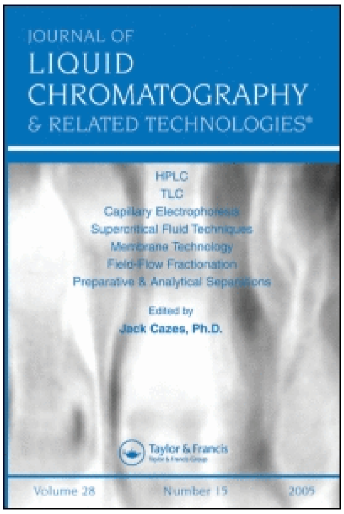

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Operational Characteristics of the Evaporative Light Scattering Detector Used in Analysis of Ethoxylated Alcohols

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To cite this Article Miszkiewicz, Witold and Szymanowski, Jan(1996) 'Operational Characteristics of the Evaporative Light Scattering Detector Used in Analysis of Ethoxylated Alcohols', Journal of Liquid Chromatography & Related Technologies, 19: 7, 1013 — 1032

To link to this Article: DOI: 10.1080/10826079608006299

URL: http://dx.doi.org/10.1080/10826079608006299

J. LIQ. CHROM. & REL. TECHNOL., 19(7), 1013–1032 (1996)

OPERATIONAL CHARACTERISTICS OF THE EVAPORATIVE LIGHT SCATTERING DETECTOR USED IN ANALYSIS OF ETHOXYLATED ALCOHOLS

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ABSTRACT

Influence of the temperature of a drift tube and flow rate of nitrogen as nebulizing gas with 100% hexane and 40% hexane plus 60% (5% H_2O in 2-propanol) as mobile phases on the respones of ELS detector for selected standards of ethoxylated alcohols was examined. For optimal conditions (1.5 dm^3 N_2/min, 50 °C) calibration curves for both mobile phases were created. The response of ELSD for standards not undergoing evaporation is mainly affected by the composition of the mobile phase.

INTRODUCTION

UV detector, most frequently used in liquid chromatography, is excellent in analysis of ethoxylated alkylphenols, mainly thanks to its compatibility with gradient mode, high sensitivity and a straight-line relationship between the response and the mass of the substance under determination. On the other hand, ethoxylated alcohols and acids which do not possess chromophore groups have to be derivatized.

Literature offers two derivatization methods. The first one is the reaction with phenyl isocyanate[1-5] and the other is the esterification of ethoxylates with 3,5-dinitrobenzoil chloride in the presence of pyridine.[6] The derivatized ethoxylates, absorbing in the UV range, are not observed to essentially change their affinity to normal-phase packings. Some elongation of retention times is observed on reversed phases due to the higher hydrophobicity of the ethoxylated alcohol molecules following their reaction with phenyl isocyanate or dinitrobenzoyl chloride. Such additional step contributes both to elongation of the analysis time and to the error of the method. Which is more, the peaks of the components determined overlap the peaks of the derivatising reagent. Unfortunately, it is also necessary for quantitative calculations to find correction coefficients for the respective derivatized oligomers, as their UV absorption depends on the length of the polyoxyethylene chain.

Traces of ethoxylated alcohols and alkylphenols are also determined using a fluorescent detector.[7,8] Alkylphenol ethoxylates can be determined without derivatization but ethoxylated alcohols with no fluorescent groups in a molecule need conversion into their appropriate derivatives in reaction with 1-anthroylnitryl. The other derivatising agents include 1-naphtyl isocyanate[9,10] and naphtoyl chlorides.[11] Because of its very low detection limit (high sensitivity) fluorescent detector is very often used in analysis of waste water samples (environmental analysis).

RI detector is not suitable for analysis of such complex mixtures (finding the distribution range), as it is only applicable under isocratic conditions[12] and have quite low sensitivity, which may be decisive in trace determination. However, it is useful in determination of the total content of PEG in ethoxylation products under isocratic conditions with either CH_3OH or CH_3CN as an organic modifier of a mobile phase.[13,14] Flame-ionisation detectors (FID) are not widely used in HPLC either, mainly because of problems with repeatability.

The most promising for HPLC is the ELS detector. Although recent versions of the ELS detector have sensitivities comparable with the RI detector, yet its applicability in the analysis of high-boiling substances using a gradient and solvents with absorptions in the UV range (tetrahydrofurane, acetone, toluene, dimethylformamide, etc.) gives it a significant advantage over all other types of detectors. Its compatibility with gradient mode may be useful in direct analysis of ethoxylation products distribution both on normal and reverse phases. The use of light scattering on aerosol particles for non-volatiles detection was first reported by Ford and Kennard who described their 'Evaporative Analyser' in 1966.[15] In 1978, Charlesworth published an excellent work[16] on the operational characteristics of the ELS detector. While the signal formation mechanism has not been studied thoroughly yet,[17-24] more and more papers are published year by year on the analysis of glycerides,[25-28] carbohydrates[29] phospholipides,[30-32] saccharides,[33] steroids,[34] surfactants,[35-37] amino acids[38] and other ones[39-42] using this detector.

