Liquid chromatography of polyethers using universal detectors V. Quantitative aspects in the analysis of low-molecular-mass poly(ethylene glycol)s and their derivatives by reversed-phase high-performance liquid chromatography with an evaporative light scattering detector

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Abstract

The performance of an evaporative light scattering detector in liquid chromatography of poly(ethylene glycol)s and their mono- and dimethyl ethers is evaluated. The aspect of quantitation is critically examined for isocratic and gradient elution. Molar mass dependence of response factors for the different homologous series is studied at different evaporator temperatures, and compared to those from isocratic elution with density and refractive index detection.

Keywords: Evaporative light scattering detector; Detection, LC; Polyethers; Poly(ethylene glycol)s

1. Introduction

Poly(ethylene glycol)s (PEGs) as well as their mono- and dialkyl ethers are used in many fields, such as technical, pharmaceutical and biochemical applications. In many of these, one has to consider the influence of the molar mass distribution (MMD) on the properties of the product. In the literature, many different methods for the determination of MMD and molar mass averages (M_w and M_n) have been described, which, however, often suffer from a lack of quantitative reliability.

Basically, three different approaches (in various modifications) can be applied, but each of them has – aside from its merits – serious drawbacks:

- Mass spectrometry (MS), preferably matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF-MS), has the advantage of excellent identification power, but quantitation may be problematic.
- Size exclusion chromatography (SEC) separates according to molecular dimensions, which simplifies the determination of the MMD, but the separation efficiency is generally poor. Moreover, considerable errors may arise from imperfect chromatographic equipment and – especially with lower-molecular-mass samples – from the assumption of a continuous MMD.
- Interaction chromatography, either by adsorption, partition or any other mechanism, in gaseous, liquid, or supercritical mobile phases can provide

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much better separation efficiency and selectivity. As gas chromatography can only be applied to lower oligomers, the typical techniques for higher oligomers are liquid chromatography (LC) and supercritical fluid chromatography. Basically, the stationary phase may be more polar (normal-phase mode) or less polar (reversed-phase mode) than the mobile phase. In both cases, a separation according to the number of ethylene oxide units can be achieved. The main problems are, however, the identification of the separated peaks and the quantitation of the analysis.

Recently, combinations of these different techniques have been successfully applied to polymer characterization. Several authors have described combinations of different modes of LC [1–5], and Pasch has also combined LC with MALDI-TOF-MS [5,6].

The problem of quantitation arises, however, as well in MS as it also does in all chromatographic techniques. In the latter case, this is due to the fact, that many of the typical high-performance liquid chromatography (HPLC) detectors are not very useful in the analysis of PEGs and their alkyl derivatives. While alkylphenol–ethoxylates allow UV detection [7], these samples do not contain any chromophoric group. Nevertheless, several authors have applied low-wavelength UV detection [8,9].

The MMDs thus obtained are, however, questionable, because in this case the detector response originates rather from refractive index (RI) differences or scattering phenomena, the molar mass dependence of which is not clear.

Derivatization of the hydroxy end groups [10–12] with various reagents allows UV, fluorescence, and electrochemical detection, but the drawbacks are obvious: first of all, the desired complete (or, at least, reproducible) degree of the reaction is not always achieved; secondly, derivatization causes problems in the separation itself, and, most of all, the basic assumption, that each molecule (bearing one or two derivatized end groups) will be seen with the same response factor, is not justified at all. In some solvents (for example dichloroethane), a considerable response is obtained from underivatized PEG at the typical absorptions wavelengths of aromatic systems (260–290 nm)!

Despite their lower sensitivity, refractive index and density detection do their job much better than UV: their response to polymer homologous series is well understood, and the molar mass dependence of response factors can be easily compensated [13–16].

On the other hand, these detectors can only be applied in isocratic elution, which is problematic in LC of higher oligomers, which typically require gradient elution.

In recent years, the evaporative light scattering detector (ELSD) [17–19] has become a very promising tool for such analytical tasks [12,20,21]. Unfortunately, the sensitivity of this instrument depends on various parameters [22] which can not always be easily controlled, and its response to polymer homologous series is not as well understood as that of RI and density detector. Moreover, the response of such an instrument is generally not linear with concentration [22,23] but can be expressed by an exponential relation [18,24]. This becomes clear, when one takes into account the complicated process of producing a signal, which shall be described and commented on briefly here.

• The eluate is nebulized by a stream of air, nitrogen or another carrier gas, the nature and flow-rate of which may affect the sensitivity. It must be mentioned, that there are basically two different designs: in the SEDEX instruments (S.E.D.E.R.E., Alfortville, France) the mobile phase is nebulized at room temperature in a special spray chamber, in which larger droplets are trapped, while in other types the entire aerosol is carried through the heated drift tube. It is clear, that the number and size of the droplets (and in the SEDEX instruments also the nebulized fraction of the eluate) will depend on the composition and the flow-rate of the mobile phase as well as on the flow-rate of the carrier gas [18,25].

• From the aerosol thus obtained, volatile components are (more or less) evaporated in a heated tube. Obviously, the degree of evaporation will depend on evaporator temperature as well as on the flow-rate of the carrier gas, which determines the time a droplet spends in the evaporator.

• At the end of the evaporator tube the particles remaining in the gas stream after evaporation of the mobile phase scatter a transversal light beam.

The intensity of the scattered light depends on number and size of the scattering particles, and should reflect the amount of non-volatile material eluted from the column within each section of the chromatogram.

Obviously, number and size of the particles depend not only on the concentration of a solute in the eluate, but also on other parameters influencing the original size of droplets formed in the nebulizer, such as surface tension, which will be strongly affected by surface active substances.

From these qualitative considerations it becomes clear, that a quantitative analysis using an ELSD is not easily achieved. Basically, a reliable quantitative analysis will require information on

1. the influence of operating conditions on detector response for a given sample
2. the dependence of response factors on chemical composition and molar mass of each oligomer (which should in turn depend on the operating conditions).

It is clear, that the response of an ELSD is affected by two groups of parameters: the first one determines the quality of the separation and can thus not freely be varied (the composition and flow-rate of the mobile phase, as well as the injected volume and concentration of the sample solution); the second one can be set by the operator (the pressure of carrier gas, which determines the gas flow, the temperature of the evaporator, and the photomultiplier gain). The influence of these parameters on the performance of an ELSD has been studied by several authors [18,22,26,27].

In this paper we have now performed some basic studies, which should give a better understanding of this promising instrument, and make it applicable to the quantitative analysis of polyethers and similar materials by gradient HPLC.

This involved the following questions:

- Which experimental conditions allow a quantitatively correct analysis?
- For which samples can accurate results be achieved?

- Is it necessary to establish a calibration for each oligomer?

2. Experimental

The measurements described in this paper were performed using the following chromatographic equipment: the mobile phase (methanol–water in different ratios, both solvents HPLC grade, from Promochem) was delivered by two JASCO 880 PU pumps, which were coupled in order to provide gradients by high pressure mixing.

In isocratic measurements, the mobile phases were methanol–water 20:80 (w/w) and 30:70 (w/w) (depending on the molecular mass of the samples), as has been described in parts 1 to 4 of this series [13,14,16,28], and the flow-rate was 0.5 ml/min, unless mentioned otherwise.

In gradient elution, solvent A was methanol–water 20:80 (w/w), solvent B was methanol–water 60:40 (w/w). The elution order of oligomers with the same number of ethylene oxide (EO) units was in both cases the same: diol<monomethyl ether<dimethyl ether. The gradient profile used in all measurements was: 0–2 min, 100% A; 2–32 min, linear to 90% B; 32–35 min, 90% B; 35–37 min, back to 100% A. (In most cases, the gradient was stopped, when all peaks had been eluted).

