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STUDY OF THE QUALITATIVE AND QUANTITATIVE PROPERTIES OF THE LIGHT-SCATTERING DETECTOR

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SUMMARY

A number of technological improvements to the design and construction of the light-scattering detector are reported. They have resulted in an improvement of the detection limits by more than an order of magnitude. The detector has thus become slightly more sensitive than the refractive index detector when the latter is used under optimum environmental conditions. The detection limits are approximatively 1 ppm in the eluent at column outlet, but the detector remains extremely unresponsive to changes in the physical environment: there is no background or baseline drift, even in gradient elution, as substantiated by chromatograms obtained for complex samples of butterfat and cod liver oil.

The noise observed with the present equipment still results mainly from the photomultiplier but in some instances a contribution due to the non-volatile content of the solvent used has been observed. It depends on the solvent batch and can be reduced to some extent by distillation and filtration of the solvents prior to use.

The detector response per unit mass of sample is almost constant, at least for compounds belonging to a given chemical group and which condense as liquids, but it is not linear. It is shown how the handling of the data for quantitative analysis can be easily achieved. A number of analyses carried out with the detector are reported.

INTRODUCTION

The response of all commonly employed liquid chromatographic (LC) detectors results from the variation of a physico-chemical property of the solvent with increasing concentration of the solutes being analysed. To achieve the very low detection limits now required, extreme sensitivity is necessary in the determination of the difference between the measurements for the pure solvent and for the column eluate. Unfortunately, there is no fundamental, important, qualitative difference between solvent and solutes in most practical instances of significance in LC. The value

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of most properties of choice is not zero for the pure solvent and a differential measurement is necessary. For this reason also, there cannot be any non-selective detector that does not respond by a more or less important baseline drift whenever the composition of the mobile phase changes, as in gradient elution.

It is well known that non-selective detectors, such as the refractive index (RI) detector, are almost or totally impossible to use when the gradient elution technique is necessary, as it is often required in the analysis of complex mixtures such as those found in lipid research. At present, the most popular detector is the UV photometer or spectrophotometer. Although it has most of the qualities requested for a good LC detector, it suffers from some significant drawbacks that restrict its applicability and, accordingly, also restrict the field of applications of LC as long as some other detector is not able to overcome them. One of the most stringent limitations of the UV detector is the great difficulty of using solvents that absorb light significantly in the UV region above *ca.* 200 nm. This necessitates the use of a differential system (double-beam instruments), and does not permit gradient elution with these solvents any better than the RI detector. Thus, useful eluents such as chloroform, acetone, methyl acetate and aromatic solvents cannot be used in any gradient elution analysis with a UV detector. There are only few alternatives at present, as most detection methods compatible with gradient elution are extremely selective. This limits the applications of reversed-phase LC to the use of a very limited number of eluents, essentially water, methanol, acetonitrile, tetrahydrofuran and their mixtures. The development of non-aqueous reversed-phase (NARP) LC is accordingly drastically limited, in spite of the interest of the technique for some applications.

The light-scattering detector, although suffering of some drawbacks that will be discussed below, offers the possibility of working with any solvent that is markedly more volatile than the solutes. The principles of this detector has already been described and some of its properties discussed[1,2]. Nebulization of the column effluent in a stream of warm gas followed by vaporization of the solvent leaves a cloud of particles consisting of the non-volatile material contained in the eluent. These particles are carried by the warm gas stream across a laser beam. Light diffracted by the particles is collected and transformed into a current used as the detector signal. Thus, a first important property of the detector is that a very low background signal will be observed, as there will be no light scattered from the laser beam by the solvent vapour. However, this requires the use of clean solvents, with only trace amounts of non-volatile impurities, and careful filtration of the suspended particles, such as the fine dust contained in the column packing material. If there is no background signal with the pure solvents, there will be no baseline drift in gradient elution, which is confirmed by our observations[1]. Accordingly, the signal baseline will be very stable and there will be no drift associated with detector start-up, ambient temperature fluctuations or gradient elution. The detector can be used with any solvent or mixture of solvents, provided they are volatile enough and their vapours do not absorb light markedly at the laser wavelength used, which is not a serious limitation.

The detector described here and in a previous paper[1] has little in common with the detector described by Jorgenson *et al.*[3] a few years ago, under the same name, except that in both instances light scattering by small particles is used as a detection principle. Jorgenson *et al.*, however, used reactions in solution to precipitate small particles in the column effluent, and used nephelometry of the mobile phase, deter-

mined from the amount of light scattered, as a measure of the solute concentration. There is no separation between the solvent and the solutes, and this detector is, to some extent, selective. The detector studied here is not selective for non-volatile substances.

The purpose of this paper is to demonstrate the wide possibilities of applications of the light-scattering detector in the analysis of fats and related products, materials for which both the RI and the UV detectors do not permit the convenient achievement of satisfactory analysis, for the following reasons: (i) in most instances the large range of retention time of the components of the sample (illustrated by the large range of molecular weight of the triglycerides, for example) demands gradient elution analysis; (ii) the most convenient mobile phases for these analyses contain acetone or chloroform, which absorb too much in the far UV region; and (iii) these compounds do not absorb significantly in the UV region above 200 nm.

The paper also describes some design improvements made recently and discusses some properties of the detector not reported previously[1].

EXPERIMENTAL

A description of the detector has already been published[1]. Some changes have been made to improve some of its characteristics, especially the signal-to-noise ratio and thus to reduce the detection limits. A scheme of the new detector design is shown in Fig. 1.

Optical system

The main changes are in the design of the light source. The laser is a Hughes 3225H-PQ 1 mW helium–neon laser, providing a polarized light beam at 632.8 nm (Hughes Aircrafts Co., Carlsbad, CA, U.S.A.). A glass window is positioned at an angle to the laser beam (*i.e.*, not perpendicular), and several diaphragms are placed along a 6 cm long tube connecting the laser exit window to the light scattering cell. The window is fastened with O-rings to provide a gas-tight seal, preventing flow of the driving gas around the window. This is important, as this gas carries in suspension the sticky particles formed by the non-volatile solutes after vaporization of the solvent. If those particles hit the window and stick to it, they scatterlight and increase the amount of stray light, and hence the background signal and the noise.

Positioning the window at a carefully selected angle and placing diaphragms along the optical path permit a marked reduction in the amount of stray light and in the divergent non-coherent radiation that always accompanies the narrow beam of coherent laser light. This was one of the most important sources of noise in our previous system[1]. Although the use of such a window is necessary to prevent the detector from being sensitive to the dust in the laboratory atmosphere, it must be realized that the polarized light of the laser beam is partially depolarized, for better or worse, when it goes across the window.

The diameter of the glass rod used as a light collector has been increased to *ca.* 5 mm. The tip of this rod has a cylindrical, concave shape and is mirror polished. This permits more efficient light collection, in a wider spatial angle. Moreover, the side walls of the rod are covered with a layer of opaque material to avoid collection of stray light or light reflected by the internal walls of the cell.

