Quantitative Analysis in the V_0 Zone. A Chromatographic Approach by Coupling HPLC with GPC

W. Winkle

Henkel KGaA, TFA Chromatographie, Postfach 1100, D-4000 Düsseldorf, FRG

Key Words

Column liquid chromatography
Reversed phase gel permeation-coupling
Polyethyleneglycol in surfactants

Summary

Quantitative HPLC methods for substances that elute in the vicinity of the V_0 peak are often characterized by poor reproducibility and/or serious systematic errors. This contribution shows that the problem can be solved when a GPC-column is coupled with the HPLC-column; the substances assembled in the V_0-Peak are developed into a new dimension (Retention volumes become shorter than V_0) und thus a separation from the V_0 zone is achieved.

The method is used to determine polyglycol in aliphatic and aromatic nonionic surfactants with a degree of ethoxylation up to 80. The products have to be soluble in methanol/water in the proportions 80/20. Polyglycols with a molecular weight up to 7000 are separated by gel chromatography according to their degree of ethoxylation, so that the mean molecular weights (number and weight average) are additionally accessible. It is found that the degree of ethoxylation in polyethyleneglycol is greater than in the associated surfactant.

I. Introduction

Quantitative HPLC methods for substances that elute in the vicinity of the V_0 peak are often characterized by serious systematic errors and/or poor reproducibility, especially when a differential refractometer is used for detection.

Systematic errors are encountered in reversed phase chromatography when the sample contains substances that are more hydrophilic than the analyte, e.g. glycol, glycerol or water. The reason is: These substances should have lower k′-values than the analyte, but they can't, if the capacity factor of the analyte equals zero, because $k' < 0$ is not possible. As a consequence each of these substances and the analyte has $k' = 0$. A separation of the analyte from more hydrophilic substances cannot be achieved and this fact leads to an overlap of the corresponding peaks.

If an RI detector is used and the mobile phase is made up of water and methanol, the water often present in the samples being analyzed gives a peak. This peak is positive if the mobile phase contains more than 51 % methanol, and negative if the eluting agent contains less than 51 % methanol. Salts, glycol, glycerol, etc., also give peaks in the V_0 zone, and thus also cause the results to be reproducibly too high or too low.

The cause of the random errors is the fact that peaks which occur in the V_0 vicinity of the chromatogram, can hardly be controlled. These peaks (components of the eluent, solvents, vacancy peaks [1], peaks caused by undesired infiltration of air during injection, peaks caused by pressure surges during injection, etc.) have no relationship to the analytes, but can positively or negatively superimpose the analyte peaks.

A determination of polyglycol in nonionic surfactants is described as an example to illustrate how the problems described above can be solved by a combination of a reversed phase HPLC column and a GPC column:

It is known that polyethylene glycol (PEG) can be separated from its monoalkyl ethers (alkyl: dodecyl, tetradecyl, i-nonylphenyl, etc.) by means of liquid chromatography with the help of a reversed phase column [2, 3]. PEG elutes in the V_0 zone and can be superimposed there by the solvent components, while the alkyl ethers, which represent the nonionic surfactants, are retarded.

In GPC columns, polyglycols can be separated from their alkyl ethers only in exceptional cases, because higher polyglycols and surfactants with a low degree of ethoxylation exhibit similar retention times.

The method described here combines the properties of both columns by extending the reversed phase column by coupling it with a GPC column. (cf. chromatographic conditions). In our example the reversed phase effects the monoalkyl ethers to reach the gel column much later than the polyglycols. The polyglycols themselves, because they have a higher molecular weight, can be separated from the solvent components water and methanol, and from other low-

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molecular hydrophilic substances by gel chromatography.

In the case of the PEG-example, it is advisable to complete the method by a special sample pretreatment with a solid phase extractor (cf. IV. Application example).

II. Experimental

II.1 Chemicals

Methanol (LiChrosolv, Merck). Deinonized water: LiChroprep RP18, 40–63 µm, Merck. Anion exchanger AG1–X2, 100–200 mesh, Cl- form, Bio-Rad 1M NaOH, dil. HNO_3, $AgNO_3$.