From the density signal, the composition of the mobile phase at any elution volume could be determined. The influence of mobile phase composition on detector response for the individual peaks shall be discussed in another paper.

The following columns were used, which were connected to two column selection valves (Rheodyne 7060):

1. Spherisorb ODS2 S3W, 3 μm, 80 Å, 100×4.6 mm
2. Spherisorb S5X C_{18}, 5 μm, 300 Å, 250×4.6 mm

For bypass measurements, a capillary (500 mm, 0.5 mm inner diameter) was also connected to the valves.

Samples were injected manually (using a Rheodyne 7125 injection valve) or using an autosampler

Spark SPH 125 Fix; in both cases, sample volumes were 50 μl.

All measurements were performed using a density detection system DDS 70 (Chromtech, Graz, Austria), which was combined either with a RI detector Bischoff 8110 or a SEDEX 45 ELSD (Sedere, France).

Nitrogen was used as carrier gas, and the pressure at the nebulizer was set to 2.0 bar for all measurements, except for those, in which the influence of pressure was studied.

Data acquisition and processing was performed using the software CHROMA (Chromtech, Graz, Austria).

Polyether samples were purchased from Fluka (Buchs, Switzerland).

3. Results and discussion

In the literature, very nice separations of PEGs and their derivatives have been described

[20,26,29,30] most of which were achieved by gradient elution using an ELSD.

For lower-molecular-mass samples, it seems obvious on the first view, that the lower oligomers must have been strongly underestimated. If this is the case, it can have several reasons, such as partial evaporation of the more volatile lower oligomers, differences in size and RI of the droplets or solid particles remaining after evaporation and so on.

3.1. Effect of evaporator temperature

The first question concerns the influence of the evaporator temperature, which seems to be the most important parameter to be set by the operator. Hence we have run the same separations (using the same sample solutions) under identical conditions, but at different evaporator temperatures. The flow-rate of the mobile phase and the pressure of the carrier gas were kept constant for all measurements described in this study, unless mentioned otherwise.

Fig. 1 shows a comparison of two chromatograms of the PEG monomethyl ether 350, which were
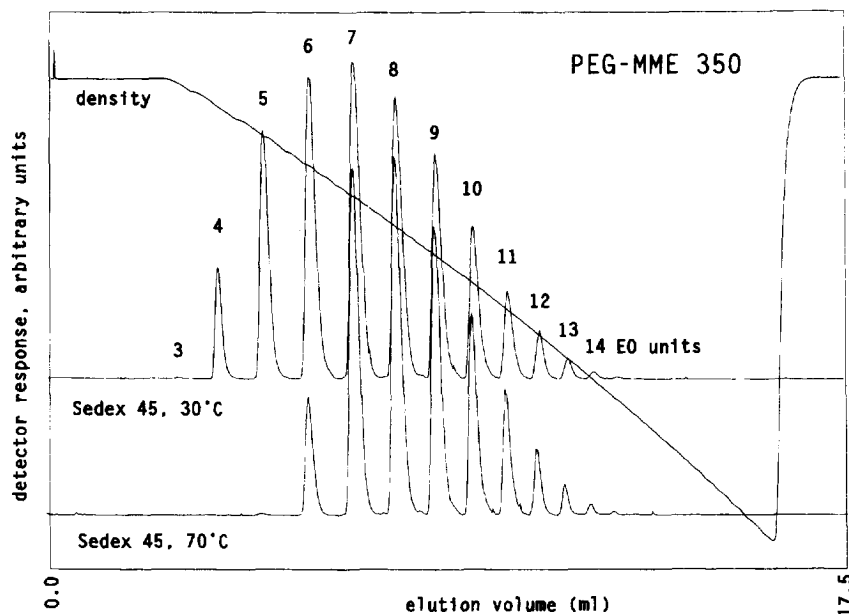

Fig. 1. Comparison of two chromatograms of the PEG monomethyl ether 350, as obtained by gradient LC on ODS2, 3 μm, 100×4.6 mm in methanol–water with different evaporator temperatures of the ELSD. Solvents: A, methanol–water 30:70 (w/w); B, methanol–water 60:40 (w/w). Gradient profile: 0–2 min, 100% A; 32–35 min, 10% A; 37 min, 100% A.
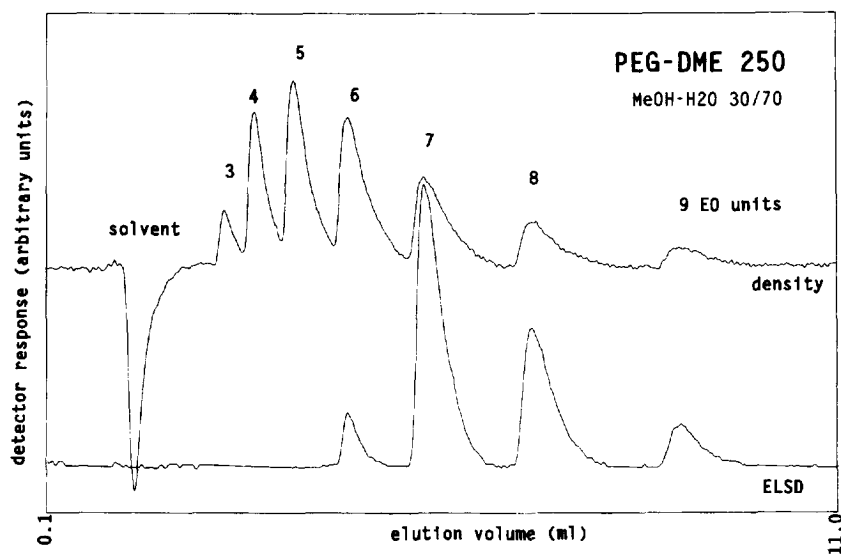
Fig. 2. Chromatogram of PEG 250 dimethyl ether, as obtained from isocratic LC on ODS2, 3 μm, 100×4.6 mm in methanol–water 30:70 (w/w) with density detector (top) and ELSD (bottom). Chromatographic conditions: flow-rate 0.5 ml/min; carrier gas pressure 2.0 bar; evaporator temperature 50°C; gain 4.
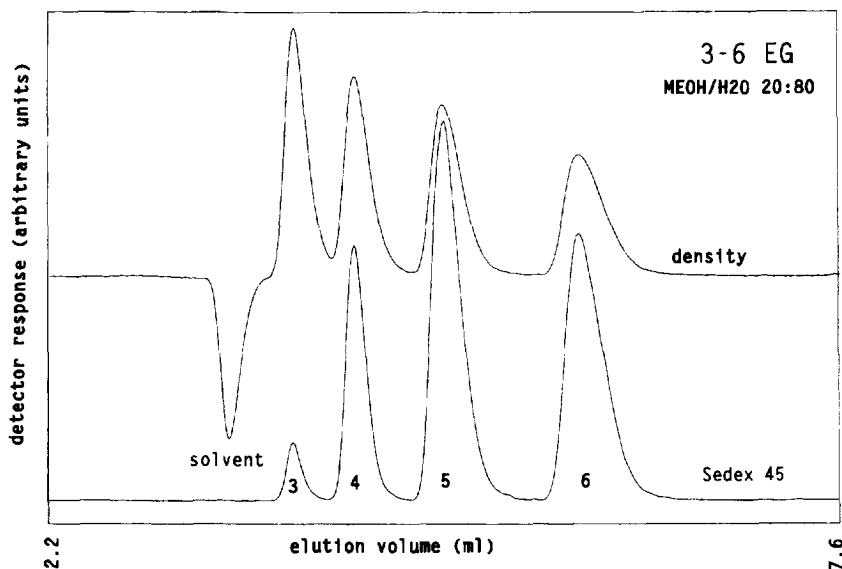

Fig. 3. Chromatogram of a mixture of tri-, tetra-, penta-, and hexa(ethylene glycol) (mass concentrations: 2.67, 2.42, 2.58, 2.68 g/l, respectively), as obtained from isocratic LC on S5X C_{18}, 25cm, 5 μm, in methanol–water 20:80 (w/w), with density (top) and ELSD (bottom). Chromatographic conditions: flow-rate 0.5 ml/min; evaporator temperature 30°C; carrier gas pressure 2.0 bar; gain 6.

obtained at evaporator temperatures of 30 and 70°C. Obviously, the lower oligomers are not detected at all at the higher temperature, which indicates, that in the analysis of lower oligomers one should use the lowest possible temperature, at which the mobile phase is sufficiently evaporated.