The ELS detector is best applicable with columns of diameters equal to or even less than 2.1 mm (narrow bore) for which the consumption of a mobile phase is drastically lower (for ϕ = 2.1 mm it is five times as low as for the widely used columns with ϕ = 4.6 mm). Beside purely economic benefits, there is also the advantage of evaporation temperature. As it is obviously easier to evaporate 0.3 cm^3/min (which is an optimum flowrate for columns with ϕ = 2.1 mm) than 1.5 cm^3/min (optimum flowrate for columns with ϕ = 4.6 mm) of a mobile phase, lower temperatures may be used in the former case without a risk of evaporation of the components of the mixture subjected to analysis.

The aim of the work is to show the correlations between the response of the ELS detector and the temperature of the drift tube and a flow rate of the nebulizing gas (N_2); Additionaly the calibration curves and the dependence of the response of the ELSD on the composition of the mobile phase for standards of ethoxylated alcohols are presented.

EXPERIMENTAL

Reagents

Hexane and 2-propanol of HPLC grade were obtained from BDH Laboratory Supplies (U.K.). High purity water was obtained through HP 661A Water Purifier (Hewlett Packard, U.S.A.). Nitrogen of technical grade (99.8%)

used as nebulizing gas was supplied by Linde Gas Poland. 1-dodecanol (C12OH) was purchased from Merck (Germany). Standards of ethoxylated alcohols were obtained either from Fluka Chemie AG (Switzerland) (Pentaethylene glycol monodecyl ether - C10EO5, Pentaethylene glycol monotetradecyl ether - C14EO5, Pentaethylene glycol monoheksadecyl ether - C16EO5, Nonaethylene glycol monododecyl ether - C12EO9) or from Nikko Chemicals Co., Ltd. (Japan) (Monoethylene glycol monododecyl ether - C12EO1, Triethylene glycol monododecyl ether - C12EO3, Pentaethylene glycol monododecyl ether - C12EO5, Heptaethylene glycol monododecyl ether - C12EO7, Pentaethylene glycol monooctadecyl ether - C18EO5); digit after EO indicate number of oxyethylene groups. All with purity at least 97 %.

Apparatus

The HPLC system consisted of a Hewlett Packard HP 1050 Liquid Chromatograph connected with ELS detector [Varex II - (U.S.A.)] by a 90 cm capillary with 0.25 mm I.D. The samples were introduced directly to detector without a chromatographic column by Waters 717 plus autosampler (Waters, U.S.A.) with 25 μL loop. The flow of a mobile phase was 0.3 cm^3/min. Channel A was hexane and B was 5% H_2O in 2-propanol. Each experimental point was an average of 5 injections. After changing a detector's working parameters (N_2 flow, drift tube temperature) the system was stabilized for at least 20 min. A personal computer with Grams/386 for Chromatography software (Galactic, U.S.A.) were used for data collection and quantification of peak areas. Sampling rate was set on 25 Hz. Results of integration were exported to Excel spreadsheet (Microsoft Corporation, U.S.A.) and after calculation of averages and standard deviations were sent to Grapher (Golden Software, U.S.A.) which was used for drawing curves and for determination of correlation parameters.

RESULTS AND DISCUSSION

Response Of ELSD For Ethoxylated Alcohols Standards

Formation of the ELS detector signal comprises three complex processes:

1. Nebulization of an effluent from the chromatographic column.

2. Evaporation of the lower boiling solvent and concentrating the higher boiling component to be determined.

3. Scattering of incident light on such aerosol particles.

For a given mobile phase, the value of the response of the ELS detector depends on three parameters, namely the following:

a) drift tube temperature,
b) linear rate of the nebulizing gas (N_2), and
c) flowrate of a mobile phase.

This paper refers only to analysis of the effect of a) and b), assuming the flowrate of a mobile phase of 0.3 cm^3/min as an optimum for chromatographic columns with $\phi = 2.1$ mm (as results from the height of the theoretical plate), and frequently recommended by manufacturers of LC equipment (Hewlett Packard, Macherey Nagel, Waters etc.).

Later in this paper, the effect of the kind of the solvent used in normal-phase separation on the detector response is investigated for the selected standards of ethoxylated alcohols. Shown below are the relationships between the ELS detector response, drift tube temperature, flowrate of the nebulizing gas (N_2) and the mass of the component determined, for limiting values of the optimal gradient used in a system of normal phases by Bear,[43] that is for 100% hexane (beginning of the gradient), and 40% hexane + 60% (5% H_2O in 2-propanol) (end of the gradient).

