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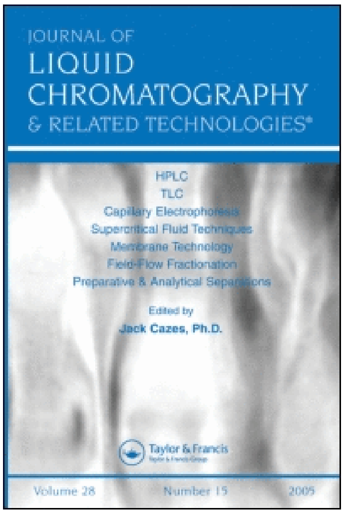

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ANALYSIS OF POLYETHERS BY ISOCRATIC HPLC WITH UNIVERSAL DETECTORS. II. QUANTITATIVE ANALYSIS AND DETERMINATION OF MOLECULAR WEIGHT AVERAGES

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Abstract:

It is shown, that LAC with coupled density and RI detection is a powerful tool in the determination of the MWD of polyethers. Polyethylene glycols and polypropylene glycols can be separated with very high resolution on a C-18 column in methanol-water under isocratic conditions. Peaks can be identified by the use of internal standards. A compensation of the molecular weight dependence of response factors is necessary only for very low molecular weight samples to yield accurate molecular weight averages. The results thus obtained can be used to evaluate the performance of SEC systems.

Introduction:

In the analysis of oligomers and low molecular weight polymers by Size Exclusion Chromatography (SEC) one has to take into account the sources of error, which arise in the determination of molecular weight averages[1-3].

1. The quality of the calibration line, whether obtained with monodisperse or polydisperse standards, limits the accuracy of the results.
2. Flow rate changes can cause severe errors, which can be reduced by the use of an internal standard.
3. Concentration effects (overloading) may influence the elution volumes.
4. Peak spreading[3] - unless corrected - will result in too high polydispersity.
5. As a complete separation of a polymer homologous series is typically not achieved, a continuous molecular weight distribution is assumed, which is problematic for low molecular weight samples[4,5].
6. An improper baseline can cause severe errors in the integration and hence in the MWD.
7. The molecular weight dependence[6,7] of response factors has to be compensated for low molecular weight samples.

As has been shown recently[8,9], polyethyleneglycols, their mono- and dimethylethers, and polypropyleneglycols can be separated very well by Liquid Adsorption Chromatography (LAC) on a reversed phase column in methanol-water under isocratic conditions.

By optimization of the mobile phase composition a separation into the oligomers almost to the baseline could be achieved in a wide molecular weight range. Using pure oligomers as internal standards, all peaks could be assigned a molecular weight[9].

As under these conditions points 1 - 5 are not relevant, the only sources of error will be the integration and the molecular weight dependence of response factors. Hence it should be possible to determine the MWD of polyethers by LAC with better accuracy than by SEC.

In this paper we describe a method, which allows a quantitative analysis of PEG and PPG by LAC under isocratic conditions, and an accurate determination of the MWD and molecular weight averages. The results thus obtained were compared to those from SEC using different stationary and mobile phases.

Molecular weight dependence of response factors:

Within a polymer homologous series response factors vary with molecular weight[6]. As we have shown previously[7], the molecular weight dependence of response factors can be described by

$$f_i = f_\infty + \frac{K}{M} \qquad (1)$$

wherein f_i is the response factor of an oligomer with the molecular weight M_i, f_∞ of a polymer with (infinitely) high molecular weight (i.e. the response factor of the repeating unit), and K is a constant describing the influence of the end groups. In a plot of f_i vs. $1/M_i$, f_∞ is the intercept and K the slope of the regression line.

At high molecular weights, the term K/M_i becomes negligible, thus leading to the common assumption of constant response factors; in the low molecular weight range, however, a correction according to equation 1 has to be made.

The limiting molecular weight, below which this is necessary, is determined by the constant K. Hence it is very important to know the parameters f_∞ and K.

