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# Use of an evaporative light scattering detector in reversed-phase high-performance liquid chromatography of oligomeric surfactants

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## ABSTRACT

The sensitivity of the evaporative light scattering detector was found to be dependent on the analyte concentration and the organic modifier concentration: under reversed-phase gradient conditions calibration is necessary for each analyte. Detection limits for alcohol and carboxylic acid homologues were determined. The advantages of helium over carbon dioxide as a nebulization gas for both volatile and non-volatile or thermolabile analytes are a $>30°C$ lower operating temperature and a five-fold better signal-to-noise ratio. High-resolution separations of polyether alkyl alcohols were effected by reversed-phase gradient high-performance liquid chromatography with ultraviolet transparent mobile phases. Evaporative light scattering detection was compared with refractive index and ultraviolet absorbance detection at 190 nm and found to be superior in signal-to-noise ratio as well as baseline drift. A column-switching system was designed to allow complete compositional analysis of technical samples of ethoxylated alkyl alcohols and their carboxylic acid derivatives.

## INTRODUCTION

Sequential evaporation and light scattering detection (ELSD) has been used mainly for triglycerides [1], (phospho)lipids [2], sugars [3] and steroids [4]. Surprisingly little information is available on the use of this technique in the detection of ionogenic oligomeric compounds, although a study of ethoxylated alcohol, alkyl ether sulphate and alkyl sulphonate surfactants has been reported [5].

In chemical manufacturing processes the analysis of oligomeric surfactants could provide very important information in control of the production parameters. For these types of compounds and reaction products reversed-phase high-performance liquid chromatography (HPLC) is the separation method of choice for quantitative analysis. The evaporative light scattering principle would appear ideally suited to these compounds since the essential requirement for this detection technique is a large difference in volatility between solvent and sample.

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## THEORETICAL

### *Nebulizer*

The mean diameter, $D_0$, of the droplets produced by a concentric nebulizer is given by the equation of Nakiyama and Tanasawa (see ref. 6)

$$D_0 = 585 \times \frac{\sqrt{\sigma}}{\Delta u \sqrt{\rho}} + 597 \left(\frac{\eta}{\sqrt{\sigma \cdot \rho}}\right)^{0.45} \left(1000 \frac{F_L}{F_g}\right)^{1.5} \quad (1)$$

where $D_0$ = mean droplet diameter ($\mu$m), $\sigma$ = surface tension of the eluent (dyne/cm), $\Delta u$ = linear velocity difference between gas and liquid (m/s), $\eta$ = viscosity of the eluent (Poise), $\rho$ = density of the eluent (g/ml) and $F_L/F_g$ = eluent/gas flow-rate ratio.

Table I gives some values of $D_0$ with different eluents. The distribution of the diameter of droplets in the primary aerosol is Gaussian or asymmetrical log-normal [7].

### *Drift tube*

At least three processes take place in the drift tube [8].

(1) Evaporation of the volatile parts of the aerosol.

(2) Precipitation on the wall of the tube.

(3) Coagulation of aerosol droplets.

The time necessary to evaporate the eluent ($t_{d_0}$) can be calculated using Charlesworth's [6] equation

$$t_d = \frac{2 \Delta H_v \rho D_0^2}{M k_f \Delta T} \quad (2)$$

where $t_d$ = time required for complete evaporation, $\Delta H_v/M$ = molar volatility (proportional to the temperature at which 1 mmHg vapor pressure is reached), $\Delta T$ = temperature difference between the nebulization gas and the surface temperature of the droplet and $k_f$ = thermal conductivity of the gas film surrounding the droplets. A decrease in response is expected above a certain temperature since the analyte (x) will evaporate in addition to the eluent (o) and an optimum is obtained at $t_{d_0} < t_d < t_{d_x}$.

The thermal conductivity close to the aerosol droplets is dependent upon the gas used. Each nebulizer gas will have a different set of optimum parameters with respect to the nebulizer, drift tube size, gas flow and temperature for each combination of

TABLE I

MEAN DROPLET DIAMETER IN THE AEROSOL

$\Delta u$ = 480 m/s; gas flow nebulizer, 7.1 l/min; eluent flow-rate, 1.0 ml/min.

| Eluent | $\eta$ (cP) | $\rho$ (g/ml) | $\sigma$ (dyne/cm) | $D_0$ ($\mu$m) |
|---|---|---|---|---|
| Acetonitrile | 0.32 | 0.78 | 29 | 8.6 |
| Methanol | 0.52 | 0.79 | 22.6 | 8.1 |
| Water | 1.00 | 1.00 | 73.0 | 11.9 |

sample, eluent and eluent flow. To minimize losses from precipitation on the wall, $t_d$ is chosen to be slightly larger than $t_{d_0}$.