Effect Of Drift Tube Temperature On ELSD Signal

Hexane as a mobile phase

The temperature effect was investigated in the range 30-120 °C and found to be significant for the response of C12OH and lower oligomers (Fig. 1). The alcohol stops being 'visible' for the ELS detector as early as above 40 °C, whereas the first homologue, C12EO1, is not detectable just above 50 °C. The value of the signal of the latter is twice as small with temperature growth from 30 to 40 °C. Up to 50 °C, the response of homologues containing not less than three oxyethylene groups is the same. For C12EO3, the value of the signal drops by half at 90°C; for C12EO5 it does at 120 °C. For C12EO9, it is not

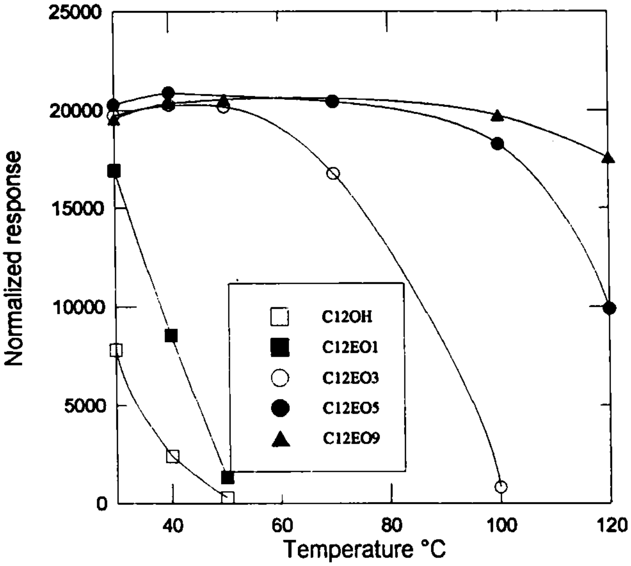

Figure 1. Relationships between the ELS detector response and the detection temperature for polyethylene glycol monododecyl ether homologue standards (mobile phase = 100 % hexane, V=1.5 N_2 dm^3/min).

temperature dependent between 30 and 100 °C. Experimental results show that for relatively volatile components, the detector signal depends on their vapor pressure.

40%Hexane + 60%(5%H_2O in 2-propanol) as a mobile phase

Unlike in the case of hexane, for a mixture 40% hexane + 60% (5% H_2O in 2-propanol) as a solvent the curves illustrating the relationship between the response of the ELS detector and detection temperature (except for the most volatile C12OH and C12EO1), have distinct maximums, namely 60 °C for C12EO3, 80 °C for C12EO5 and 110°C for C12EO9. Contrary to the highly volatile hexane, to evaporate 2-propanol which has lower volatility the drift tube is heated to a higher temperature, obviously producing an effect on the course of evaporation of the ethoxylates. The process is earliest commenced (even before complete evaporation of 2-propanol) for ethoxylated alcohols with the shortest polyoxyethylene chains (Fig. 2). C12EO1 stops being visible to the ELS detector above 50 °C, C12EO3 does above 100 °C and C12EO5 does above 130 °C. All the curves are convex parabolas ($y=ax^2+bx+c$, with $a<0$).

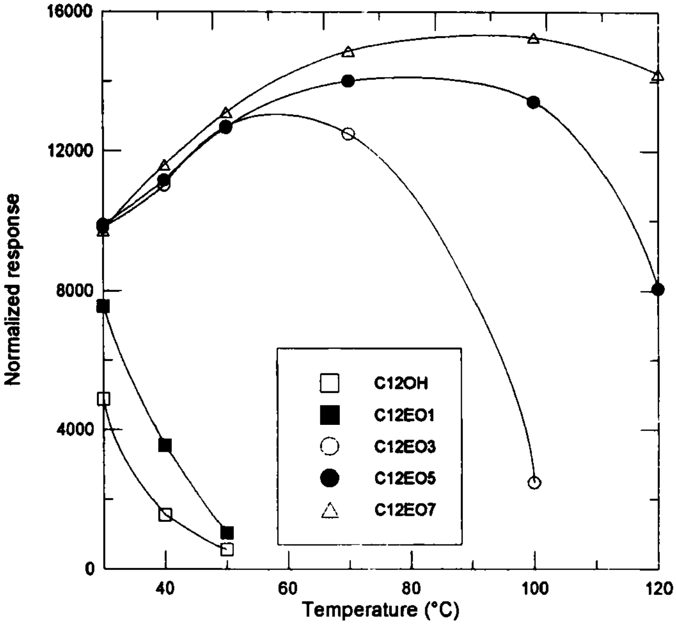

Figure 2. Relationship between the ELS detector response and the detection temperature for polyethylene glycol monododecyl ether homologue standards (mobile phase = 40 % hexane + 60 % (5 % H_2O in 2-propanol, V =1.5 dm^3 N_2 /min).