Provided that each peak in a chromatogram can be assigned a molecular weight, the corresponding response factor can be calculated using equation 1. For low molecular weight samples this is rather easy, as long there are pure oligomers available, which can be used as internal standards. In the case of PEGs the oligomers up to the heptamer are commercially available, and from samples with overlapping MWDs one can identify higher oligomers.

For PEGs with higher molecular weights, however, a mobile phase composition is required, which doesn't allow a separation of the low oligomers, hence one will have to use a well defined standard with an appropriate degree of polymerization. Such samples can be prepared by a Williamson ether synthesis[10,11].

Experimental :

For these investigations, a density detection system DDS 70 (commercially available from A.PAAR KG, Graz, Austria) was used, which has been developed in our group. It was combined with a Bischoff 8110 RI detector, and connected to a MS-DOS computer for data acquisition and processing. The entire system has been described in full detail in previous communications[12,13]. Data acquisition and processing was performed using the software CHROMA, which has been developed for the DDS 70.

LAC measurements were performed on a Spherisorb ODS 2 column (5µ , 250 * 4.6 mm) in isocratic mode using methanol-water (30:70 - 90:10 w/w) as mobile phase at a flow rate of 0.5 ml/min, which was maintained by a JASCO 880 PU HPLC pump.

The solvents used were HPLC grade (Merck LiChroSolv). Samples were injected using a Rheodyne injection valve equipped with a 50 µl loop, the concentration range was 4 - 8 g/l. The integration results from LAC-measurements were written to ASCII-files and transferred to a spreadsheet (Quattro Pro), in which the molecular weight averages were calculated.

SEC measurements in water (HPLC grade, Merck) were performed on a TSK G 1000 PW column (300 * 7.8 mm) at a flow rate of 1.0 ml/min using the same instrumentation as above. SEC measurements in $CHCl_3$ (HPLC grade, Rathburn) were performed on a set of 3 Phenogel 300 * 4.6 mm columns (1*500 + 2*100 Å) at a flow rate of 1.0 ml/min, which was maintained by a Gynkotek 300 C HPLC pump. A SICON LCD 201 RI detector was coupled to the density detector for these measurements. Samples were injected using a VICI injection valve equipped with a 100 µl loop, the concentration range was 2 - 4 g/l.

Molecular weight averages from SEC measurements were calculated within the software package CHROMA. The software includes also a deconvolution procedure, which allows the separation of overlapping peaks.

Polyoxyethylene and polyoxypropylene samples and the lower oligomers (DP 2 to 6) were purchased from FLUKA and used without further purification.

The higher ethylene oxide oligomers were prepared by a Williamson ether synthesis[10,11] and purified by preparative SEC[11].

Results and discussion:

As we have already shown in part 1 of this series, polyethylene glycols can be separated very well by LAC under isocratic conditions, and each peak can be assigned a molecular weight by the use of internal standards.

Fig.1 shows a chromatogram of PEG 300 obtained in methanol-water 30:70, Fig.2 shows a comparison of chromatograms of PEG 600 and the 18-mer, which were obtained in methanol-water 40:60.

As can be seen, the oligomers are sufficiently separated, and each peak can be identified. Hence the only sources of error remaining are the integration error (which should be small, because the peaks are rather narrow) and the molecular weight dependence of response factors.