This is also confirmed by another example shown in Fig. 2: in an isocratic separation of PEG 250 dimethyl ether, which was performed with a combination of a density detector and an ELSD (operated at 50°C), the latter detects only half of the peaks! This effect is also found for the "native" PEGs, but to a lesser extent, because the volatility of the diols is lower than that of the corresponding mono- and dimethyl ethers (see Fig. 6).

In Fig. 3, a chromatogram of a mixture of tri- to hexa(ethylene glycol) (mass concentrations: 2.67,

2.42, 2.58, 2.68 g/l, respectively) is shown, which was obtained by isocratic elution in methanol–water 20:80 (w/w) with coupled density and ELSD at an evaporator temperature of 30°C. (This mixture was also used as standard sample for studying the influence of other operating parameters). As can be seen, lower PEG oligomers are underestimated by the ELSD even under these conditions.

In Figs. 4 and 5, the peak areas of the individual oligomer peaks obtained from gradient elution of two different PEG monomethyl ethers (PEG-MME 350 and 550, respectively) at different temperatures are plotted versus their molar mass: while for PEG-MME 350 temperatures higher than 40°C result in severe errors, there is almost no difference between 30 and 50°C for the higher-molecular-mass sample (PEG-MME 550).
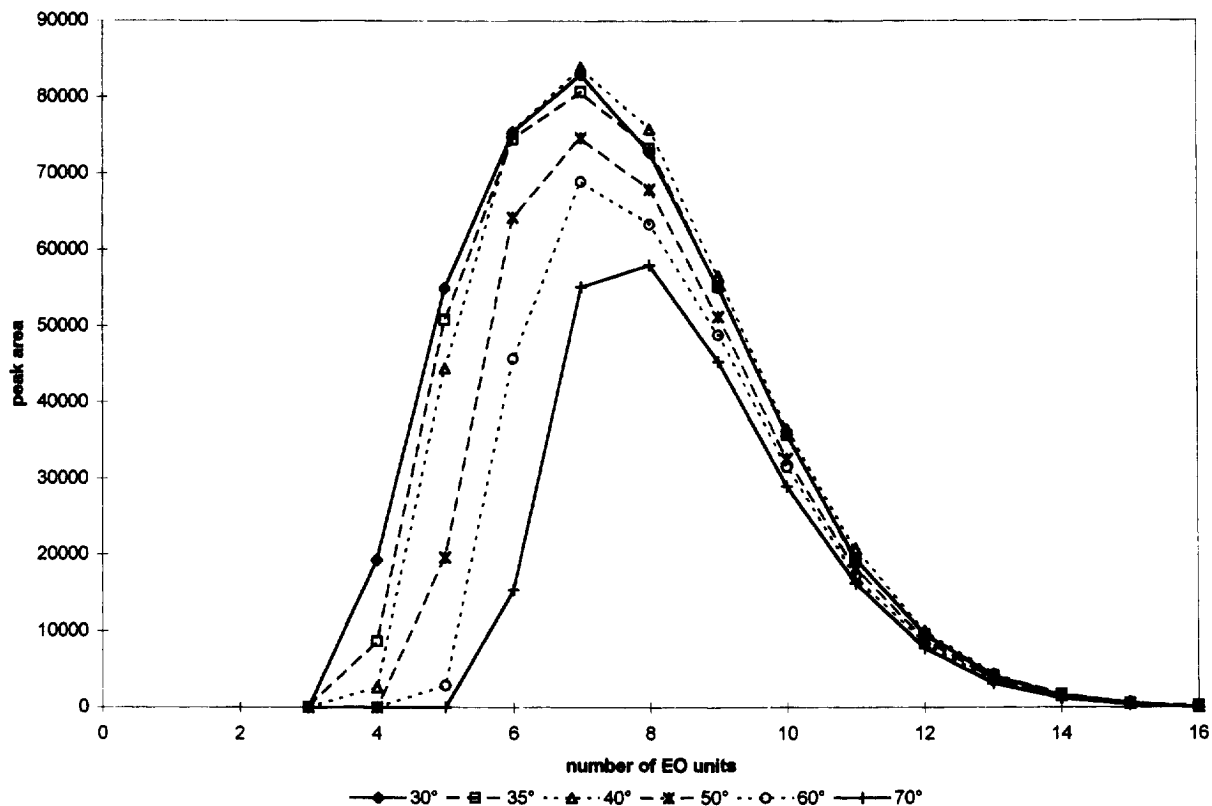

Fig. 4. Peak areas of individual oligomers in PEG 350 monomethyl ether, as obtained from gradient LC with ELSD at different evaporator temperatures, conditions as in Fig. 1.
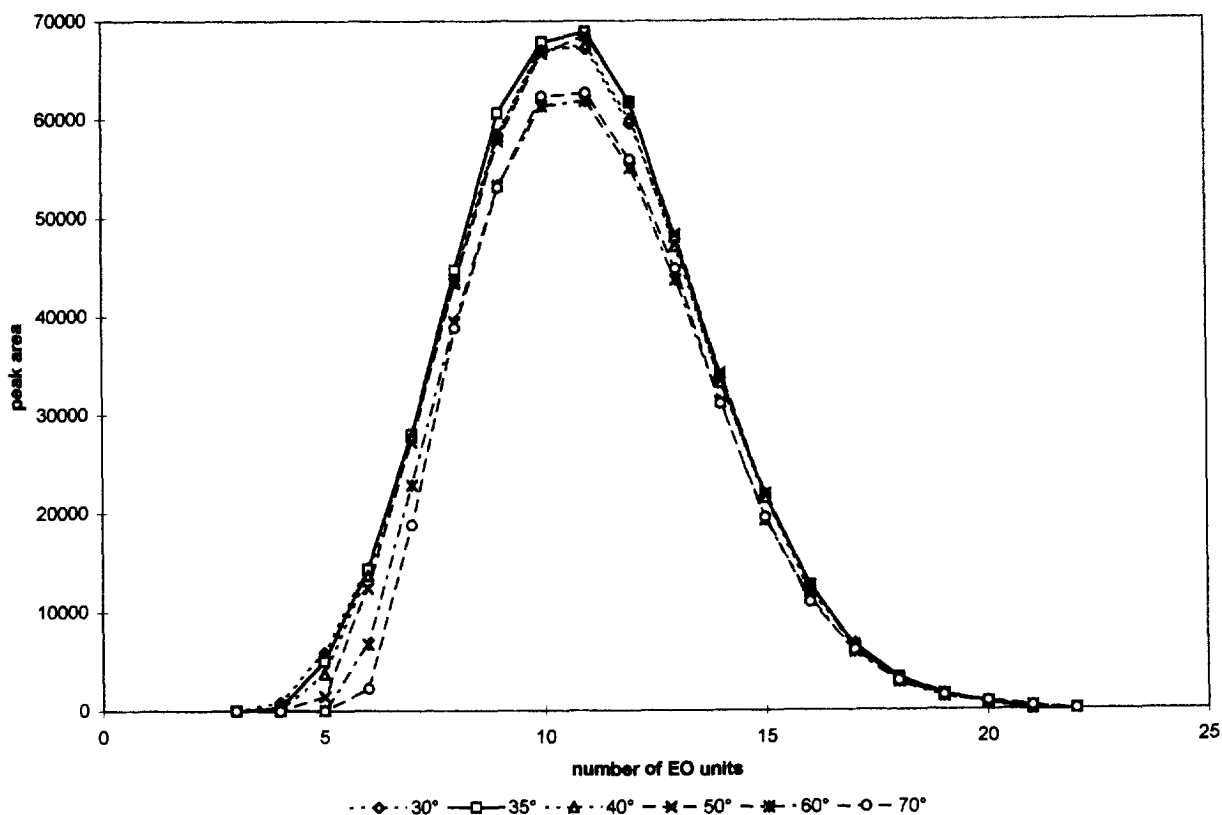
Fig. 5. Peak areas of individual oligomers in PEG 550 monomethyl ether, as obtained from gradient LC with ELSD at different evaporator temperatures, conditions as in Fig. 1.