Monoethylene-, diethylene- and tetraethylene glycol: Shell, BASF, Polymer Laboratories. Polyethylene glycols for GPC calibration: Polymer Laboratories. Monoalkyl ethers of defined oligoglycols: Nikko Chemicals, Tokyo. Nonionic surfactants: Commercial products of BASF, Henkel, Marl-Hüls.

II.2 Apparatus

HPLC.

Isocratic pump:	Spectra Physics, model 8810
Detector:	UV detector with variable wavelength, Differential refractometer (Shimadzu, model RID-6A) in this order, positioned after the column(s)
Sample metering:	Automatic sample metering unit (Gilson, model 231–401)
Data system:	Chromstation-AT (Spectra Physics) consisting of ChromJet SP4400 integrators, IBM-AT, LABNET networking

Sample preparation.

Solid-phase extractor:	Vac-Elut, manufacturer ICT
Chromatography column:	Glass column 350 × 18 mm
Extraction columns:	Own manufacture, using a 10 ml one-way syringe from Henke-Sass Wolf

II.3 Chromatographic Conditions

Stationary phases.

1. LiChrosorb RP18 5 µm, 125 × 4 mm, Merck CAT 50433, column no. 906290
2. Ultrahydrogel 120, 300 × 7.8 mm, Waters in this order, if linked in sequence.

Mobile phases.

1. Methanol (80)/water (20) (v/v) Standard system
2. Methanol (75)/water (25) (v/v) In special cases (cf. text)

Apparatus settings.

1. Flow rate:	1 cm^3/min
2. Temperature:	Room temperature, approx. 22 °C
3. Metered volumes:	100 µl
4. Wavelength:	280 nm

II.4 Solid Phase Extraction

Preliminary treatment of the ion exchanger. The anion exchange resin (cf. II.1) is delivered in the Cl^- form and has to be converted to the OH^- form. To do this, a chromatography column is filled with the resin and washed with 1M NaOH until it is free of chloride ($AgNO_3/HNO_3$ proof). Then the exchange resin is washed with water, removed from the column and suspended in methanol.

Making the cartridges (extraction columns). The one-way syringe (cf. II.2) is filled with some cotton wool, 4 to 4.5 g LiChroprep RP18, cotton wool and 2 cm^3 exchange resin suspension. The cartridge filling is washed three times with methanol, each time with 5 cm^3, then three times with 5 cm^3 of the HPLC eluent.

Extraction. 2 cm^3 of the stock solution of the sample (approx. 10 % in the eluting agent) are transferred to the cartridge. After the soltuion has been siphoned off into a measuring flask (10 cm^3), the cartridge is washed twice, each time with 4 cm^3 eluent. Then the flask is filled up to the calibration mark with eluent.

III. Fundamentals of the Process

III.1 Elution zones

The basic equations used in HPLC to calculate the retention volume V_i of the substance i

$$V_i = V_0 (1 + k_i') \quad (1)$$

$$k_i' = \frac{\text{Mass of the substance i in stationary phase}}{\text{Mass of the substance i in the mobile phase}} \quad (2)$$

lead to the conclusion that the dead volume V_0 cf a column is the smallest observable retention volume because, according to Eq. (2), $k_i' \geq 0$ with respect to all substances i.

The possibility to be described here, namely that of quantitatively evaluating peaks in the V_0 zone of an HPLC column, begins with the statement that V_0 is not the smallest elution volume.