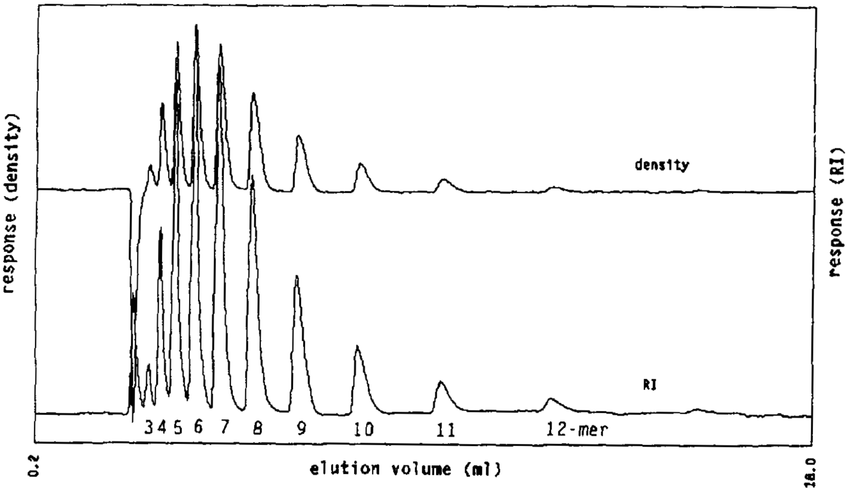

Fig.1: Density and RI-trace of a chromatogram of PEG 300 obtained on ODS 2 in MeOH-H_2O 30:70.

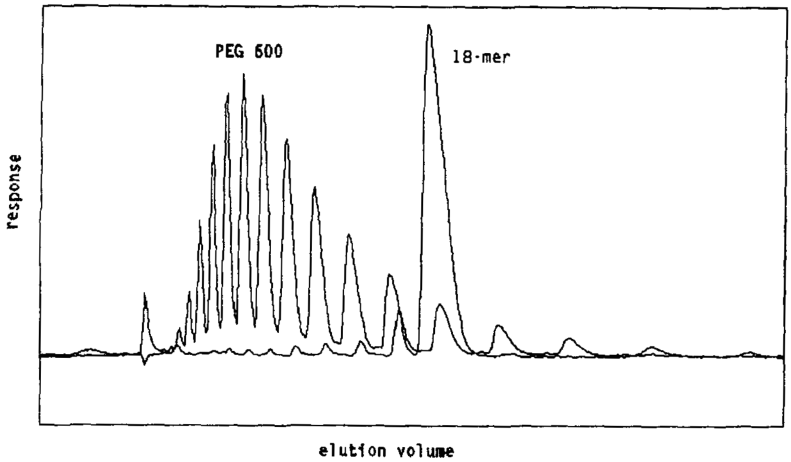

Fig.2: Comparison of two chromatograms (RI-trace) of PEG 600 and the 18-mer of ethylene oxide, which were obtained on ODS 2 in MeOH-H_2O 40:60.

In a previous paper[7] we have described three different approaches to determine f_∞ and K in SEC, two of which can be applied in LAC:

a) If a sufficient number of monodisperse oligomers is available, f_∞ and K can be determined by linear regression.

b) If this is not the case, dual detection can provide the desired informations: f_∞ and K for both detectors can be determined using a non-linear least square fit. If the peaks are only partially separated, a deconvolution procedure may be used to determine the peak areas for each oligomer.

c) In SEC, an iteration procedure can be used to determine K, if f_∞ is known from samples with high molecular weight.

In LAC of polyethylene glycols, approach a and b can be applied, because a sufficent number of monodisperse oligomers is available, for polypropylene glycol only approach b can be used. The parameters $f_{D,\infty}$, $f_{R,\infty}$, K_D, and K_R in eq.1 for density and RI detection, respectively, were determined for each mobile phase composition - as described in a previous communication[7] - from the chromatograms of monodisperse oligomers by linear regression as well as by a nonlinear least square fit from chromatograms of polydisperse samples, obtained with coupled density and RI detection.

Fig.3 shows a plot of the response factors $f_{D,\infty}$ and $f_{R,\infty}$, respectively, vs. 1/M for PEGs in a mobile phase composition of MeOH-H_2O 40:60.

Tab.1 shows the corresponding parameters $f_{D,\infty}$, $f_{R,\infty}$, K_D, an K_R for two mobile phase compositions, which were obtained from linear regression (approach a) and from the non-linear least square fit (approach b). As can be seen, the results from both approaches agree quite well.

