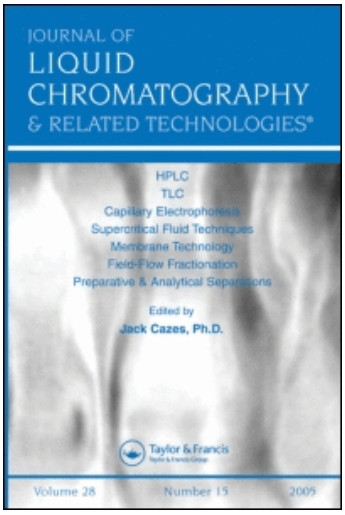

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EFFECT OF THE WAVELENGTH OF THE LASER BEAM ON THE RESPONSE OF AN EVAPORATIVE LIGHT SCATTERING DETECTOR

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ABSTRACT

The effects of the wavelength of the laser beam on the response of an evaporative light scattering detector (ELSD) are discussed. Data characterizing the response of the detector and its dependence on the sample size have been collected for six solutes, using a pulsed dye laser as light source. The experimental results suggest that there is little influence of the wavelength on the intensity of the scattered light. On the other hand, the noise decreases in proportion to the wavelength of the incident light beam. Thus, the detection limit (at constant value of the signal to noise ratio) decreases with decreasing wavelengths. The performance of the ELSD improves when a short wavelength is used.

INTRODUCTION

In a previous paper ([1]), we have discussed the effects of the nature of the solvent and solutes on the response of an

Evaporative Light Scattering Detector (ELSD). We have investigated and compared the performance of two nebulizer configurations for a fixed wavelength of the laser beam used as light source. Our conclusion was that the ELSD is not a truly mass sensitive detector. Like for practically all other detectors for chromatography (the only exception remains the gas density balance, see ref. [2]) the response factors depend on the nature of the compounds analyzed. The amount of light scattered by the droplets formed depend on the nature of these compounds. Also, because the size distribution of the droplets formed depends on the nature of the solvent used as mobile phase, the response factor changes with the solvent. One important parameter was not studied in our previous work, the influence of the wavelength of the incident light beam, which is scattered by the particles of the dry aerosol.

The present paper describes a new version of the ELSD developed in our laboratory. This new detector is based on the use of a pulsed dye laser with a variable wavelength. It was expected that the amount of light scattered would increase with decreasing wavelength and that the performance of the detector would be improved.

The prediction of a relationship between the response factor of the ELSD for a certain compound and the wavelength of the incident light beam is a complex problem, for a combination of reasons. First, the particle size distribution in the aerosol generated by common nebulizers is not well known, and it influences considerably the scattered intensity ([1]). Secondly, as shown by previous results obtained with dyes, the response

factor decreases when the compound absorbs light at the wavelength of the incident light beam ([1]). The effect is sometimes very strong. This observation certainly justifies the development of an ELSD using a light beam with a variable wavelength. With such a detector, the response factor of certain compounds could be either increased or decreased by adjusting the light beam wavelength, depending on the problem to solve. But on the other hand, this means that the spectral dependence of the response factor will be a function of the absorption spectrum of the compound considered. Thirdly, the amount of scattered light is a function of its incident wavelength. This effect will combine with the previous one, and the result may be very difficult to predict, at least for compounds having strong absorption bands. Finally, the response factor depends on the intensity of the primary light beam. With dye lasers it is not easy to predict, nor to measure in a chromatography laboratory, the change in intensity of the light beam when a dye is replaced by another one.

The particle size distribution of the aerosols generated by pneumatic nebulizers, either of concentric or cross-flow design, is very wide ([1]). Thus, the light scattering process in the ELSD is very complex. The intensity of the scattered light beam results from the superimposition of several phenomena described by the theories of Rayleigh and Mie, by reflection or by refraction. It can be affected by the size distribution of the aerosol particles, by their chemical composition, by the intensity and the wavelength of the incident light beam.

In general, the light from a discrete source is scattered ([3,4]) by small particles randomly distributed in a medium of refractive

index different from their own one. There are several processes which can be involved in this phenomenon. Their relative importance depends essentially on the ratio of the diameter, d, of the particle to the wavelength, , of the incident light beam. Rayleigh scattering is predominant when the ratio d/ is smaller than 0.1, Mie scattering becomes preponderant when d/ is greater than 0.1 and smaller than 10. Beyond a ratio of 10, the classical theory of reflection applies. The intensity of Rayleigh scattering increases very rapidly with increasing particle diameter, and is proportional to the reverse of the fourth power of the light beam wavelength. The Mie scattering intensity of a spherical particle increases as the second power of the wavelength (^{4}).

