutes with seven different solvents using concentric and cross-flow nebulizers. The experimental results suggest that the influence of the solvent on the properties of a dried aerosol are not properly accounted for by the equation of Nukiyama and Tanassawa. A modification of the droplet size distribution of the aerosol takes place, probably during the vaporization of the solvent. The amount of scattered light depends strongly on the molar absortivity of the solute at the light beam wavelength. Accordingly, the detector response depends on the properties of the solvent and the solute. The ELSD is not a mass detector and calibration is required for quantitative analysis.

INTRODUCTION.

The aim of this paper is to study of the influence of solvent properties (surface tension, density, viscosity) and solute

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properties (refractive index, melting point) on the signal of an evaporative light scattering detector (ELSD) in liquid chromatography. The performance of this detector has already been described (1-7). Interesting developments have been reported but no cohesive model has yet been developed. No comprehensive study of the influence of experimental parameters on the signal has been published. In other areas, such as atomic spectrometry (8-11) or mass spectrometry (12), some authors have demonstrated the effect of the surface tension of the sample solution on the analytical response through the modification in the droplet size distribution. There are reasons to believe that similar effects should take place with the ELSD.

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The different parts of the ELSD, such as the nebulizer, the drift tube and the light scattering cell have their own properties and influence differently the detector response. We experience intricate processes involving aerosol generation, redistribution and condensation, as well as the detection of a heterogeneous aerosol by light scattering. The influence of the solvent is most significant during aerosol formation and evolution, as is the solute in the detection cell.

In the theoretical section, equations describing the phenomena of nebulization and light scattering are examined. In the experimental section the properties of two conventional pneumatic nebulizers, of concentric and cross-flow designs respectively, and especially their relative sensitivity to the nature of the solvent, are studied. Finally, a qualitative comparison between the results obtained for different samples is presented.

THEORETICAL

I Nebulizer.

The column effluent is nebulized into a primary aerosol which is dried in a drift tube where the solvent vaporizes. The aerosol droplets may merge during their transit. The main characteristics of the performance of the combination of a nebulizer and a drift tube are: 1) The average particle diameter of the aerosol at the system outlet, 2) the polydispersity of this aerosol, and 3) the amount of solution entering the optical cell.

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The properties of aerosols prepared by nebulization of an analyte solution have been extensively studied, especially in atomic spectroscopy (8-16). Grigor'ev et al. (9) have shown that the droplet size distribution of the primary aerosol can be represented by the equation:

$$f(D) = a \cdot D^{n} \cdot e^{-b D^{s}} \qquad (1)$$

where a, n, b and s are constants and D is the aerosol droplet diameter. Gustavsson (13,14) has suggested other equations which are similar to equation 1. All these equations consider the droplet size distribution to be a log-normal Gaussian distribution, centered on the average aerosol droplet diameter D_0. Mourey et Oppenheimer (3) have developed a different model based on an asymmetrical log-normal distribution, using the upper-limit droplet size distribution of Mugele and Evans (15) and assuming that D_0 can be calculated from the Nukiyama and Tanassawa (16) empirical equation:

$$D_0 = \frac{585\ \sigma^{\frac{1}{2}}}{(v_g - v_l)\ \rho^{\frac{1}{2}}} + 597 \left(\frac{\mu}{(\sigma\ \rho)^{\frac{1}{2}}}\right)^{0.45} \left(\frac{1000\ Q_l}{Q_g}\right)^{1.5} \quad (2)$$

where σ is the liquid surface tension of the solution (dyne/cm), ρ is the solution density (g/ml), μ its viscosity (poise), $v_g - v_l$ is the difference between the gas and liquid velocities in the nebulizer (m/s), and Q_l/Q_g is the ratio of liquid to gas volumetric flow rates. At constant gas and liquid flow rates, the average droplet diameter is a function of the solution surface tension, density and viscosity.

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Previous publications (9,10,13,14) have shown good correlation between calculated values and experimental results derived from measurements of the primary droplet size distribution directly at the outlet of the nebulizer. Equation 2 is applicable for liquid densities between 0.7 and 1.2 g/ml, surface tensions between 19 and 73 dyne/cm and liquid viscosities between 0.003 and 0.5 P. In addition, this equation is only valid when the average droplet diameter (D_0) is in the range 15-90 µm. Briton (17) has shown that equation 2 is valid for concentric and cross-flow nebulizers as well, when using gases having supersonic velocities (460-680 m/s) expanded in Laval nozzles.

This last restriction means that the first term of equation 2 depends mainly on $(\sigma/\rho)^{\frac{1}{2}}$, i.e, on the nature of the solution, since the ratio $585/(v_g - v_l)$ will have to remain approximately constant and v_g is very large compared to v_l. On the other hand, the second term of the right hand side of equation 2 can be adjusted by the analyst over a considerable range by changing the ratio Q_l/Q_g.

In the case of the ELSD, the droplets pass from the nebulizer into a drift tube where the solvent is rapidly evaporated. Different phenomena may take place: 1) Some of the aerosol droplets can be lost by direct precipitation on the walls of the drift tube; 2) Some droplets may coagulate, which contributes significantly to an increase of the average droplet size and of the settling velocity (9); 3) In the case of ionic solutions, the formation of an aerosol ionic distribution has been suggested (10) but this applies to the ELSD only very rarely. Grigor'ev et al. (9) have derived an expression for the aerosol coagulation rate as a function of the aerosol concentration in the stream, Q_l/Q_g. The amount of coagulated droplets increases with decreasing gas flow rate.

From equation 2 and the aerosol coagulation rate, we conclude that, at constant eluent flow rate there will be a gas flow rate for which the average droplet size will be at a maximum. This is in agreement with published results showing the existence of optimum liquid and gas flow rates at which the response is at a maximum. An essential feature of the aerosol in the ELSD, however, is a constant modification of the droplet size distribution when the flow rate changes. It is important to point out that equation 2, derived by Nukiyama and Tanassawa (16), is valid only for the primary aerosol, not for the aerosol emerging from the drift tube, which has undergone losses by precipitation and changes in size distribution by coagulation, and for which no other equation has yet been proposed.

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II Drift tube.

This is where the solvent contained in the droplet is vaporized, where particles or droplets of pure solute are produced. In order to distinguish the droplets at the drift tube entry and the particles or the droplets of pure solute at the drift tube exit, we use 'droplet' in the first case and 'particle' in the second. The length and diameter of the tube as well as its temperature must be optimized to achieve quick vaporization and minimum loss of particles by condensation on the wall.

The following equation has been proposed by Charlesworth (1) to calculate the time, t_d, for the solvent of the droplet to be completely vaporized:

$$t_d = 2 \Delta H_v \rho D^2 / M k_f \Delta T \qquad (3)$$

where ΔH_v is the latent heat of vaporization of the solvent, ρ is the liquid density, D is the initial droplet diameter, M is the molecular weight of the solvent, k_f is the thermal conductivity of the gas film surrounding the droplets, and ΔT is the difference between the air temperature and the surface temperature of the droplet. Unfortunately, this last temperature depends on the rate of vaporization of the droplet and cannot be calculated directly. It is important to note that this equation is also applicable to the solute, and that if the temperature in the drift tube is too high, a reduction of the particle size and, consequently, a loss of signal are observed.

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III Light scattering cell.

Light scattering in the ELSD is an extremely complex process because of the existence of several parallel mechanisms of light scattering, of the wide particle size distribution, of the radial energy distribution in the light beam, and of the nature of the particle surface , which may be spherical (liquid analytes) or non-spherical (solid analytes).

There are four major processes of light scattering: Rayleigh scattering, Mie scattering, reflection and refraction. According to Charlesworth (1), the relative importance of each one of these processes depends on the ratio of the diameter (d) of the particle to the wavelength (λ) of the incident light. Rayleigh scattering is predominant when the ratio d/λ is smaller than 0.1, Mie scattering becomes preponderant when d/λ is greater than 0.1.

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The diameter of the particles entering the detector cell is related to the droplet diameter at the nebulizer outlet by the equation:

$$d = D_0 \, (C/\rho_a)^{1/3} \qquad (4)$$

where C is the concentration of the non-volatile material in the original droplet and ρ_a the density of the analyte. The relationship neglects, however, the effects of the various phenomena resulting in a redistribution of the size distribution of the aerosol droplets.