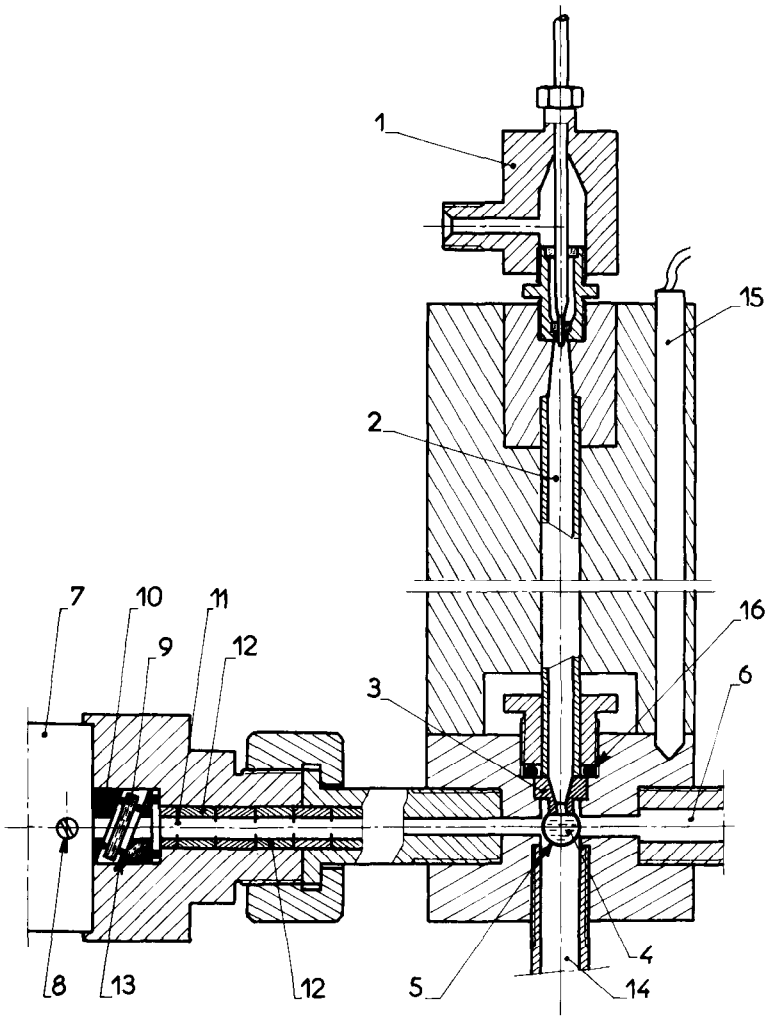

Fig. 1. Schematic diagram of the modified light-scattering detector. 1 = Nebulizer (identical with that shown in ref. 1); 2 = drift tube; 3 = nozzle, for focusing droplets into the laser beam; 4 = glass rod (scattered light collector); 5 = opaque coating of the glass rod; 6 = outlet to "Raleigh horn" (to absorb laser light and avoid back-scattering); 7 = helium–neon laser; 8 = laser shutter; 9 = glass window; 10 = mounting of the glass window; 11 = apertures, to eliminate divergent non-coherent light; 12 = spacers for the diaphragms; 13 = sealing O-rings; 14 = exhaust for the driving gas and suspended particles (valve not shown); 15 = heating cartridge; 16 = sealing O-ring.

Pneumatic system

The nebulizer and the drift tube of the previous design remain unchanged. The end of the drift tube, however, has been modified and has the shape of a nozzle. This forces almost all particles that leave the drift tube to fly across the laser beam, thus increasing the detector signal.

The exhaust from the cell is vented through a valve, which permits control of the pressure inside the cell. It was observed that if the working pressure is above

atmospheric pressure, the detection limits decrease, probably because the diffusion of the particles out of the laser beam decreases. It is also possible that an increase in the pressure results in the formation of larger droplets, and we have shown previously[1] that the response increases with the droplet average diameter.

Careful temperature control of the detector cell was arranged, as it was demonstrated previously that the detector response is a function of the cell temperature.

Other instrumentation

A Model M-6000 A pump, a Model 660 solvent programmer and, for comparative studies, a Model R 401 RI detector and a Model 440 UV detector (all from Waters Assoc., Milford, MA, U.S.A.) were used.

Injections were carried out using a Rheodyne 7120 injection valve (Rheodyne, Berkeley, CA, U.S.A.), equipped with a 5-μl loop, sometimes without a column, for direct study of the detector response.

Chromatographic columns

A small-bore 200 × 2 mm I.D. stainless-steel column was packed in the laboratory with LiChrosorb RP-18, 5 μm (Merck, Darmstadt, F.R.G.). For the achievement of some separations, such as those of butter fats and marine oil triglycerides, a 250 × 4.0 mm I.D. HIBAR LiChrospher 1000 CH-18/2 (5 μm) column (Merck) was used.

For comparison between the performances of the light-scattering detector and those of other detectors, an Altex (Berkeley, CA, U.S.A.) 15 cm × 4.6 mm I.D. column packed with Ultrasphere ODS (5 μm) was used.

All solvents were of analytical-reagent grade, filtered before use on a Millipore filter and degassed in an ultrasonic bath.

Comparison with other detectors

The stream splitter was used with a regular 4.6 mm I.D. column. The flow-rate was about 1 ml/min, and the splitting ratio was 10% to the light-scattering detector. The other detector was connected to the waste line with a short capillary tube (5 cm × 0.25 mm I.D.).

RESULTS AND DISCUSSION

Because the light beam of the laser used is polarized, there is a new parameter to optimize, namely the angle between the plane of light polarization and the direction in which the scattered light is collected. The influence of the other parameters has also been studied in detail, to determine the effect of the various improvements made in the design.

Background current, noise and drift

The dark current (laser on, but shutter off) is slightly reduced, with a noise of 9 pA for a photomultiplier polarization voltage of −630 V. With the laser on and a stream of driving gas of 4.5 l (STP)/min, the background current and the noise are increased to 4.2 nA and 160 pA, respectively (with the previous design they were 200 and 10 pA, respectively). Hence the background current has been increased 20-fold.

This does not affect the stability of the baseline, which does not drift under any circumstance. The noise has been increased 16-fold, but the detection limits have also been decreased markedly. We observed, however, that a reduction in the polarization voltage of the photomultiplier used (372 PM from Hammamatsu) decreased the noise faster than the signal. The trade-off is that a larger gain of the amplifier becomes necessary and the time constant of the system increases. When a time constant below 0.2 sec is not required, however, the detection limits are markedly improved. Therefore, with a polarization voltage of −350 V, the background current becomes 10 pA when the laser shutter is off (dark current of the photomultiplier) and 67 pA with the shutter on. In this last case the noise is only 0.6 pA. A change in either the driving gas or the eluent flow-rate below 0.3 ml/min does not affect visibly the noise or the background signal, provided freshly distilled acetone is used. With less pure solvents the background current and noise increase, as discussed below.

With the present design, the noise originates most of the time from instabilities of the polarization voltage of the photomultiplier or the photomultiplier itself. A significant component of that noise has a frequency of 100 Hz. In some instances, however, it was observed that some significant noise appeared when the solvent bottle was changed. Methanol was observed to be prone to give that effect more often than acetone. We noticed also that some batches of solvent have a larger dry residue than others, and a strong correlation between the increase in the noise and the amount of dry residue was observed. Careful distillation of the faulty solvent eliminated the problem. For example, when pure acetone for liquid chromatography is used, the background current is typically 11 pA larger than when no solvent is pumped. The noise is then between 1 and 1.5 pA. When carefully distilled acetone is used, there is no shift in the background current and no change in noise when the solvent flow to the nebulizer is stopped or started again. With freshly distilled methanol, the results are the same as with non-distilled acetone. It seems to us that if a significant reduction in the noise originating in the measuring system is achieved, non-volatile solvent impurities will rapidly become the major source of noise.

With organic solvents at a flow-rate of 0.20–0.30 ml/min or lower, and with a temperature of the driving gas and drift tube of 35 and 45°C, respectively, the background current and noise are not significantly different from the values observed without solvent flow. When the solvent flow-rate increases, both background current and noise increase, because of increasingly incomplete vaporization of the solvent. At a flow-rate of 4.5 l/min, it takes a few milliseconds for a droplet to fly between the nebulizer and the detector cell, which does not leave much time for solvent vaporization, whereas it would take a solvent flow-rate of *ca.* 1 and 1.5 ml/min for acetonitrile and methanol, respectively, to saturate the stream of driving gas. Hence it is not surprising to see that solvent vaporization is not complete at flow-rates above about 0.3 ml/min.

The use of a longer drift tube is an alternative solution to permit the achievement of complete solvent vaporization at flow rates between *ca.* 0.3 and 1 ml/min. We did not try it, however.