A comparison between the particle size range of aerosols generated by concentric or cross-flow nebulizers (^{1}) and the wavelengths accessible for light scattering experiments (see Table I) shows that both types of light scattering can be expected, so the predominant phenomenon will depend on the shape of the particle size distribution. For large average diameters, and especially at large analyte concentrations, the predominant process will be described by the Mie theory of light scattering. For small particle diameters, and at small analyte concentrations, Rayleigh light scattering will become predominant. It is important to emphasize that, when the analyte concentration is reduced and becomes closer to the detection limit, the predominant light scattering process will pass progressively from the Mie scattering to the Rayleigh scattering. Similarly, for the particles of a given diameter, the predominant process of light scattering will pass progressively from the Mie

TABLE I

Expected Nature of the Light Scattering Phenomenon

d (nm)	Wavelength (nm) 330	400	500	700	
20	0.06	0.05	0.04	0.03	Rayleigh Scattering
40	0.12	0.10	0.08	0.06	
60	0.18	0.15	0.12	0.09	
80	0.24	0.20	0.16	0.11	
100	0.30	0.25	0.20	0.14	Mie Scattering
120	0.36	0.30	0.24	0.17	
140	0.42	0.35	0.28	0.20	
160	0.48	0.40	0.32	0.23	

scattering to the Rayleigh scattering when the light wavelength increases.

This observation has some potentially very important consequences. The intensity of the scattered light in the Rayleigh region decreases when the wavelength increases. On the contrary, in the Mie region, it increases with increasing wavelength. This means that for small concentrations, the response factor increases with increasing wavelength, i.e., the detection limit increases when the wavelength decreases. The sensitivity of the detector would not be improved when a blue or a UV light beam is used to replace the red one which is currently used on our instrument.

The theory of light scattering does not seem able to predict the performance of an ELSD using a pulsed dye laser beam with a variable wavelength, because the aerosol formation and its redistribution is too complex and it is not possible to predict correctly the particle size distribution. Accordingly, an experimental investigation of the effect of the wavelength on the performances of the detector was in order.

E X P E R I M E N T A L

The principle of the ELSD and the basic design of the instrument have been described previously ([1,3,5,6,7,8]). Only the laser, the detection cell, the photo-cell and the amplifier have been changed compared to the instrument used previously ([1]). A schematics of the optical system is shown on Figure 1.

For this study no chromatographic column was used. The sampling system, using a Rheodyne valve (Rheodyne, Berkeley, CA, model 7010) with a pneumatic actuator (Rheodyne, Berkeley, CA, model 5701) and a solenoid valve kit (Rheodyne, Berkeley, CA, model 7163) was connected to the detector through a 30 cm long, 0.25 mm I.D. tube. Sample volume was uniformly 10 l. The peaks obtained were somewhat unsymmetrical; their elution lasted approximately 15 seconds.

A pneumatic cross-flow nebulizer has been used as described previously (1). The scavenger nitrogen gas flow rate in the nebulizer was 1.7 l/mn.

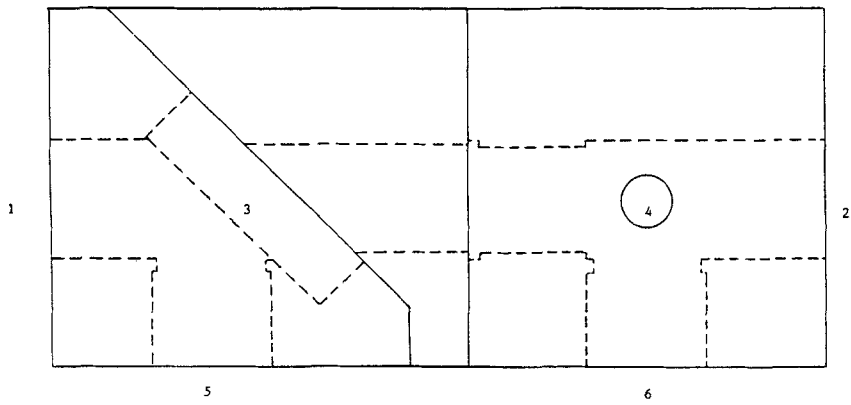

Figure 1. Schematics of the optical system of the detector used. 1 - Laser beam inlet. 2 - Laser beam outlet (to light trap). 3- Fused silica beam splitter. 4 - Inlet of the gas stream from the drift tube. 5 - Reference photo-cell. 6 - Measure photo-cell.