From these parameters, the response factor of each oligomer was calculated according to eqn.1 for both detectors. As an example, Fig.4 shows the experimental and calculated response factors for each oligomer in MeOH-H_2O 40:60 plotted vs. their molecular weight.

Obviously, the molecular weight dependence of response factors has to be taken into account only for the lowest oligomers, for molecular weights above 300 the response factors can be considered to be constant.

In Tab. 2, the parameters $f_{D,\infty}$, $f_{R,\infty}$, K_D, and K_R, as obtained from non-linear regression, are given for polypropylene glycols in in a mobile phase composition of MeOH-H_2O 70:30.

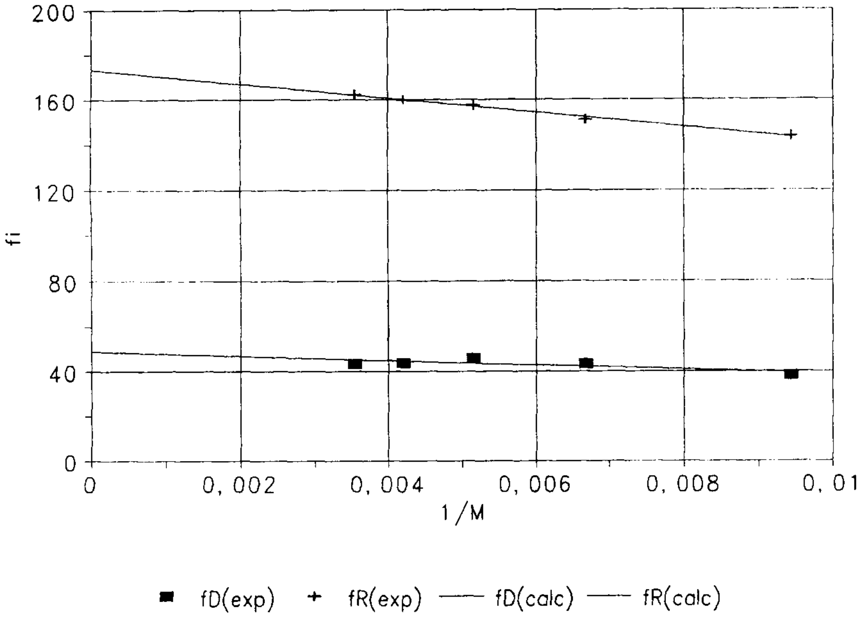

Fig.3: Response factors of polyethylene glycols in methanol - water 40:60 at 25.0°C as a function of 1/M

Tab.1: $f_{D,\infty}$, $f_{R,\infty}$, K_D, and K_R for polyethylene glycols in two mobile phase compositions, as determined using linear regression (approach a) and non-linear least square fit (approach b)

% MeOH	approach	$f_{D,\infty}$	K_D	$f_{R,\infty}$	K_R
30	a	39.10	589.5	154.57	-3326.9
30	b	39.58	881.1	157.08	-2969.2
40	a	38.39	1390.9	173.34	-3208.6
40	b	38.60	1580.4	171.29	-3228.1