An increase in the temperature of the driving gas and the drift tube would help markedly, but the final temperature must be controlled properly, otherwise serious losses of the most volatile components of the mixture will take place. Such losses occur even for compounds whose vapour pressure is rather low at the driving gas

temperature, as demonstrated by previous results (see Fig. 5 in ref. 1, and the decrease in response for methyl myristate above 30°C, while the response for methyl oleate increased slightly). It takes only 725 ng of a compound with a molecular weight of 300 to saturate a gas volume of 4.5 l if its vapour pressure is 10 μTorr: the detector temperature should be kept close to ambient. Only a carefully designed and accurately carried out temperature profile could achieve total solvent vaporization, while producing a driving gas still almost saturated in solvent, and thus minimize the sample losses.

Accordingly, rather than design such a programme or increase the temperature of the drift tube, and still face solute losses, we preferred to use either small-bore LC columns or a stream splitter at the column outlet, in order to be able to work at a low enough solvent flow-rate to the detector drift tube. The detection limits, in terms of concentration, are unchanged, so there is a decrease in sensitivity only when the sample is limited.

Detection limit

With the modification described above, the injection of 150 ng of methyl behenate gives a peak height of 8.5 nA, with a peak width of 33 μl, resulting in a detection limit of 22 ng under the chromatographic conditions used for the experiment (flow-rate *ca.* 0.30 ml/min, methanol as mobile phase). The resulting detection limit for methyl behenate is 5 ng/sec, and corresponds to a concentration in the mobile phase at column outlet of *ca* 1 ppm (w/v), measured as described previously.[1]

The detection limit for fatty acid methyl esters of lower molecular weight, which on vaporization of the solvent give liquid droplets instead of solid particles like methyl behenate, is about three times larger than that of methyl behenate. For example, it is 4.5 ppm for methyl oleate. Hence this represents an improvement in the detector sensitivity by about a factor 10 over the previous design[1].

Relationship between response and sample size

As shown in Fig. 2, a plot of peak area *versus* sample size in logarithmic coordinates is linear over a ratio of sample sizes exceeding 500:1. The slope of this straight line, however, is 1.69, a value significantly different from those reported previously, *i.e.*, 1.81 in our previous work[1] and 1.0 by Charlesworth[2]. We do not know whether this change in slope is associated with (i) the replacement of the previous laser by one that gives a polarized light beam, (ii) an increase in the angle within which the scattered light is collected by the new device or (iii) the use of a nozzle at the exit of the drift tube into the light scattering cell. All these changes could have affected the response mechanism of the detector, but the most important effect is probably a change in the size distribution of the droplets formed.

In fact, there is no reason to observe response curves such as those in Figs. 2 and 3, which are linear in double logarithmic coordinates, are all parallel and have a slope between 1 and 2. If the ratio of the particle size to the wavelength of scattered light is between 0.1 and 10 (the so-called Mie region) the amount of scattered light is proportional to a power of the particle diameter which is close to 4 (ref. 4). If the ratio is smaller (the so-called Rayleigh region), the amount of scattered light is proportional to the power 6 of this diameter[4]. In fact, there are transition regions where the dependence is intermediate, the power varying with the particle diameter.

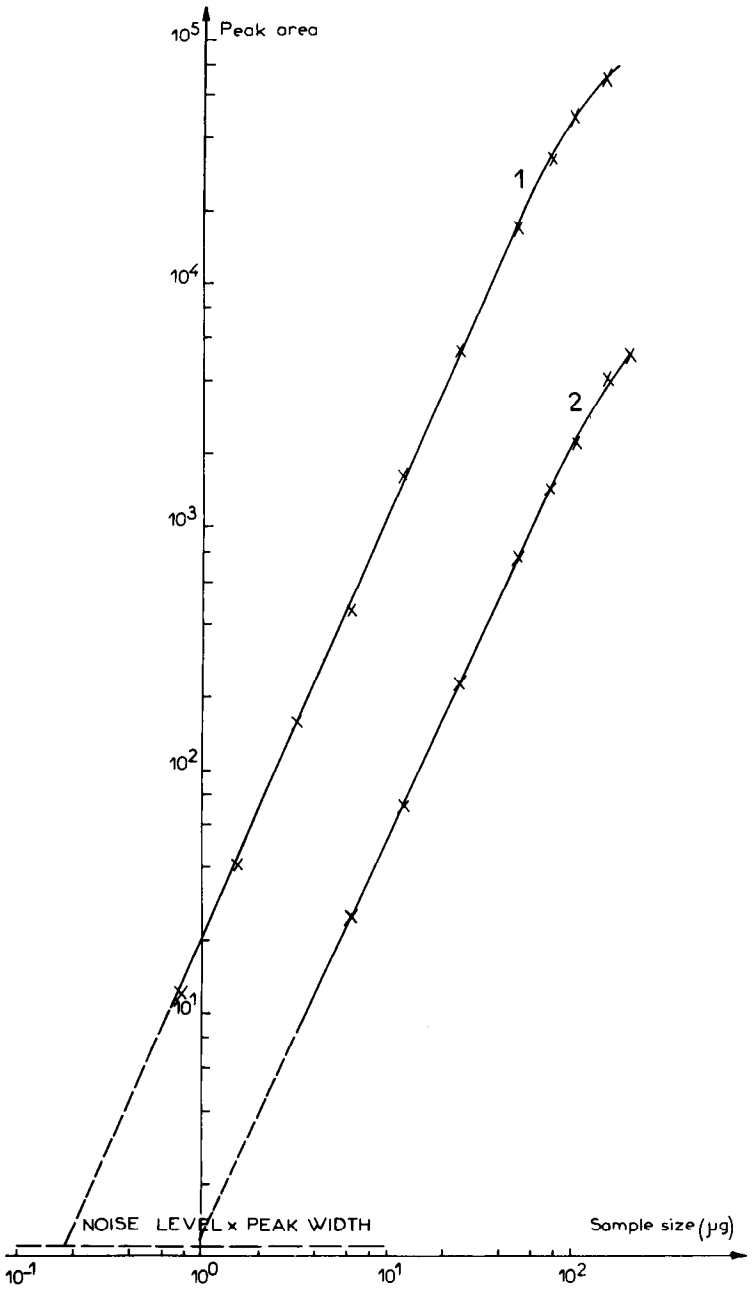

Fig. 2. Detector response: variation of peak area with sample size. Sample: dioctyl phthalate. Solvent: methanol. Direct connection of injection valve to detector. Driving gas flow-rate: 1, 4.5 l/min; 2, 9.0 l/min.

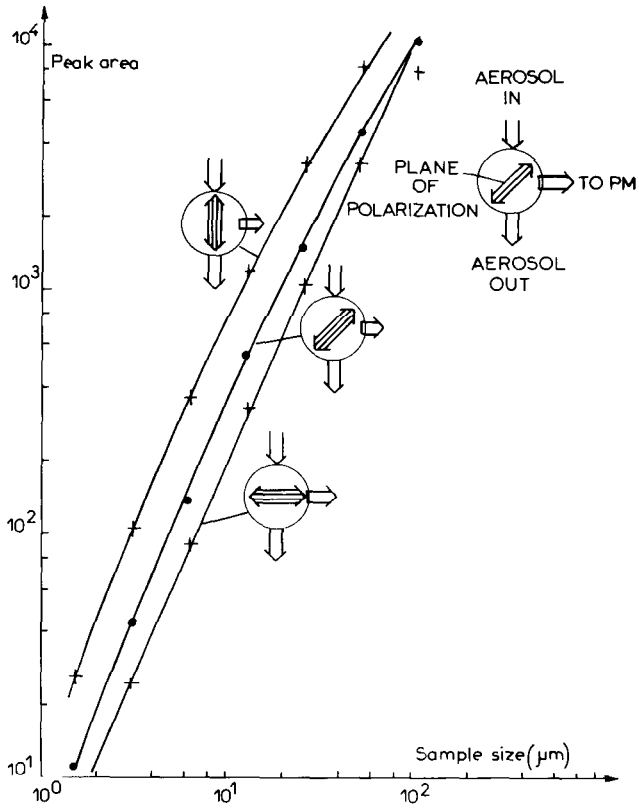

Fig. 3. Influence of the orientation of the light polarization plane on the detector response. Sample: dioctyl phthalate. Solvent: methanol. Driving gas flow-rate: 4.5 l/min.

