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Review

High-performance liquid chromatography and detection of polyethers and their mono(carboxy)alkyl and -arylalkyl substituted derivatives[1]

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Abstract

This review deals with high-performance liquid chromatographic (HPLC) separation techniques and detection methods for a wide variety of polyether derivatives, including alkyl-, arylalkyl-, carboxyalkyl-substituted polyethylene glycols mainly applied as non-ionic surfactants (NIS), and native (underivatized) polyethylene glycols (PEGs), polypropylene glycols (PPGs) and polybutylene glycols (PBGs) widely used in either industrial or pharmaceutical applications. Normal-phase (NP) and reversed-phase (RP) systems have been considered for NIS, the NP technique being the most suitable for separation according to the number of ethoxylate units, whereas separation according to the hydrophobic alkyl chain is favored on RP stationary phases. At variance with NIS mainly RP–HPLC is applied for native (underivatized) polyethers of the PEG, PPG and PBG type. Ion-exchange chromatography (IEC) is still a minor technique for the separation of PEG derivatives, which exploits the capability of the 1,2-dioxoethylene moiety to form complexes with potassium ions. Liquid chromatography under critical conditions (LCCC) is the method of choice for separation of polyethers according to their chemical composition and is preferably applicable to copolymers built up from different components. A multitude of detection principles substantially differing in either selectivity or sensitivity has been successfully used for signal monitoring, with detection by measurement of refractive indexes (RI), UV absorption, fluorescence and responses from evaporative light scattering of "solid" droplets being the most prominent. Nevertheless, UV detection still dominates because of the existence of the phenyl chromophor in many NIS and the ease of derivatization of polyethers lacking an inherent aromatic moiety with a large variety of chromophoric agents. Additionally, well-established methods are available for low wavelength UV detection below 200 nm even for the native polyethers.

Keywords: Reviews; Stationary phases, LC; Mobile phase composition; Detection, LC; Polyethers; Polyethylene glycols; Surfactants; Polypropylene glycols; Polybutylene glycols

Contents

[1]This paper is dedicated to Professor Hinrich Cramer on the occasion of his 65th birthday.

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1. Introduction

During the last two decades polyethers and their monoalkyl and -arylalkyl derivatives have gained more and more interest in different fields of technical as well as pharmaceutical and biochemical application. In particular polyethylene glycol (PEG) derivatives are extensively used as essential additives in non-ionic surfactants and wetting agents (NIS) in laundry and industrial cleaners, solubilizers in enhanced oil recovery, ingredients in the cosmetic and food industries, emulsifiers in pharmaceutical preparations and solubility enhancers in biochemical membrane technology [1]. Owing to their low toxicity, the native PEGs were also used in studies of intestinal permeability and adsorption in man [2–5]. Their covalent coupling to proteins and enzymes yields extensive changes in protein pharmacology, immunogenicity and enzymatic actions [6,7]. Furthermore they are used as stationary phases in gas chromatography (GC) and, recently, have played an increasing role as surface-coatings of reversed-phase materials in the synthesis of so-called semi-permeable stationary phases (SPS materials) [8–14]. Other recent applications of polyethylene glycols in biochemistry and bio-organic chemistry were opened by the synthesis of poly-rotaxanes containing a polyether axis threaded with a multitude of cyclodextrin rings [15,16], which may have implications for biomolecular recognition, molecular machines and material science, novel crown ether based catenanes [17,18]; linearly linked crown ether segments as essential structural elements of macromolecular chains [19] and polyethylene glycol oligodesoxynucleotide hybrid molecules used for DNA recognition [20]. In particular, during the last few years polyether derivatives have gained further importance for evaluation of the metabolic fate of NIS by high resolution techniques in investigations of environmental protection. Extensive work has been addressed to this aspect, primarily concerning extraction and separation of sludge samples in urban sewage plants due to the increasing consumption of NIS for industrial and domestic use.

Polypropylene-1,2-glycol (≅ methylethylene glycol) derivatives represent either the base materials for flexibilizers or polyurethane prepolymers for the use in structural adhesives as well as rigid foams. The polypropylene-1,2-glycol (PPG) di- and triamines sold under the trade name Jeffamine com-

prise a special class of PPGs, which are synthesized from propylene oxide (PO) and 1,2-propylene glycol (PG), yielding the linear PPG amines and/or glycerol/trimethylolpropane, yielding the branched PPG amines, and used as intermediates in the synthesis of polyamides, polyurethanes, polyureas, epoxy curing agents and flexibilizers [21].

The polybutylene-1,4-glycols (PBGs), i.e. polyoxybutylenes or polytetrahydrofurans, which possess a substantially more flexible tetramethylene chain between the two ether oxygens, as compared with PEG and PPG, are used as flexibilizers and tougheners in formulated epoxy-based systems (e.g. adhesives), and also find application as long-chain α,ω-dialcohol components in the synthesis of polyurethane fibres, polyester and polyurethane plastics, or as starting materials in the synthesis of cross-linked polyurethane casting elastomers.