Substances i with the same value of the capacity factor k_i' can have different retention volumes V_i, in the case that $k_i' = 0$. This is known to be a consequence of the morphology of the stationary phases, whose pores can be penetrated by "small" molecules, but not by "large" ones (size exclusion or gel permeation). The basic equations used in GPC are [4]

$$V_i = V_Z + (V_T - V_Z) \cdot K_i \quad 0 \leq K_i \leq 1 \quad (3)$$

where

V_Z = Interstitial volume
V_T = Total volume of the mobile phase in the column
V_P = Pore volume of the stationary phase

$$V_P = V_T - V_Z \quad (4)$$

If – in a first approximation [5] – V_T and V_0 are regarded as being equal, Eq. (3) becomes $V_i = V_Z + (V_0 - V_Z) \cdot K_i$, i.e. for substances whose k'_i values are zero, V_i ranges from V_Z to V_0. According to Eq. (1), retention volumes V_i of substances whose k'_i values are greater than zero can have values between V_0 and ∞.

In this paper, the interval $V_Z \leq V_i \leq V_0$ will be referred to as the exclusion zone, and the interval $V_0 < V_i < \infty$ will be referred to as the HPLC zone (cf. Figure 1), because the emphasis of the applications is on the GPC column in the exclusion zone, and on the HPLC column in the HPLC zone.

As a consequence of the considerations presented here I would like to propose a splitting of Eq. (1) according to

$$V_i = \begin{cases} V_0 (1 + k'_i), & \text{if } k'_i > 0 \text{ (HPLC)}; 0 < k'_i < \infty \\ V_z + (V_0 - V_z) \cdot K_i, & \text{if } k'_i = 0 \text{ (GPC)}; \quad 0 \leq K_i \leq 1 \end{cases} \quad (5)$$

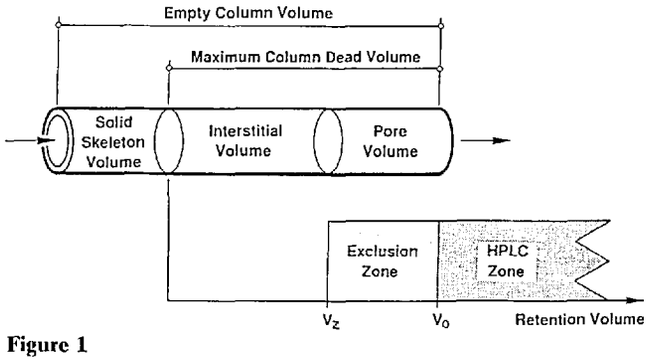

Figure 1
Elution zones in relationship to the structure of a column.

III.2 HPLC/GPC Coupling

Coupling a GPC column with an HPLC column effects a tailor-made leftward shift of the exclusion zone of the HPLC column.

If the analyte elutes in the V_0 vicinity of the HPLC column, and the sample matrix at elution volumes with a lowest value $V_{min} > V_0$, V_{min} can be adjusted so that it is at least as big as the pore volume of the GPC column. With this presumption the retention volume of the matrix cannot slip into the dead volume of the column combination, even under unfavorable conditions.

This means: The elution sequence of the HPLC column

1.) Analyte + eluting agent components + pressure peaks + ...
2.) sample matrix

is converted with the column combination to the elution sequence

1.) Analyte
2.) eluting agent components + pressure peaks + ...
3.) matrix.

By the separation from the eluent components etc. now the analyte is determinable.

III.3 RP18 Column

If 80 % methanol is used in the mobile phase, methanol, water and D_2O elute after 1.49 ± 0.03 min or cm^3. If the dead volume of our apparatus is taken into consideration, (0.4 ml between injector, UV-detector and the measuring cell in the RI detector), a retention volume of 1.09 ± 0.03 cm^3 results. This value is a good approximation of the column dead volume V_0 [5].

The interstitial volume V_Z was determined with a dextran ($M_W = 2 \times 106$). A mixture of methanol/water in the proportions 30/70, in which dextran is soluble giving a clear solution, was chosen as the mobile phase [6].

Result: $V_Z = 0.62$ cm^3 (corrected to take account of the dead volume of the equipment).