Under our experimental conditions, the amount of scattered light is proportional to the power 1.69 of the sample size. As the nebulizer forms droplets of solution of constant diameter during elution of a solute, the number of droplets formed per unit volume of solution is constant. Accordingly, the volume of the droplets of solute obtained after vaporization of the solvent is proportional to the solute concentration. This volume is also proportional to the cube of their diameter, and the amount of light scattered in our detector is proportional to the power $1.69 \cdot 3 = 5.07$ of their diameter, which is intermediate between the values observed in the Mie and the Rayleigh regions. It is thus not surprising that the response curve is not linear in logarithmic coordinates over a wide range of sample size, and that some changes in the equipment design result in a small change in the power argument.

At large sample sizes, the response begins to level off. This effect is not due to saturation of the photomultiplier or its electronics, as it occurs for about the same sample size under various experimental conditions when the response is markedly different. It may be related to the large variations in the intensity of the scattered light in different directions as predicted by the Mie theory. This theory shows that the spatial distribution of the logarithm of the intensity of scattered light has the shape of a daisy, the number of petals and their orientation being a function of the

ratio of the droplet diameter to the light wavelength, as well as of the complex refractive index of the particles. In the present case, the light wavelength is constant but, as the concentration increases continuously, the intensity of light scattered in the direction of the collector increases and then decreases, to rise again and oscillate a number of times. Admittedly, the size distribution of the droplet diameter and the integration effect of the light collector whose entrance slit admits light rays coming from a certain range of directions combine to blur the phenomenon to some extent and possibly replace the oscillations by a saturation effect.

A change in the number of droplets generated per unit volume of eluent could also explain the saturation effect. It is difficult to justify such a change, however. The surface tension of the eluate must vary markedly to explain it, and it does not seem possible that the surface tension of an organic solvent could be decreased to a large enough extent by the low concentration of a fatty acid ester reached in these experiments. Saturation occurs under the conditions in Fig. 2 for a concentration of *ca.* 3000 ppm only.

It is interesting to compare our results with those of Jorgenson *et al.*[3], although they used precipitation to prepare particles of solute, suspended in the mobile phase. As shown in Fig. 5 in their paper, the response of their detector is not linear either. A plot of the square root of the response *versus* sample size is curved towards the sample size axis, which shows that the peak area increases slightly more slowly than the square of the sample size. Their data would be compatible with a response proportional to the power 1.7–1.8 of the sample size, the same exponent as that observed here, for a similar phenomenon. It is difficult to comment further at this stage as the average size of the particles obtained by "salting out" precipitation is not known. The detection limits achieved were also in the ppm range.

Influence of driving gas flow-rate on detector response

As shown in Fig. 2, the response varies rapidly with the driving gas flow-rate. It has been observed, in agreement with previous results[1], that the detector signal-to-noise ratio is maximum for a driving gas flow-rate of approximately 4.5 l/min. The existence of a maximum detector response for some intermediate value of the driving gas flow-rate is easy to explain. The comparison between the two curves in Fig. 2 shows that the response mechanism does not change when the flow-rate of the driving gas is varied. With increasing gas flow-rate, but constant eluent flow-rate, the diameter of the droplets formed decreases, whereas their number increases proportionately[2]. As the amount of scattered light increases more rapidly than the cube of the particle diameter[1–3], an increase in the driving gas flow-rate must decrease the detector response beyond some optimum value, as at very low flow-rates nebulization and/or eluent vaporization would not be achieved properly.

At a constant sample size, doubling the flow-rate over the near optimum value of 4.5 l/min results in a reduction of the detector response by a factor of 24, which is an enormous change. If the variation were linear, this would mean that in order to ensure a reproducibility of 2.5% in the peak areas, the fluctuations of the driving gas flow-rate should be kept below about 0.1%. Fortunately, the flow-rate has an optimum value around 4.5 l/min, and a peak area reproducibility of 1–2% can be achieved with fluctuations of flow-rate between 5 and 10%. This is not a drastic specification.

A consequence of the considerable influence of the droplet diameter on the amount of light scattered is that only the larger droplets contribute to the signal. The remainder of the sample, contained in the smaller size droplets, is wasted. Accordingly, care should be exercized to achieve as narrow as possible a size distribution during nebulization of the column effluent. A jet nebulizer is probably not the most appropriate device, and better results could be achieved with a diaphragm. A system similar to that used in LC–mass spectrometry, but with a wider hole, in the 10–20 μm range, would permit the production of a stream of droplets with a narrow size distribution. This possibility is under investigation.

Influence of laser beam polarization on detector response

The laser used delivers a polarized light beam. The polarization plane can be rotated easily which changes the detector response markedly, as demonstrated in Fig. 3.

These results show that the lowest response but the widest dynamic linear range are obtained when the direction of light collection is in the polarization plane. These are the conditions corresponding to Fig. 2. When the polarization plane is rotated, the response increases while the noise remains unchanged. Depending on the exact conditions, an increase in the response by a factor of up to 5 can be observed, which could be useful for trace analysis, if proper calibration is carried out.

Analogous results have been obtained for a number of different compounds. In all instances the response curves are similar, with identical slopes of the straight lines. Further, only very small differences are observed in the response for compounds that are not volatile at the drift tube temperature, but condense as a liquid at this temperature. For volatile compounds the response is small or nil, whereas for compounds that condense as solids it may be much larger[1].

All other data reported in this paper were obtained with the light collector placed in the polarization plane of the light beam.

Determination of column efficiency

There is apparently a serious difficulty in deriving quantitative data corresponding to the peak size or shape from profiles obtained using a non-linear detector, and relating them to column properties or to the sample size.

As observed above and previously[1], the apparent or recorded peak area, A_a, is related to the sample size by the following relationship:

$$A_a = am^x \tag{1}$$

where x is the slope of the response line (1.69 with the present detector), m is the mass of compound injected in the column and a is the response factor. It is simple to relate the actual concentration profile to the response profile recorded on a chromatogram if the peak profile is Gaussian, an approximation which is often satisfactory.

If the concentration profile at column outlet, $C(t)$, is given by the classical equation

$$C = C_0 e^{\frac{(t - t_R)^2}{2\sigma^2}} \tag{2}$$

where C_0 is the maximum solute concentration, t_R the retention time and σ the peak time standard deviation, and if the detector response is such that at any time the signal, y, is related to the solute concentration by

$$y = iC^x \tag{3}$$

where i is the response factor, then the recorded peak is also a Gaussian curve, because of the properties of the exponential. Only the standard deviation is changed. Combination of eqns. 2 and 3 gives

$$y = iC_0^x e^{-\frac{x(t-t_R)^2}{2\sigma^2}} \tag{4}$$

The apparent standard deviation, σ_a, is related to the true value by

$$\sigma_a^2 = \frac{\sigma^2}{x} \tag{5}$$

Accordingly, the column efficiency appears larger than it really is, the apparent plate number being equal to the actual number multiplied by x.

The compatibility between eqns. 1 and 3 is not obvious. It is related to the fact that the peak profile is Gaussian, and would probably extend to an exponential convoluted Gaussian, which gives it a general character in the field of chromatography. For a Gaussian profile, it is easy to show that the constants a (eqn. 1) and i (eqn. 3) are related by

$$a = \frac{i}{\sqrt{x}F^x} \cdot \frac{1}{(\sigma\sqrt{2\pi})^{x-1}} \tag{6}$$

where F is the mobile phase flow-rate.

If the column is apparently more efficient, with a plate number larger for isolated peaks, its real separation power has not changed, however. In the case of interfering peaks, the signal observed being the sum of those resulting from the concentration profiles of the two compounds, we observe that the peaks appear to merge less rapidly than usual when they become closer to each other (*cf.*, Fig. 4).