Synthesis starting from either polymerization of ethylene oxide (EO) and PO under basic conditions yielding the corresponding polyethers with two free α,ω-hydroxyl endgroups, or reaction in the presence of aliphatic alcohols and alkyl substituted phenols yielding the monoalkylated or -arylalkyl derivatives, gives a more or less characteristic oligomer distribution, which largely depends on the OH^-/alkylene oxide or $alkoxy^-$/alkylene oxide ratio. In contrast, polybutylene glycols are prepared by ring-opening polymerization of tetrahydrofuran, which gives rise to a wide variety of oligomers.

The common feature of all polyether derivatives consists in their more or less wide synthesis-dependent oligomer distribution, which in turn, requires very efficient techniques for exhaustive characterization, not only with respect to the degree of polymerization but also with concern to the different endgroups (e.g. nonylphenyl-, octylphenyl-, alkyl groups of different chain length etc.). It is obvious that the chemical and physico-chemical properties are largely influenced by either length or structure of the polyether chain as well as the endgroups, and for this reason, largely depend on the ratio of hydrophobic to hydrophilic structural segments. In particular, in NIS this ratio is primarily governed by the length and chemical structure of the alkyl or arylalkyl chain attached to a terminal hydroxy group of the polyether and the mass ratio of these hydrophobic substituents vs. the more hydrophilic polyether backbone. In contrast, in the less polar polybutylene glycol (PBG) derivatives, the tetramethylene group is responsible for its "inherent" hydrophobicity as evidenced by reversed-phase HPLC.

Until modern high-performance liquid chromatography became an efficient tool for oligomer and polymer characterization, spectrophotometric methods based on complex formation between non-ionic surfactants and different reagents [22–24], potentiometric titration [25] and atomic absorption spectroscopy [26,27] played the major role in polyether analysis. Rather novel mass spectrometric techniques not directly coupled to a chromatographic separation step, such as direct inlet field desorption mass spectrometry (FD-MS) [28], fast atomic bombardment mass spectrometry (FAB-MS) [29], electrospray mass spectroscopy (ES-MS) [30], time-of-flight secondary ion mass spectroscopy (TOF-SIMS) [31] and matrix assisted laser desorption ionization Fourier transform mass spectroscopy (MALDI-FT-MS) [32] can be used with high efficiency when the pure samples are to be analyzed, but have drawbacks when applied to complex matrices. In these cases prior chromatography is essential. Nevertheless, the high resolution MS techniques provide monitoring of individual polyether oligomers with molecular mass (M_r)>5000 at which peak resolution by use of chromatographic methods either substantially decreases or is not possible. However, polyethers may also occur in complex matrices and in mixtures of different types of polyethers, and only high-performance chromatographic separation techniques combined with mass-spectrometric detection fulfil the requirements of optimum characterization of the corresponding derivatives. Despite its unsurpassable peak resolution, gas chromatography (GC) [33–37] and GC coupled to mass spectrometry (GC–MS) [38–40], preponderantly used in environmental analysis of NIS, are only applicable when polyethers with a low degree of polymerization are to be detected. In general, all samples with M_r>600 are extensively excluded from the measurement, except where special high-temperature techniques (HT-GC) are applied extending the range of M_r to about 800. Although size-exclusion chromatography (SEC) [41–56] covers the whole M_r range and yields sufficient separation of oligomers below M_r 600,

signal resolution decreases more and more when M_r exceeds 600. Thin-layer chromatography (TLC) [35,57–69] proves to be superior to SEC and GC, in particular in the M_r range>600, and the automated multiple development (AMD) procedure [66,70–72], simulating the gradient technique in HPLC, provides a further extension of the method, nevertheless it does not achieve the separation potency and peak resolution of supercritical fluid chromatography (SFC) [73–79] and HPLC. Indeed, the latter alternatives represent the methods of choice for analysis of non-volatile polyether derivatives with M_r>600 and, in particular, SFC provides excellent separation of oligomers [73–79]. Nevertheless, HPLC is still preferred due to the simplicity of the experimental design and the ease of column and mobile phase handling.

A large variety of either mobile or stationary phases for high resolution chromatography of polyether oligomers has been described in the literature. Among the systems for liquid chromatography, normal-phase adsorbents and pure organic and preponderantly water-immiscible solvents; reversed-phase matrices and mixtures of water-miscible organic solvents and water; and ion-exchange materials with solutions of aqueous organic solvents containing inorganic salts, were successfully used.

Monitoring of signal responses from the column effluent comprises a further essential aspect and the choice of the appropriate technique markedly depends on the expected concentration range of the polyether samples. A variety of alternatives for detection, which show large differences in either selectivity and sensitivity is available, and in some cases, native polyethers lacking an inherent chromophor have to be derivatized with a chromophoric agent in order to provide the required high sensitive signal monitoring.

The aim of this review is to survey the most recent developments in polyether separation, with major focus on the choice of the appropriate stationary phase materials and suitable mobile phases. Further attention is given to the method of detection, which in turn is largely dependent on the level of polyethers to be determined in either industrial, biological and environmental samples. The latter aspect presents a great challenge for the analyst because the total polyether response is composed of a large number of individual contributions from the oligomers and thus very low limits of detection are often required considering the very small concentrations involved.

Sample preparation and extraction procedures for polyethers from complex biological and environmental matrices will not be considered in the review.