When a single particle flies through the center of a Gaussian laser beam, for which the radial energy distribution is Gaussian,

which is the case of our apparatus, the scattered light signal has a Gaussian profile intensity given by the expression (18):

$$I(t) = I(0)\, e^{-2(t-t_0)^2/(w/v_p)^2} \qquad (5)$$

where $I(t)$ is the time-dependent light scatter signal, t_0 is the time when the particle passes through the axis of the laser beam, $I(0)$ is the scattered light intensity when the particle is at the center of the beam, w is the laser beam spot size, and v_p is the velocity of the particle. $I(0)$ is a function of the laser design, its power and wavelength, and of the size and nature of the particle.

A study of the dependence of scattered light intensity (18,19) on the particle diameter shows that for particles of very narrow size distribution, the amount of scattered light depends not only on the particle size but also on the direction, e.g., it is not the same in the forward direction and at right angle. For example, with the 442 nm beam of a helium-cadmium laser, the plots of the logarithm of the scattered light intensity versus the logarithm of the particle diameter for different samples of monodisperse particles, with diameters ranging from 90 nm to 1.09 μm, are straight lines for light scattered in either direction (i.e., forward and right angle). These lines intersect. For right angle collection, the slope is 2.1. It is smaller than the slope observed for forward collection. This suggests that right angle collection is more suitable for the detection of very small particles, smaller than 350 nm in diameter.

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With our apparatus, the average size of the droplets formed in the nebulizer is between 5μm and 15μm. In the detector cell, the average particle size for a solute at a 1 ppm concentration will be between 50 nm and 150 nm. This is just at the limit between the Rayleigh and the Mie regions of light scattering. Right angle collection will be preferable.

IV Response of the Evaporative Light Scattering Detector (ELSD).

As has been firmly established by various series of experimental results (2-5,7), the detector response is not linear. It, rather, follows the equation:

$$A = a\,C^{b} \qquad (6)$$

where A is the peak area ($\int I(t).dt$) and a and b are numerical coefficients. As a result, the plot of the peak area versus the sample size in logarithmic coordinates is linear with slope b, at least in some range of sample size. All experiments have shown b to be between 1 and 2 and to depend strongly upon the nebulizer dimensions and the design of the apparatus.

V Application of the theory to the apparatus design.

The previous theoretical discussion leads to a few conclusions regarding the design and operation of the nebulizer. In order to maximize the response factors, we need to produce solvent droplets as large as possible. The upper limit will be set by the requirements of properly operating the nebulizer under steady conditions and not losing droplets by condensation on the drift tube wall. For example, the nebulizer will be operated at the

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lowest gas flow rate that is compatible with steady state behavior, since both terms of equation 2 increase with decreasing gas flow rate; on the other hand, the gas velocity must remain supersonic in the nebulizer. If the scavenger gas flow rate is too low, huge droplets are occasionally formed, they vaporize incompletely and result in spikes in the detector response. In order to achieve supersonic velocity with a very small volume flow rate, we need a small nebulizer with very narrow tubes.

In order to maximize the amount of light scattered by small particles, i.e., to maximize the detector response, we collected the scattered light perpendicular to the incident beam, which seems to be optimum for the size of the particle to be detected after the results of Zarrin et al. (18).

Mourey and Oppenheimer (3) have shown good agreement between the experimental response factors and the values calculated on the basis of equation 2 and the Mie theory of light scattering. They also showed that the range of validity of equation 6 is very narrow. In logarithmic coordinates, the response plot is sigmoidal. These results assume that fast, complete solvent vaporization is achieved without droplet coagulation. Such a result may be explained by the wide diameter of the drift tube of the instrument used and its large detection limit (3).

The response factors also depend on the nature of the mobile phase (specifically, on its density, viscosity and surface tension), and, to some extent, on the refractive index of the solute. However, there are little or no experimental data

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available, regarding the influence of the nature of the solvent and the solute on the response factors, to confirm or deny these predictions.

In order to study these effects, we have measured the response factors of six solutes with different properties (UV absorbance, refractive index and melting point), using two conventional nebulizers, with concentric and cross-flow designs, respectively, and seven organic solvents classically used in liquid chromatography.

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EXPERIMENTAL

The ELSD has been described previously (2,4,5). Only the data acquisition system has been changed, by incorporation of a microcomputer, and use of two different nebulizers.

The gas stream was carefully filtered prior to its admission into the nebulizer. Its flow rate was controlled and measured. It was heated at a controlled temperature, adjustable between 25 and 100°C. In this work, the drift tube temperature was maintained at 60°C, which is sufficient to permit complete vaporization of methanol, the most difficult solvent to vaporize in the group of solvents studied (Table I).

The light beam used was produced with a 1 mw He-Ne laser (Hughes Aircraft Co, Carlsbad, CA; Wavelength: 632 nm). The scattered light was collected with an optical fiber, its power was measured by a photocell. The light beam was perpendicular to the particle stream.

The signal was acquired with an IBM PC microcomputer (IBM Co, Kingston, NY) connected to the amplifier, through an analog-to-digital I/O board (Ref# DT2801, Data Translation, Inc., Malborough, MA) and general purpose software (Labtech Notebook, Laboratory Technologies Co., Wilmington, MA). The acquisition frequency was 20 Hz. A Hewlett-Packard 3392A integrator (Hewlett-Packard Co., Avondale, PA) was connected directly to the photocell.

For this study no chromatographic column was used. The sampling valve (Reodyne, Berkeley, CA, model 7125) was connected to the detector through a 30 cm long, 0.25 mn I.D. tube. Sample volume was 10 µl. The peaks obtained were somewhat unsymmetrical; their elution lasted approximately 15 seconds.

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Two different nebulizers have been studied. The concentric nebulizer was built after the same design as those which were previously used (2,4,5). A 0.007" I.D., 1/32" O.D. capillary tube carried the liquid stream to the nozzle. The gas stream arrived through a concentric tube 0.04" I.D., 1/16" O.D.. The tip of the liquid feeder was placed inside the gas tubing, at an adjustable distance of between 1/16" and 1/2" from its end. With this nebulizer, the scavenger gas flow rate was 2.4 l/mn.

The cross-flow nebulizer uses a 0.007" I.D., 1/32" O.D. capillary tube as the solvent port, positioned perpendicular to a 0.02" I.D., 1/16" O.D. stainless-steel capillary tube connected to the gas inlet. The scavenger gas flow rate in this nebulizer was 1.0 l/mn.

The solvents used were chromatography grade, from J.T. Baker (J.T. Baker Chemical Co., Phillipsburg, NJ). The solvent properties are given in Table I. Solvent was delivered at a uniform flow rate of 0.3 ml/mn by a chromatographic pump (Waters Assoc., Milford, MA, model 6000A), equipped with a pulse dampener. All solutes obtained from Sigma Chemical Co. (St Louis, MO) were 99% pure. The solute references are: pyrene [No. P-2146], anthracene [No. A-3885], arachidic acid methyl ester [No. A-3881], stearic acid methyl ester [No. S-5376], oil red EGN [No. 0-2003], oil blue N [No. 0-8376].

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Table I : Coefficients of equation 2 for different solvents with the concentric nebulizer.

Solvent	Viscosity (cP) *	Density (g/ml) *	Surface Tension dyne/cm *	Equation 2 ** 1 st term	Equation 2 ** 2 nd term	Droplet Average Diameter (µm)
n-Heptane	0.38	0.684	19	9.60	1.20	10.80
Ethyl Acetate	0.45	0.924	23.6	9.21	1.15	10.36
Acetone	0.31	0.787	23	9.85	1.02	10.87
Benzene	0.65	0.879	28.5	10.37	1.32	11.69
Chloroform	0.53	1.498	27	7.73	1.08	8.81
Acetonitrile	0.32	0.783	29	11.09	0.98	12.07
Methanol	0.52	0.791	22.6	9.74	1.29	11.03

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

** Flow rates, gas: 2.4 l/mn , liquid: 0.3 ml/mn
Flow velocities, gas: 321 m/s , liquid: 0.5 m/s

DISCUSSION

I - Influence of the nature of the solvent on the response.

Equation 2 shows that the nature of the solvent determines, to some extent, the average size and the distribution of the droplets generated by the nebulizer and, thus, the value of the response factor.

a - Response of the detector with the concentric nebulizer.

The value of the two different terms of equation 2 are reported in Table I for each solvent and at the scavenger gas and mobile phase flow rate used. The estimates of the average droplet diameter are also given in the table. Under the experimental conditions selected, the first term is most significant.

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Samples of the standard solutions for different concentrations, ranging from 0.05 g/l to 1.6 g/l, were injected in the 10 µl loop of the sampling valve. In almost all cases more than 11 samples of different sizes were injected (exception, oil blue in acetonitrile). The peak area was determined and plotted versus the sample size, on a log-log scale. The response data are reported in Table II, as the best estimates of the slope and intercept of each straight line. The response for oil blue in heptane was too small to be measured, even at large concentrations and no data are supplied.