Standards containing shorter polyoxyethylene chains give 'narrower', while those with longer polyoxyethylene chains give 'wider' parabolas. 'Narrow' parabolas for the 'lower' standards show that evaporation of 2-propanol is accompanied by simultaneous evaporation of an ethoxylate. The left branches of the curves may indicate incomplete evaporation of 2-propanol which 'dilutes' the surfactant causing weaker light scattering which results in poor response. It may well be that the parabolic shape of the curves, other than for hexane, is under the influence of hydrogen bonds formed between the surfactants and 2-propanol.

For both extreme values of the gradient, 50 °C was selected as an optimum temperature. Such temperature is high enough to effect complete hexane evaporation while preventing evaporation of the homologues being determined (except C12OH and C12EO1). At such temperature, the

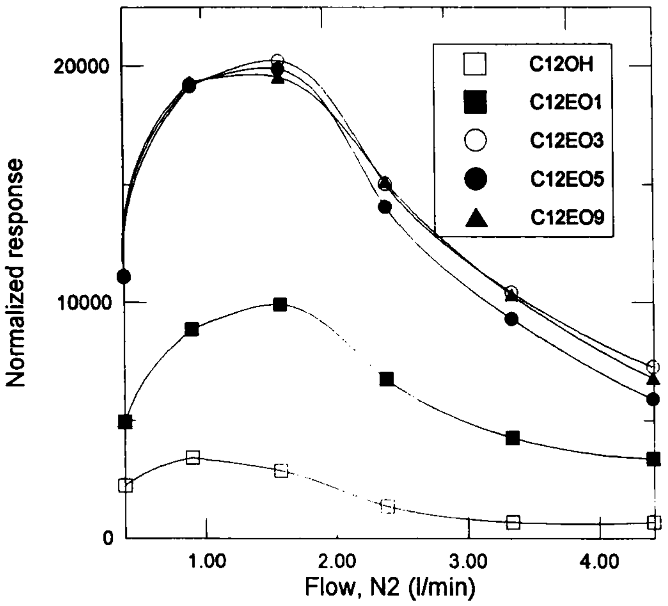

Figure 3. Relationship between the ELS detector response and the flowrate of N_2 for polyethylene glycol monododecyl ether homologue standards (mobile phase = 100 % hexane, T_{det}=50°C).

evaporation of 40% hexane + 60% (5% H_2O in 2-propanol) is probably incomplete and the resulting signal is lower than for hexane but, the higher homologues (C12EO3 and C12EO5) are not evaporated either.

Effect Of Volume Flow (Linear Velocity) Of Carrier Gas (N_2) On ELSD Signal

Hexane as a mobile phase

The same standards of ethoxylated alcohols (C12OH, C12EO1, C12EO3, C12EO5, C12EO7, C12EO9) were used to analyze the effect of linear velocity of N_2 on the value of the ELS detector response. The course of all the curves is similar to Gaussian curve with a maximum around 1.5 dm^3/min (35 mm N_2) and a distinct upward slope on the left side of the curves up to the maximum, which is followed by a drop of the value of the response with increasing linear

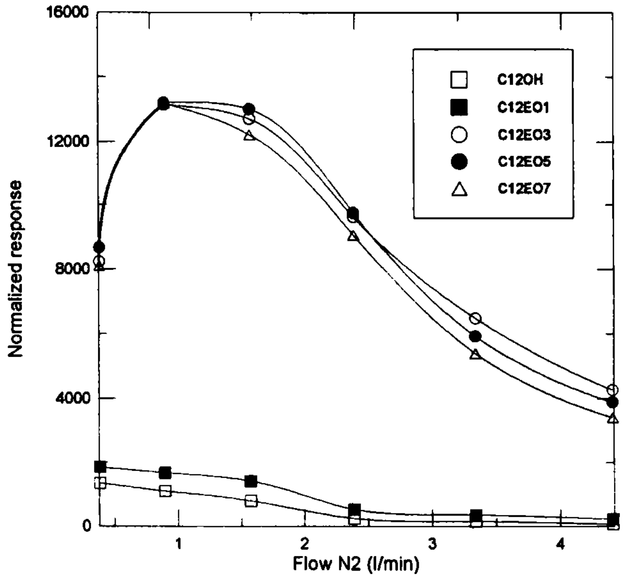

Figure 4. Relationship between the ELS detector response and the flowrate of N_2 for polyethylene glycol monododecyl ether homologue standards (mobile phase = 40 % hexane + 60 % (5% H_2 O in 2-propanol, T_{det} =50°C).

velocity of the nebulizing gas. (Fig. 3). The curves seem to indicate that the detector signal is dependent on the size of the aerosol particles of the ethoxylates rather than on their quantity, since with a growth in the linear velocity of the nebulizing gas, the quantity of the aerosol particles is inevitably higher while their size is smaller. Because the detector's signal also declines with an increase of linear velocity, the detector response is proportional to the particle size (right branches of the curves). The course of the left side of the curves at such low detection temperature (50 °C) and poor eluate nebulization may indicate incomplete evaporation of hexane which 'dilutes' the substances determined and cause poorer light scattering. Moreover, for C12EO3 and higher oligomers the course of the curves is almost identical. On the other hand, the value of the response at a maximum for C12EO1 is twice as small as for the oligomers referred to above, and almost ten times as small for C12OH (Fig. 3).