2. Mobile and stationary phases for HPLC of polyethers

2.1. General considerations

In general, the more polar the polyether backbone of NIS the more polar must be the stationary phase used for separation of oligomers. Alexander et al. [80] have applied either normal-phase (NP) or reversed-phase (RP) materials for separation of nonylphenol-ethoxylates (NPEOs) and found the lower the carbon load of C_{18}-matrices (ranging from approx. 3 to about 23%) the better the resolution. In particular, when a 3% carbon load C_{18}-adsorbent was used, the chromatographic pattern more or less approximated to that obtained with pure underivatized silica gel. The increased resolution of the latter stationary phase can be interpreted by the greater number of exposed silanol groups that provided polar adsorption sites and thus a decrease in column hydrophobicity. The resulting increase of interactions between the polar ethoxylate moieties of NPEOs and the hydrophilic sites of the column matrix will thus be associated with better oligomer resolution on pure silica.

Jandera [81] evaluated that in reversed-phase systems the separation selectivity of the individual oligomers of NIS is mainly determined by size and polarity of the repeat structural unit, but the influence of a bulky and polar structural residue should also be taken into consideration. Native (underivatized) PEGs elute in the order of increasing size due to a concomitant increase of the interactive surface area available for solute–matrix interactions, whereas in contrast, e.g. ethoxylated nonylphenols are eluted in the order of decreasing size due to a relative decrease of polar structural segments and thus providing a

concomitant increase of overall sample hydrophobicity. On the other hand, the same author found that in normal-phase systems, the separation selectivity depends on the adsorption energy and on the adsorbed area of the oligomers. As a consequence, retention of alkyl- or alkylphenyl substituted ethoxylates increases with increasing number of repeating EO units in NP chromatography due to the increase of the dominating hydrophilic moiety of the molecule, which strongly adsorbs onto polar surfaces. Furthermore, in an oligomeric series the selectivity is affected far more significantly by the nature of the polar organic solvent than by its concentration [81]. However, when native PEGs are subjected to NP-HPLC on bare silica gel or polar bonded-phase materials, strong retention occurs and the oligomers can only be eluted from the adsorbents with difficulty or not at all.

The investigations of Alexander et al. [80] implied the use of more polar adsorbents for separation of NIS according to the number of EO units, whereas in contrast, the alkyl and/or alkylphenyl substituents exert substantial influences on hydrophobic retention and thus afford separation according to the structural properties of the unpolar moieties. As shown by Jandera [81], RP-materials are less suited for adequate separation of NIS according to the number of repeat structural units, and yield marked "signal compression" at lower retention times. In this case oligomers elute in the range of decreasing number of EO units and peak resolution is mainly influenced by interactions between the hydrophobic alkyl or carboxyalkyl substitituents and the unpolar stationary phase. On the other hand, only RP-HPLC gives adequate insight into either distribution or isomerism resulting from, e.g., branching of alkyl and/or arylalkyl side chains. Thus, the final choice of an appropriate chromatographic system essentially depends on the specific problem encountered in the analysis. When samples with a broad oligomer distribution are to be analyzed, separation of individual oligomers will be the better the larger the M_r difference between two oligomers and, as a logical consequence, peak resolution decreases with increasing number of repeat units. Nevertheless, separation of more than 50 oligomers can be achieved, and combination of high resolution chromatography with mass spectrometry will further contribute to the exhaustive characterization of polyethers with a wide range of M_r distribution.

However, it should be taken into account, that high-molecular-mass NIS of the polyethoxylate type are increasingly adsorbed onto the stationary phase and can only be eluted from it by use of polar organic modifiers, such as ethanol and 2-propanol, but unfortunately all these oligomers are merged into one peak without any individual signal resolution [76]. In these cases the reversed-phase technique will be the method of choice, as will be shown in Section 2.2.2.

In a study of reversed-phase separation of PEG derivatives, Melander et al. [82] reported that the dependence of oligomer retention on the number of EO units is reversed at a particular concentration of organic modifier, but their investigations cannot be generalized and in a lot of cases retention follows the regular and expected behavior. The authors assumed a two-state model for PEGs, which can adapt a "zig-zag" and a "helix" form. Their results imply that samples containing polydisperse polyethoxylate (PEO) chains can elute as single peaks regardless their chain lengths. However, both (different) endgroups and the use of different organic modifiers may largely affect the "zig-zag/helix" equilibrium and thus, precise and detailed predictions of retention behavior remain uncertain.

Similarly Okada [83] investigated the conformation of dialkylated PEGs (R=methyl, ethyl, butyl) in order to eliminate the effects of terminal hydroxy groups on retention and physico-chemical behavior in solution. He found that the trans conformer is less stable than the corresponding gauche conformer in polar media, in particular for PEG derivatives with large EO numbers, whereas conformational changes are less influenced by the chain length of the terminal hydrocarbon substituent. Furthermore, he observed that retention of the trans conformer increases with increasing EO number, while retention of the gauche conformer decreases. For this reason, the interaction of both effects may contribute to unexpected retention behavior in special cases.

Finally, in most applications gradient LC will be superior to isocratic elution due to the wide range of M_r of PEG derivatives in real samples, but neverthe-

less both modes of separation are appropriately considered in this review.