For 41 series of experiments, the lowest value of the correlation coefficient R^2 is 0.930 (methyl stearate in acetone); in 28 cases R^2 is equal to or larger than 0.99. In all cases the standard deviation on the response slope is below 8.5% (worst

Table II : Response of the Light Scattering Detector. Results of the log-log correlations of the response for the concentric nebulizer.

Parameter	Pyrene	Anthrace	Me.Stear	Me.Arach	Blue oil	Red oil
			- n-HEPTANE -			
Ordinate	6.13	5.95	5.33	5.78		5.67
Std Dev.	0.15	0.17	0.11	0.12		0.11
R^2	0.9881	0.9837	0.9933	0.9915		0.9926
# Measur	18	18	15	17		14
# Deg Fr.	16	16	13	15		12
Slope	1.13	1.06	1.30	1.13		1.36
Std Dev.	0.031	0.034	0.029	0.027		0.033
			- ETHYL ACETATE -			
Ordinate	4.54	4.54	4.47	4.93	4.63	5.07
Std Dev.	0.18	0.20	0.15	0.18	0.08	0.09
R^2	0.9860	0.9767	0.9878	0.9651	0.9942	0.9960
# Measur	15	15	15	11	12	15
# Deg Fr.	13	13	13	9	10	13
Slope	1.45	1.26	1.29	1.15	1.35	1.41
Std Dev.	0.047	0.054	0.039	0.073	0.032	0.024
			- ACETONE -			
Ordinate	5.87	4.98	5.76	5.74	6.02	5.70
Std Dev.	0.19	0.12	0.26	0.18	0.20	0.17
R^2	0.9713	0.9882	0.9305	0.9689	0.9404	0.9879
# Measur	15	13	14	15	15	18
# Deg Fr.	13	11	12	13	13	16
Slope	1.06	1.24	0.89	0.99	0.77	1.23
Std Dev.	0.050	0.041	0.070	0.049	0.054	0.034
			- BENZENE -			
Ordinate	5.36	4.70	4.43	5.21	4.74	5.31
Std Dev.	0.054	0.11	0.059	0.12	0.10	0.088
R^2	0.9983	0.9889	0.9969	0.9900	0.9931	0.9958
# Measur	16	13	11	14	14	17
# Deg Fr.	14	11	9	12	12	15
Slope	1.12	1.13	1.32	1.20	1.19	1.12
Std Dev.	0.012	0.036	0.024	0.034	0.028	0.018

(continued)

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Table II (continued)

	- CHLOROFORM -					
Ordinate	5.24	4.82	4.65	4.70	4.21	5.32
Std Dev.	0.18	0.087	0.14	0.15	0.095	0.12
R^2	0.9785	0.9923	0.9854	0.9834	0.9920	0.9902
# Measur	14	12	11	11	12	15
# Deg Fr.	12	10	9	9	10	13
Slope	1.20	1.17	1.49	1.41	1.26	1.17
Std Dev.	0.051	0.032	0.060	0.061	0.035	0.032
	- ACETONITRILE -					
Ordinate	4.05	3.81	3.68	4.82	3.53	4.27
Std Dev.	0.078	0.090	0.086	0.20	0.092	0.15
R^2	0.9974	0.9960	0.9940	0.9659	0.9865	0.9903
# Measur	15	15	12	15	9	15
# Deg Fr.	13	13	10	13	7	13
Slope	1.48	1.36	1.30	1.01	1.23	1.47
Std Dev.	0.020	0.023	0.032	0.052	0.054	0.040
	- METHANOL -					
Ordinate	4.46	2.50	3.31	6.09	3.18	3.69
Std Dev.	0.11	0.18	0.24	0.086	0.24	0.090
R^2	0.9934	0.9784	0.9612	0.9970	0.9351	0.9943
# Measur	15	12	11	15	12	14
# Deg Fr.	13	10	9	13	10	12
Slope	1.33	1.43	1.47	1.50	1.10	1.11
Std Dev.	0.030	0.067	0.098	0.022	0.092	0.024

Conditions :

Temperature - 60°C
Scavenger Gas (Nitrogen) Flow Rate - 2.4 l/mn
Eluent Flow Rate - 0.3 ml/mn

case, oil blue in methanol); in 19 cases (out of 41) the standard deviation is less than 3%. Similar results are obtained for the standard deviations of the ordinate intercepts. This indicates that, under the conditions where the measurements were carried out, equation 6 accounts correctly for the response.

On the other hand, the results obtained are in disagreement with the predictions drawn from equation 2. There is wide scatter of

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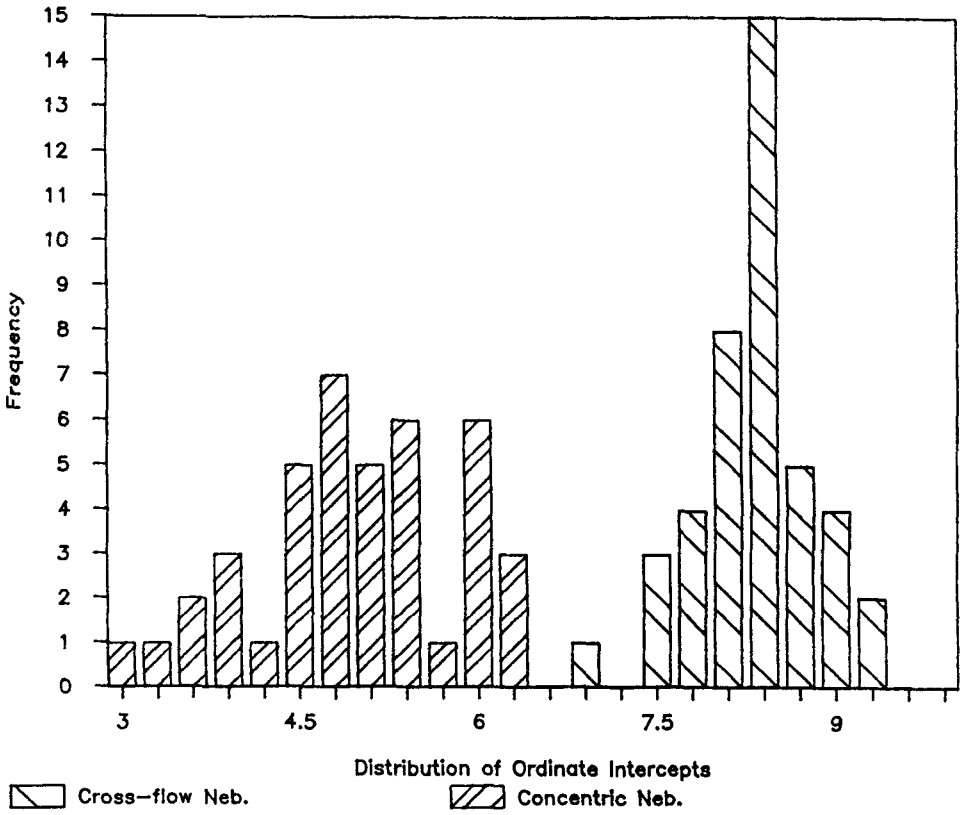

Figure 1. Distribution of the ordinate intercept of the LSD response of a concentric and a cross-flow nebulizer. All combinations of 6 solutes and 7 solvents (see Tables II and III).

the response ordinates (Equation 6, log(a)) for the various combinations of solutes and solvents investigated (see Figure 1) These ordinates are the response for 1 µg samples. Their logarithms vary from 2.5 (anthracene in methanol) to 6.1 (pyrene in n-heptane), a ratio of 1 to 850. For pairs of similar compounds, such as anthracene and pyrene or methyl stearate and

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methyl arachidonate, the ratio of the ordinates changes markedly from solvent to solvent. For anthracene/pyrene, the ordinate ratio varies from 91 (methanol) to 1.00 (ethyl acetate). For methyl arachidonate/methyl stearate, the response ratio varies from 600 (methanol) to 0.95 (acetone), methanol excluded, the ranges of variation of these ordinate ratios are still 7.8 (acetone) to 1.00 and 13.7 (acetonitrile) to 0.95, respectively. For a given solvent, the range of ordinate intercept varies from 0.60 (ethyl acetate, a factor 4 for the responses) to 1.29 (acetonitrile, a factor 20) and 3.9 (methanol, a factor 8000).

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Equation 2 predicts that a plot of the ordinate intercept versus the droplet size would be a straight line for the concentric nebulizer (See Table I). The experimental data do not seem to exhibit any trend nor correlation (See Figure 2), even if we eliminate the data for methanol. Although the behavior of this last solvent seems to be extreme, it is not out of line.