In Fig. 6, the relative peak areas for the tetra- to hexamers obtained at different temperatures are shown (area at temperature T/area at 30°C). As can be seen, considerable amounts of the lower oligomers are already lost at 35°C. In the analysis of low-molecular-mass samples, one has thus to apply the lowest reasonable evaporator temperature in order to minimize vaporization of the lower oligomers. In practice, temperatures below 30°C can hardly be controlled in commercially available instruments. Moreover, complete evaporation of the mobile phase is not achieved at such low temperatures. Hence all subsequent measurements were performed at an evaporator temperature of 30°C. In order to eliminate any effects of mobile phase composition (which will be the subject of another

paper), we have applied isocratic elution throughout these investigations.

3.2. Effect of photomultiplier voltage (gain)

One of the parameters to be set by the operator of an ELSD is the voltage of the photomultiplier (gain), which determines the sensitivity of the detector. It is clear that the sensitivity has to be matched to the sample size and to the concentration of the individual components in the sample: a too low sensitivity will not allow an accurate determination of minor peaks, while a too high value will mean that the main peaks will be higher than the maximum input voltage of the AD-converter, i.e., generally 1024 mV. As is shown in Fig. 7(upper panel), the dependence of peak areas
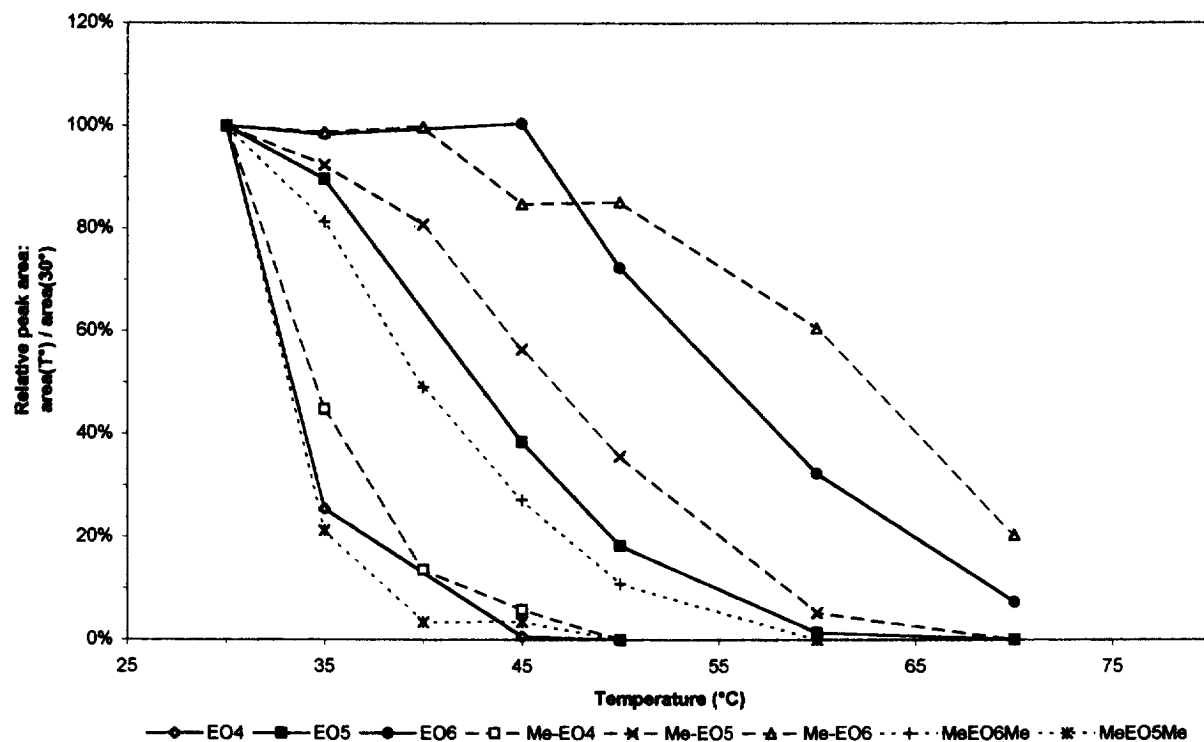
Fig. 6. Relative peak areas of the tetra-, penta-, and hexamers in PEG 200, PEG-MME 350, and PEG-DME 250, as obtained from gradient LC with ELSD at different evaporator temperatures, chromatographic conditions as in Fig. 1.

on the gain value is not a linear one. If peak areas are plotted on a logarithmic scale (Fig. 7(lower panel)), there is roughly a linear dependence, but with deviations at higher values of gain (as has already been pointed out by Van der Meeren et al. [22]).

3.3. Effect of carrier gas pressure

As has been pointed out by several authors [18,22,27], the peak area obtained for a given amount of sample at a given flow-rate of the mobile phase will depend on the applied pressure, which determines the flow-rate of the carrier gas and the size of the droplets As this influence may be different for the individual oligomers, we analyzed a mixture of tri-, tetra-, penta- and hexa(ethylene glycol) (in equal mass concentrations) by isocratic elution under the same conditions except for the pressure of the carrier gas (for all other measure-

ments, carrier gas pressure was set constant at 2.0 bar).

In Fig. 8 the peak areas obtained for the individual oligomers are shown as a function of carrier gas pressure. Obviously, increasing the pressure results in a loss of sensitivity for the oligomers with $n>4$, while the opposite seems to be true for the trimer.