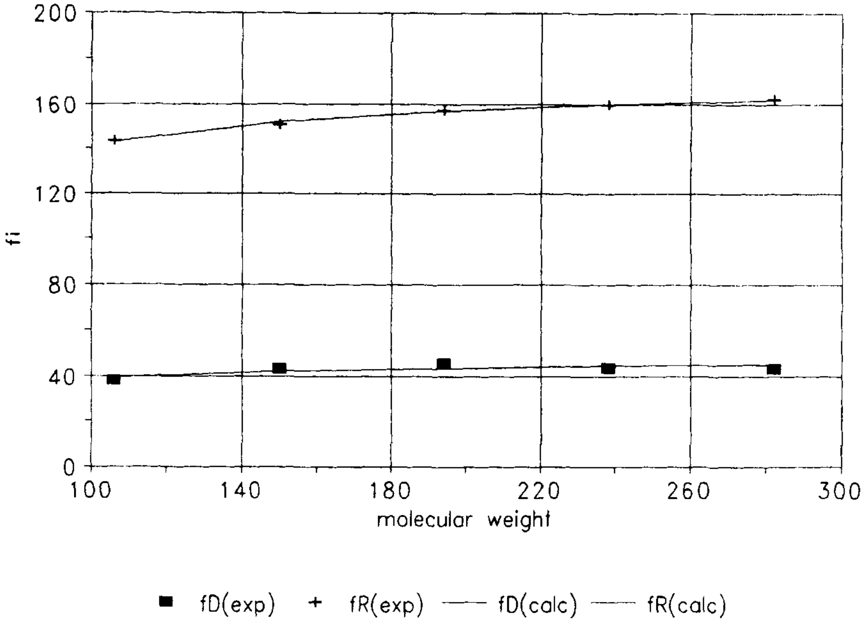

Fig.4: Response factors of polyethylene glycols in methanol- water 40:60 at 25.0°C as a *function of molecular weight*

Tab.2: $f_{D,\infty}$, $f_{R,\infty}$, K_D, an K_R for polypropylene glycols, as determined using non-linear least square fit

% MeOH	approach	$f_{D,\infty}$	K_D	$f_{R,\infty}$	K_R
70	b	32.67	484.73	166.02	-3132.3

Tab.3: Molecular weight averages of polyethylene glycol 300, as determined by LAC on ODS 2 in MeOH-H_2O 30:70, without and with compensation of molecular weight dependence of response factors

	without compensation		with compensation	
	Density	RI	Density	RI
M_w	322	327	324	325
M_n	299	304	301	302
M_w/M_n	1.079	1.075	1.078	1.076

Tab.4: Molecular weight averages of polyethylene glycol 400, as determined by LAC on ODS 2 in MeOH-H_2O 30:70, without and with compensation of molecular weight dependence of response factors

	without compensation		with compensation	
	Density	RI	Density	RI
M_w	405	407	406	406
M_n	380	384	384	382
M_w/M_n	1.064	1.061	1.061	1.062

Tab.5: Molecular weight averages of polyethylene glycol 600, as determined by LAC on ODS 2 in MeOH-H_2O 40:60, without and with compensation of molecular weight dependence of response factors

	without compensation		with compensation	
	Density	RI	Density	RI
M_w	615	619	616	619
M_n	589	594	591	593
M_w/M_n	1.044	1.042	1.044	1.042

Tab.6: Molecular weight averages of polypropylene glycol 425, as determined by LAC on ODS 2 in MeOH-H_2O 70:30, without and with compensation of molecular weight dependence of response factors

	without compensation		with compensation	
	Density	RI	Density	RI
M_w	439	441	440	440
M_n	418	421	419	420
M_w/M_n	1.051	1.049	1.050	1.050