The drift tube temperature was maintained at 60°C, which is sufficient to permit complete vaporization of the solvent used, acetone (chromatography grade, from J.T Baker Chemical Co., Phillipsburg, NJ). The solvent was delivered by a chromatographic pump (Waters Assoc., Milford, MA, Model 6000A), at a constant flow rate of 0.3 ml/mn. All solutes, obtained from Sigma Chemical Co. (St Louis, MO), were 99% pure. Solute references are: pyrene [N°. P-2146], anthracene [N°. A-3885], arachidic acid methyl ester [N°. A-3881] (AAME), stearic acid methyl ester [N°. S-5376] (SAME), oil red EGN [N°. O-2003], oil blue N [N°. O-8376].

The light beam was produced with a 1.6 mw nitrogen laser (Laser Science Inc., Cambridge, MA, model VSL-337 N°. 337000) which gives a 3 nsec light pulse at 337 nm, with a 40 kw peak power and a 0.1 nm spectral band-width. The pulse frequency is adjustable

from 1 to 20 Hz. We have used an external trigger, built in the laboratory, working at 20 Hz. The nitrogen laser pumped a dye laser with a tunable output from 360 to 700 nm (Laser Science Inc., Cambridge, Ma, model Dye Laser Module N°. 337120). The dye laser used a 1 cm quartz dye cell and one of five dyes, all from Laser science; BBQ (wavelength 366-395 nm, peak gain 386 nm, N°.337876), Coumarin 440 (wavelength 416-473 nm, peak gain 437 nm, N°.337860), Coumarin 481 (wavelength 460-518 nm, peak gain 481 nm, N°.337852), Coumarin 540A (wavelength 508-627 nm, peak gain 542 nm, N°.337840), DCM (Wavelength 620-680 nm, peak gain 655 nm, N°.337820). For this study, the wavelengths were adjusted at peak gain.

The light scattering cell is a pipe to the side of which is connected the measure photo-cell which collects the scattered light. The signal is the differential measure from a reference photo-cell and the measure cell. The former photo-cell is placed before the entry of the sample in the cell, and receives light reflected by a beam splitter on fused silica, fixed at a 45° angle from the laser beam (See Figure 1). Because of the dimensions of the laser beam (height 3 mm, width 8 mm) the internal diameter of the light scattering cell is 1 inch. Both cells are placed in order to obtain the same optical distance covered. The signals are measured at the maximum of power during about 0.5 ns, the intensity of the signal being approximately constant during this time. The ratio of the signals is measured through a Log-AntiLog converter with a time constant adjustable from 0.5 to 3 s, built in the laboratory.

D I S C U S S I O N

We first describe the characteristics of the laser beam obtained. Then we discuss the performances of the new ELSD and its potential as a detector for liquid chromatography.

I - Measure of the scattered light.

The measurement of the amount of scattered light and its reproducibility were a major problem because of the instability of the laser beam in terms of power, focalization and optical path. The laser used was not the most suitable for that kind of experiments.

With the type of pulsed laser used, the peak power at constant wavelength is not reproducible within better than about 5%. In order to measure the absolute response, we need to use an ideal target. The width of this target is equal to the width of the laser beam and its height is equal to the dimension of the optical cell, in order to scatter a constant fraction of the light beam. Then, the noise is very important, because of the important pulse to pulse fluctuations of the pulse energy. With a differential measurement, the noise due to these power fluctuations is reduced by a factor 20. If the target is narrow, about 10% of the width of the laser beam at a given position into the optical cell, the noise increases resulting from fluctuations of the optical path length. When the width of the target is equal to the dimension of the optical cell and its height is 10% of the height of the laser beam, the noise is approximately equal

to the one obtained with an ideal target but the intensity of the scattered light is not constant along the target. From these observations, we conclude that we should not expect an identical response for particles which have an equal diameter but are travelling at different distances from the axis of the detector cell. This is so because the amount of light scattered by a particle is a function of its position in the optical cell and of the time since the origin of the pulse. But since we measure the signal of the photo-cells for a constant time, which is at least 10 times larger than the real acquisition time, we obtain a reproducible chromatographic signal.