Coagulation increases with $F_L/F_g$.

*Light scattering cell*

Without precipitation and coagulation the average diameter, $d$, of the aerosol droplets in the light scattering cell is proportional to the analyte concentration, $c$, in the eluent [7]

$$d \sim D_0 \left(\frac{c}{\rho_a}\right)^{1/3} \tag{3}$$

where $\rho_a$ = density of the analyte. With a concentric nebulizer and an analyte concentration of 1 ppm, the lower limit of Mie scattering ($\lambda/20 < d < 2\lambda$) is reached.

The intensity of scattered light, $I$, can generally ea expressed by [9]

$$I = kN\, d^2 \left(\frac{d}{\lambda}\right)^y$$

in which $k$ is a constant, $N$ is the number of particles in the scattering volume and $y$ decreases from 4.0 (Rayleigh scattering) to $-2.2$ with increasing $d/\lambda$ ratio. Thus at constant $\lambda$ and $N$ the intensity of scattering increases with $d^p$ ($p < 6$) so, according to eqn. 3 with $c^q$ ($q < 2$):

Decreasing the gas flow-rate increases $D_0$ and coagulation should enhance the response even further.

An optimum is reached when the minimum gas flow approaches $t_d = t_{d_0}$.

At $d \geq 2\lambda$ refractive index and reflection effects become important [6] and diminish the sensitivity so that the total response curve is sigmoidal.

The effect of the type of eluent on the response is complex and no simple relation has been demonstrated [8].

*Calibration curve and detection limit*

At lower concentrations the log–log plot of analyte concentration *versus* response is predominantly linear. The detection limit can be determined by

$$m_{LOD} = \left(\frac{2\, x\, N}{H}\right)^{1/x} m \tag{4}$$

where $m_{LOD}$ = detection limit (g), $m$ = injected mass of analyte (g), $N/H$ = ratio of the noise to the peak height and $x$ = slope of the calibration curve.

## EXPERIMENTAL

A Gilson modular gradient pumping system (Villiers-le-Bel, France) was used for HPLC in several configurations.

Gilson 231/401 or Marathon (Spark, Emmen, The Netherlands) autosamplers

were employed to introduce samples onto 250 × 4 mm I.D. or 100 × 4 mm I.D. Nucleosil 120-5$C_{18}$ reversed-phase columns (Macherey-Nagel, Düren, Germany).

The ELSD tested was the Varex Mark II (Varex, Rockville, MD, U.S.A.). UV absorbance detection was performed with an HP 1040 diode array detector (Hewlett-Packard, Waldbronn, Germany) and refractive index (RI) detection with an HP 79877A refractometer (Hewlett-Packard) thermostatted at 35°C. Water was Milli-Q quality (Millipore, Bedford, MA, U.S.A.) and LiChrosolv acetonitrile was obtained from Merck (Darmstadt, Germany).

The technical surfactant samples were a gift from Chem-Y (Emmerich, Germany) and consisted of mainly (A1) $C_{12}H_{25}$–O–$(C_2H_4O)_n$–$CH_2$–COOH, (A2) $C_{14}H_{29}$–O–$(C_2H_4O)_n$–$CH_2$–COOH, (A3) $C_{12}H_{25}$–O–$(C_2H_4O)_n$–H and/or (A4) $C_{14}H_{29}$–O–$(C_2H_4O)_n$–H, in which $n$ ranges from 0 to approximately 25.

## RESULTS AND DISCUSSION

### *Calibration curves under reversed-phase gradient conditions*

From neat organic solvent experiments it is known that the sensitivity of the ELSD can be mobile phase- (ref. 1 *versus* refs. 4 and 8) and concentration- (ref. 5 *versus* refs. 4 and 6) dependent. We investigated the extent to which the dependence of sensitivity during reversed-phase gradients of water to acetonitrile [4] might complicate quantitation.