2.2. Chromatography of alkyl-, arylalkyl- and monocarboxyalkyl-substituted polyethylene glycols

2.2.1. Normal-phase liquid chromatography (NPLC)

Normal-phase high-performance liquid chromatography (NP-HPLC) is carried out on pure silica gel and on the so-called bonded-phase matrices, containing substituents, such as 3-cyanopropyl-(CN), 3-aminopropyl-(NH_2) and 2,3-dihydroxypropyl (diol), covalently bound to the silica gel base materials. Whereas separations on native silica gel are usually performed with pure organic solvents, and small amounts of water are often added in order to accelerate the adsorption–desorption equilibrium to improve reproducibility of retention times and peak shapes [84,85], the latter three stationary phases are increasingly used in typical reversed-phase high-performance liquid chromatography (RP-HPLC). Nevertheless their chromatographic behavior as "deactivated" silica gel provides new perspectives, in particular for resolution of PEG derivatives, due to the presence of significantly polar anchor groups, and thus allows a more pronounced "fine-tuning" of the eluent composition in order to achieve the required separation efficiency. Although native silica gel is still used for PEG derivatives, it is increasingly being replaced by CN, NH_2 and diol matrices. In this context, it should be emphasized that the bonded-phase materials are not as sensitive towards traces of water in organic solvents, as e.g., silica gel and, for this reason, retention times and peak shapes exhibit excellent reproducibility making them suitable for long-term application without marked loss of chromatographic performance.

Although in a lot of applications isocratic elution yields excellent resolution of polyethers with a low to medium degree of oligomerization, the higher oligomers are often truncated, as can be seen from their increasing peak tailing and thus more and more merge with the baseline. This drawback can easily be overcome by the use of a solvent gradient, which is the main method for polyether separation. In most cases the less polar mobile phase components consist of alkanes (e.g. *n*-hexane, *n*-heptane and cyclohexane), whereas methanol, ethanol, acetonitrile, 2-propanol and tetrahydrofuran are mainly used as the polar eluents. Due to insufficient mixibility or existence of a mixing gap by using methanol and acetonitrile in combination with alkanes or cycloalkanes, a third component effecting solvent compatibility has to be added, and tetrahydrofuran (THF), dichloromethane and chloroform have been successfully applied. In contrast, ethanol and 2-propanol being more lipophilic compared to methanol and acetonitrile, dissolve in both aliphatic and cycloaliphatic solvents at any volume ratio. In particular when using silica gel, i.e., the most polar stationary phase, elution is often started with a significant percentage of dichloromethane or chloroform instead of pure alkane and thus, no additional "compatibility modifier" is required, but a third eluent is often used for chromatographic "fine-tuning". An overview of optimization strategies for HPLC separation of ethoxylated alkylphenol surfactants by use of pure silica gel and NH_2-stationary phases has recently been published by Márquez et al. [86].

The following part presents a chronological compilation of NP-HPLC for separation of NIS of the ethylene glycol type with respect to the number of ethoxylate units.

In 1978 Van der Maeden et al. [87] described excellent separation of octylphenol ethoxylate (OPEO) oligomers on a NH_2 bonded phase by gradient elution with THF in hexane (solvent A) and 10% water in 2-propanol (solvent B), and up to 20 different base-line resolved peaks were observed as shown in Fig. 1. Similar results, but with better resolution of minor peaks attributable to structural heterogeneities in the octyl side chain of OPEOs on the same column material, using a gradient of two mixtures of isooctane–dichloromethane–methanol, which markedly differed in the concentration of the dichloromethane modifier, was reported by Rothman [88].

Escott et al. [89] used gradient and isocratic elution on pure silica gel with hexane–ethanol mixtures, whereas separation on a CN matrix was performed isocratically with mixtures of THF, ethanol and small amounts of water. The authors found that both systems are very similar with respect to their separation efficiency.

Bogatzki and Lippmann [90] separated fatty al-

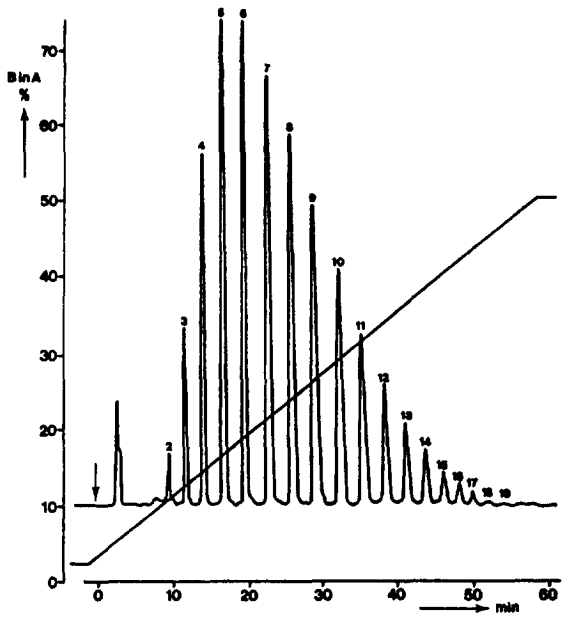

Fig. 1. GE–HPLC chromatogram of an ethoxylated octyphenol on a μBondapak NH_2 column. Peaks: 2=oligomer with 2 EO units; 3=3 EO units; etc. Conditions: solvent gradient from 2% to 50% of solvent B in A in 60 min; solvent A is 20% THF in *n*-hexane; solvent B is 10% water in isopropanol; sample size, 200 μg in 20 μl of solvent A; flow-rate, 1 ml/min; UV detection at 280 nm, 0.5 a.u.f.s. (From Ref. [87], with permission).

cohol ethylene oxide adducts on a NH_2 matrix by isocratic elution with heptane–THF and achieved separation according to their degree of ethoxylation.