If we change the wavelength using the dye laser, another complication arises from the very variable yield of the dye laser, depending on the nature of the dye used. The maximum power of the dye laser beam is several orders of magnitude smaller than that of the UV laser beam used to pump the dye laser. This phenomenon makes it difficult to compare directly results obtained at different wavelengths. Comparisons between relative responses are easier and, for this purpose, we have used methyl stearate as a reference solute.

II - Performances of the ELSD.

It has been shown by many previous authors ([1,2,4-7]) that the response of the ELSD is exponential. The peak area, A, is proportional to a certain power, a, of the analyte concentration, C:

$$A = b \cdot C^{a} \qquad (1)$$

where a and b are numerical constants, function of the nature of the analyte, of the mobile phase ([1]) and of the other experimental conditions. The results obtained are summarized Table II, where we give the values of the coefficients a and b obtained by fitting the experimental results (peak area versus the concentration of the injected sample) on equation 1, for an sample sizes ranging from 1 to 8 g.

In double logarithmic coordinates, log b is the intercept of a straight line whose slope is a. b is also the response for a 1 g sample. Typically, the value of a is around 1. In a former work (1), we found for a cross-flow nebulizer values of the slope ranging from 1.2 to 0.8.

In the present study, the square of the correlation coefficient is better than 0.90 for 34 calibration curves, except those corresponding to the blue oil at 440 nm, the red oil at 481 nm, the methyl arachidonate at 540 nm and the blue oil at 655 mn. For these compounds at these wavelengths, the responses were too small to be measured with any accuracy. The dyes used absorb light strongly at these wavelengths, which explains easily the observation ([1]). The phenomenon is more difficult to explain with methyl arachidonate. For these 30 calibration curves the slopes measured range from 0.75 to 1.87, with an average value of 1.14. The standard deviations on these slopes are all smaller than 15%, 19 are smaller than 10% and 6 smaller than 5%. The ordinates of these 30 calibration curves range from 1.58 to 3.08, with an average value equal to 2.51. The standard deviations of these ordinates are all smaller than 2.9% and 29 are smaller than 2%.

TABLE II

Calibration Data for different solutes and wavelengths

	Pyrene	Anthr.	SAME	AARE	Red Oil	Blue Oil
			Wavelength = 337 nm			
Ordinate	2.43	3.08	2.62	2.93		
Std Dev. %	0.90	1.14	0.78	0.45		
R^2	0.993	0.960	0.992	0.996		
# Measur	6.0	6.0	6.0	6.0		
Slope	1.41	0.94	1.22	1.07		
Std Dev. %	4.22	10.21	4.59	3.33		
			Wavelength = 386 nm			
Ordinate	2.38	2.21	1.78	2.43	2.34	2.40
Std Dev. %	0.99	1.35	2.89	1.35	1.34	1.51
R^2	0.984	0.980	0.964	0.960	0.952	0.953
# Measur	6.0	6.0	6.0	6.0	6.0	6.0
Slope	1.01	1.13	1.44	0.87	0.76	0.89
Std Dev. %	6.34	7.16	9.67	10.27	11.16	11.04
			Wavelength = 440 nm			
Ordinate	2.46	2.76	2.30	2.65	2.55	2.96
Std Dev. %	0.79	1.28	1.39	0.61	1.36	1.36
R^2	0.993	0.972	0.981	0.990	0.977	0.800
# Measur	6.0	6.0	6.0	6.0	6.0	6.0
Slope	1.26	1.13	1.24	0.87	0.80	0.43
Std Dev. %	4.16	8.48	7.00	5.05	11.79	25.03
			Wavelength = 481 nm			
Ordinate	2.72	2.94	2.50	2.81	2.66	2.78
Std Dev. %	1.26	1.02	1.56	1.35	2.77	1.00
R^2	0.973	0.979	0.972	0.955	0.842	0.960
# Measur	6.0	6.0	6.0	6.0	6.0	6.0
Slope	1.11	1.12	1.24	0.95	0.92	0.75
Std Dev. %	8.34	7.31	8.55	10.80	21.69	10.16

T A B L E I I (cont'd)