3.4. Effect of mobile phase flow-rate

It is clear, that the flow-rate of the mobile phase will also have an effect on sensitivity. Complete nebulization will at best be achieved at very low flow-rates, while in the range typically used in HPLC (0.5–1.0 ml/min) the eluate will be nebulized only partially. This is indeed the case, as can be seen from Fig. 9, which shows the normalized peak areas (area/sample size) in a mixture of PEG oligomers ($n=3-6$, as above) as a function of flow-rate. Again there is a difference between the lowest oligomers and the
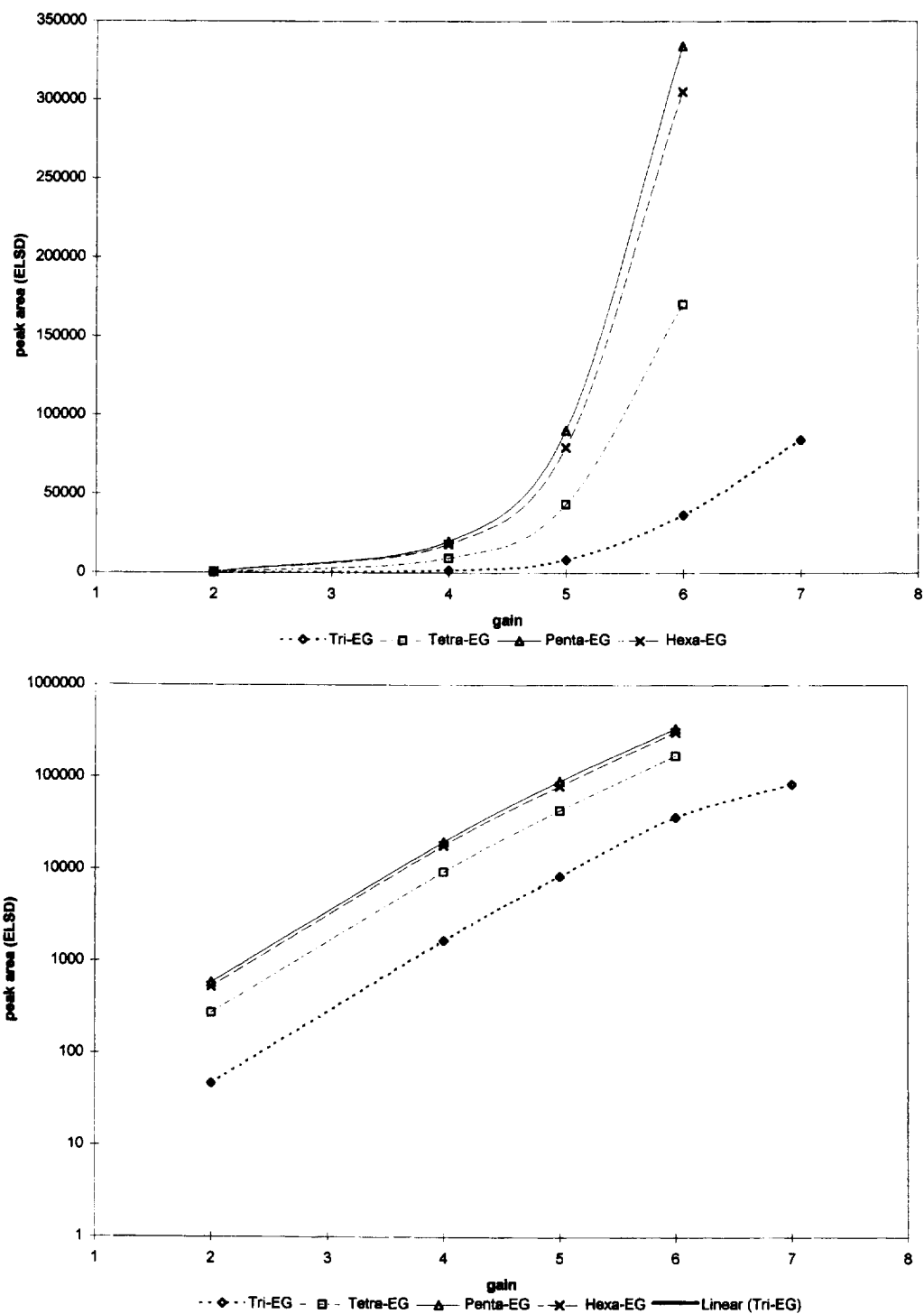
Fig. 7. Influence of photomultiplier voltage (gain); (upper panel) normal scale, (lower panel) logarithmic scale); sample and chromatographic conditions as in Fig. 3.
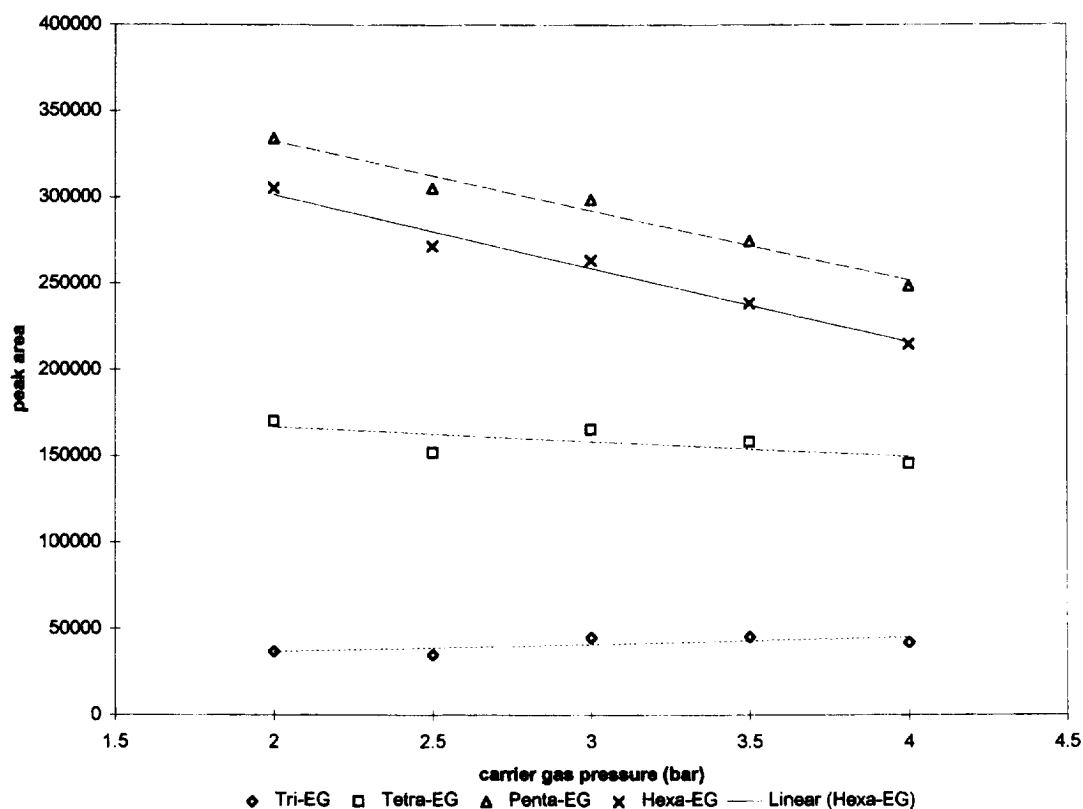
Fig. 8. Influence of carrier gas pressure; sample and chromatographic conditions (except for pressure) as in Fig. 3.

higher ones ($n>4$). An interpretation of these findings is not easy: the pressure of the carrier gas as well as the flow-rate affect the number and size of the droplets formed in the nebulizer, and their fraction reaching the drift tube, for each oligomer to a different extent, which obviously depends on molar mass. The nature of this dependence is, however, rather complex.

3.5. Linearity of detector response

As has been discussed in several papers, linear calibrations should be rather the exception than the rule for the ELSD: in general, the linear range – if there is one, at all – of such a detector is rather narrow. In most cases, the non-linear behavior can be described by an exponential equation

$$x_i = a \times m_i^b \tag{1}$$

where the signal x_i is related to the mass m_i of a sample component in the corresponding interval (at a given flow-rate) by the constants a and b. (It is clear that an exponent $b=1$ will be found in linear calibrations.)