Gradient elution of fatty acid ethoxylates on bare silica gel stationary phases with *n*-hexane–2-propanol–methanol was applied by Aserin et al. [91] and up to more than 20 different oligomers could be adequately separated.

In a study focusing on identification of trace amounts of fatty alcohol or alkylphenol ethoxylates in river sediment, Kudoh et al. [92] subjected their ω-1-anthroyl derivatives, being amenable for subsequent fluorescence detection, to gradient HPLC on silica gel starting with *n*-hexane, which is subsequently replaced by ethanol–THF–water. Whereas fatty acid ethoxylates caused some problems on bare silica gel due to interferences arising from the excess of derivatization agent, and in this case the reversed-phase technique proving to be superior (see Section 2.2.2), OPEOs and NPEOs, which due to the existence of a chromophor/fluorophor did not require derivatization, exhibited excellent separation properties on bare silica gel stationary phases.

Alexander et al. [80] investigated the influences of salt and water on separation of NIS. Addition of small amounts of sodium chloride in the m*M* range affords lower retention times. In the same way, small amounts of water greatly decreased either retention or resolution of oligomers. The latter effect can be ascribed to a de-activation of active silanol sites by physically adsorbed water. When both, salt and water are added to the mobile phase, a further reduction in retention and signal resolution was observed.

A lot of work has been dedicated to the analysis of polyether derivatives in studies of biodegradation and environmental protection. For evaluation of the metabolic fate of NIS in river water, Levsen et al. [93] applied an NH_2 column and a gradient of *n*-hexane–THF (solvent A) and 10% water in 2-propanol (solvent B) and found that the NPEOs are biodegraded to the homologue with two EO units within three days, while no significant degradation of the corresponding dinonylphenolethoxylate is observed within the same period.

Ahel and Giger [94,95] and Marcomini and Giger [96], used special techniques, such as "exhaustive steam distillation" [94] or "gaseous stripping" into ethyl acetate [95] to extract NIS with a large M_r distribution from waste water, sewage sludge and river soil and applied the extracts to subsequent gradient HPLC on NH_2 phases with *n*-hexane–2-propanol mixtures. Peak-splitting of the signals of the individual NPEO oligomers, which in contrast, is not observed on the chromatographic pattern of OPEO, reveals structural heterogeneity within the nonyl side chain. This fact is attributable to the manufacturing process of 4-nonylphenol, starting from dimerized propylene and phenol and, therefore, consisting of a complex mixture of isomers with differently branched nonyl substituents. In contrast, 4-octylphenol mainly consists of 1,1,3,3-tetramethylbutylphenol (95%). Fig. 2 reveals the side chain isomerism between NPEO and OPEO. In a similar way, Holt et al. [97] applied pure silica gel and NH_2 stationary phases and gradients of methyl *tert.*-butylether (MTBE) containing 0.1% acetic acid (solvent A) and acetonitrile–methanol (95:5) containing 0.1% acetic acid (solvent B) for analysis of alkylphenol ethoxylates in environmental samples.

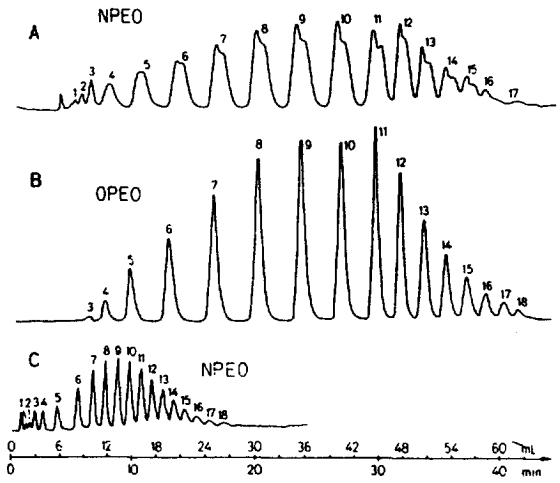

Fig. 2. Normal-phase high-performance liquid chromatograms of non-ionic surfactants of the APEO type: (A and C) Marlophen 810 (NPEO); (B) Synperonic OP10 (OPEO). Peak numbers refer to numbers of ethoxy units. The HPLC columns were 250 mm×4.6 mm I.D., 10-μm LiChrosorb-NH_2 (A, B) and 100 mm×4 mm I.D., 3-μm Hypersil APS (C). Injected samples contained 2 μl of solutions containing 100 μg/l of surfactant in ethyl acetate (A, B) and 20 μg/μl of surfactant in a mixture of 2-propanol–*n*-hexane (20:80) mixture (C). (From Ref. [95], with permission).