	Pyrene	Anthr.	SAME	AARE	Red Oil	Blue Oil
Wavelength = 540 nm						
Ordinate	2.25	2.43	2.68	2.77	2.27	1.58
Std Dev. %	1.07	2.63	1.60	2.85	2.41	1.99
R^2	0.989	0.922	0.933	0.534	0.927	0.992
# Measur	6.0	6.0	6.0	6.0	6.0	6.0
Slope	1.27	1.20	0.87	0.46	1.06	1.87
Std Dev. %	5.15	14.51	13.44	46.67	14.00	4.55
Wavelength = 655 nm						
Ordinate	2.59	2.41	2.43	2.73	2.92	3.09
Std Dev. %	1.59	1.55	1.44	1.49	0.66	1.57
R^2	0.969	0.987	0.982	0.960	0.993	0.860
# Measur	6.0	6.0	6.0	6.0	6.0	6.0
Slope	1.25	1.79	1.43	1.09	1.21	0.65
Std Dev. %	8.93	5.65	6.69	10.17	4.31	20.15

As it is apparent from the data in Table III, the effect of the wavelength of the laser beam is very important for the blue oil, and much less important for the other solutes. This results from the values calculated for the standard deviations of the ordinates and slopes of the responses for each solute at the different wavelength. The larger these standard deviations, the stronger the variation of the response with the light wavelength. For the blue oil the standard deviations on the slopes and intercepts are around 46% and 23%, respectively. For all the other solutes the standard deviations on the slopes and intercepts are inferior to 21% and 10%, respectively. We see a similar effect on Figures 2 and 3. The intercept ordinates of all solutes seem to vary in the same direction, although with different amplitudes. On the other hand, it does not seem that there is much of a correlation between the value of the slope and the wavelength.

TABLE III

Average ordinate and slope for different solutes over the range of wavelength studied

	Pyrene	Anthr.	SAME	AARE	Red Oil	Blue Oil
Ordinate	2.47	2.64	2.39	2.71	2.52	2.46
Std Dev. %	6.1	11.7	12.5	6.3	9.9	22.7
Slope	1.21	1.21	1.24	0.97	0.95	1.04
Std Dev. %	10.7	21.5	15.3	10.3	20.0	46.2

The standard deviations are calculated for the response data of each solute over the range of wavelengths investigated. These values indicate the effect of the wavelength of the laser beam on the response.

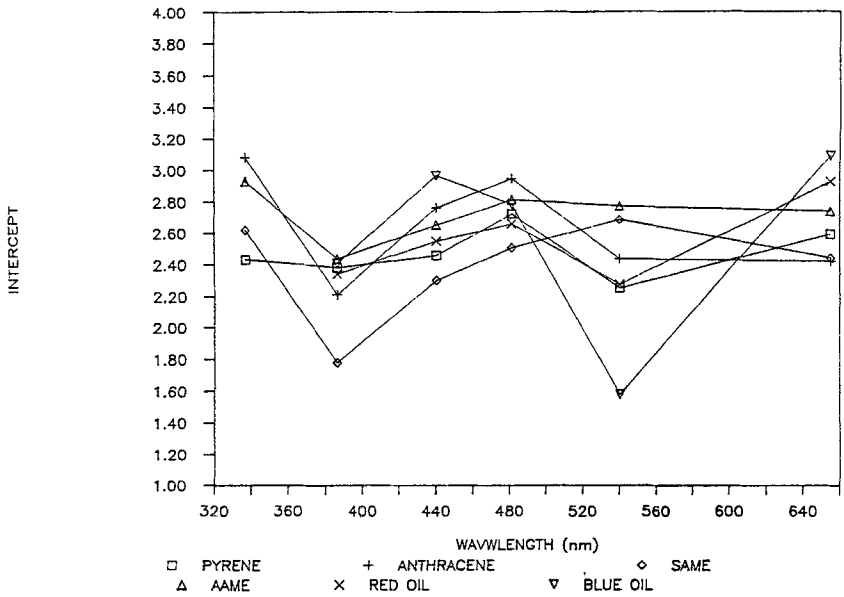

Figure 2. Plot of the ordinate of the LSD response curve (equation 1 in double logarithmic coordinates) versus the wavelength of the laser beam, for six different analytes.