Furthermore, the large values of the ordinate tend to be associated with small values for the slope, and conversely, showing that there is some compensation between the two terms. The actual range of the responses measured for samples between 2 and 10 µg is not very large, as reported by previous authors. It is certainly smaller than the separate analysis of ordinates and intercepts would lead to conclude. Nevertheless, it is quite significant, much too large to justify the reputation of the detector as a mass detector.

b - Response of the detector with the cross-flow nebulizer.

The concentrations of the solutions used were smaller, because the responses obtained were larger than with the concentric

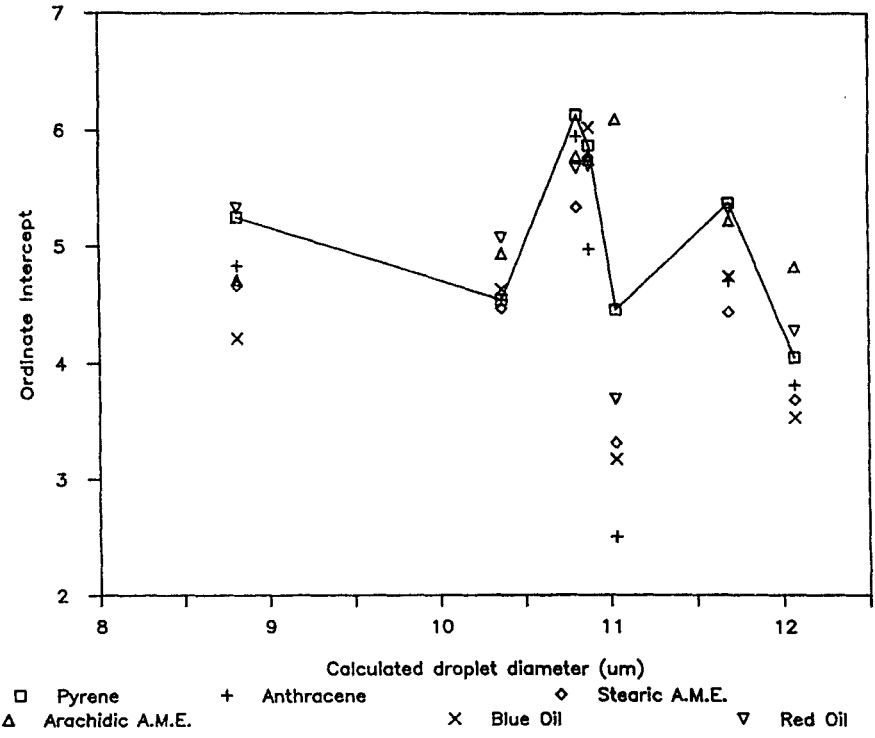

Figure 2. Plot of the ordinate intercept of the response curve for the concentric nebulizer versus the droplet diameter derived from Equation 2. All combinations of 6 solutes and 7 solvents (see Tables II and III). The ordinate intercept is the response for a 1µg sample.

nebulizer, between 3 mg/l and 400 mg/l, corresponding to sample sizes between 0.03 and 4 µg. We have calculated the parameters of the same linear correlation, between the logarithm of the peak area versus the logarithm of the sample size. The results are reported in Table III.

The square of the correlation coefficient is always larger than 0.93 (worst case, Methyl Stearate in Acetone). In 28 cases out of

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Table III: Response of the Light Scattering Detector. Results of the log-log correlations of the response for the cross-flow nebulizer.

Parameter	Pyrene	Anthrace	Me.Stear	Me.Arach	Blue oil	Red oil
			- n-HEPTANE -			
Ordinate	8.58	8.38	8.41	8.82	7.22	9.12
Std Dev.	0.10	0.16	0.14	0.15	0.12	0.15
R^2	0.9963	0.9918	0.9938	0.9904	0.9933	0.9924
# Measur.	24	24	23	23	24	24
# Deg Fr.	22	22	21	21	22	22
Slope	1.08	1.12	1.14	0.98	0.93	1.09
Std Dev.	0.014	0.021	0.019	0.021	0.016	0.020
			- ETHYL ACETATE -			
Ordinate	8.41	8.22	8.14	8.26	7.97	8.86
Std Dev.	0.24	0.16	0.094	0.17	0.074	0.11
R^2	0.9827	0.9927	0.9969	0.9867	0.9975	0.9959
# Measur.	22	24	21	21	21	24
# Deg Fr.	20	22	19	19	19	22
Slope	1.08	1.15	1.18	1.03	1.03	1.09
Std Dev.	0.032	0.021	0.014	0.027	0.011	0.014
			- ACETONE -			
Ordinate	8.54	8.39	8.38	8.48	8.26	9.06
Std Dev.	0.15	0.079	0.071	0.12	0.10	0.10
R^2	0.9896	0.9977	0.9983	0.9949	0.9953	0.9962
# Measur.	24	24	24	24	24	23
# Deg Fr.	22	22	22	22	22	21
Slope	0.89	1.00	1.05	1.09	0.93	1.02
Std Dev.	0.019	0.010	0.009	0.016	0.013	0.013
			- BENZENE -			
Ordinate	8.20	7.85	7.94	8.13	8.05	8.75
Std Dev.	0.25	0.15	0.088	0.30	0.23	0.14
R^2	0.9787	0.9899	0.9965	0.9570	0.9687	0.9932
# Measur.	21	20	17	21	18	23
# Deg Fr.	19	18	15	19	16	21
Slope	1.19	1.11	1.24	0.98	1.02	1.12
Std Dev.	0.040	0.026	0.018	0.047	0.046	0.020

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	- CHLOROFORM -					
Ordinate	8.30	8.15	8.16	8.25	7.92	8.95
Std Dev.	0.14	0.14	0.11	0.16	0.049	0.13
R^2	0.9946	0.9887	0.9947	0.9928	0.9988	0.9948
# Measur	23	21	20	24	21	24
# Deg Fr.	21	19	18	22	19	22
Slope	1.21	0.94	1.12	1.17	0.99	1.11
Std Dev.	0.019	0.023	0.019	0.021	0.007	0.017
	- ACETONITRILE -					
Ordinate	7.80	7.66	7.55	8.02	7.57	8.30
Std Dev.	0.14	0.13	0.12	0.26	0.17	0.11
R^2	0.9923	0.9941	0.9947	0.9678	0.9783	0.9937
# Measur	21	17	19	18	17	20
# Deg Fr.	19	15	17	16	15	18
Slope	1.12	1.14	1.17	0.96	0.83	1.03
Std Dev.	0.022	0.022	0.020	0.044	0.032	0.019
	- METHANOL -					
Ordinate	7.96	7.46	7.48	7.62	6.77	8.10
Std Dev.	0.18	0.22	0.21	0.24	0.21	0.18
R^2	0.9896	0.9795	0.9790	0.9727	0.9631	0.9867
# Measur	24	22	18	21	20	21
# Deg Fr.	22	20	16	19	18	19
Slope	1.11	0.90	1.05	1.01	0.78	1.06
Std Dev.	0.024	0.029	0.038	0.039	0.036	0.028

Conditions :

Temperature - 60°C
Scavenger Gas (Nitrogen) Flow Rate - 1 l/mn
Eluent Flow Rate - 0.3 ml/mn

42, it is larger than 0.99. The relative standard deviations of the slope of the plots are all less than 5%. 9 of them only are larger than 3% and 17 larger than 2% (including all six for methanol). The reproducibility of these slopes is much better than with the concentric nebulizer. Similarly the standard deviation on the ordinate intercept is smaller than 3%. The cross-flow nebulizer gives results which are both more precise and more sensitive than the concentric nebulizer.

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The distribution of the ordinate intercept (responses for a 1 µg sample) is narrower than for the concentric nebulizer. It ranges from 6.8 for oil blue in methanol to 9.12 for oil red in n-heptane, a ratio of 210 (see Figure 1). For almost all solutes, the response decreases in the order: acetone = heptane> ethyl acetate> chloroform> benzene> acetonitrile> methanol. The exceptions are minor (e.g. pyrene in acetonitrile and methanol).

The ratio of the largest to the smallest response for the four colorless solutes varies between 1.46 (acetone) and 3.2 (methanol), which is considerably less than with the concentric nebulizer.

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The distribution of the slopes is also narrower than with the concentric nebulizer. It also tends to compensate for the effect of the intercept on the response. As a consequence, the influence of the solvent on the response is rather small between 0.1 and 1 µg.