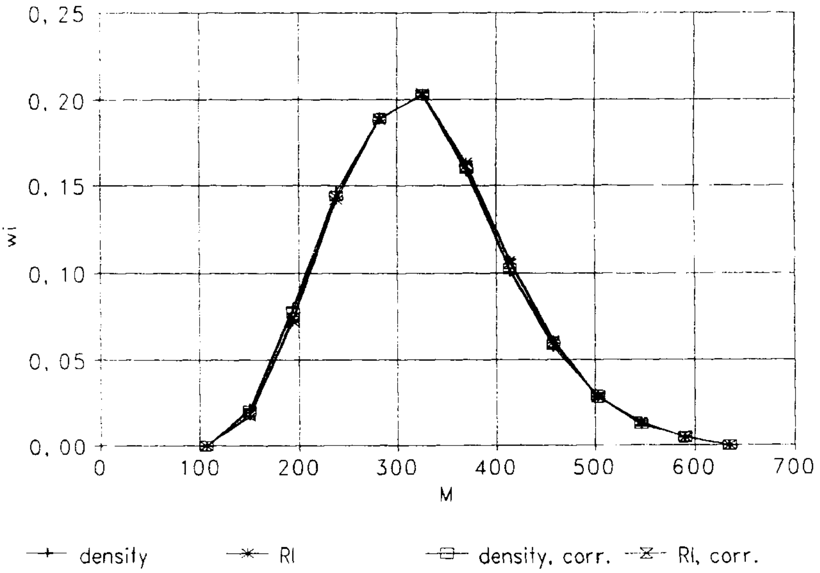

Fig.5: Mass distribution of polyethylene glycol 300, as determined by LAC with density and RI detection, without and with compensation for molecular weight dependence of response factors. Mobile phase: methanol- water 30:70

In order to evaluate the influence of the integration error and the molecular weight dependence of response factors on the MWD, we have calculated the MWD of several PEG and PPG samples from LAC data and compared the results obtained without and with correction, as is shown in Tab. 3 - 6.

In Fig. 5 - 8, the corresponding mass distributions are shown.

It is evident, that in the mobile phases used in this communication, a compensation for molecular weight dependence improves the accuracy of molecular weight distributions only for very low molecular weight samples.

The effect of the integration error is apparently more important, as can be seen from Tab.5 and Fig.7: the results from the density and RI detector are slightly different because of uncertainties in the baseline.

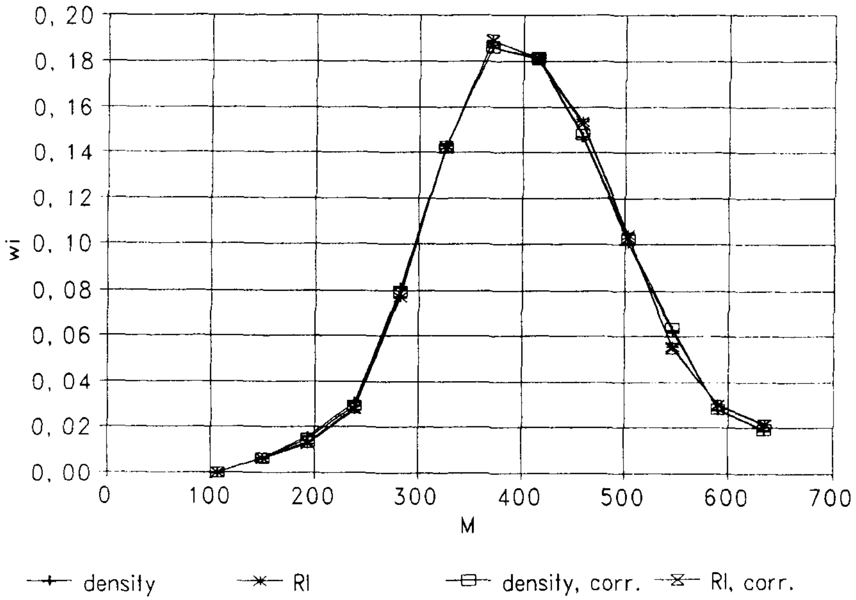

Fig.6: Mass distribution of polyethylene glycol 400, as determined by LAC with density and RI detection, without and with compensation for molecular weight dependence of response factors. Mobile phase: methanol- water 30:70