Let C_1 and C_2 be the concentrations of solutes 1 and 2, respectively, in the effluent of the column, and assume that the response factor, i, is the same for the two compounds. This seems to be the general situation provided that solutes condense in the liquid form and do not crystallize. The detector signal is now the result of the coelution of the two compounds and is given by the equation

$$y = i(C_1 + C_2)^x \tag{7}$$

because the response is related by a power relationship, as expressed in eqn. 4, to the total amount of solute eluting from the column. The underlying assumption is the additivity of volumes of the two solutes in the droplets formed after vaporization of the solvent. This is true in general, as the mixing volume results in only a negligible correction, to a first approximation. When the two compounds are non-miscible, or

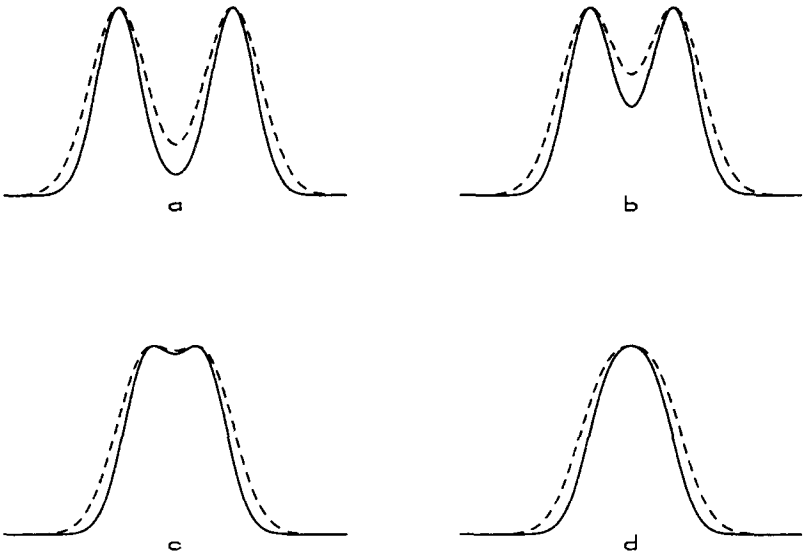

Fig. 4. Influence of the resolution between two peaks on the profile of a doublet. Simulated chromatograms for a doublet of two Gaussian peaks of equal height and standard deviation. Solid lines, light-scattering detector signal with $x = 1.69$; broken lines, signal of a linear detector. Intervals between individual peak maxima: (a) 4; (b) 3; (c) 2.2; (d) 1.8 sec.

condense as solid particles, there may be some unexpected spurious responses, but we have not encountered such a case, in spite of extensive use of the detector.

Eqn. 7 gives a non-linear relationship and explains why, as shown in Fig. 4, the resolution recorded on the chromatogram appears better than it really is.

Determination of peak height and width at half maximum concentration

From eqns. 3 and 4, it results that the height of the peak of a given compound, analysed with the same column but recorded with a linear detector, would be

$$h = j\,(h_a)^{1/x} \tag{8}$$

where h is the "true" peak height, now proportional to the concentration of solute at peak maximum, h_a is the actual height measured on the chromatogram obtained with the light-scattering detector and j is a proportionality coefficient. Accordingly, the peak width at half-height, *i.e.*, at the height corresponding to half the maximum solute concentration, is the width at the height $h/2$. It should thus be measured on the chromatogram at the height $h_a/(2)^{1/x}$.

From the explanation in the previous section, the real plate number can be calculated either by dividing by x the plate number calculated from the width at the actual mid-height on the chromatogram, or by using the conventional equation with the width measured at the height $h/(2)^{1/x}$.

Quantitative analysis

The area of a Gaussian curve is proportional to the product of its height and

its standard deviation. It has just been shown that the apparent standard deviation is too small by a factor of $\sqrt{x}$ (*i.e.*, 1.30), while the peak height is proportional to the power $x = 1.69$ of the maximum concentration, C. The apparent peak area is therefore given by

$$A_a = bC_0^x \cdot \frac{\sigma}{\sqrt{x}} \tag{9}$$

where b is a proportionality coefficient. The actual peak area observed with a linear detector would be given by

$$A = b'C_0\sigma \tag{10}$$

The proportionality coefficients b and b' are different. Combination of eqns. 9 and 10 gives the following relationship between A and A_a:

$$A_a = b''A^x \cdot \frac{\sigma^{(1-x)}}{\sqrt{x}} \tag{11}$$

which shows that the actual peak area is proportional to the power $1/x$ of the apparent area, *i.e.*, that eqn. 1 is valid as long as the peak standard deviation is constant. Hence eqn. 1 permits easy quantitative determinations from the area obtained from electronic integrators, except that the programme of these integrators for area addition and allocation cannot be used, as they are based on the assumption of a linear response.

In Fig. 5, the product of peak width at half-height and the peak height to the power $1/x$ is plotted against sample size for dioctyl phthalate. This is equivalent to plotting the power $1/x$ of the area given by an electronic integrator, as the peaks obtained in this study are Gaussian, and accordingly the peak width at half-height (or at any given fraction of the height) is proportional to the standard deviation. The plot is now linear over the same range as the plots in Fig. 2, but the slope is unity. The straight line does not pass exactly through the origin, but very close to it, which is not unusual and corresponds to an error compatible with the precision of experimental determination.

Measurements of corrected peak area as a function of sample size, as carried out to obtain the data in Fig. 5, were repeated for dioctyl phthalate. A straight line passing through the origin, within experimental error, was again obtained. The slope of this line was not significantly different from that reported in Fig. 5.

In order to check the reproducibility of quantitative data supplied by the light-scattering detector and the precision that can be expected in quantitative analysis, systematic measurements were made on a few compounds. The results are reported in Table I. A reproducibility of *ca.* 1% can be achieved on a routine basis. The systematically low value found for the concentration of dibutyl phthalate is probably due to small losses through partial vaporization in the drift tube.

Use of the light-scattering detector with wide-bore columns

As explained above, excellent results were obtained with various small diameter columns, with flow-rates ranging from 0.03 to 0.30 ml/min. Above this value, noise

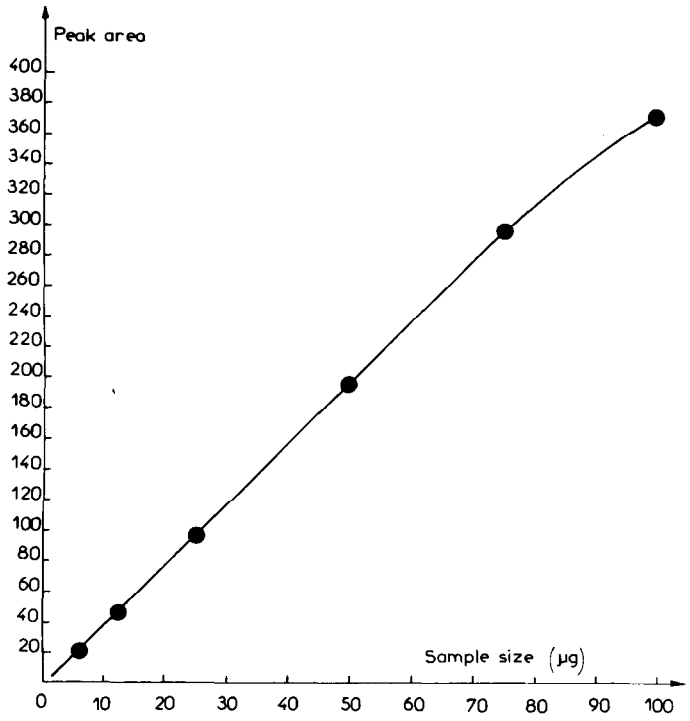

Fig. 5. Plot of corrected peak area *versus* sample size. The area is obtained as the product of the peak width at half-height and the actual recorded peak height to the power $1/x$. This is equivalent to using the peak width at half the maximum peak concentration, as both widths are proportional to the peak standard deviation. A similar result is obtained by using the power $1/x$ of the area count supplied by an electronic integrator. Deviation from linearity at large sample sizes is observed for the same sample size as was observed in Fig. 2, which is expected as it comes from a change in the detection mechanism. Column: 200 × 2 mm I.D., LiChrosorb RP-18, 5 µm. Sample: dioctyl phthalate. Solvent: methanol, 0.2 ml/min. Sample volume: 5 µl.