These results show that there is no correlation between the average droplet size in the primary aerosol and the measured relative responses. Equation 2 cannot be used to predict responses. But, on the other hand, several conclusions can be drawn: 1) the relative response supplied by the concentric nebulizer and the cross-flow nebulizer are similar, 2) previous observations (3,9,10,14) regarding the apparent anomalous behavior of the solvent are confirmed, presumably because of the failure of the Nukiyama and Tanassawa equation (16), 3); this failure could be attributed (9,10,14) to an aerosol

redistribution, probably by a combination of coagulation and wall precipitation, resulting in modification of the constants in the particle size distribution.

c - Comparison between the performance of both nebulizers.

The characteristics of the response obtained with the cross-flow nebulizer varies with the nature of the solvent in a much narrower range than the response characteristics of the concentric nebulizer. The distribution of the ordinate intercept (Figure 1) is narrower. On the average, the intercepts are larger. The distribution of the slope (see Figures 3a and 3b) is narrower. On the average the slopes are smaller, which compensates in part the effect of the intercept. For the cross-flow nebulizer, the average slope is 1.05 and the relative standard deviation 10%, instead of 1.23 and 14% respectively for the concentric nebulizer.

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The use of a cross-flow nebulizer brings the ELSD much closer to the ideal mass sensitive detector, while still falling short of achieving an identical response for all compounds.

The detection limits are also lower with the cross-flow nebulizer than with the concentric nebulizer (See Figures 4a and 4b). With the former they are between 3 and 20 ng, on the average 7 ng (See example, Figure 5). With the latter, they are between 60 and 1,000 ng. The range is larger again, and the average value is more than 20 times larger.

d - Dynamic linear range.

The concentration range investigated is imposed either by the solute solubility in the solvent used and the amplifier

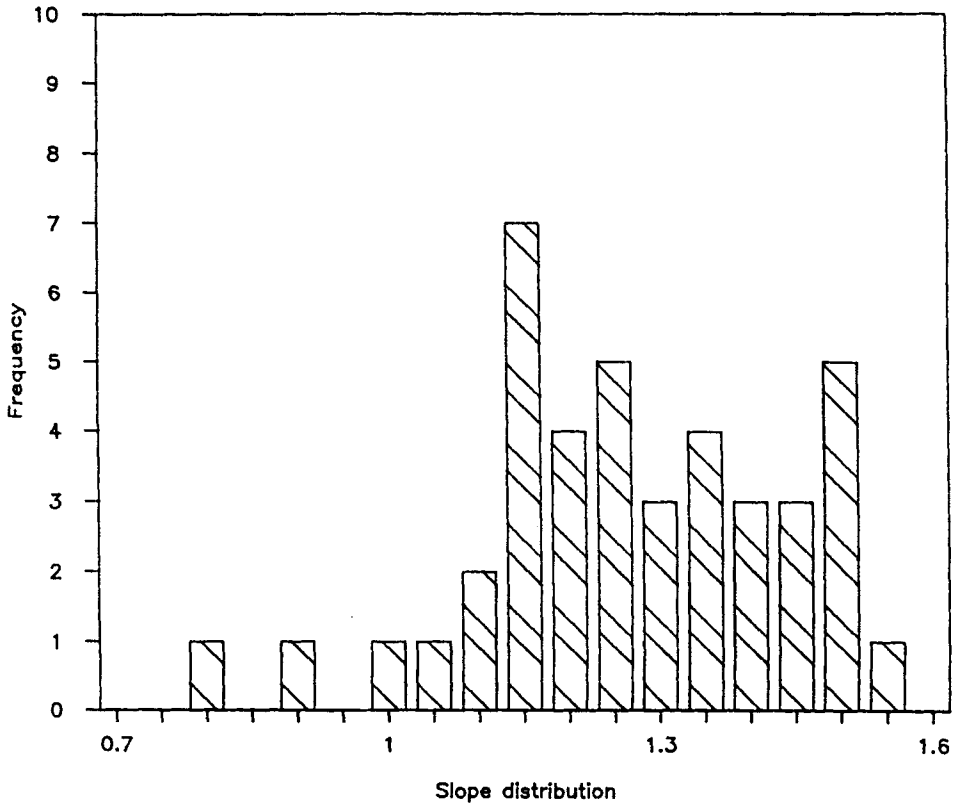

Figure 3. Distribution of the slope of the detector response for a concentric nebulizer (Figure 3a) and a cross-flow nebulizer (Figure 3b). All combinations of 6 solutes and 7 solvents (see Tables II and III).

saturation on the one hand (high concentration), and by the detection limit on the other hand (low concentration). The higher concentration used is limited by the solubility of the probe solutes with the concentric nebulizer and by the amplifier saturation with the cross-flow nebulizer. The dynamic linear range obtained from an ELSD using a cross-flow nebulizer could be widened by changing the amplifier.

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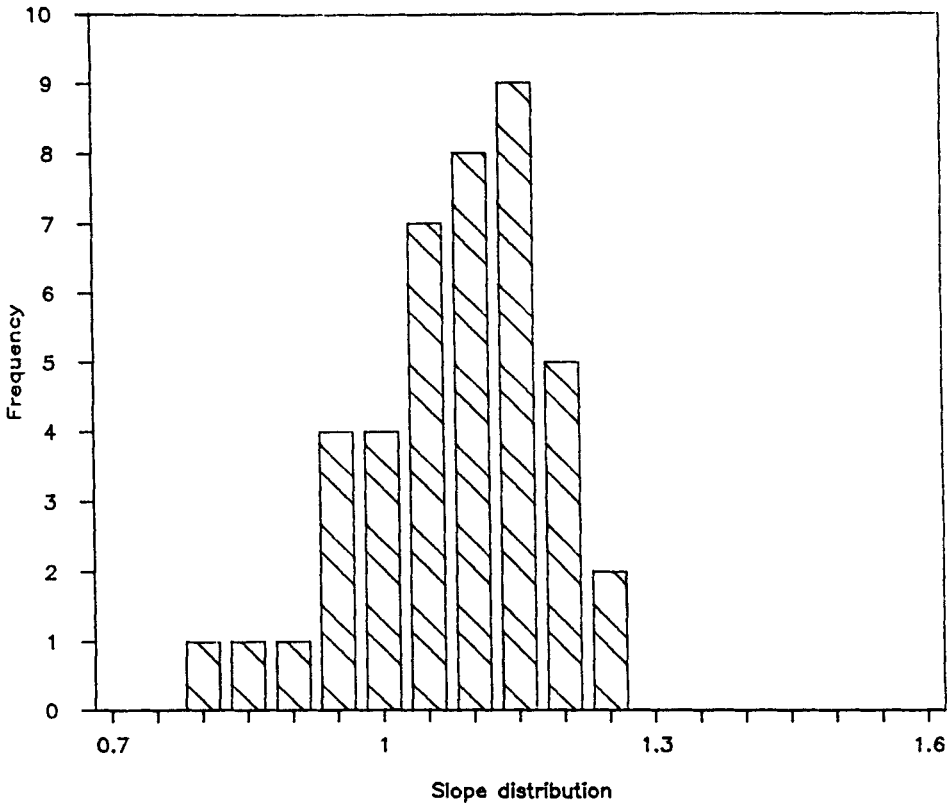

FIG. 3 (continued)

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II Influence of solvent and gas flow rates.

In order to probe further into the extent of the failure of equation 2, we have determined the response of the detector for different values of the scavenger gas and mobile phase flow rates.