Polyglycols with a molecular weight of approx. 440 elute in the column dead volume V_0 of the reversed phase, without penetrating the exclusion zone. Substances with a molecular weight between 440 and approx. 4000 elute with k′ values < 1, still in the vicinity of V_0 in the HPLC zone of the column. Above MG 4000 an appreciable delay sets in [Table I] and the peaks become markedly asymmetrical. This effect is reinforced if the methanol content of the mobile phase is reduced from 80 % to 75 % (Figure 2).

Fatty alcohols and their EO adducts become more easily separable from the polyglycols as the carbon chain length increases (Table I).

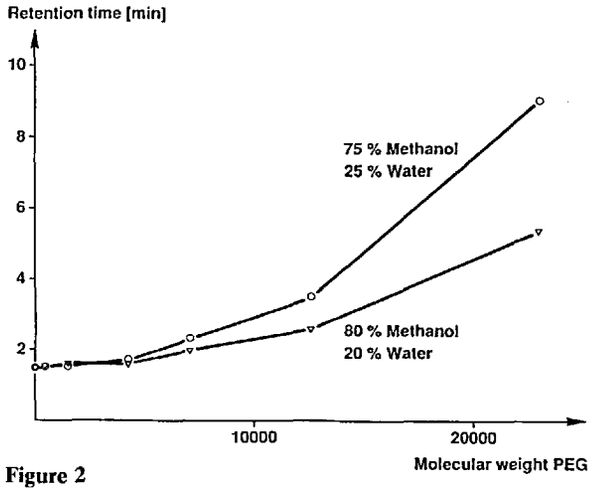

Figure 2
Retention times of PEG with RP18 column.
Column: LiChrosorb RP18, 125 × 4 mm; eluent: As stated in the figure; flow: 1 cm^3/min.

Table I. Retention times.

Analyte	MW	Retention Times		
		RP18	GPC	RP18+GPC
H_2O	18	1.49	10.75	11.76
CH_3-OH	32	1.49	10.81	11.77
H-(OC_2H_4) -OH	62	1.48	10.58	11.70
H-(OC_2H_4)$_2$-OH	106	1.48	10.10	11.23
H-(OC_2H_4)$_4$-OH	194	1.48	9.40	10.55
Polyethylenglycol	440	1.51	8.37	9.51
Polyethylenglycol	1470	1.59	6.94	8.19
Polyethylenglycol	4250	1.59	6.22	
Polyethylenglycol	7100	1.99	5.85	7.51
Polyethylenglycol	12600	2.59	5.76	8.10
Polyethylenglycol	23000	5.35	5.76	10.55
Phenyl-(OC_2H_4)$_1$-OH	138	1.83	12.32	13.59
Phenyl-(OC_2H_4)$_2$-OH	182	1.83	11.38	13.67
Phenyl-(OC_2H_4)$_3$-OH	226	1.83	10.66	12.01
Phenyl-(OC_2H_4)$_4$-OH	270	1.83	10.11	11.48
Phenyl-(OC_2H_4)$_5$-OH	314	1.83	9.68	11.07
Phenol + 30 EO	1400	1.97	7.11	8.71
Octanol	130	2.57	9.43	11.70
Decanol	158	3.77	9.10	12.78
Dodecanol	186	6.30	8.98	15.35
Dodecyl-(OC_2H_4)$_2$-OH	274	7.45	8.50	16.03
Tetradecanol	214	11.98	8.33	21.03
Tetradecyl-(OC_2H_4)$_2$-OH	302	14.08	8.43	22.62
Hexadecanol	242	23.23	8.57	32.45
Hexadecyl-(OC_2H_4)$_2$-OH	330	27.75	8.20	36.50

III.4 Ultrahydrogel Column

An eluting agent containing 80 % methanol was used with the Ultrahydrogel column. This percentage is outside the operating range specified by the manufacturer (max. 50 %), but nevertheless the column characteristic survived that of the reversed phase column.

High methanol content was chosen on grounds of solubility.