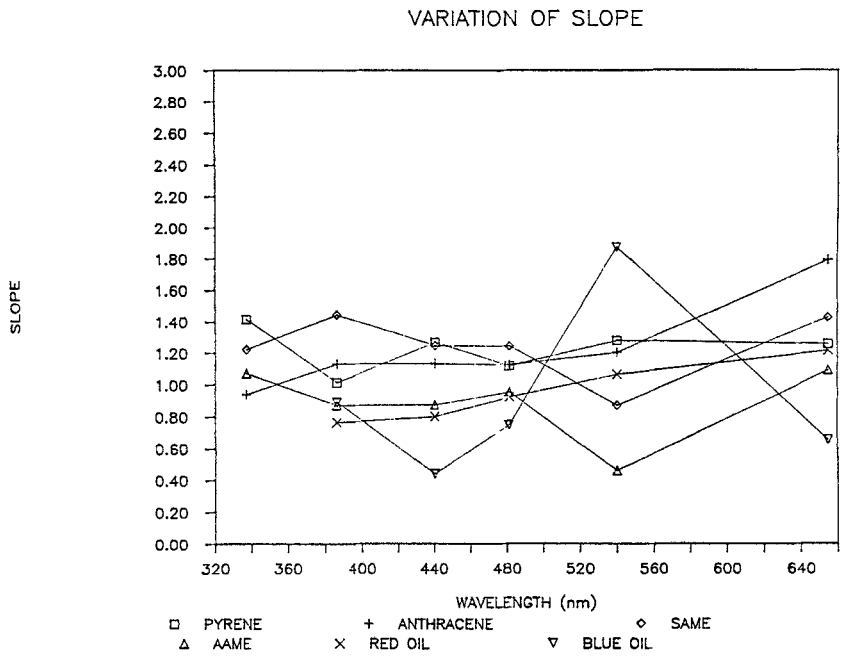

Figure 3. Plot of the slope of the LSD response curve (equation 1 in double logarithmic coordinates) versus the wavelength of the laser beam, for six different analytes.

In order to eliminate the influence of the variation of the power of the dye laser beam with the wavelength, we have normalized the response data by reference to the response of methyl stearate (SAME), a compound which does not absorb in the spectral region studied. Figures 4 and 5 show the plots of the normalized response ordinates and slopes, respectively, versus the laser beam wavelength. The curves are simultaneously above and then under the reference line, indicating of a trend which is not independent of the solute, however. For example, the response for methyl arachidonate does not vary proportionally to that of methyl stearate, although the two solutes are so similar. The

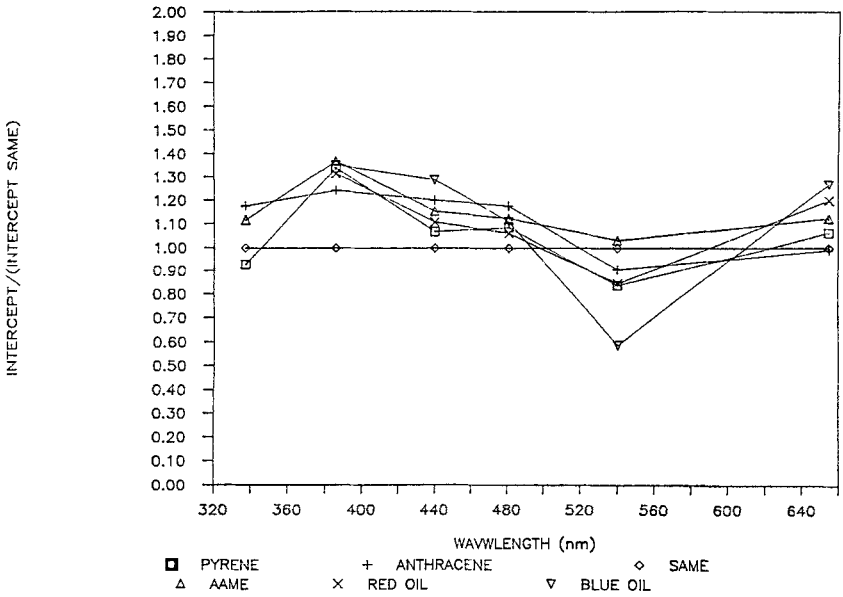

Figure 4. Plot of the normalized ordinate of the LSD response curve (equation 1 in double logarithmic coordinates) versus the wavelength of the laser beam, for six different analytes. Reference: methyl stearate.

variation of the normalized ordinate intercept of methyl arachidonate is identical to that of the other solutes (Figure 4). We can see the same behavior with the slope of the calibration curves (Figure 5). The response of the blue oil is strongly modified by the change of the wavelength. Unfortunately the variation of the ordinate and the slope are not monotonous and we cannot derive any general conclusion regarding the effect of the wavelength of the laser beam on the behavior of the detector response.