Stolyhwo et al. (4) have already shown that the detector response varies qualitatively as predicted by equation 2 when the gas

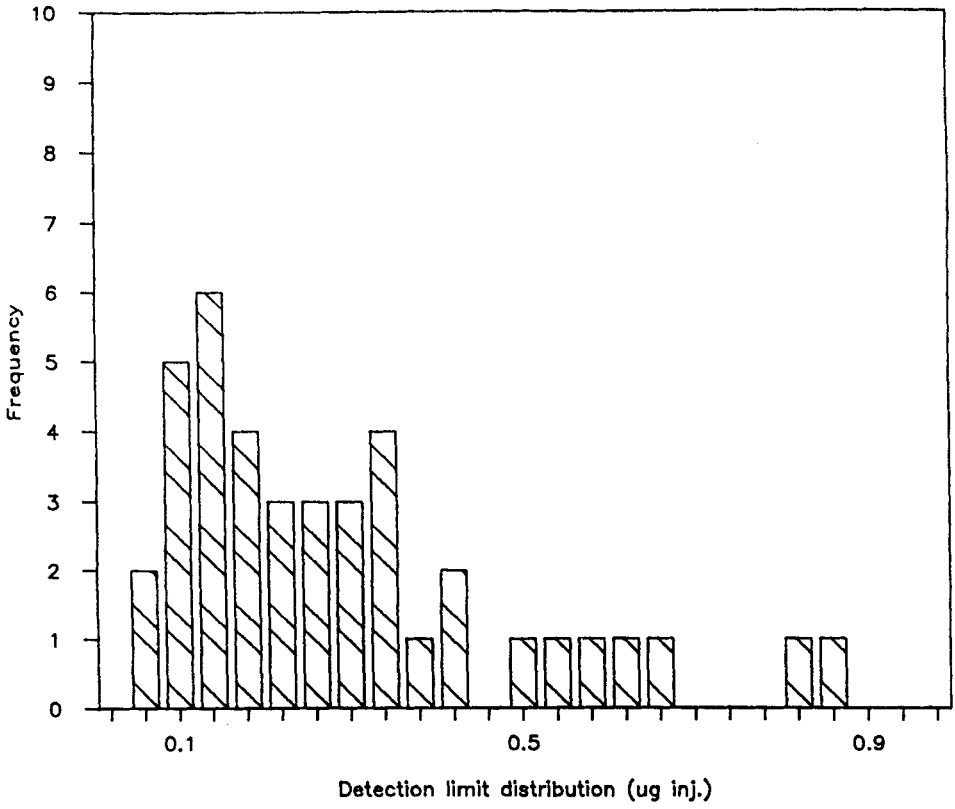

Figure 4. Detection limits of the LSD for a concentric nebulizer (Figure 4a) and a cross-flow nebulizer (Figure 4b). All combinations of 6 solutes and 7 solvents (see Tables II and III).

velocity is sonic. When the gas flow rate increases, the detector response for a sample of given size increases first, reaches a maximum when the gas velocity becomes sonic and then decreases further with increasing gas velocity. In this experiment, the solvent flow rate was kept constant, so the response was only function of the variation of the gas flow rate.

The first term of equation 2 is related to the difference between solvent and gas velocities. It decreases when the gas velocity

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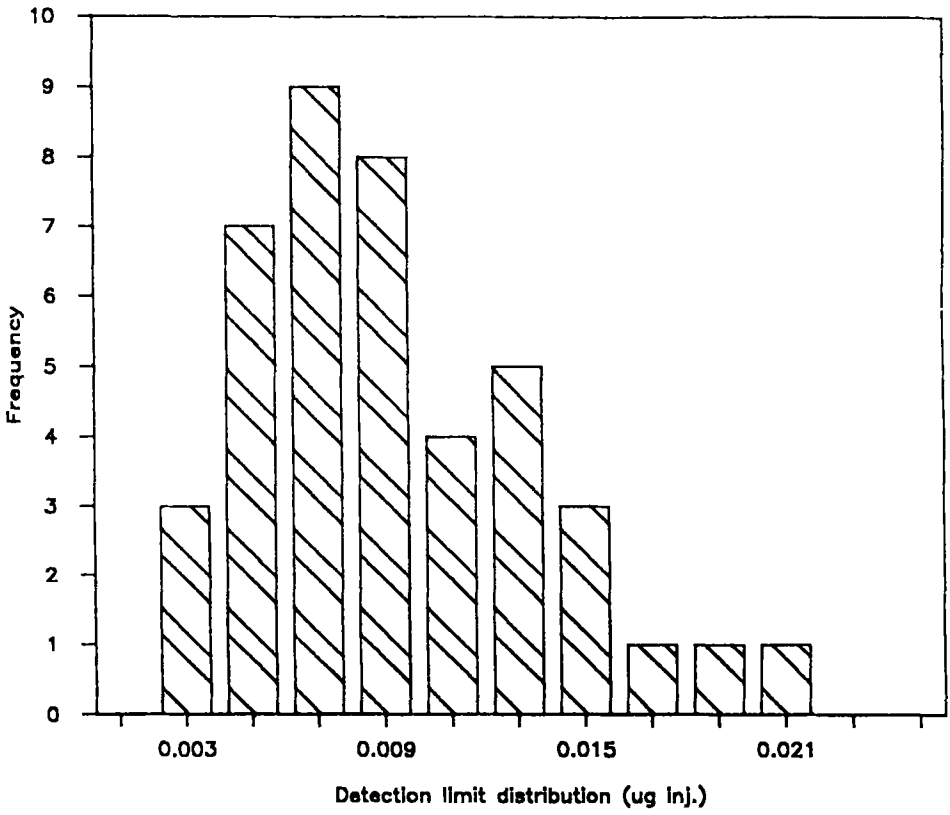

FIG. 4 (continued)

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increases. As a first approximation we can neglect the influence of the solvent velocity since it is around 40 cm/s while the gas velocity is around 300 m/s. The second term of equation 2 is related to the flow rate ratio. It increases when the solvent flow rate is increased or when the gas flow rate is decreased. Accordingly, the average droplet diameter increases with increasing solvent flow rate and with decreasing gas flow rate.

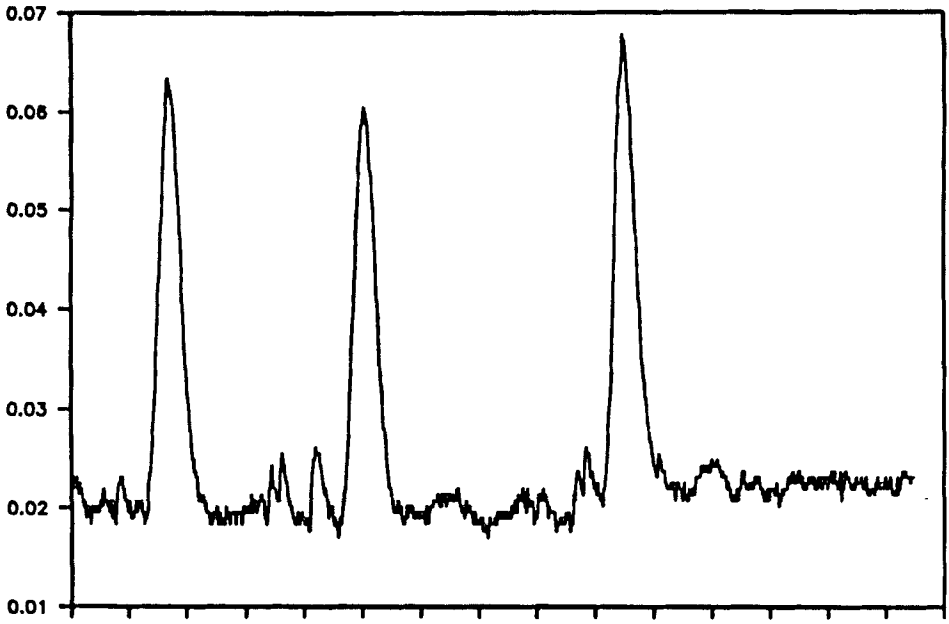

Figure 5. Peaks of pyrene (15.6 ng. inj.) obtained with a cross-flow nebulizer. Solvent: n-heptane, 0.3 ml/mn. Scavenger flow rate: 1ml/mn. Drift tube temperature: 50°C.

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We have measured the detector response for Pyrene, with n-Heptane as solvent. The solvent flow rate was varied between 0.1 and 1.0 ml/mn and the gas flow rate between 1.50 and 3.5 l/mn (velocity between 200 and 450 m/s). Only the concentric nebulizer is used in these experiments. The results are listed in Table IV.

a - Influence of the gas flow rate on the detector response.

Figure 6 shows a plot of the detector response versus the gas flow rate, at constant solvent flow rate. Two types of response

Table IV : Influence of the gas and solvent flow rates on the detector response for the concentric nebulizer.

Solvent flow rate ml/mn	Gas flow rate (l/mn)	3.18	2.7	2.35	1.86	1.5
	Gas velocity (m/s)	426	361	314	249	201
0.1	R	43.3	59.2	50.0	70.7	33.9
0.2	E	98.1	143.7	88.6	91.0	58.2
0.3	S	148.2	219.1	190.8	112.3	70.5
0.4	P	167.1	261.4	207.1	145.6	178.0
0.5	O	243.8	286.2	221.1	243.8	343.2
0.6	N	236.8	315.5	217.3	417.0	543.4
0.7	S	204.7	259.3	267.7	621.0	331.7
0.8	E	228.1	334.7	554.4	712.4	377.2
0.9		221.6	339.2	645.2	722.0	391.2
1.0	(mv) *	542.8	691.8	740.6	772.1	400.6

Conditions :

Temperature - 60°C
Eluent - Heptane
Solute - Pyrene (400 mg/l)

* The response is the concentration plateau height resulting from the injection of a constant solute concentration.

curves are obtained. When the solvent flow rate is below 0.7 ml/mn (Figure 6a) the curves have no marked maximum and the response does not vary much. Equation 2 is certainly not valid. Above 0.7 ml/mn (Figure 6b), the detector response reaches a maximum when the gas velocity becomes sonic and then decreases, as predicted by equation 2.