Column data measured (corrected to take account of the dead volume of the apparatus):

$V_Z = 5.36\ cm^3$ (retention volume of PEG 23 000)

$V_0 = 10.5 \pm 0.1\ cm^3$ (retention volume of methanol and H_2O)

$V_P = V_0 - V_Z = 5.14\ cm^3$

The volume V_P is available for separation of the analyte from the solvent components methanol and water.

On its own, however, the gel column would not be a suitable solution for the application problem (separation of polyglycol from its monoalkyl ethers and from solvent components), because, for example, tetraethylene glycol and octanol, or diethylene glycol tetradecyl ether and PEG 440 superimpose (Table I).

III.5 RP18/GPC Column Coupling

When the two columns are coupled, the resultant retention time is the sum of the two individual retention times (Table I).

The following separation zones can therefore be postulated from the technical data of the individual columns:

	V_Z [cm^3]	V_0 [cm^3]	
RP18	0.62	1.09	Measured exclusion zone
GPC	5.36	10.5	Measured exclusion zone
RP18 + GPC	5.98	11.59	Expected exclusion zone
		> 11.59	Expected HPLC zone

The polyglycols are separated in the exclusion zone of the column combination in accordance with their molecular size, up to a molecular weight of 7100 (cf. Figure 3). The retention times then increase. A definite relationship between retention time and molecular weight can be made, for molecular weights up to 4000 (approx. 90 EO units). This is fully sufficient for determination in most nonionic surfactants. In addition very recent investigations show that the inflection point at MW 4000 can be elevated to 7000, if LiChrospher 100 RP8 is chosen instead of the LiChrosorb RP18 column, which is based upon a silica gel with 60 Å.

The pore volume of 5.14 cm^3 of the gel column, and the retention volume of the fatty alcohols in the reversed

phase (Table I) ensure that tetradecanol and all higher fatty alcohols cannot fall into the dead volume of the column combination, even under unfavorable conditions. In fact, however, dodecanol is already well separated from V_0. Octanol and the solvent fall together (Table I).

Whereas the number of peaks in the exclusion zone corresponds to expectations, one additional peak is observed in the HPLC zone of the column combination (Figure 4). This is probably the result of vacancy effects [5]. Example: injection of dodecanol.

Retention times (min):

RP18	1.50 (t_0)	6.30 (dodecanol)	–
GPC	11.0 (t_0)	8.98 (dodecanol)	
RP18 + GPC	12.10 (t_0)	15.35 (dodecanol)	17.33 (vacancy?)

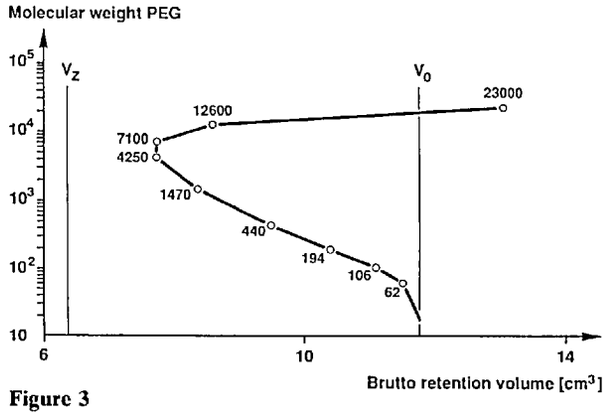

Figure 3

Combination of RP18 and GPC column:
Elution of PEG in exclusion zone and HPLC zone.
(Numbers 62,106 etc represent molecular weight of oligomers; retention volumes include an extra column volume of 0.4 cm^3.
Columns: LiChrosorb RP18, 125 × 4 mm + Ultrahydrogel 120, 300 × 7.8 mm; eluent: Methanol (80) / Water (20) (v/v); flow: 1 cm^3/min.