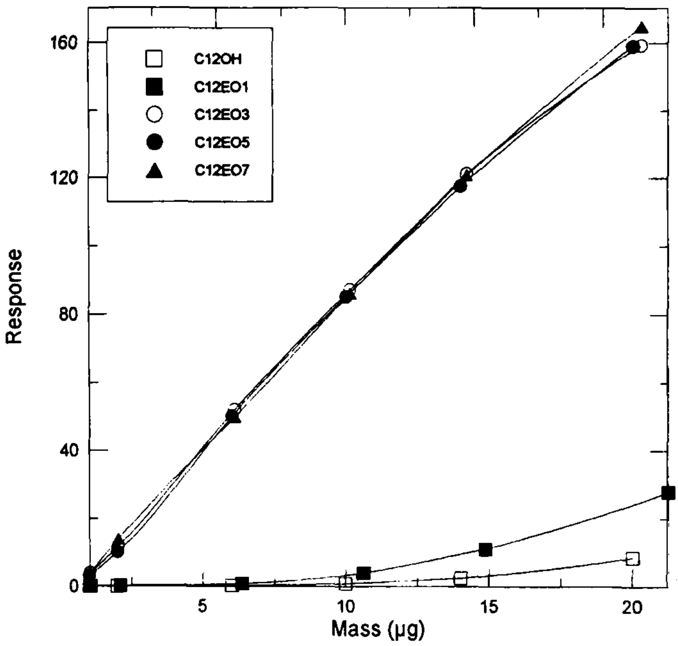

Figure 5. Calibration curves of polyethylene glycol monododecyl ether homologue standards with the same lengths of hydrophobic and different lengths of hydrophilic chains (mobile phase = 100 % hexane, V =1.5 dm^3 N_2 /min, T_{det} =50°C).

40 % Hexane + 60% (5% H_2O in 2-propanol) as a mobile phase

At 50 ^{o}C, the signal of all the ethoxylated alcohols (except C12OH and C12EO1) dissolved in 40% hexane + 60% (5% H_2O in 2-propanol) is the same; so the effect of the flowrate of the nebulizing agent was analyzed at such temperature. The resulting relationships are shown in Fig. 4.

No differences are observed in the course of the curves, though the maximum signal is about 13000, much lower than the maximum for hexane (20000).

For both extreme values of the gradient, an optimum flowrate of the nebulizing gas (in other words, the highest detector response) is 1.5 dm^3 N_2/min (35 mm), so the study was continued for that flowrate of N_2.

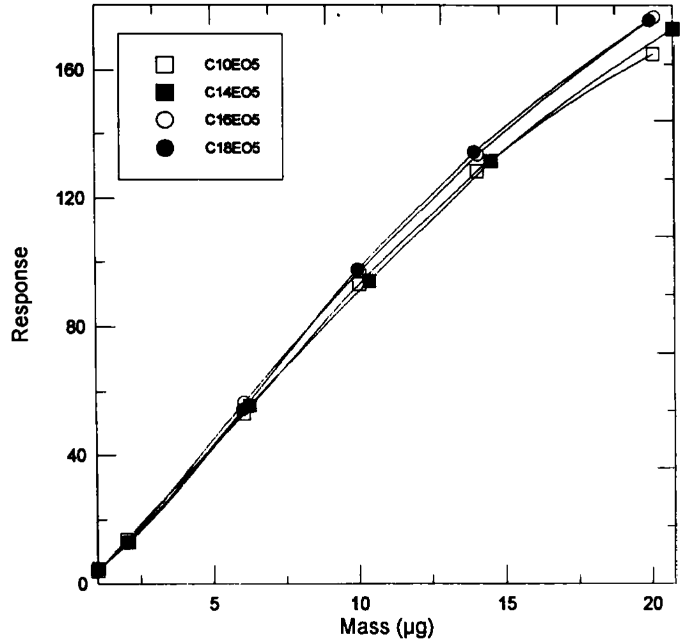

Figure 6. Calibration curves of pentaethylene glycol alkyl ether homologue standards with the same lengths of hydrophobic and different lengths of hydrophilic chains; (mobile phase = 100 % hexane, V=1.5 dm^3 N_2/min, T_{det}=50°C).