The constants a and b can be determined easily from the logarithmic form

$$\log(x_i) = \log(a) + b \times \log(m_i) \tag{2}$$

In a plot of $\log(x_i)$ versus $\log(m_i)$ the exponent b is obtained from the slope and the constant a (which is closely related to the response factor f_i) from the intercept of the regression line.

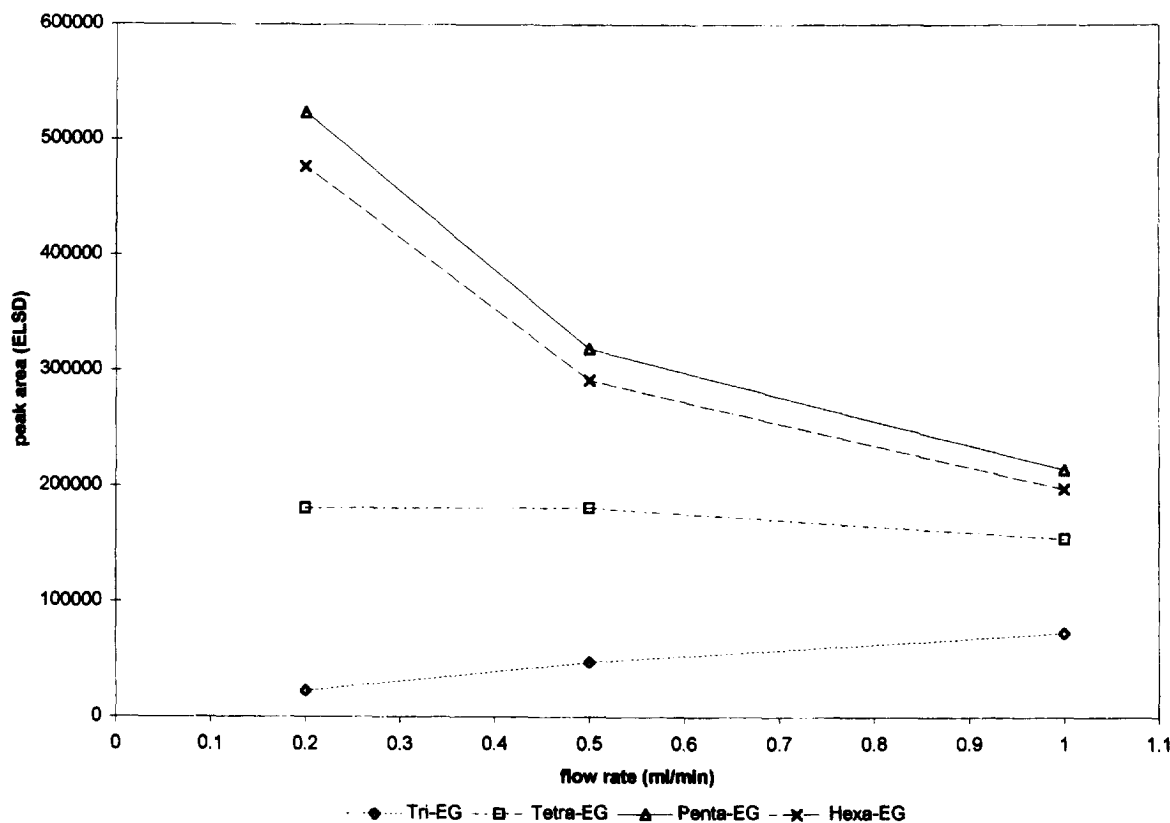As both parameters (a and b) may depend on

Fig. 9. Influence of mobile phase flow-rate; sample and chromatographic conditions (except for flow-rate) as in Fig. 3.

temperature as well as molar mass, we have determined them for hexa(ethylene glycol) and PEG 1000 by bypass measurements in methanol–water 20:80 (w/w) at two different temperatures. While density and RI detection show a wide linear range (Fig. 10), this is not the case for the ELSD. In the case of hexa(ethylene glycol), linear regression yields a quite good correlation ($R^2 = 0.9942$)), but the intercept is far from zero (slope = 2352.2, intercept = −12525!), which will result in severe errors at low sample concentrations. As can be seen from Fig. 11 and Table 1, an exponential fit is much more appropriate.

Even at different values of gain, the exponent b is fairly constant.

There remains still the question, whether the parameters a and b depend on temperature and on

molecular mass, and if so, to which extent. Fortunately, there is no difference between hexa(ethylene glycol) and PEG 1000, and even for the lower oligomer the same calibration function can be used at evaporator temperatures of 30 and 50°C, as can be seen from Figs. 12 and 13. The corresponding data are given in Table 1. These results agree quite well with the findings of Koropchak et al. who studied the linearity of ELSD for PEG in aqueous SEC [24].

3.6. Influence of end groups on detector response

The next question concerned the effect of molar mass (or, in other words, of the different end groups). In the case of density and RI detection, this dependence can be expressed by a simple relation: the
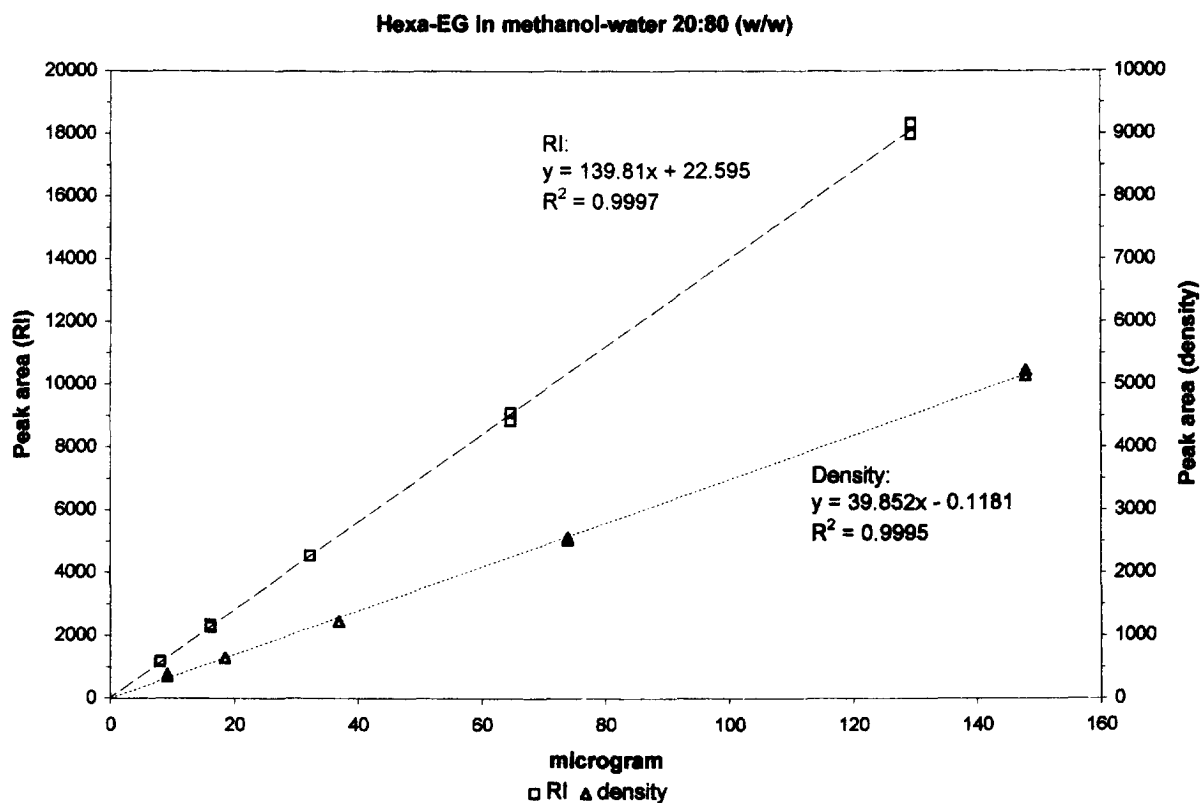
Hexa-EG in methanol-water 20:80 (w/w)