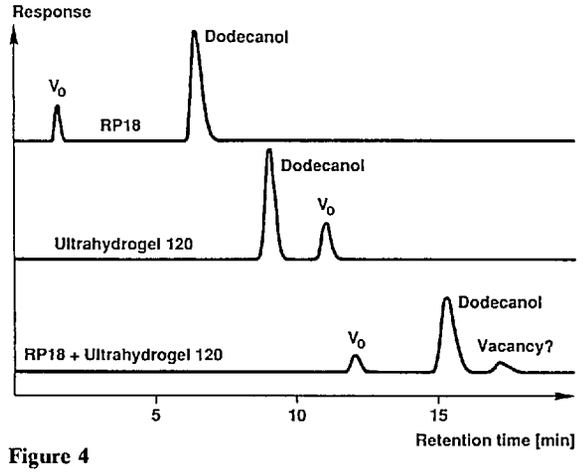

Figure 4

Combination of RP18 and GPC column: Appearence of an additional peak.
Eluent: Methanol (80) / Water (20) (v/v); flow: 1 cm^3/min; columns: As stated in the figure.

The peak at 17.33 corresponds to the sum of the dodecanol peak (RP18; 6.30 min) and the V_0 peak of the GPC column (11.0 min). It can be assumed that the dodecanol leaving the reversed phase HPLC column and entering the GPC column is in a solvent environment with a different composition to that of the eluting agent. This "other" solvent is separated from the fatty alcohol in the GPC column and elutes after dodecanol, on account of its lower molecular weight.

IV. Application Example: Polyglycol in Nonionic Surfactants

Monoalkyl ethers of polyglycol are manufactured from alcohols and ethylene oxide in the presence of a sodium methylate catalyst. In particular, ethylene oxide adducts manufactured from long chain alcohols (C12 to C18) or from octyl or nonyl phenol have become important as nonionic surfactants used in the manufacture of detergents.

As by-product of this reaction polyethylene glycol (PEG) is formed. This can be determined by liquid-liquid distribution between n-butanol and a 5 % aqueous $NaHCO_3$ solution [8], or by reversed phase chromatography [2, 3].

The wet chemistry method generally gives results that are too low [9]. The HPLC method was the object of several interlaboratory tests, and gave a wide spread of results [10]. As explained above, the reason for this is superimposition of polyglycol peaks with the positive or negative V_0 peak, the size and sign of which are determined by fortuities or systematic disturbances.

When a GPC column is coupled, as described in III.4, the polyglycols elute sooner than t_0, so that the desired separation from the components of the eluting agent is achieved without impairing the separation of the EO adducts: Figure 5: nonyl phenol + nEO, Figure 6: octyl phenol + nEO, Figure 7: coconut alcohol + nEO.

Aromatic polyglycol ethers can contain phenylglycol ethers formed during the synthesis of the alkyl phenol [11]. Depending on their degree of ethoxylation, they generally fall in the exclusion zone (Table I), where they can be recognized as non-PEG components with the help of a UV detector. For this reason, all chromatograms of this contribution were produced with sequentially coupled UV and RI detectors. Unfortunately this measure results in poorer resolution and poorer detection limits.

Because the separated polyglycols with molecular weights up to 7000 exhibit GPC or size exclusion behavior (Figure 3), the molecular weights (number average) of the polyglycols can be determined with the help of PEG calibration standards (Table II). In this context, it is noticeable that these molecular weights are higher than the degree of ethoxylation of the associated surfactant.

In comparison with the aromatic EO adducts, the fatty alcohol ethoxylates, mainly those with chain lengths C_{16} and C_{18}, exhibit especially long retention times. The resulting long analysis times can be avoided, when the C_{16}, C_{18} surfactants are eliminated from the sample

Table II. Content and molecular weight of PEG in technical nonionic surfactants.