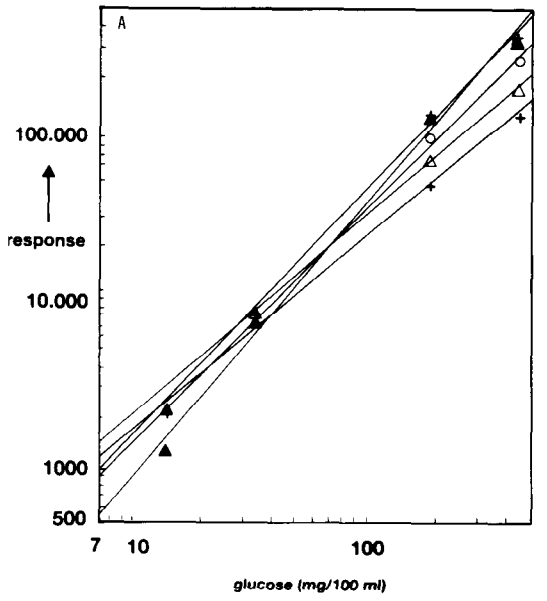
The temperature and nebulizer gas flow-rate producing maximum response of the ELSD varied with acetonitrile concentration such that at higher acetonitrile concentration an optimum was obtained at lower temperature. However, it was found that under operating conditions this effect was completely obscured by the dependence of the sensitivity on the analyte concentration.

Fig. 1A gives the sensitivity normalized to that for 75% acetonitrile for differing amounts of a non-volatile test compound: glucose. This figure demonstrates that the interdependence of sensitivity, acetonitrile concentration and analyte concentration necessitates the use of calibration curves for analytes under the exact gradient conditions used and that the combined effects could create an illusion of excessive compound dependency [4,10]. At low analyte concentrations the sensitivity difference could be explained by the dependence of the mean particle diameter on the eluent (eqn. 1), water giving larger droplets than acetonitrile (Table I) and thus a larger sensitivity (eqn. 3). The reversal at high analyte concentrations could mean that the temperature and gas flow are suboptimal in this case (see Fig. 1B).

The obligatory use of calibration curves for quantitation of each analyte under the exact analytical conditions is in our opinion a major drawback of this type of detector: quantitation without standards is not possible.

### *Detection limits for alcohols and carboxylic acids*

Detection limits with the ELSD are usually given for long-carbon-chain fatty alcohols and acids, these being economically important classes of compounds, the detection limits of which are less than a microgram. We explored the use of the ELSD in the detection of smaller carboxylic acids and alcohols. Table II shows a clear inverse trend, relating the temperature where the analyte has a vapor pressure of 1 mmHg (or molecular weight) to the detection limit (as defined in eqn. 4). Also the slope of the



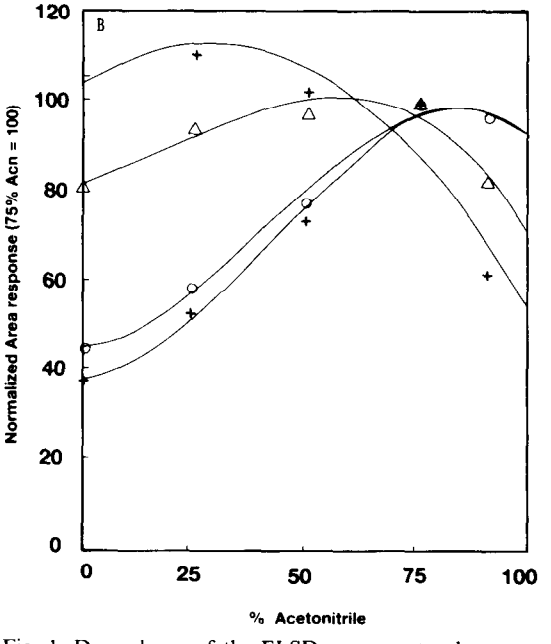




Fig. 1. Dependence of the ELSD response to glucose on the acetonitrile concentration in the eluent Nebulizer temperature: 125°C. Gas (helium) flow-rate: 7.1 l/min. (A) +, 0%; △, 25%; ○, 50%; +, 75%; △ 90% acetonitrile. (B) +, 13.9; △, 32.4; ○, 181; +, 439 mg/ml glucose.