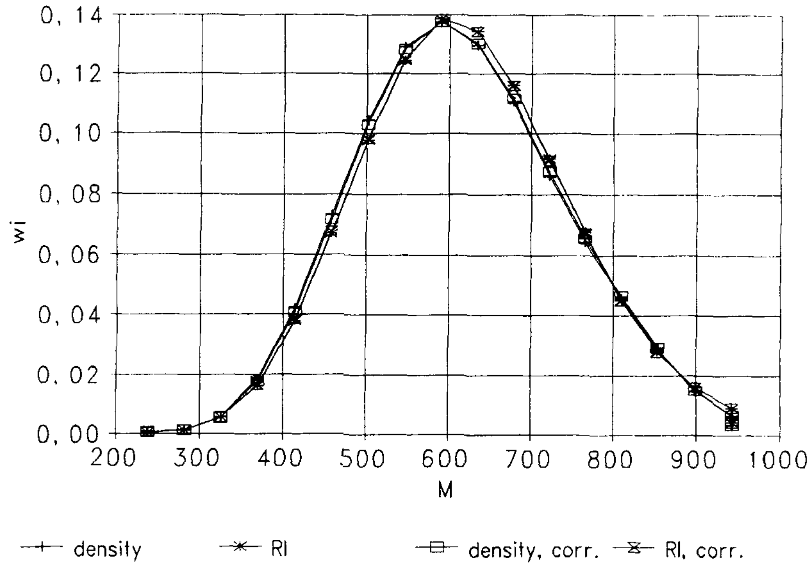

Fig.7: Mass distribution of polyethylene glycol 600, as determined by LAC with density and RI detection, without and with compensation for molecular weight dependence of response factors. Mobile phase: methanol- water 40:60

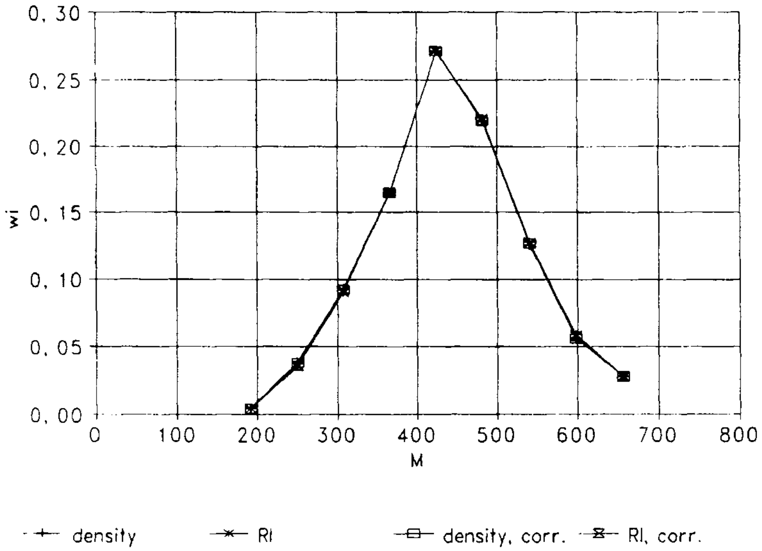

Fig.8: Mass distribution of polypropylene glycol 425, as determined by LAC with density and RI detection, without and with compensation for molecular weight dependence of response factors. Mobile phase: methanol- water 70:30

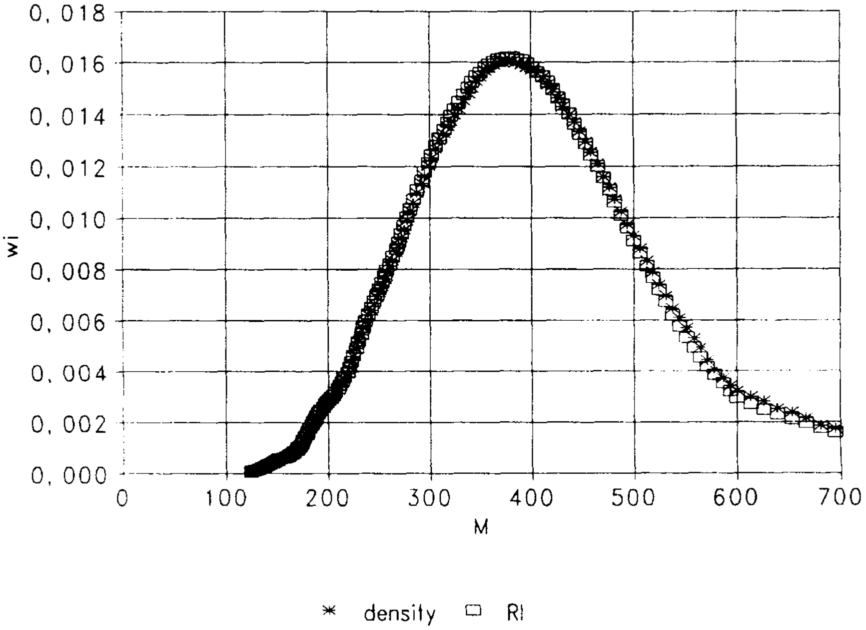

Fig.9: Mass distribution of polyethylene glycol 300, as determined by aqueous SEC on TSK G 1000 PW