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b - Influence of the solvent flow rate on the detector response.

If the gas velocity is kept constant, the average droplet diameter depends practically only upon the second term of equation 2, and increases as the power 1.5 of the solvent flow rate. Figure 7 shows that the response tends to increase with increasing solvent flow rate but most curves exhibit a weak maximum. The defective behavior of the nebulizer, at velocities below sound (curve at 1.5 l/mn) is illustrated by the sharp maximum.

c - Correlation between the detector response and the calculated average droplet diameter.

Figure 8 shows a plot of the detector response versus the average droplet diameter derived from equation 2. This graph shows clearly than there is no correlation between the detector response and the droplet average diameter. Most important is the fact that the apparent size distribution of the droplet population is different with each solvent flow rate.

At best, equation 2 qualitatively describes the influence on the response of the gas velocity at a given solvent flow rate, in a very restricted range of velocities. The gas velocity must be sonic or supersonic, in any case. But, the combined influence of the solvent and the gas flow rates on the detector response cannot be correlated with the results of the Nukiyama and Tanassawa equation (16).

III - Influence of the nature of the solute on the response.

We have measured the detector response for three kinds of compounds, two poly nuclear aromatic hydrocarbons (PNA) which

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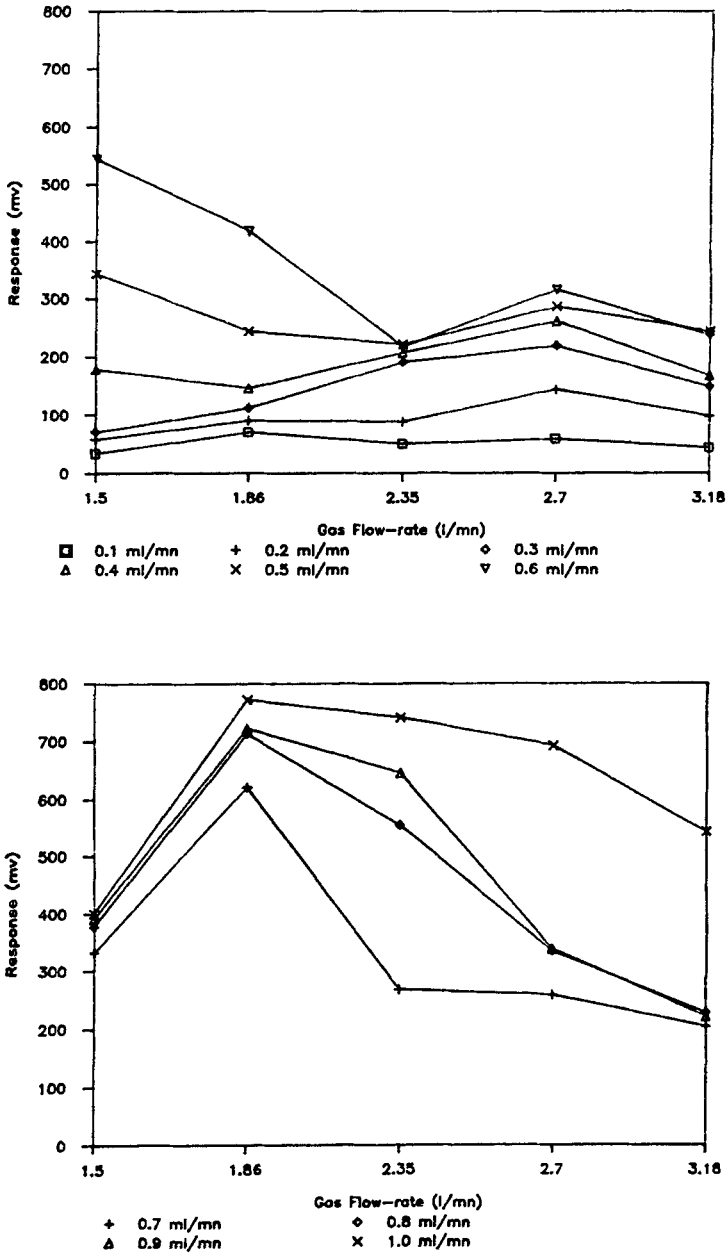

Figure 6. Detector response versus gas flow rate for several solvent flow rates. Concentric nebulizer.

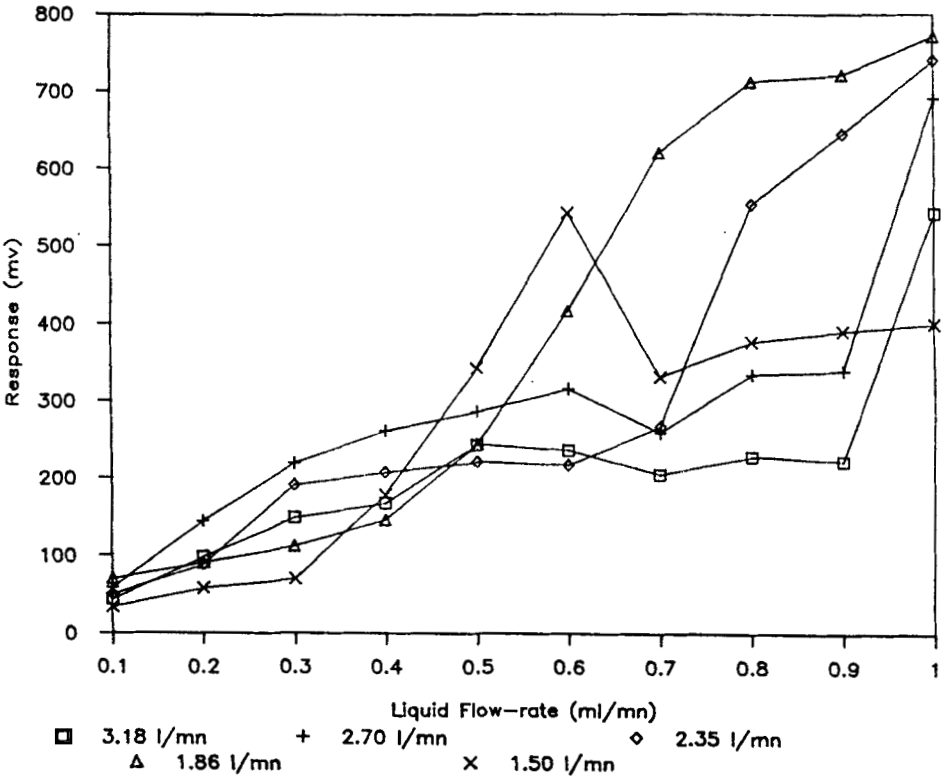

Figure 7. Detector response versus solvent flow rate for several gas flow rates. Concentric nebulizer.

have strong ultra-violet absorbance, two fatty acid methyl esters which do not absorb above 210 nm and two coloring oils which have strong absorbances in part of the UV-visible domain (see spectra Figures 9a and 9b). The blue oil has a strong absorbance (ε=1000) at 637 nm, the wavelength of the laser beam, while the red oil does not absorb at this wavelength. The melting points of the PNA's and the oils are higher than 150°C. Those of the fatty acid methyl esters are lower than 60°C.