The noise of the detector signal decreases with decreasing wavelength. At very low values of the signal/noise ratio, the

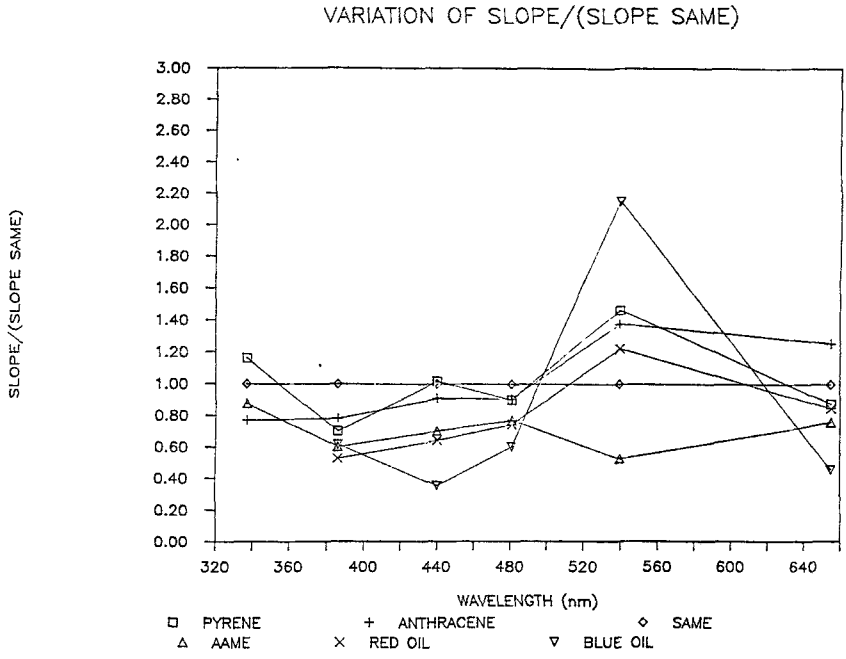

Figure 5. Plot of the normalized slope of the LSD response curve (equation 1 in double logarithmic coordinates) versus the wavelength of the laser beam, for six different analytes. Reference: methyl stearate.

droplet size is very small, the ratio d/ is also small and we reach the Rayleigh scattering. The detection limit for each solute is given Table IV. Figure 6 shows a plot of the detection limit of each solute versus the wavelength of the laser beam. It shows a wide distribution of these detection limits. The average detection limit for all wavelengths studied is equal to 0.69 g, with a standard deviation around 37%. The smallest variation are observed with pyrene, anthracene, methyl stearate and arachidonate, and the red oil, for which the standard deviation is equal or inferior to 33%. The highest variation is observed

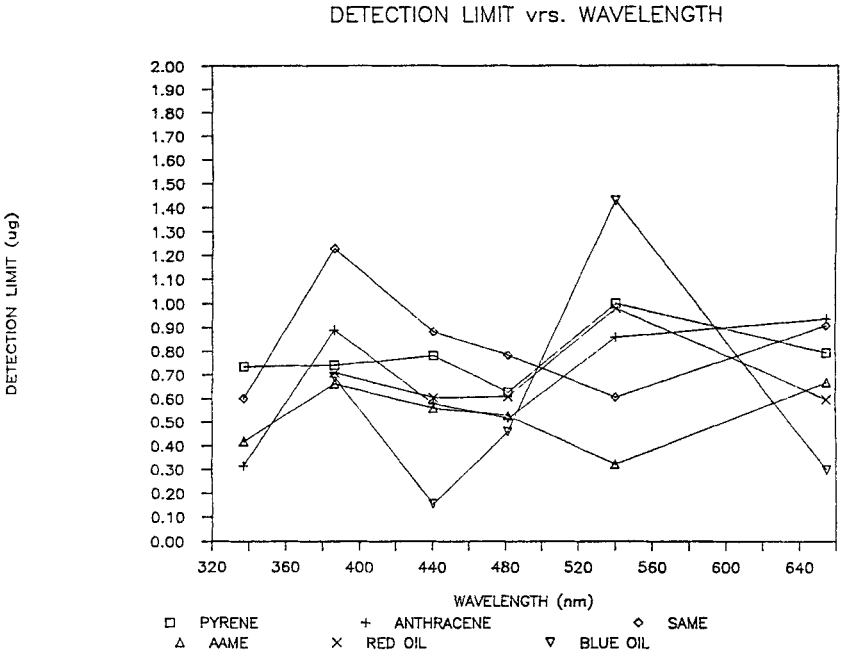

Figure 6. Plot of the detection limit for six different analytes versus the wavelength of the pulsed dye laser beam.