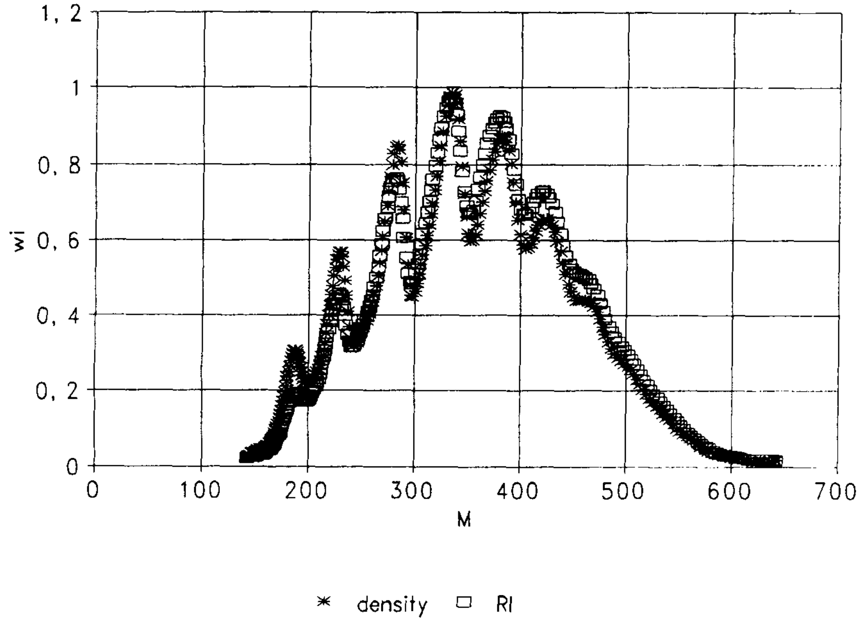

Fig.10: Mass distribution of polyethylene glycol 300, as determined by SEC on 3 Phenogel columns (1*500 + 2*100Å) in $CHCl_3$

Tab.7: Molecular weight averages of polyethylene glycol 300, as determined by aqueous SEC on TSK G 1000 PW, without and with compensation of molecular weight dependence of response factors

	without compensation		with compensation	
	Density	RI	Density	RI
M_w	334	329	332	326
M_n	301	297	299	295
M_w/M_n	1.109	1.105	1.110	1.107

Tab.8: Molecular weight averages of polyethylene glycol 300, as determined by SEC in $CHCl_3$ on a set of 3 Phenogel columns (1*500 Å + 2*100 Å, 300 * 4.6 mm each), without and with compensation of molecular weight dependence of response factors

	without compensation		with compensation	
	Density	RI	Density	RI
M_w	323	338	327	321
M_n	296	313	300	294
M_w/M_n	1.092	1.079	1.091	1.090

A comparison with SEC data (Fig.9 - 10) shows, that the molecular weight dependence of response factors can be neglected in water (Tab. 7), while it has to be taken into account in chloroform (Tab. 8). Molecular weight averages agreed quite well, but - as expected - higher polydispersities were found in SEC.

Conclusions:

LAC with coupled density and RI detection proves to be a more powerful tool than SEC in the determination of the MWD of oligomers. Under the conditions presented in this paper, PEG and PPG can be separated with very high resolution. With a compensation of the molecular weight dependence of response factors, accurate molecular weight averages can be calculated, which can be used to evaluate the performance of SEC systems.

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