	Polyethyleneglycol		
	Number Average Molecul. Weight	Mole EO	Content [%]
Saturat. fatty alcohol $C_{12,14}$ + 6EO	970	22	1.5
Saturat. fatty alcohol $C_{12,14}$ + 30EO	3140	71	3.7
Saturat. fatty alcohol $C_{12,14}$ + 50EO	4200	95	10.3
Saturat. fatty alcohol $C_{16,18}$ + 80EO	4400	100	12.0
Unsatur. fatty alcohol + 38EO	2700	61	5.8
Nonylphenol + 3EO	780	18	0.7
Nonylphenol + 10EO	1200	27	1.5
Nonylphenol + 20EO	1900	43	3.6
Nonylphenol + 28EO	2800	63	4.9
Nonylphenol + 60EO	4300	98	11.8
Octylphenol + 10EO			3.9
Octylphenol + 25EO	2400	55	4.2
Octylphenol + 40EO	3600	82	15.3

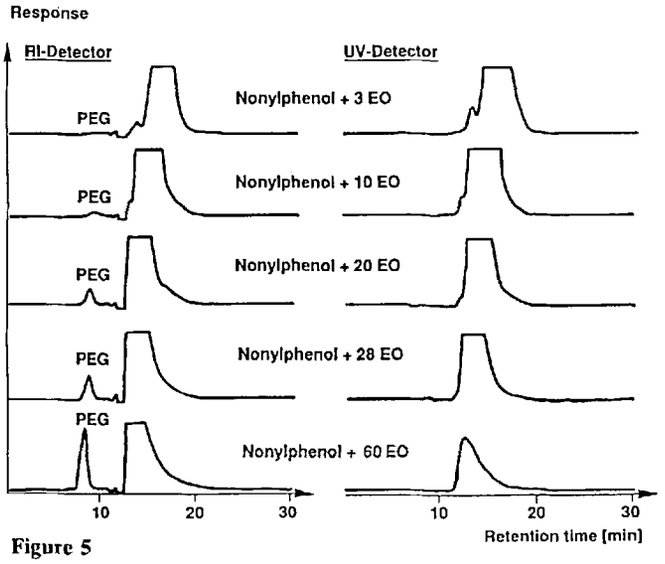

Figure 5

Chromatograms of Nonylphenol + n EO.
Columns: LiChrosorb RP18, 125 × 4 mm + Ultrahydrogel 120, 300 × 7.8 mm; eluent: Methanol (80) / Water (20) (v/v); flow: 1 cm^3/min; detectors: UV (280 nm) + RI, in this order, linked in sequence.

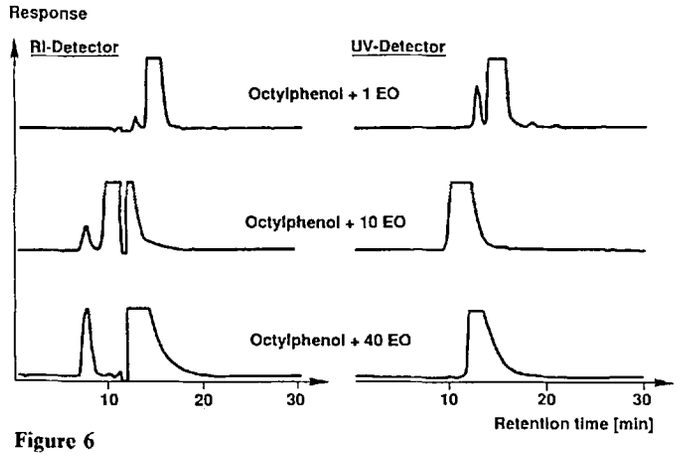

Figure 6

Chromatograms of Octylphenol + n EO. Conditions: As in Figure 5.

by means of a reversed phase solid phase extractor. Aliphatic carboxylic acids such as lactic acid and acetic acid, which are used to neutralize the catalyst after EO addition, superimpose with very low molecular oligoglycols in the exclusion zone. They can be removed by an anion exchange resin before the HPLC/GPC.

A cartridge method for simultaneous removal of long chain fatty alcohol ethoxylates and of carboxylates is described in detail in II.4. Figure 8 shows the effect of this sample pretreatment on the chromatograms. In some cases there are several peaks in the exclusion zone. If available, an LC/MS coupling with a thermospray interface can be used to identify the correct, i.e. PEG, peak [7]. In the interests of good quality control, this operation should not be dispensed with, especially as it only needs to be carried out once.