TABLE II

CALIBRATION AND PHYSICAL DATA OF CARBOXYLIC ACIDS AND ALCOHOLS

| Compound | Limit of detection ($\mu$g) | Slope of calibration curve | Temperature for 1 mmHg vapor pressure (°C) | MW |
|---|---|---|---|---|
| $C_{17}H_{35}COOH$ | 0.48 | 1.18 | 173.7 | 284.5 |
| $C_{13}H_{27}COOH$ | 1.20 | 1.58 | 142.0 | 228.4 |
| $C_{11}H_{23}COOH$ | 1.96 | 1.73 | 121.0 | 200.3 |
| $C_8H_{17}COOH$ | 8.75 | 1.94 | 108.2 | 158.2 |
| $C_7H_{15}COOH$ | 19.5 | 1.94 | 92.3 | 144.2 |
| $C_{18}H_{37}OH$ | 0.90 | 1.48 | 150.3 | 270.5 |
| $C_{16}H_{33}OH$ | 1.13 | 1.52 | 122.7 | 242.5 |
| $C_{12}H_{25}OH$ | 3.81 | 1.62 | 91.0 | 186.3 |
| $C_{10}H_{21}OH$ | 21.1 | 1.67 | 69.5 | 158.3 |

calibration curve is increased with more volatile compounds. The sensitivity, however, never approached that of the $C_{18}$ analyte. This is all consistent with less prominent evaporation of larger droplets after removal of the solvent.

*Helium as a nebulizer gas*

In an attempt to improve detection limits for more volatile, polar and thermolabile compounds, helium was studied as a nebulizer gas. At similar gas velocities the five-fold larger thermal conductivity of helium relative to air, nitrogen or carbon dioxide allowed a lower vaporization temperature (at 1 ml/min water *ca.* 25°C lower, see Fig. 2A), thus enabling more volatile analytes like glutaric acid ($T_{1\ mmHg}$ = 155.5°C) to be analysed (see Fig. 3A and B).

Increasing the nebulizer gas flow to a maximum of 20 l/min helium at 10 atm for this brand of instrument decreased the droplet size of the aerosol and therefore the response. However, the lower temperature range limit was extended by a further 15°C.

Depending on the volatility of the analytes, the helium flow-rate on this detector could be adjusted to 20 l/min and the optimum temperature thus determined. Non-volatile analytes had to be measured at the lowest possible nebulizer gas flow ($> t_{d_0}$) at the highest temperature permitted by the analyte. At equal flows helium gives a much larger response for a non-volatile analyte than carbon dioxide (see Fig. 2B).

The optimum for carbon dioxide was found to be at a much lower gas flow-rate than shown in Fig. 2, but not as low as the helium flow, so that the carbon dioxide response never approached the maximum response with helium.

*Ethoxylated alkyl alcohols and carboxylic acid derivatives*

A comparison of ELSD with UV and RI detection for ethoxylated alkyl alcohols A3 and A4 (see Experimental section) is shown in Fig. 4A, B and C. The signal-to-noise ratio in UV and RI detection was found to be inferior to that with the ELSD. Moreover the larger baseline drift at 190 nm made UV detection difficult so the gradient had to be adapted and sensitive RI detection was rendered impossible, so that Fig. 4C had to be made with an isocratic eluent.