Separation of alkyl ethoxylates up to the 30-mer was reported by Zeman [98] using isocratic HPLC with *n*-hexane–2-propanol–water–acetic acid mobile phases and a diol matrix. This analytical system can further be extended to the separation of ethoxylation products derived from fatty acids, fatty acid monoethanolamides and alkylphenols.

Determination of NIS used in tertiary oil recovery was performed by Desbène et al. [99,100] either isocratically or by gradient HPLC on amino, cyano and diol bonded silica gel with mixtures of *n*-heptane–dichloromethane–methanol [99] and/or *n*-heptane–dichloromethane–2-propanol [100]. Whereas methanol was used as the polar mobile phase modifier for CN and amino phases, 2-propanol was applied on a diol matrix. The aminopropyl column allows separation of NIS according to the number of EO units and the nature of the fatty alcohol chain. Nevertheless, the authors stated that the less polar CN matrix proved to be slightly better than the amino phase for the study of NIS. Furthermore, the diol matrix shows superiority over the other two other phases when NIS with a high degree of oligomerization are to be analyzed and, as already observed on the NH_2 column, at least partial separation according to both EO number and fatty alcohol chain length is achieved.

In the analysis of NIS of the OPEO and NPEO type in liquid pesticide formulations, Schreuder and Martijn [101] applied a prior solid-phase extraction (SPE) step for sample concentration, followed by linear gradient HPLC with hexane–THF (solvent A) and 10% water in 2-propanol (solvent B) on an aminopropyl stationary phase for separation with respect to the number of EO units.

Base-line separation of more than 20 individual NPEO oligomers on an aminopropyl matrix using gradient elution with hexane–2-propanol–water mixtures was effected by Bear [102]. Furthermore, the same author separated mixtures of linear C_{12} and C_{14} ethoxylates, not only with respect to the number of EO units but also, at least partially, to the nature of the fatty alcohol substituent as depicted in Fig. 3. However, when using branched C_{13} ethoxylates, he effected only separation according to the number of EO units, and separation with respect to side-chain isomerism could not be achieved [102].

Excellent interlaboratory reproducibilities of arylalkyl ethoxylates subjected to HPLC on both NH_2 and diol columns by application of either different isocratic or gradient systems prepared from *n*-hexane–2-propanol–water and different flow-rates as well as sample volumes and sample concentrations, respectively, was reported by Zeman [65].

Extensive investigations to compare various stationary phases, such as aminopropyl, cyanopropyl, diol and pure silica gel materials with respect to their capabilities for ethoxymer separation were performed by Jandera et al. [103]. The authors applied both isocratic and gradient techniques and found that chromatographic performance of unmodified silica is improved when ethanol–propanol–aliphatic hydrocarbon ternary mobile phases are used. Due to their substantially lower polarity with respect to bare silica, diol and nitrile stationary phases require a lower content of propanol for reasonable ethoxymer peak resolution, whereas in propanol-rich eluents, a mixed retention mechanism occurs yielding a non-linear increase in log k' values with increasing number of EO units. However, as expected, higher proportions of propanol have to be used for elution

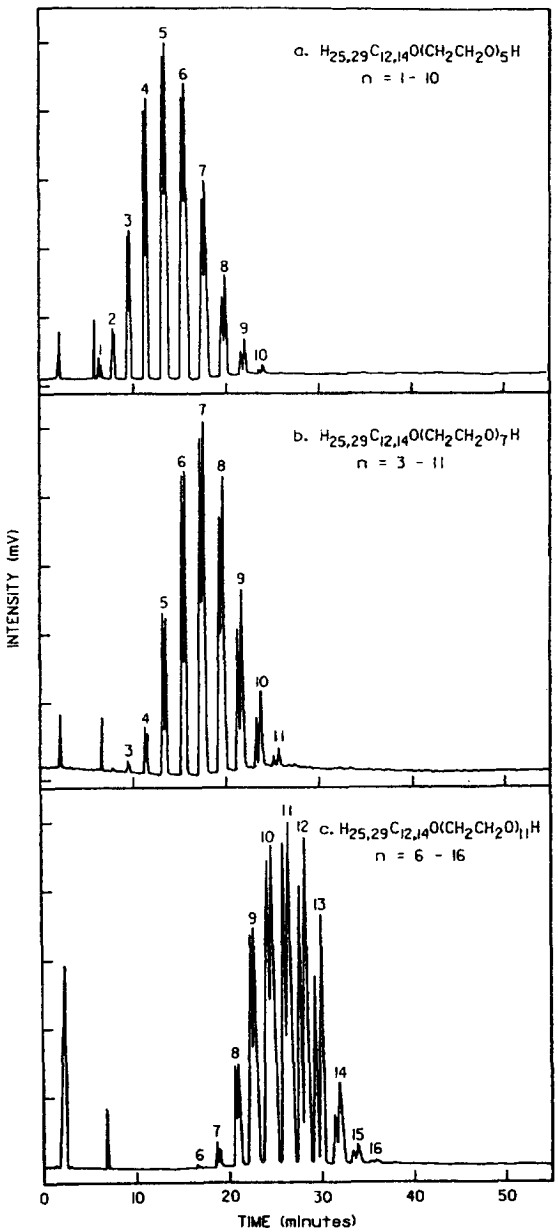

Fig. 3. HPLC analysis of linear alkylethoxyalcohol oligomers: (a) AE5; (b) AE7; (c) AE11. (From Ref. [102], with permission).

of higher ethoxymers on the aminopropyl matrix due to its higher polarity compared with the CN and diol materials. Unfortunately, the latter stationary phases exhibit significant peak tailing with increasing number of EO units, as also observed on silica gel. As a consequence, small amounts of very polar additives, such as acetic acid, 2-methoxyethanol or even water, must be used in order to overcome this drawback. Tailing effects on silica gel can be markedly suppressed by replacement of propanol by ethanol. Nevertheless the NH_2 column shows the best separation characteristics of all adsorbents investigated [103].