Up to a degree of ethoxylation of 60 to 80, the PEG determination is not affected by the presence of its alkylether in the sample (Figures 5 to 7). No samples with a higher degree of ethoxylation were available. Surfactants with a low degree of ethoxylation (1 to 3) pose problems of poor solubility, poor separation of the glycols from the V_0 peak, and low PEG content.

Surfactants with a short carbon chain (C8, C10) elute in the vicinity of V_0 and superimpose low glycols.

Poor solubility can be combatted by working at higher temperatures (40–50 °C). The high temperature GPC unit from WATERS is especially suitable for this purpose. Surfactants can be prevented from penetrating the exclusion zone by reducing the methanol content of the mobile phase to 75 %.

The following data were obtained for the method under optimal conditions (RP18 column, 5 µm, 250 × 4 mm, 30 °C, cartridge, only RI detector):

Sample	Mean value % PEG by weight	Variation coefficient	Number of measurements	Recovery
C_{12}, C_{14} alcohol+7EO with added PEG 800	6.1	1	6	100 %
Nonyl phenol+7EO	0.5	3	6	–
Octyl phenol+25EO[1.)]	4.2	2	6	

1.) Eluting agent: methanol/water = 75/25 (v/v)

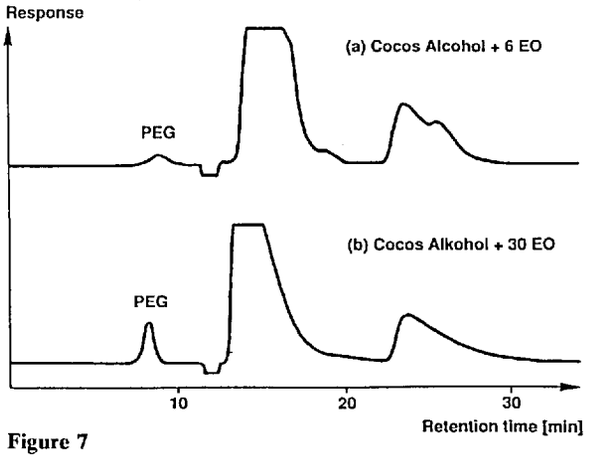

Figure 7
Chromatograms of fatty alcohol + n EO. Conditions as in Figure 5 (shown is the signal of the RI-detector).

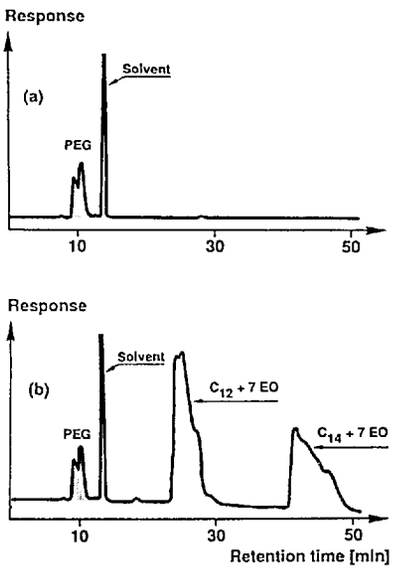

Figure 8
Chromatograms of C_{12}/C_{14}-fatty alcohols + 7 EO.
a.) Sample Pretreatment with Solid Phase Extraction.
b.) Without Solid Phase Extraction.
Conditions: As in Figure 7.

Recovery: The sample C_{12}, C_{14} was freed of PEG by semi-preparative HPLC in a reversed phase. Approx. 6 % PEG 800 was added. Calibration was carried out with a PEG 800.

V. Outlook

The method of quantitating substances, which elute in the V_0 zone, by coupling HPLC with GPC is not limited to polymer analytes or aqueous GPC with reversed phases. The coupling of normal phases with polystyrene GPC columns can be applied to the analysis of apolar substances in the presence of more polar compounds. The molecular weight of the analytes should, however, be higher than the molecular weight of the solvents, which form the eluent.

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