Dependence Of ELSD Response On The Mass Of Ethoxylated Alcohol

Hexane as a mobile phase

The relationships between the ELS detector response and the mass of ethoxylated alcohols and C12OH are shown in Figures 5 and 6 (at an optimum drift tube temperature and optimum flowrate of the nebulizing gas: 50 °C, 1.5 dm^3/min). The curves were ploted in a range 1-20 μg. Both for standards of ethoxylated alcohols having the same lengths of their hydrophobic and different lengths of their hydrophilic chains (C12EO3, C12EO5, C12EO7, C12EO9), as well as the same hydrophilic and different hydrophobic chain lengths (C10EO5, C12EO5, C14EO5, C16EO5, C18EO5), the detector response is the same within the margin of error and is not linear in the studied range 1-20 μg.

Table 1

Exponential Correlation Parameters ($Y=b*X^a$) for Ethyxylanted Alcohols for Hexane as a Mobile Phase

	Correlation Parameter		
Ethoxylate	**a**	**b**	**R^2**
C12EO3	1.2451±0.13407	4.52994±2.103E-05	0.98986
C12EO5	1.3107±0.14793	3.79955±3.0457E-05	0.98889
C12EO7	1.22077±0.14434	4.81896±2.8247E-05	0.98785
C10EO5	1.2396±0.16074	4.87505±4.2735E-05	0.98628
C14EO5	1.21462±0.11137	5.09298±1.0409E-05	0.99266
C16EO5	1.26393±0.1493	4.80086±3.195E-05	0.98786
C18EO5	1.25772±12728	4.87743±66.34E-05	0.99107

The average values of a and b for all standards are 1.2500 (σ-0.0298) and 4.6850 (σ=0.3926) respectively.

The only exceptions are C12OH and C12EO1 standards, having the highest volatility and undergoing evaporation in the drift tube under detector working parameters (Fig. 5).

Even the earliest measurements with ELS detectors showed that the relationship between the detector response and the mass of various components is non-linear,[43] though the results shown in a double-logarithmic scale tended to form straight lines with slopes of about 1.82.[44] So the function of the detector response should be of the exponential type:

$$Y=b*X^a$$

where: a - response coefficient.

The parameters of those correlations are shown in Table 1.

40% Hexane + 60%(5%H_2O in 2-propanol) as a mobile phase

Calibration curves of the detector response were found for the mixture of 40 % hexane + 60% (5% H_2O in 2-propanol) as a solvent in the same optimum conditions (1.5 dm^3/min, 50°C) as for hexane. The curves were also ploted in a

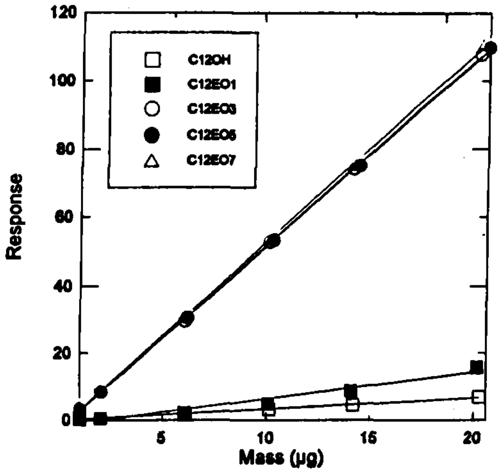

Figure 7. Calibration curves of polyethylene glycol monododecyl ether homologue standards with the same lengths of hydrophobic and different lengths of hydrophilic chains (mobile phase = 40% hexane + 60 % (5% H_2O in 2-propanol, V=1.5 N_2 dm^3/min N_2, T_{det} = 50°C).

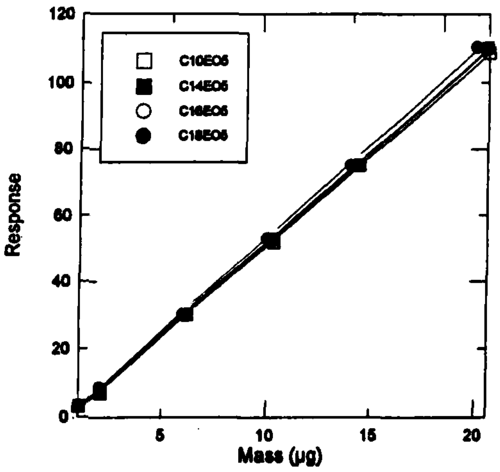

Figure 8. Calibration curves of pentaethylene glycol alkyl ether homologue standards with the same lengths of hydrophilic and different lengths of hydrophobic chains (mobile phase = 40% hexane + 60 % (5 % H_2O in 2-propanol, V=1.5 N_2 dm^3/min, T_{det} = 50°C).