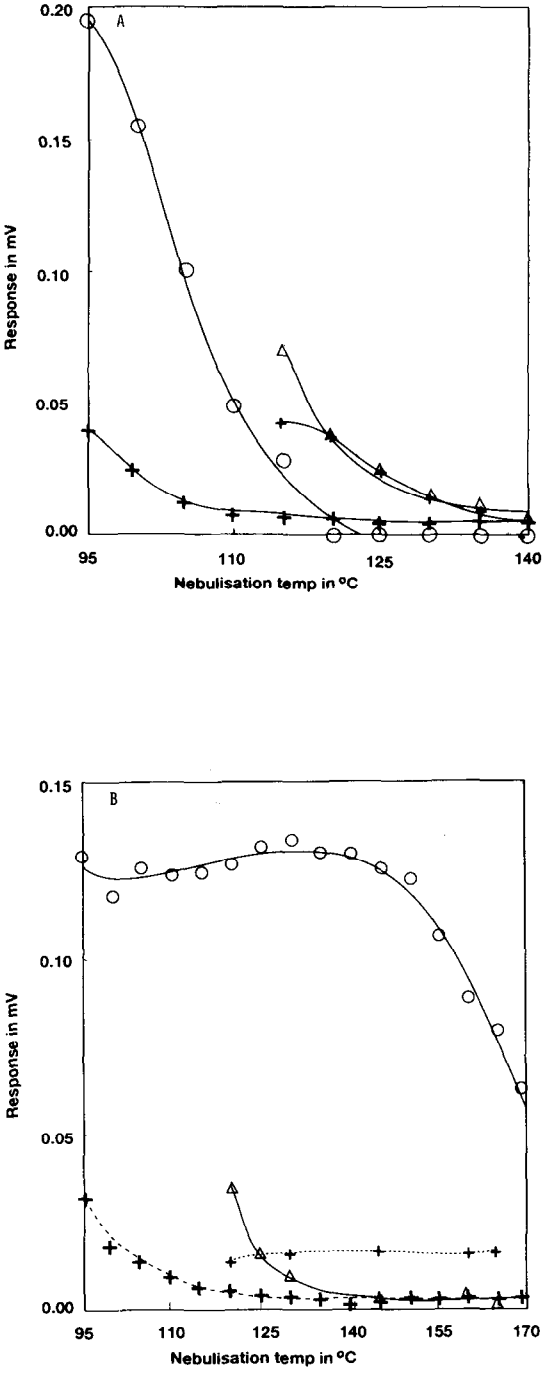



Fig. 2. Variation of the response and noise of the ELSD with temperature for carbon dioxide and helium. Nebulization gas flow-rate: 6.4 l/min. +, Signal carbon dioxide; △, noise carbon dioxide; ○, signal helium; +, noise helium. (A) Glutaric acid. (B) Glucose.

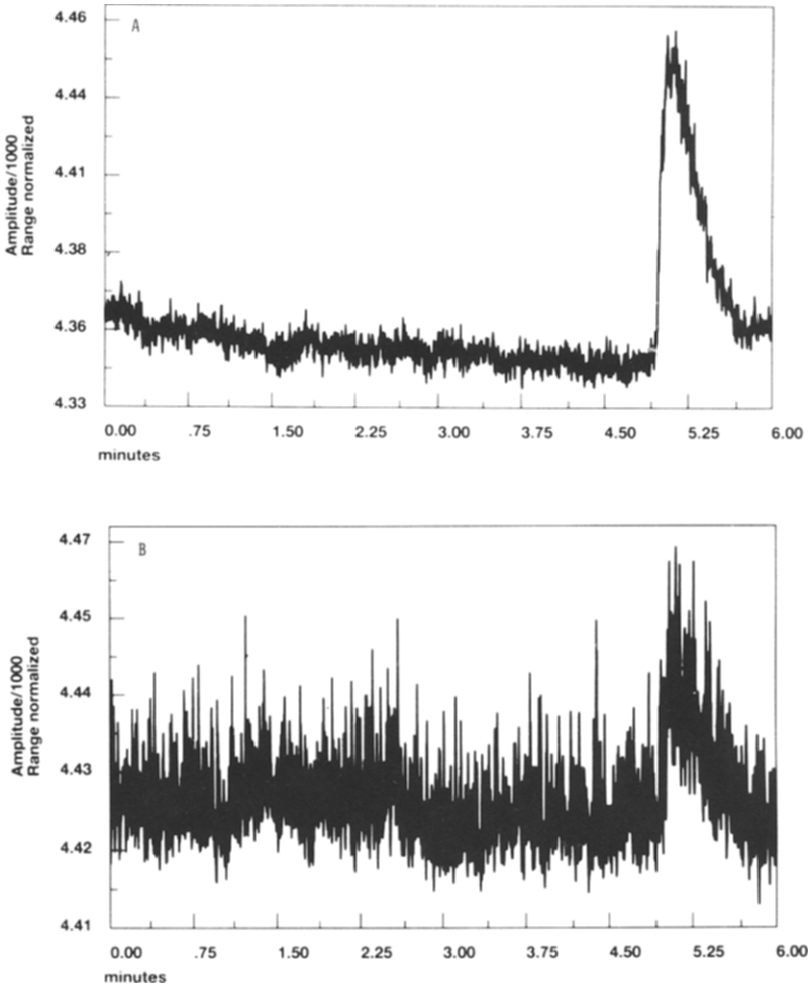



Fig. 3. Comparison of carbon dioxide and helium as a nebulization gas for glutaric acid. Column: 100 × 4 mm Nucleosil 120-5$C_{18}$. Mobile phase: 0.1% aqueous acetic acid. Flow-rate: 1.0 ml/min. Injection: 20 $\mu$l containing 90 $\mu$g glutaric acid. (A) $T_{neb}$ = 105°C, 6.4 l/min helium. (B) $T_{neb}$ = 125°C, 6.4 l/min carbon dioxide.