TABLE I

QUANTITATIVE ANALYSIS OF A SIMPLE MIXTURE

Column: 200 × 2 mm I.D., LiChrosorb RP-18, 5 µm. Peak area measured with a Hewlett-Packard 3390A integrator. Results are averages of five analyses. The relative standard deviation obtained for the same analysis on the UV detector is 0.86%.

Compound	*Peak area reproducibility (%)*	*True amount (%)*	*Measured amount (%)*
Dibutyl glycol phthalate	0.56	33.3	31.7
Dioctyl phthalate	1.38	33.3	34.6
Methyl stearate	1.51	33.3	33.4

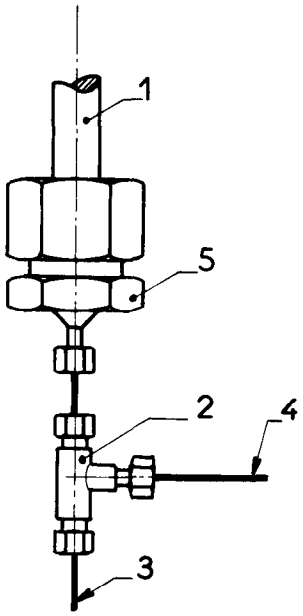

Fig. 6. Stream splitter for the light-scattering detector. This splitter connects the column exit to the nebulizer of the detector and has a near-zero dead volume, because of the flow-rate of the mobile phase. 1 = Chromatographic column; 2 = splitter; 3 = capillary connection to the nebulizer; 4 = capillary connection to a parallel detector or waste; 5 = connecting union. The splitting ratio is controlled by adjusting a valve downstream of the parallel detector and the exhaust valve of the detector (component 14 in Fig. 1).

begins to increase with all organic solvents. To solve this problem, we preferred to keep the flow-rate to the nebulizer at a maximum value of 0.3 ml/min when working with wider diameter columns, and to use a stream splitter between the column and the nebulizer.

This splitter is shown in Fig. 6. Careful adjustment of the valves on the line 4 and on the exhaust of the detector (*cf.*, Fig. 1, component 14) permits an easy selection of the proper value of the splitting ratio. The quantitative results obtained with this system are as good as those obtained with small-bore columns. The sensitivity could be increased for high-molecular-weight compounds by working without a splitter at a higher drift tube temperature, but we have no data on this point.

Comparison with other detectors

Samples of dioctyl phthalate were injected into the column, as explained under Experimental.

The response curves of dioctyl phthalate for both the light-scattering and the UV detectors are plotted in Fig. 7. The detection limit of the UV detector for this strongly UV-absorbing compound is about 2 orders of magnitude lower than that of the light-scattering detector, which is not surprising.

The result would, of course, be the opposite if fatty acid methyl esters or triglycerides were used as samples (see the chromatograms below). From these results,

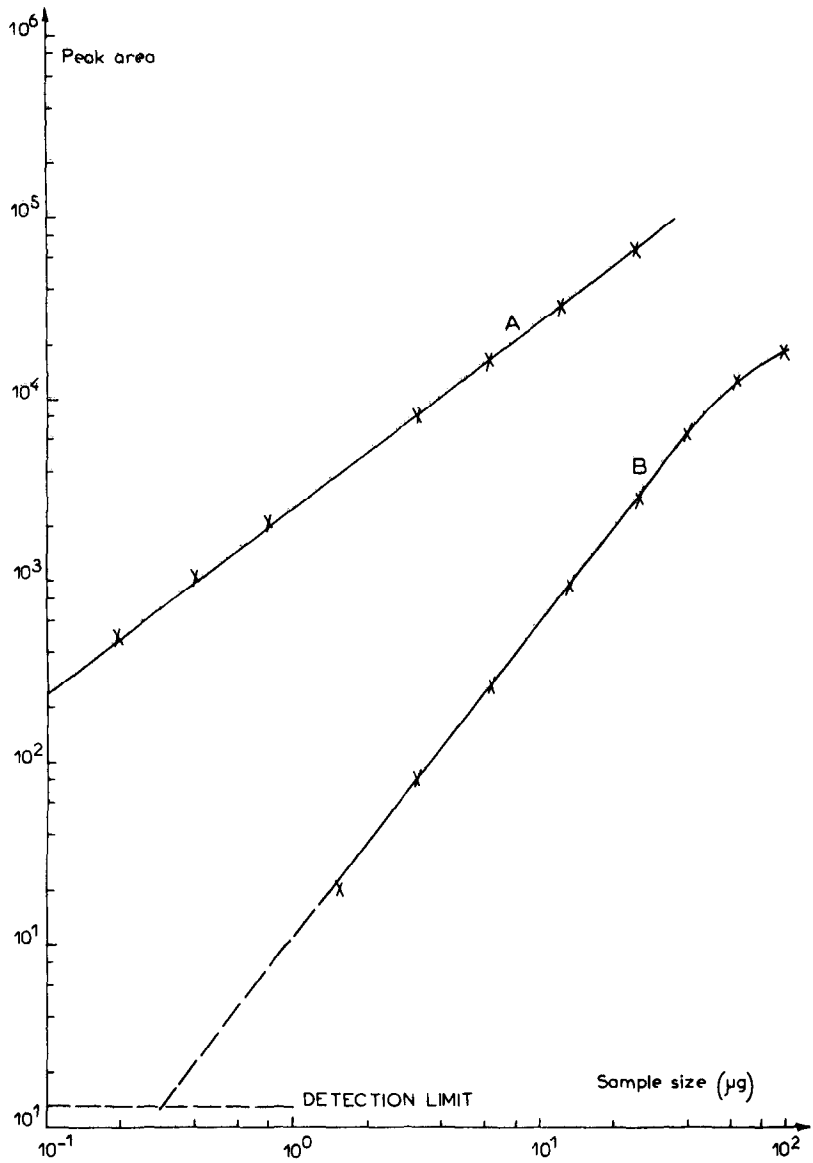

Fig. 7. Comparison between the responses of the UV detector (A) and the light-scattering detector (B). Sample: dioctyl phthalate. Solvent: methanol. Flow-rate: 1 ml/min. Splitting ratio: 10% to the light-scattering detector, 90% to the UV detector. Column: 150 × 4.6 mm I.D., Ultrasphere ODS, 5 μm. The detection limit with the UV detector is approximately 1 ng.

and the molar absorptivity of dioctyl phthalate (ε = 1065 l mol^{-1} cm^{-1} at 254 nm), it follows that the two detectors have comparable detection limits for compounds with ε values of *ca.* 10 l mol^{-1} cm^{-1}.

It is interesting that the true efficiency measured for the column is 5400 plates with the UV detector and 5900 plates with the light-scattering detector. Obviously the splitter has been optimized for the light-scattering detector.

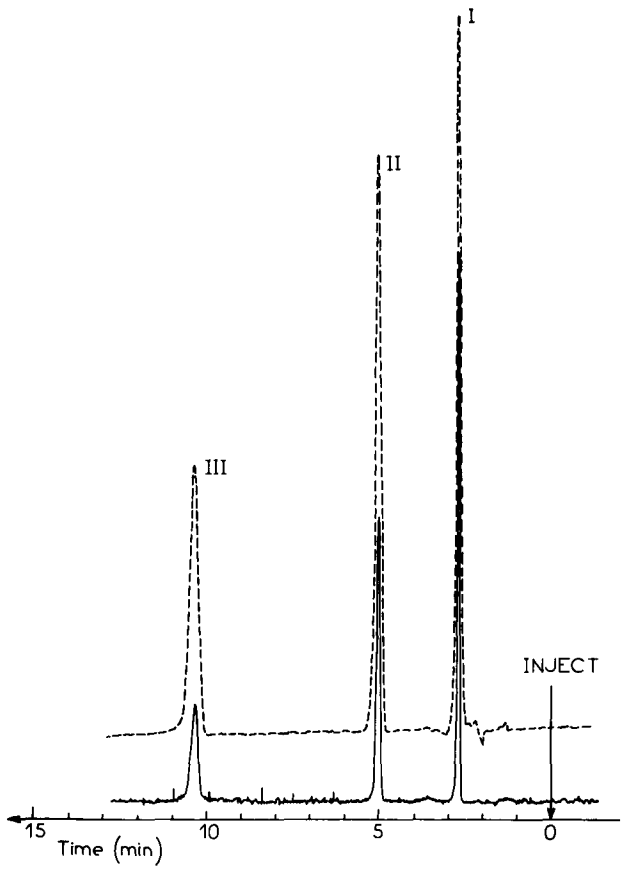

Fig. 8. Comparison between chromatograms obtained with the RI detector (solid line) and the light-scattering detector (broken line). Sample: mixture of equal masses of dibutyl glycol phthalate (I), dioctyl phthalate (II) and methyl stearate (III). Column and chromatographic conditions as in Fig. 7 and Table I.