Fig. 10. Linearity of density and RI detection in bypass measurements of hexa(ethylene glycol) in methanol–water 20:80 (w/w). Conditions: flow-rate 0.5 ml/min; sample volume 50 μl.

response factor f_i of an oligomer with the molar mass M_i is given by

$$f_i = f_\infty + \frac{K}{M_i} \qquad (3)$$

wherein f_∞ is the response factor of a polymer with infinite molar mass (or at least a very high one) and K is a constant reflecting the influence of the end groups. This well-known equation describes the molar mass dependence of any specific property. In the case of the ELSD, the molar mass dependence of response factors is, however, not very likely to follow such a simple relation.

Fig. 14(a and b) shows a comparison of the response factors for PEG in isocratic LC, as obtained from bypass measurements in methanol–water 30:70. Of course, this mobile phase can not be used

for the separation of higher oligomers, but the trend is obvious: above a molar mass of several hundreds, the response factor of the ELSD becomes fairly constant.

This is however, not the case for lower oligomers. While in the case of density and RI detection a compensation of molar mass dependence can easily be performed using Eq. (3) (Fig. 14b), individual calibrations are required for the ELSD.

As monodisperse standards are only available for PEGs up to $n = 7$, and for the ethers up to 3 or 4, a direct calibration can not be obtained.

As will be shown in another paper, an indirect calibration using polydisperse standards works very well: if these samples can be characterized by isocratic LC with coupled density and RI detection (as has already been described in previous communications [13,14,16,28,31]), one may calculate the
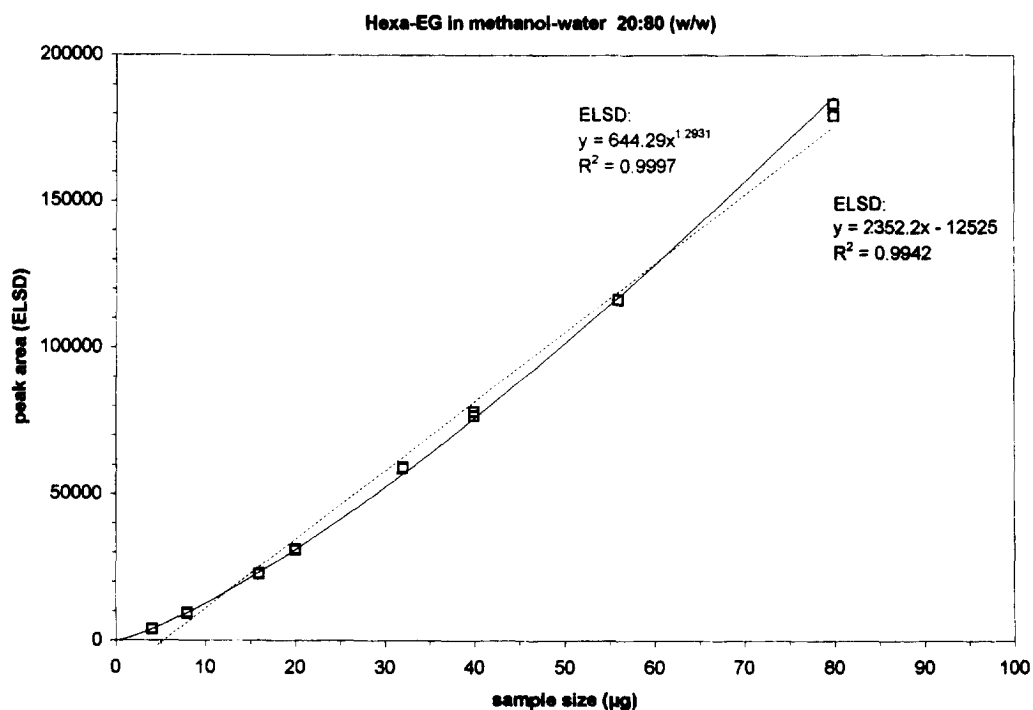
Fig. 11. Calibration of ELSD for hexa(ethylene glycol). Mobile phase: methanol–water 20:80 (w/w), flow-rate 0.5 ml/min, sample volume 50 μl. Carrier gas pressure: 2.0 bar. Evaporator temperature: 30°C. Gain: 6.

absolute amount for each oligomer in a sample, and therefore the response factor of the ELSD.

4. Conclusions

The ELSD is a very useful instrument in LC of polyethers, because it is more sensitive than other detectors, which may be applied, and – most of all – it allows gradient elution. On the other hand, the experimental conditions have to be optimized and the performance and applicability of the instrument must be carefully evaluated.

Acknowledgments

Table 1

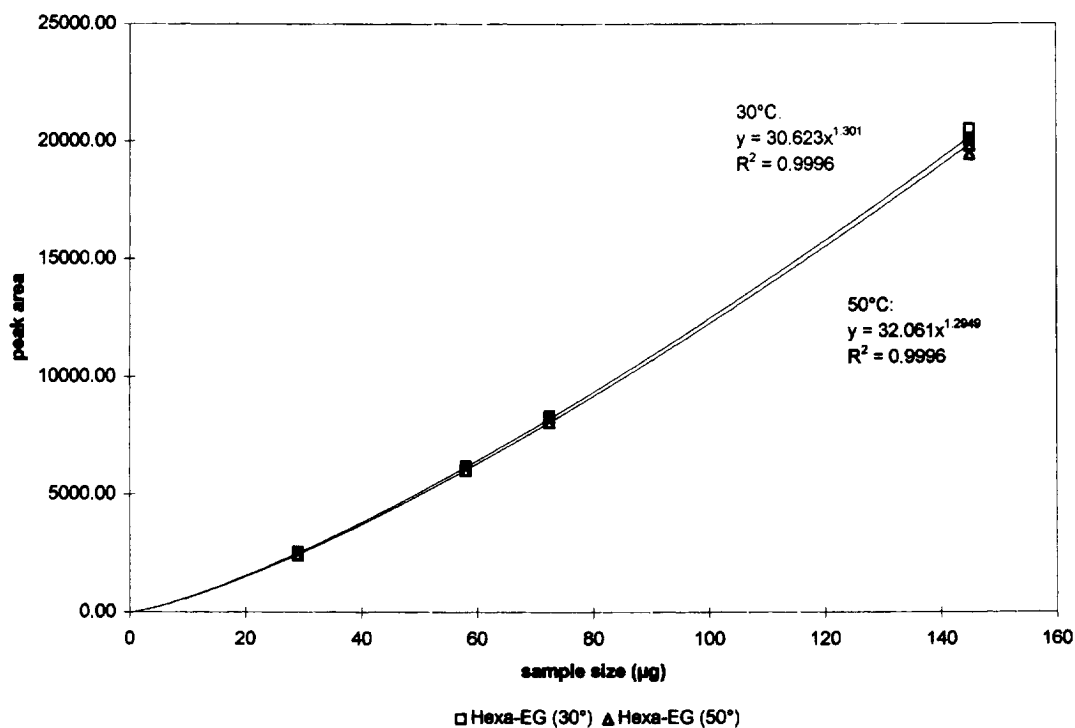| Sample	Evaporator temperature (°C)	Gain	a	b	R^2
Hexa(ethylene glycol)	50	4	32.06	1.2949	0.9996
Hexa(ethylene glycol)	30	4	30.62	1.3010	0.9996
Hexa(ethylene glycol)	30	6	644.29	1.2931	0.9997
PEG 1000	30	6	633.29	1.2997	0.9982