The ELSD provided a reasonably predictable but non-equivalent response of a series of oligomers with reversed-phase eluent, in contrast to that found with organic solvents [5].

Fig. 5A and B show the maximum response for the most abundant oligomer to be at $\geq$150°C. However, the volatility of the dodecyl triethoxylate oligomer was limited to an operational temperature of 80°C, where accurate compositional analysis [coefficient of variation (C.V.) = 2.8% ($n$ = 13), C.V. = 3.6% ($n$ = 7)] was afforded. A complete analysis of technical samples containing mixtures of both the ethoxylated alkyl alcohols and their carboxylic acid derivatives would prove useful for detailed reaction modification studies.

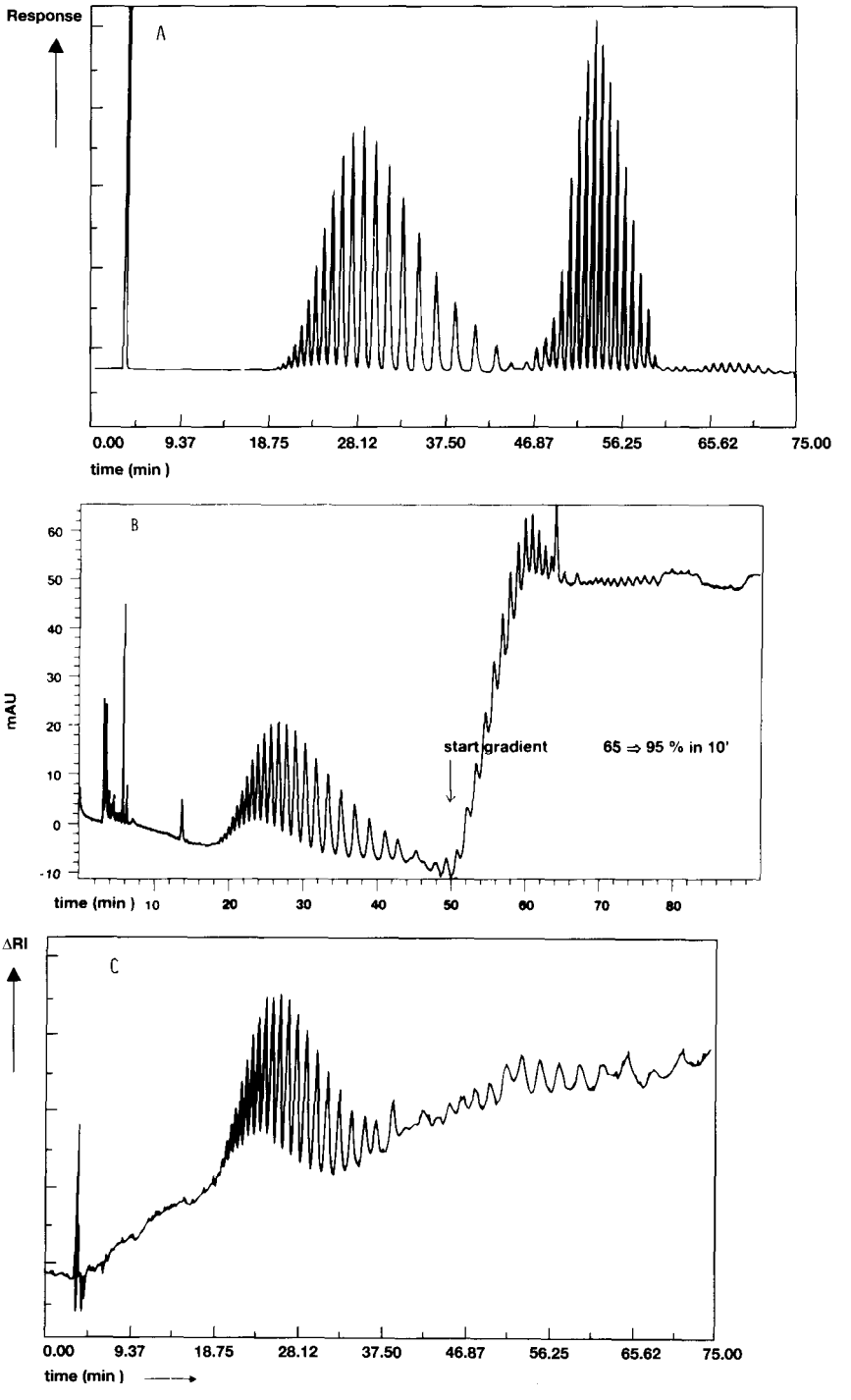
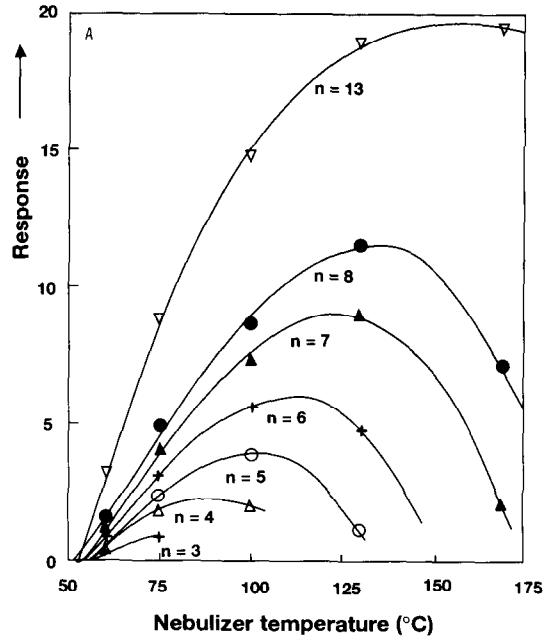