In another series of experiments, the performance of the light-scattering detector were compared with that of the RI detector. The two chromatograms obtained for the same mixture as was used to collect the data used for Table I are reproduced in Fig. 8. The efficiency measured from the RI detector trace is only 4600 plates, a consequence of the large cell volume (10 μg) and of the heat exchanger volume (*ca.* 15 μl). The responses of the two detectors are similar and their detection limits are close: for methyl stearate in pure acetone, the detection limit of the RI detector, after a long and careful stabilization, is 3 ppm. Erratic drift of the baseline is observed at this sensitivity, and in current analytical applications the practical detection limit is closer to 10 ppm.

Another important aspect of a detector performance, especially in modern HPLC using the fast LC columns now widely available, is the detector response time. With the light-scattering detector, this time is so short that it is difficult to measure with our equipment, as we do not have a fast enough injection system to be able to

see an actual contribution of the detector. We estimate the time constant to be 100 msec, a large part originating in the amplifier of the photomultiplier signal. With the RI detector, the time constant in modern equipment is typically between 0.5 and 1 sec at low sensitivity (large signal attenuation factor), and can become as large as 10 sec at maximum sensitivity[5,6]. Here again the advantage of the light-scattering detector is obvious.

It must be emphasized that the data in Table I were obtained directly from the corrected peak areas, without any calibration as the concentrations were derived from the peak areas by internal normalization, whereas it is impossible to obtain an acceptable quantitative analysis this way with the RI detector. This detector must be calibrated for all compounds, an additional task that does not seem to be necessary with the light-scattering detector.

The detection limits achieved with the two detectors are nevertheless comparable when the RI detector is operated under stable conditions. There is, however, a fundamental difference between them, as the RI detector is a true differential detector, its signal being the difference between two very large effects due to the pure solvent and the column eluent, whereas the light-scattering detector is not a real differential detector, as there is no or only a negligible signal for the pure solvent, and so it does not need a reference solvent. Therefore, when gradient elutions are contemplated, the RI detector gives such a strongly drifting baseline that it is not possible to record it and at the same time actually see the peaks.

Similarly, a moderate temperature drift makes the RI detector signal almost impossible to use, whereas it does not affect the signal of the light-scattering detector.

Examples of applications

Among the important classes of compounds that have to be analysed, lipids have an unusual situation. They are vital products, of large economic consequence, and their analysis is difficult to achieve because of the complexity of the mixtures and the number of isomers and homologous. No satisfactory solution has yet been developed for the want of a suitable LC detector. Gradient elution reversed-phase LC, preferably NARP, would permit an easy separation of most of these samples, but this precludes the use of the RI detector. In addition, triglycerides, which account for almost all of the components, have no UV chromophore active above *ca.* 210 nm. Accordingly, most analyses are carried out using gas chromatography, which is difficult, especially for the high-molecular-weight triglycerides, or even impossible, as for the important class of phospholipids, which cannot withstand high temperatures.

Some examples of triglyceride analyses are given Figs. 9–12. Although the triglycerides of rape seed oil can also be separated in isocratic analysis, the analysis shown in Fig. 9 was carried out with gradient elution. On the other hand, the sample of palm oil whose separation is shown in Fig. 10 could not have been analysed except with gradient elution. The three groups of peaks shown are characteristic of this sample, and this feature appears to be unusual.

The analysis of butterfat triglycerides (Fig. 11) has been improved by the use of a more efficient column than the previous one[1]. The separation of marine oil (Fig. 12) is incomplete, but this is an extremely complex sample, and already more than 150 peaks have been separated. The use of a more efficient column will permit the separation and identification of a large number of compounds.

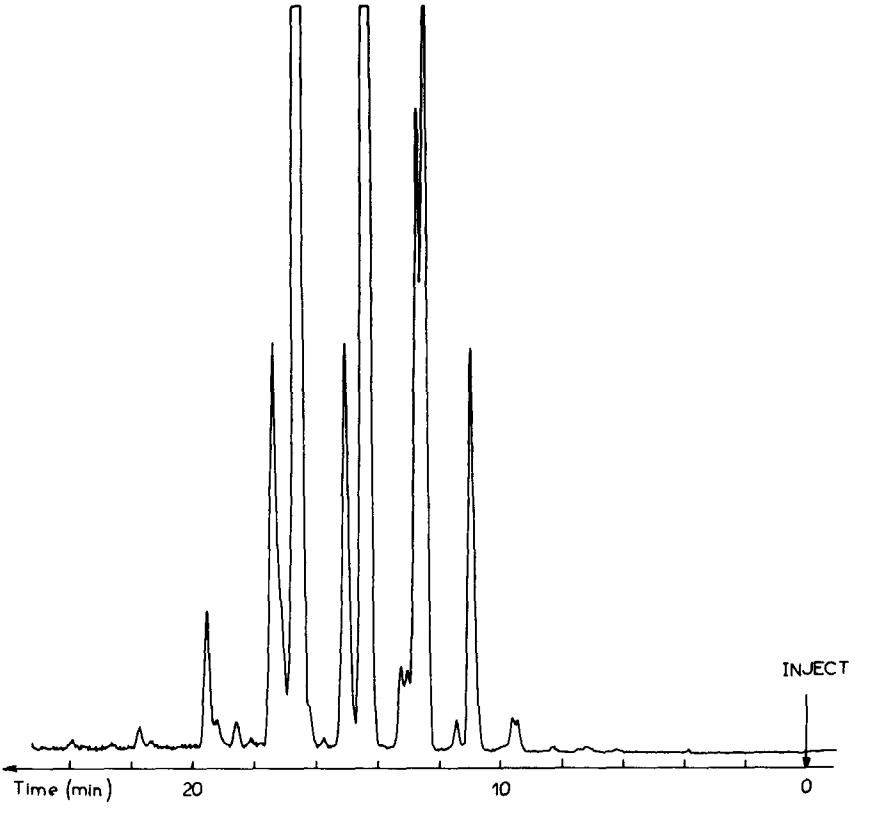

Fig. 9. Separation of a sample of rape seed oil. Column: Hibar, 250 × 4 mm I.D., LiChrospher RP-18, 5 μm. Mobile phase: linear gradient of acetone–acetonitrile from 50:50 to 99:1, 0.8 ml/min. Gradient time: 25 min. Splitting ratio: 10% to the light-scattering detector. Sample size: 0.5 mg.