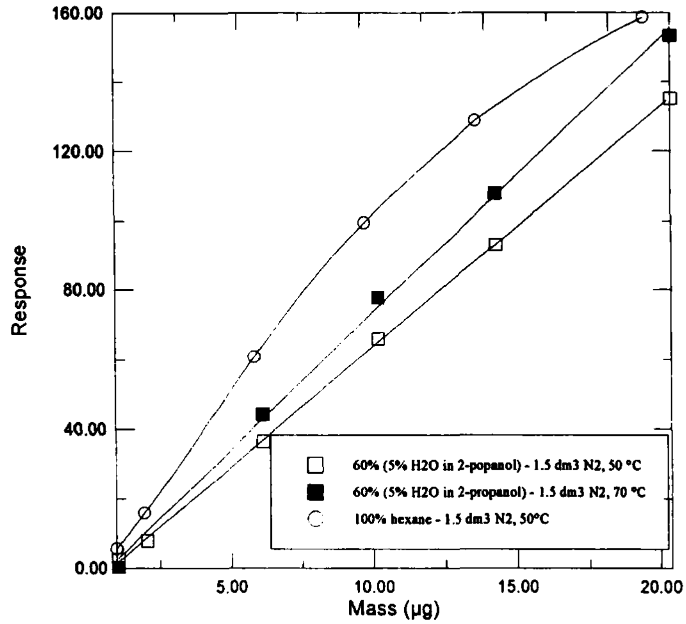

Figure 9. Comparison of the ELS detector response for C12E07 under various operating conditions.

range 1-20 µg. Contrary to hexane in the range under examination, all the curves are straight lines (Fig. 7 and 8). The most volatile standards - C12OH and C12EO1, which are evaporated at 50°C show lines with much lower slopes (0.3329 and 0.8022 respectively). At the same time however, as previously observed for the curves showing the dependence of the detector response on the drift tube temperature and nebulizing gas flowrate, the value of the signal was about 30% smaller than it was for hexane. Linear correlation parameters of the standards above are shown in Table 2.

Dependence Of ELSD Response On The Composition Of The Mobile Phase

Fig. 9 illustrates the differences in the run of the calibration curves for C12EO7 for hexane and 40% hexane + 60% (5% H_2O in 2-propanol) as a mobile phase. For the latter system, the calibration curve was plotted at 50 and 70 °C. As the standard is not evaporated at 70 °C while the mixture 40% hexane + 60% (5% H_2O in 2-propanol) is undoubtedly better evaporated than

Table 2

Linear Correlation Parameters (Y=a*X+b) forEthoxylated Alcohols for 60 % (5 % H_2O in 2-propanol) as a Mobile Phase

	Correlation Parameter		
Ethoxylate	**a**	**b**	**R^2**
C12EO3	5.4691±0.0813	-2.81351±0,07083	0.99985
C12EO5	5.45463±0.0800	-2.76133±0.07194	0.99981
C12EO7	5.56501±0.0740	12.66079±0.04936	0.99973
C10EO5	5.43627±0.087553	-3.35764±0.10297	0.99976
C14EO5	5.50475±0.0947	-3.31288±0.13873	0.99982
C16EO5	5.49277±0.1178	-2.76655±0.32474	0.99960
C18EO5	5.62852±0.1056	-2.83525±0.19375	0.99969

The average values of a and b for all standards are: 5.5073 (σ=0.0627 and -2.9297 (σ=0.2618) resepectively.

in 50°C, the run of the this curve is closer to the one plotted for hexane. The straight-line shape of the curve obtained for 40% hexane + 60% (5% H_2O in 2-propanol) as a mobile phase is quite far (about 20% in the middle section of the range) from the curve plotted for hexane which is 'positively deflected' from it while both at low and high concentrations, the responses are very close for the two mobile phases.

Since the ELSD responses for ethoxylates non-evaporating under optimal detection conditions (V= 1.5 dm^3 N_2/min, T_{det} = 50 °C) are statistically the same and only dependent on the composition of a mobile phase, the arbitrarily selected C12EO7 was used to create calibration curves for 10, 20, 30, 40, 50, 60% (5% H_2O in 2-propanol) as a mobile phase (with hexane complement). As expected, an increase in the content of 2-propanol in a mobile phase was accompanied by a decrease of the slopes of the plotted lines with simultaneous 'intensification' of the linear shape of the curves, indicated by greater linear correlation coefficients (Fig. 10).