The successful use of ternary hexane–chloroform–2-propanol mobile phases in gradient HPLC was described by Brossard et al. [76] for separation of C_{18} fatty alcohol ethoxylates on a diol matrix (Fig. 4) and Zhou et al. [104] on a diol, as well as an aminopropyl column for alkylphenol ethoxylates. Brossard et al. [76] additionally used *n*-hexane–2-propanol–water mixtures in order to circumvent the chlorinated hydrocarbon-invoked baseline drift in UV detection. Furthermore, they recommend replacement of dichloromethane by chloroform due to its stronger "donor" character, whereas 2-propanol is preferred to methanol, because it allows a better control of retention.

Nitschke and Huber [105] separated NPEO oligomers and the phenylisocyanate derivatives of alkylethoxylates, with gradients of hexane–dichloroethane (solvent A) and acetonitrile–2-propanol (solvent B) on a bare silica gel column matrix after

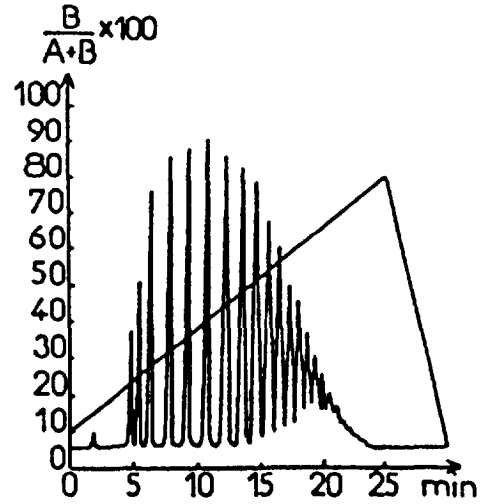

Fig. 4. Brij 76, $C_{18}H_{37}(OCH_2CH_2)_{10}OH$. Chromatographic conditions: column, LiChrospher 100 Diol (125 mm×4 mm I.D.); mobile phase, linear gradient from 90% to 20% A in 25 min, A=hexane, B=chloroform–2-propanol (98:2); flow-rate, 1 ml min^{-1}; detection, ELSD. (From Ref. [76], with permission).

extraction of environmental samples by "gaseous stripping" into ethylacetate [95].

Using a nitrophenyl stationary phase, operated at normal-phase conditions with hexane–dichloromethane–methanol mobile phases either in the isocratic or gradient mode, Desbène and Desmaizières [106] achieved separation of fatty alcohol ethoxylates and arylalkylethoxylates up to EO numbers of about 80. Under these experimental conditions, the superiority of the nitrophenyl matrix over a "conventional" CN phase is impressively demonstrated in Fig. 5, showing excellent separation of PEG non-ionic surfactants.

Rather novel mobile phase combinations for separation of NPEOs on unmodified silica gel were reported by Anghel et al. [107]. For this purpose, gradients of e.g. *n*-hexane–diethyl ether (solvent A), *n*-hexane–diethyl ether–dioxane–ethanol–2-propanol–water–acetic acid (solvent B) were used, and satellite signals, partially fused with the main oligomer peaks, reveal substantial molecular heterogeneity in the lipophilic side chain moieties as shown in Fig. 6.

Recently, Martin [108] described a ternary gradient system of hexane, chloroform and methanol and an aminopropyl column for separation of C_{10}, C_{12} and C_{14} alkyl ethoxylates. Besides the discrimination according to the number of EO units, the author observed signal resolution with respect to side-chain isomerism of the alkyl endgroups, and very complex signal patterns are obtained when extensively branched alkyl chains were used for surfactant preparation (see Fig. 7).

Forgács and Cserháti [109] reported on the first

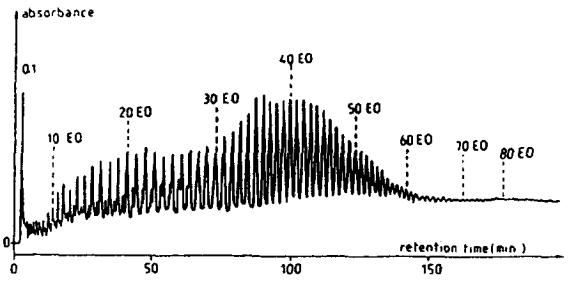

Fig. 5. Analysis of a mixture of non-ionic POE surfactants KM25 (10^4 ppm, w/w) and Cetalox AT (10^4 ppm, w/w) as esters by normal-phase partition chromatography. Conditions as in Fig. 3, except temperature, 45°C. (From Ref. [106], with permission).