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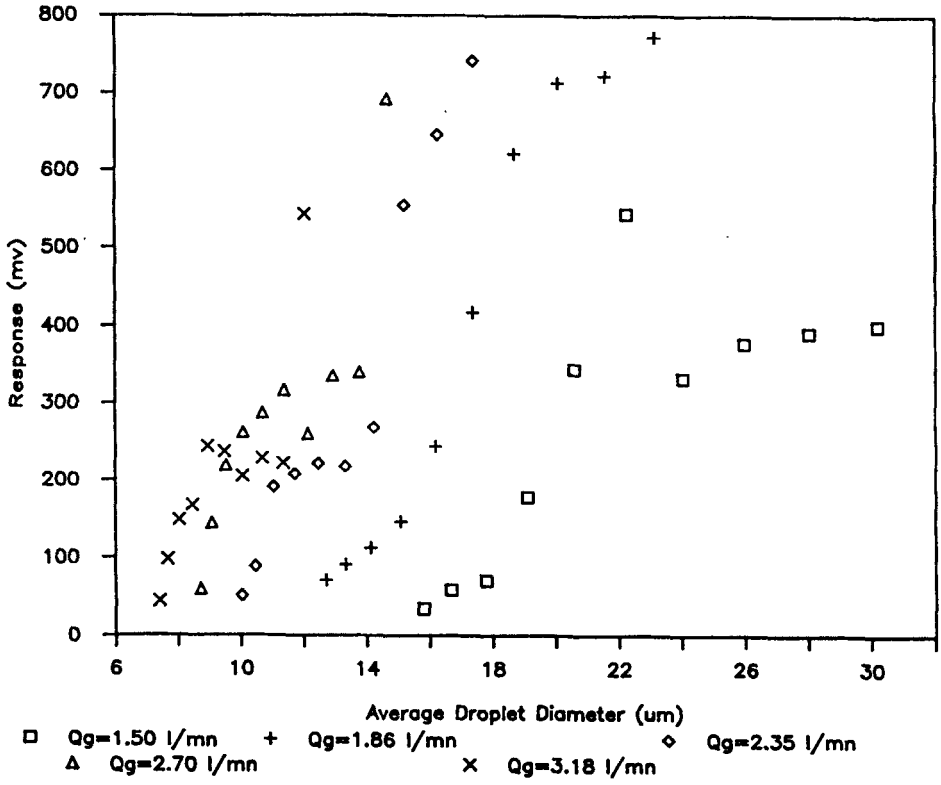

Figure 8. Detector response versus calculated average droplet diameter. Concentric nebulizer.

a - Concentric nebulizer.

The data, especially the response slope (equation 6, coefficient b), are too scattered and the response of the detector is too small to permit a meaningful discussion of the influence of the nature of the solute on the response obtained with an ELSD using a concentric nebulizer. We note only that the blue oil is hard to detect. We can assume that this occurs because the strong absorbance of the oil blue reduces drastically the amount of scattered light. For the other solutes, the influence of the nature of the solute is too weak to be measured.

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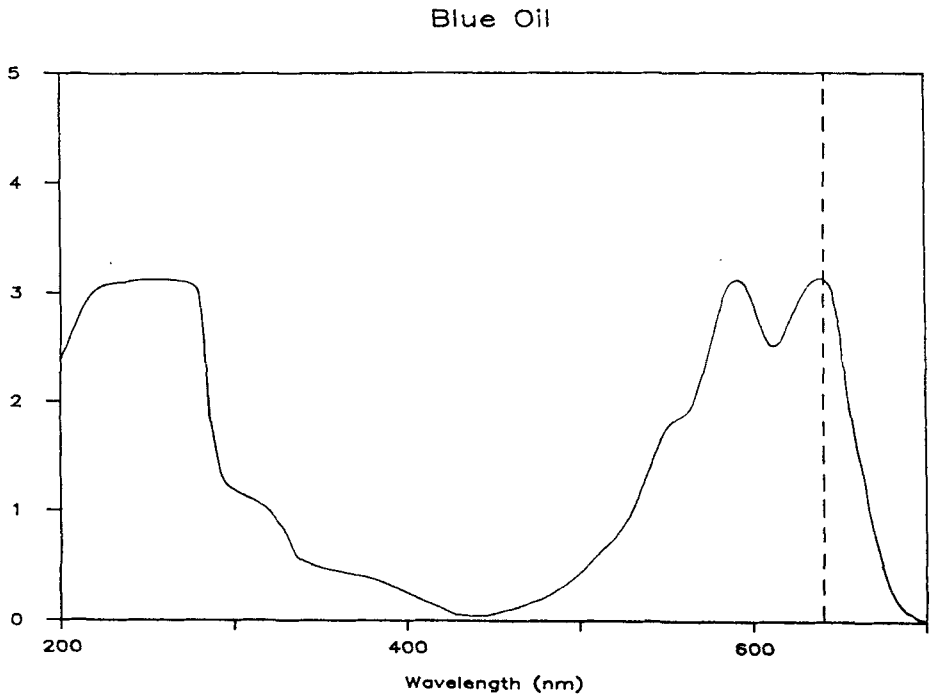

Figure 9. Oil Blue absorbance spectrum (Figure 9a) and oil red absorbance spectrum (Figure 9b), 1 g/l in n-heptane.

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b - Cross-flow nebulizer.

The influence of the nature of the solute is obvious but limited to the coloring oils. The responses for pyrene and anthracene and for stearic acid methyl ester and arachidonic acid methyl ester are very similar but they are quite different from those for the oil red and the oil blue. As expected, the response for the blue oil is also very small with this nebulizer.

Regarding the other solutes, no changes in response can be associated with differences in refraction indices nor to

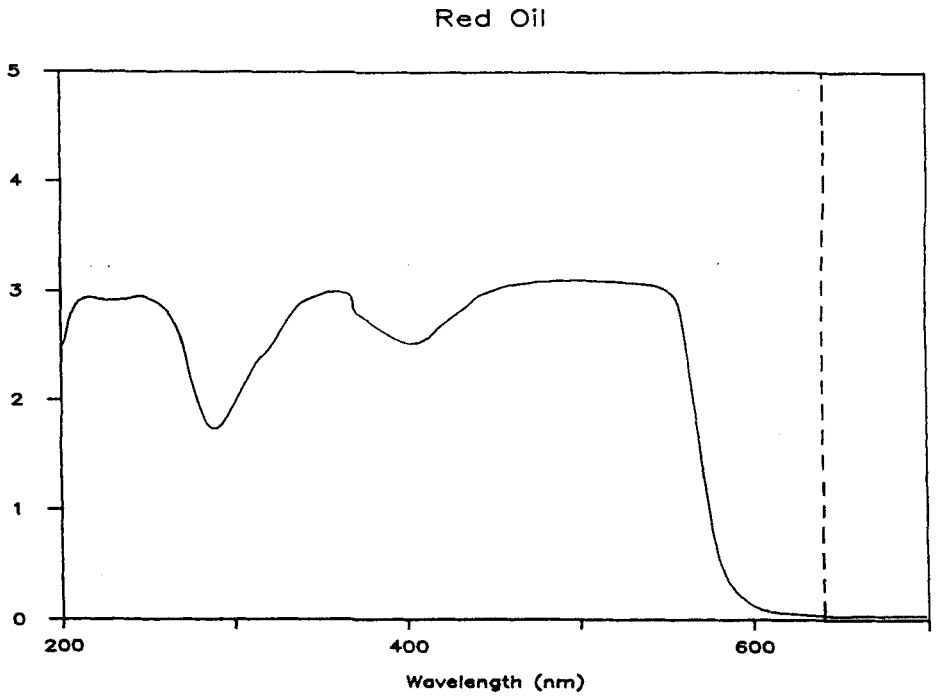

FIG. 9 (continued)

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differences in the physical states of the particles causing changes in the mechanism of light scattering and the appearance of reflection. In fact, many organic compounds will not precipitate and will form nonspherical crystalline particles when the solution is evaporated below their melting point. The proportion of solid particles depends on the probability of seeing a crystal germ formed in each droplet during its residence in the ELSD.

According to a former publication (1), the total amount of scattered light appears to depend but weakly upon the refractive index of the solute. The strong effect of solute absorbance indicates that the ELSD response may depend also upon the chemical structure of the solute. It would be much more so, should a UV beam be used for light scattering measurements.

CONCLUSION

Our experimental results show, in agreement with previous publications (1,3), that there is a definitive influence of the solvent properties on the response of the ELSD. This influence is not properly accounted for by the Nukiyama and Tanassawa equation (16). This can be explained by a probable change of the droplet size distribution during their migration through the drift tube. The aerosol which enters the light scattering cell is not a "primary" aerosol, as it is supposed to be according to equation 2, but is rather, a "tertiary" aerosol. The results obtained can be explained only by assuming a strong dependence of the phenomena leading to a change of the droplet size distribution with the nature of the solvent.

The apparent disagreement between our results and those of Mourey and Oppenheimer (3) may be explained in two ways: 1) their drift tube was much wider than ours, 2)the detection limits of their detector larger than ours, i.e., the density of particles in the gas stream is much lower and it could be that agglomeration of aerosol particles is negligible with their instrument and significant with ours. Also, the relationship between amount of

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light scattered and average size of the droplets is not obvious. The phenomenon is not linear. Changes in the standard deviation of the particles size distribution could partly explain our results, but there is no way to relate, at present, the effect of the nature of the solvent upon this standard deviation.

Finally, the chemical structure of the solute can strongly influence the amount of scattered light. Consequently, the light scattering detector is not a true mass detector as it has sometimes been called. Calibration is required for all quantitative applications of the ELSD as it is for all other HLPC detectors.

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CREDIT

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