T A B L E I V

Detection limit (g) for different solutes

as a function of the wavelength of the light beam.

Wave-length (nm)	Pyrene	Anthr.	SAME	AAME	Red Oil	Blue Oil
337	0.73	0.31	0.60	0.41		
386	0.74	0.89	1.23	0.66	0.71	0.69
440	0.78	0.58	0.88	0.56	0.60	0.15
481	0.62	0.51	0.78	0.52	0.61	0.46
540	1.00	0.86	0.60	0.32	0.98	1.43
655	0.79	0.93	0.91	0.67	0.59	0.30

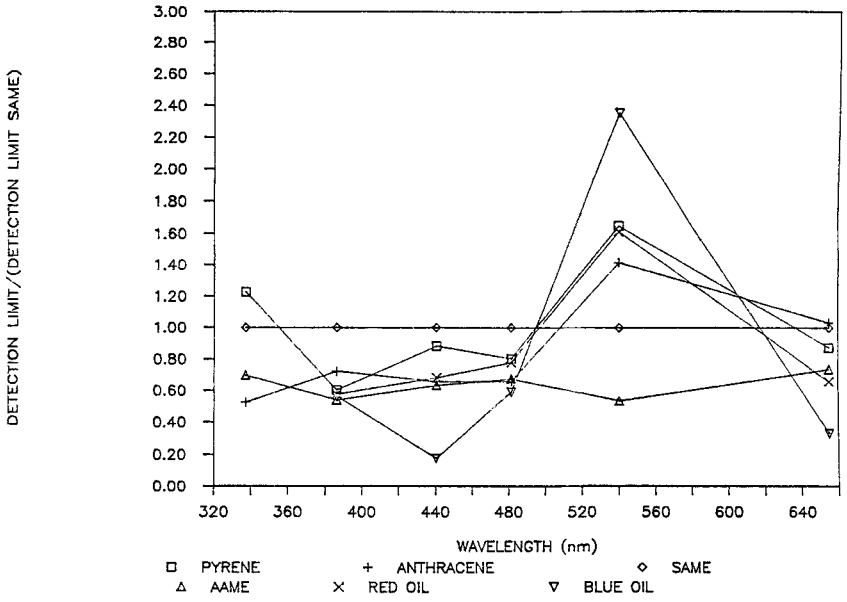

Figure 7. Same as Figure 6, but detection limit relative to methyl stearate.

with the blue oil, in which case the standard deviation is around 70%. This result is related to the strong influence of the wavelength on the response of the blue oil.

The detection limits reported here are higher than those obtained with a laser at fixed wavelength and published earlier (around 10 ng, ref. 1). This is due to the strong dispersion of the light intensity across the cross-section of the laser beam. Figure 7 shows a plot of the ratio of the detection limit of one solute to the detection limit of methyl stearate versus the wavelength of the laser beam. The detection limits tend to be relatively better at small wavelengths and to increase with increasing

wavelength, except for methyl arachidonate. For this compound, the detection limit remains approximately constant, about 40% lower on the average than that of methyl stearate. This result is not surprising, since both compounds are very similar.

CONCLUSION

The use of a pulsed laser beam with a variable wavelength as the light source of an evaporative light scattering detector has not permitted to cross the border line between the two regions where different light scattering processes take place, the Mie and Rayleigh scattering and to operate effectively in the latter area. The effect of the change of the incident beam wavelength on the ELSD response is not constant from one solute to another one, which confirms the dependence of the response on the nature of the analyte, but the magnitude of the change of the response factor with a change of the wavelength in the domain studied remains small. When methyl stearate is used as a reference, the ordinates of the calibration curves are superior to 1.00 for the short wavelengths and inferior for higher wavelengths. The slopes are smaller than unity and increase with increasing wavelength. The noise also increases with increasing wavelength. When the wavelength increases, the detection limits relative to methyl stearate increases. At low wavelengths they are smaller than unity; they become larger at high wavelengths.

From these observations, it results that the detector performance, in term of signal to noise ratio, is better at short wavelengths. In the general case, where a monochromatic light

source is used to operate an ELSD, the properties of the solutes influence their response factor. Accordingly, in order to perform accurate quantitative analysis, a calibration curve is preferable to the use of an internal standard or a mass calibration.

C R E D I T

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