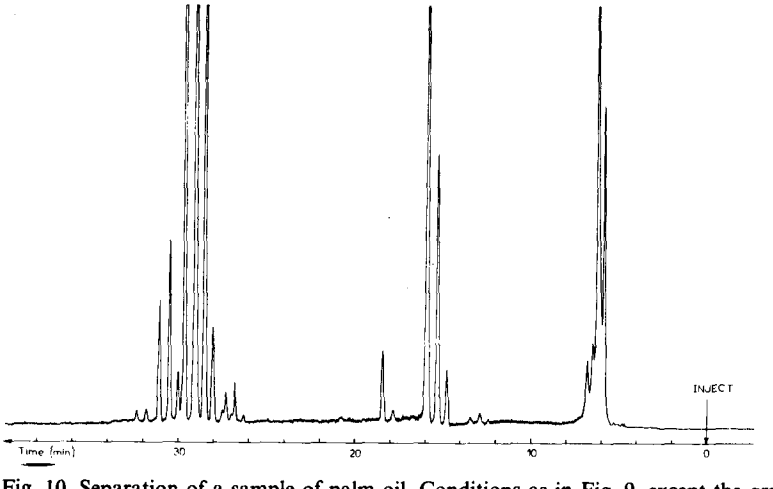

Fig. 10. Separation of a sample of palm oil. Conditions as in Fig. 9, except the gradient starts at 40:60 and the flow-rate is 0.7 ml/min.

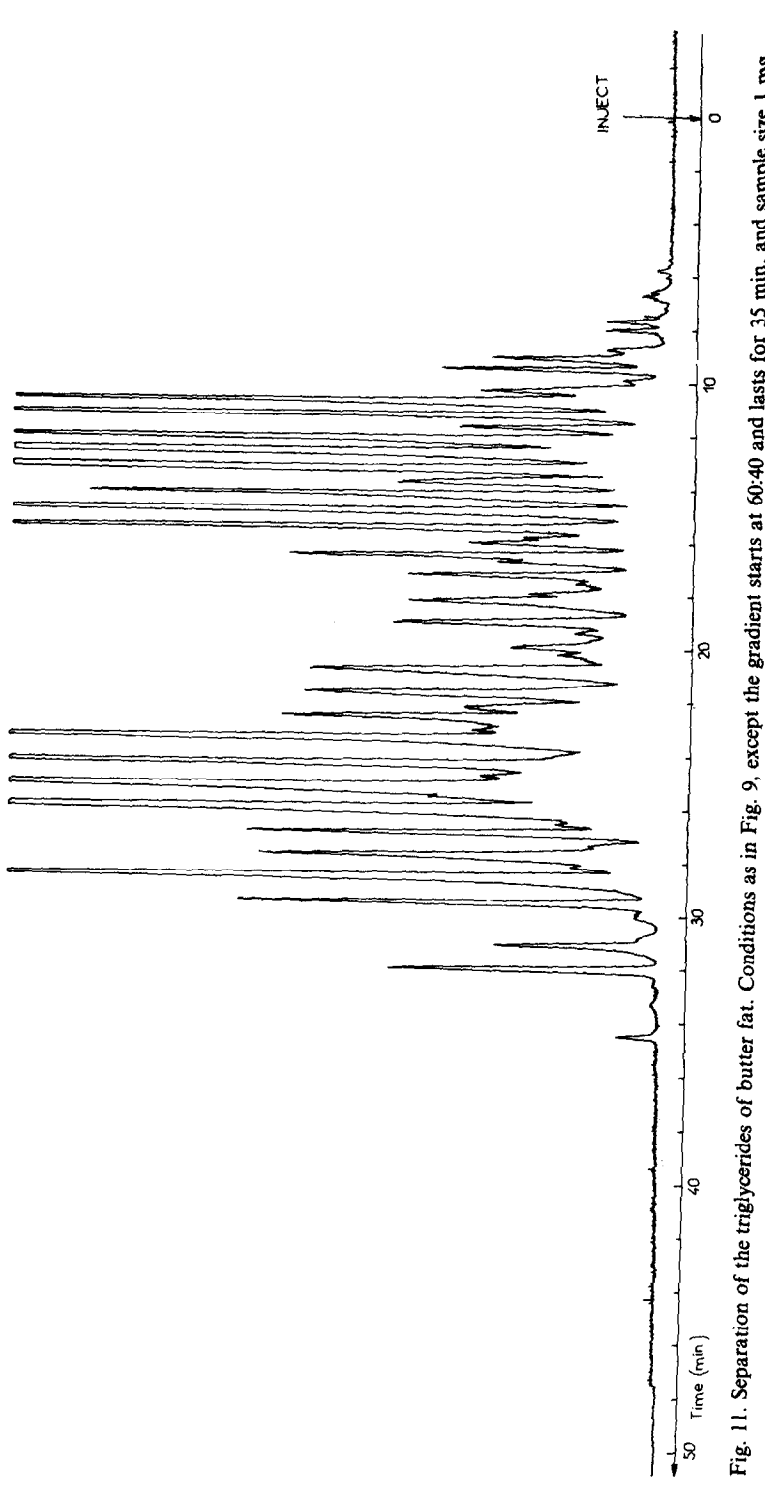

Fig. 11. Separation of the triglycerides of butter fat. Conditions as in Fig. 9, except the gradient starts at 60:40 and lasts for 35 min, and sample size 1 mg.

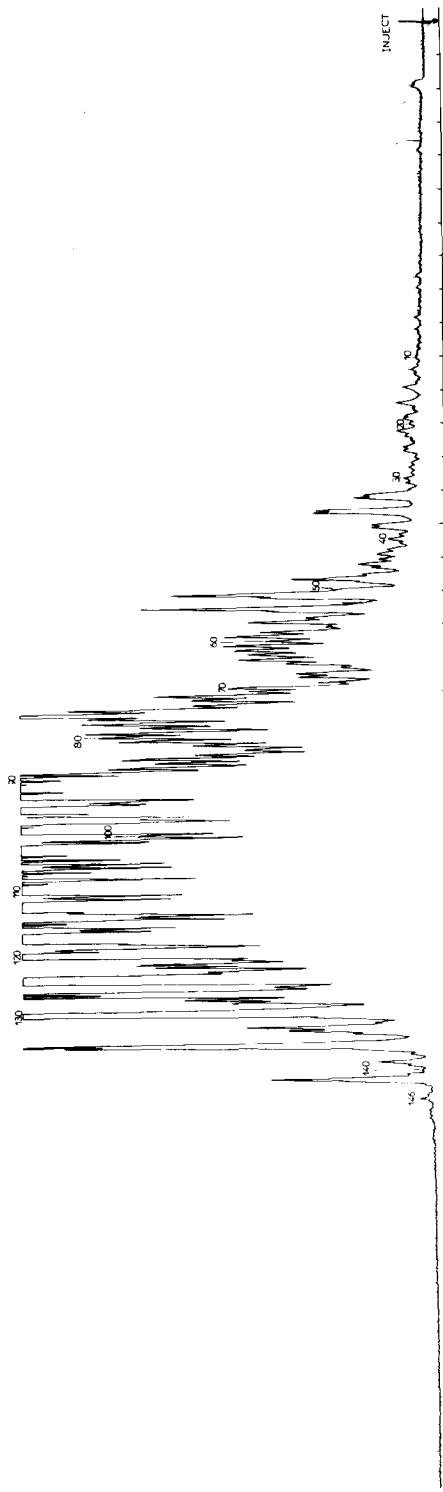

Fig. 12. Separation of a sample of cod liver oil. Conditions as in Fig. 9, except the gradient starts at 40:60 and lasts for 59 min, flow-rate 0.7 ml/min and sample size 1 mg.

Very few analyses of this kind have been carried out previously, and the use of the light-scattering detector seems to be very promising in this field, as well as for phospholipids, polysaccharides and related compounds.

CONCLUSIONS

Although still in an early stage of development, the light-scattering detector compares favourably with the RI detector, which has been in use in LC for over 15 years and has experienced considerable technological development. At present, the weak points in the design are the quality of the light beam, the unstable, non-coherent radiation emitted at the same time as the laser beam, resulting in too much noise, the performance of the photomultiplier used and the quality of the solvents. The optimization of the nebulizer, possibly replacing the spray nozzle with a diaphragm, to obtain larger droplets with a narrow droplet diameter distribution would be another possibility for improving the signal-to-noise ratio, this time by increasing the signal.

Finally, the origin of the non-linear response should be sought, and also the parameter(s) that control the value of x (eqn. 1), in order to ascertain the basis of the correction for this non-linearity.

All these problems are currently under investigation.

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