Fig. 12. Temperature effect on calibration of ELSD: hexa(ethylene glycol) at 30° and 50°C, gain 4, other conditions as in Fig. 11.
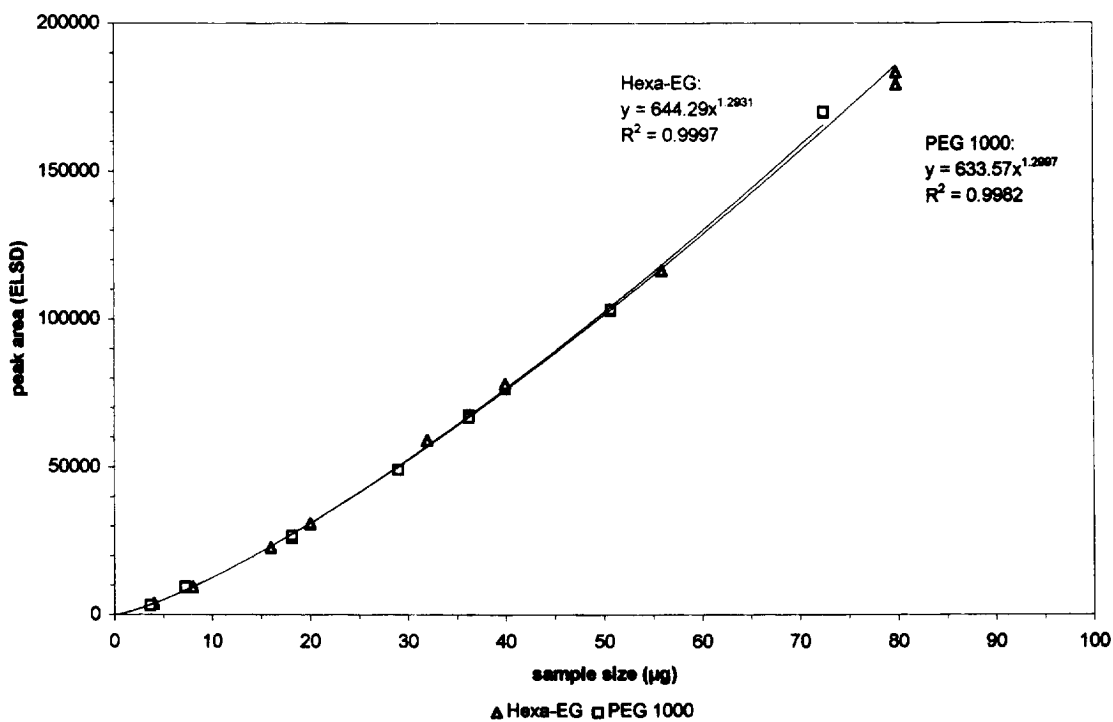

Fig. 13. Calibration of ELSD for hexa(ethylene glycol) and PEG 1000 at 30°, gain 6, other conditions as in Fig. 11.
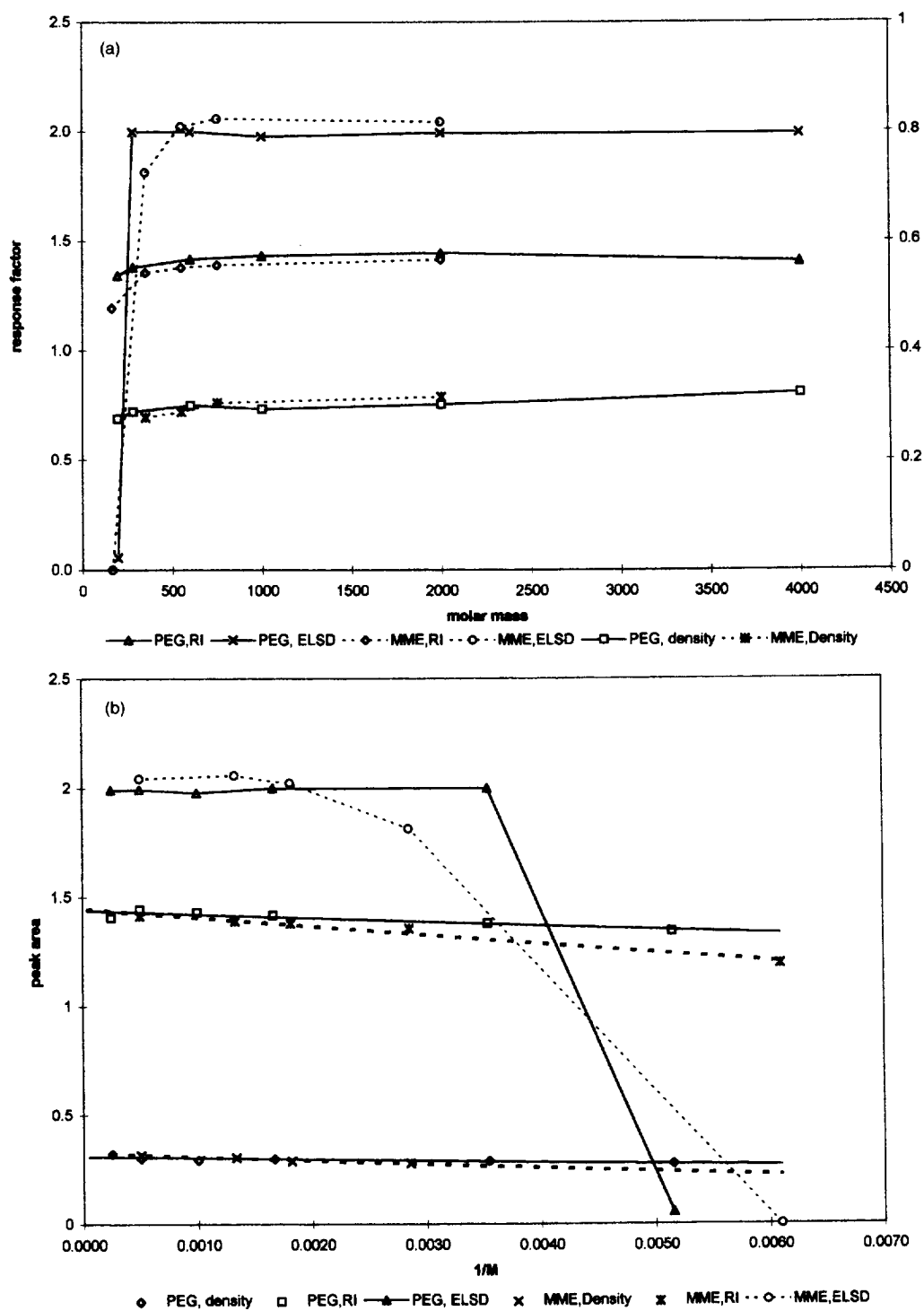
Fig. 14. Response factors of PEG in isocratic LC with ELSD, density, and RI detection, as obtained from bypass measurements in methanol–water 30:70 (w/w), flow-rate 0.5 ml/min, sample volume 50 μl. Conditions: carrier gas pressure 2.0 bar; evaporator temperature 50°C; gain 4.

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