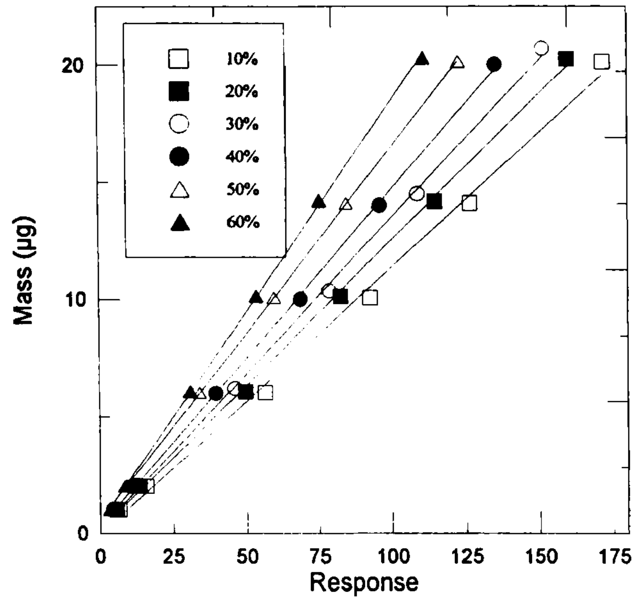

Figure 10. Mass=f(response) for various compositions of a mobile phase for heptaethylene glycol monododecyl ether C12EO7(V=1.5 dm^3 N$_2$/min, T$_{det}$=50°C).

Fig. 11 illustrates the dependence of the slopes of the curves shown in Fig. 10 on the composition of a mobile phase (% B) and retention time. In the range under examination, the relationship is illustrated by a parabola and may be described by the equation:

$Y = 1.4143*10^{-5}*X^{2} + 2.78094*10^{-4}*X + 1.11003*10^{-1}$ (R^2_a=0.997097, R^2_b= 0.971322) for the composition of a mobile phase (where X= %B), and

$Y = 1.68331*10^{-5}*X^{-2} + 1.92203*10^{-4}*X + 1.110186*10^{-1}$ (where X=t_r) for retention times.

The latter shows a direct relationship between the composition of a mobile phase and the correction coefficient by which the peak area of a given homologue with a specific retention time, t_r, is to be multiplied to obtain its quantity expressed in µg.

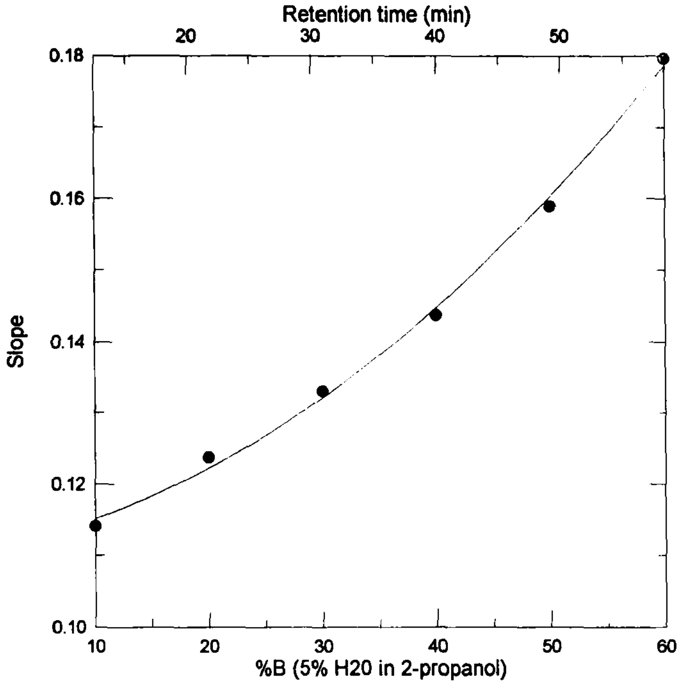

Figure 11. Relationships between the slope of the straight lines shown in Figure 13 and the concentration of (5 % H_2O in 2-propanol) in hexane and retention times.

SUMMARY

A study carried out with the use of an ELS detector indicates that the ELSD response, in the range under examination (valuable from the practical point of view) is the same for all studied standards of ethoxylated alcohols (except for C12OH with C12EO1, and C12EO2 which have the lowest boiling points), whereas the UV-VIS detector gives a response dependent on the length of the hydrophilic chain. However, the detector signal for these standards depends to a significant extent on the composition of a mobile phase and is the greatest for 100% hexane under optimum conditions (N_2 flowrate and drift tube temperature). The run of the calibration curves for a mobile phase containing 2-propanol is linear, while for hexane is linear in log-log scale with a response coefficient equal to 1.25. The difference between the response for 100% hexane and a mobile phase containing 2-propanol, probably caused by different evaporation of these solvents, is much smaller for higher detection temperatures (>70 °C), at which on the other hand, standards of the lower ethoxylated alcohols undergo evaporation.

ACKNOWLEDGEMENT

The authors are grateful to Dr. Lesiński from Waters-Sp. 20.0. for providing 717 plus autosampler (Waters, U.S.A.) for this work.

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Received October 20, 1995
Accepted November 10, 1995
Manuscript 4013