Fig. 4. Comparison of ELSD (A), 190-nm UV (B) and RI (C) detection techniques for alkylethoxylates A3 and A4. Column: 2 × (250 × 4 mm I.D.) Nucleosil 120-5$C_{18}$. Eluent: (A) 0.1% aqueous acetic acid; (B) acetonitrile. Gradient: 65% acetonitrile for 38 min then 90% acetonitrile for 10 min; at 75 min back to 65% acetonitrile for 1 min. Flow-rate: 1.0 ml/min. Injection: 20 $\mu$l containing 1.2 mg sample. (A) $T_{neb}$ = 100°C, 2.9 l/min carbon dioxide. (B) $\lambda$ = 190 nm (4 mm band width), $\lambda_{ref}$ = 500 nm (100 nm, band width). (C) Isocratic separation at 65% acetonitrile, detector sensitivity setting 8.



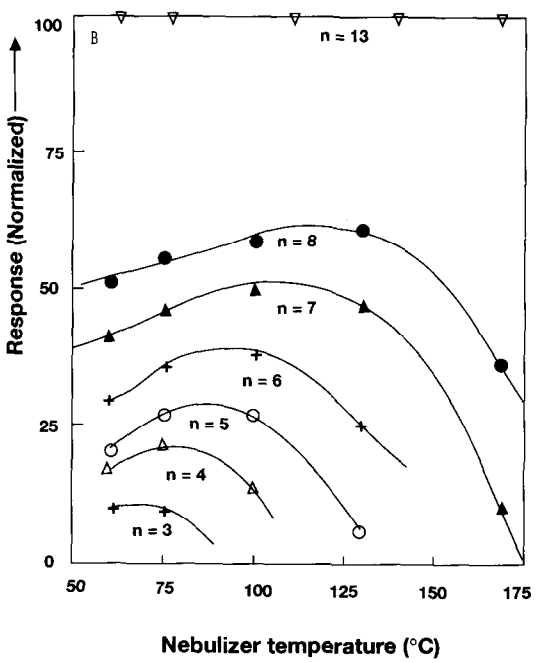




Fig. 5. Response of polyethylene glycol monododecyl ether surfactant homologues (A3) as a function of nebulizer temperature. For chromatographic conditions, see Fig. 4A. Helium flow-rate: optimized. (A) Absolute response. (B) Response normalized to that of tridecaethoxylated dodecanol.

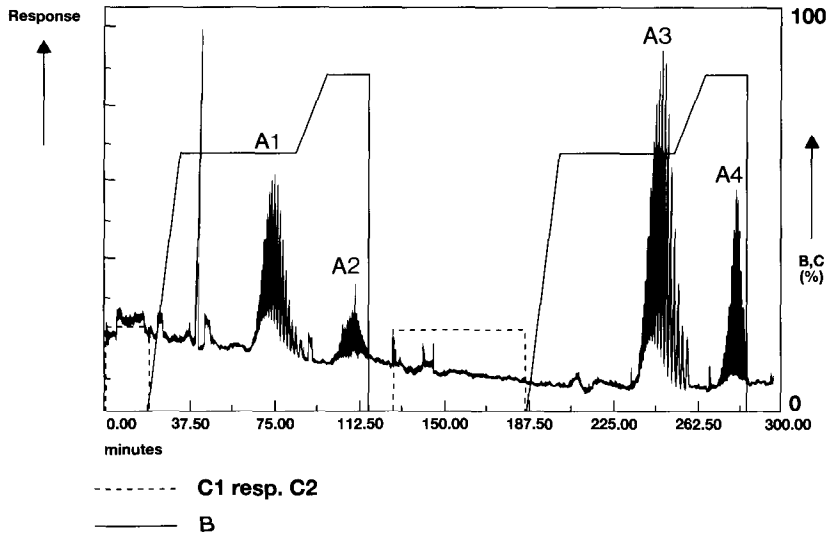



Fig. 6. Separation of the ethoxylated alkyl alcohols (A3 and A4) and their carboxylic acid derivatives (A1 and A2) by automated column-switching HPLC. Sample size: 1.2 mg. Trapping column: 100 × 4 mm I.D. Nucleosil 120-5$C_{18}$. Separation columns: one 50 × 4 mm I.D. plus two (250 × 4 mm I.D.) Nucleosil 120-5$C_{18}$. Mobile phases: (A) 1% formic acid in water; (B) acetonitrile; (C1) 65% acetonitrile, 35% 50 m*M* ammonium bicarbonate; (C2) 82% acetonitrile, 18% 50 m*M* ammonium bicarbonate. Total mobile phase flow-rate: 1.0 ml/min. The trapping column is only eluted with mobile phase C; mobile phases A and B are introduced between the exit of the trapping column and the 4-ml mixing coil. Detector settings: nebulizer temperature 100°C; nebulizer gas flow 18.4 l/min helium.

Unfortunately, however, these compounds coelute under ion suppression (*e.g.*, pH 2.3) conditions. At neutral pH the alcohols displayed asymmetric peaks. Consequently a decreasing pH and increasing organic modifier gradient would also appear futile.

The desired result was provided by column switching, effectively trapping the alcohol derivatives in a 100 × 4 mm I.D. first column, at neutral pH and low organic modifier concentration, and allowing the separation of the carboxylic acids on a second column set.

Since the separation of the alcohols was not started before completion of the carboxylic acid separation, a total time of about 5 h was required and was consequently performed automatically (see Fig. 6).

Fig. 6 shows the separation of the ethoxylated alkyl alcohols (A3 and A4) and their carboxylic acid derivatives (A1 and A2). The carboxylic acid derivatives were separated first, the separation being complete in 2 h. After column switching the separation of the ethoxylated alkyl alcohols could be performed and was also completed in 2 h.

Each peak cluster represents a distribution of ethoxylated derivatives of one type of hydrophobic moiety (*e.g.*, $C_{12}H_{25}$–O–), and each peak within the peak cluster reflects oligomers of differing degrees of ethoxylation.

In order to alleviate the stringent demands of this high-resolution separation on the extra, second column set, peak broadening, the sample fraction eluting from the

first column was further diluted in a 4-ml mixing coil —determined to be the minimum volume— with low-pH acetonitrile-free mobile phase.

This concentration by dilution may only be applied when the increase in capacity factor ($k'$) on the second column set is much larger than the dilution factor —the pH and modifier change for the carboxylic acid derivatives was found to be more effective than just the modifier jump for the alcohols.

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