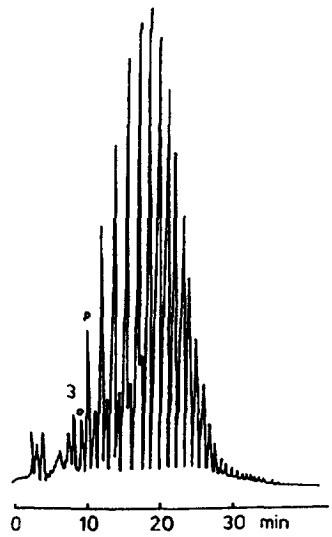

Fig. 6. Chromatogram of NPE_{10}. Eluent A, *n*-hexane–diethyl ether (80:20, v/v); eluent B, *n*-hexane–diethyl ether–dioxane–ethanol–2-propanol–water–acetic acid (10:15:50:20:5:1:0.25, v/v). Gradient: 10–95% B in 40 min. (From Ref. [107], with permission).

application of an alumina column for separation of nonylphenol ethoxylates in isocratic chromatography with ethyl acetate–*n*-hexane mixtures. They achieved separation according to the number of EO units, whereas in contrast, isomerism in the side-chain of the nonyl substituent was not accomplished.

2.2.2. Reversed-phase liquid chromatography (RPLC)

Although RP-HPLC is the method of choice for separation of NIS according to the length and chemical structure of the alkyl, arylalkyl or carboxyalkyl substituents, an increasing number of papers deals with efficient chromatographic procedures based on separation with respect to the number of ethoxylate units, which are described in more detail in Section 2.2.3. As referred to in Section 2.1, high-molecular-mass NIS strongly adsorb onto normal-phase matrices and are only elutable with strong polar solvents lacking any individual peak resolution [76]. So, for this reason RP chromatography will be complementary to the NP technique.

As stated in Section 2.1, NIS are eluted in the order of decreasing number of EO units on reversed-phase packings [81]. Nevertheless, it should be

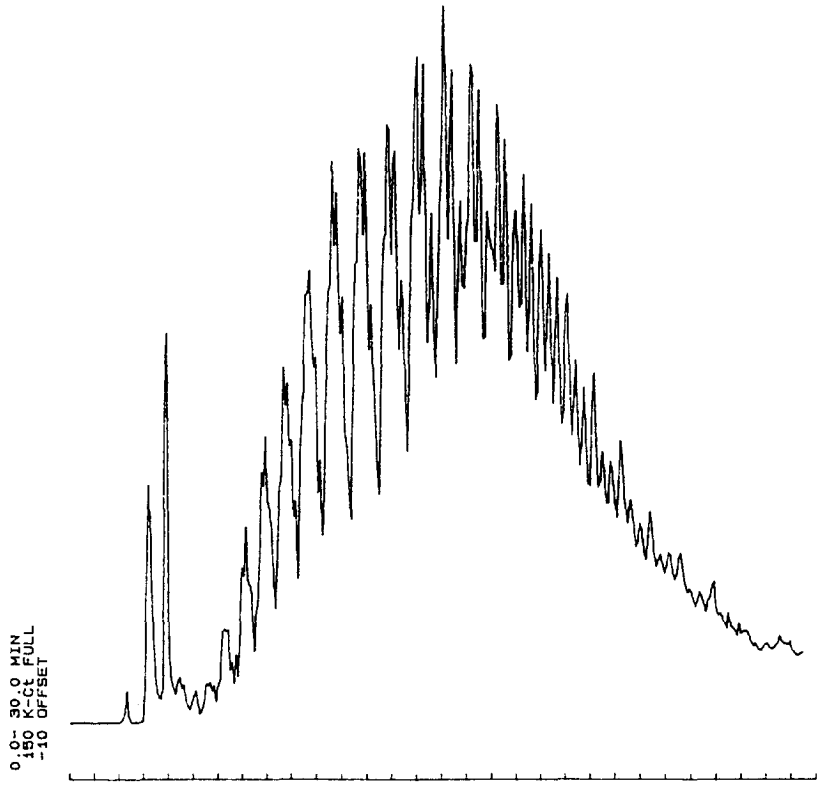

Fig. 7. HPLC profile for the ethylene oxide condensate of a fatty alcohol with a complex alkyl chain. (From Ref. [108], with permission).

emphasized again that Melander et al. [82] observed a "biphasic" retention behavior in the case of phenyloligoethylene glycols. As expected, the authors observed that in aqueous organic solvents, rich in organic modifier, the larger oligomers are eluted before their smaller homologues, whereas the opposite is true with hydro-organic solvents lean in organic modifier. As mentioned in Section 2.1, this effect was mainly attributed to conformational changes of the solute molecules as the consequence of the exposure of different sites for interaction with the stationary phase in organic solvents, either rich or poor in water content. The authors performed gradient separation of OPEOs with acetonitrile–water mixtures on either C_8 or C_{18} materials.

Nozawa and Ohnuma [110] reported on investigations of 3,5-dinitrobenzoylated monododecyl ethoxylates on a C_2 stationary phase by isocratic elution with an acetonitrile–water mixture, finding that retention of ethoxymers increases with decreasing number of EO units.

Schulz et al. [45] used acetonitrile–water gradients and a C_{18} column for investigation of macromonomers obtained by reaction between either native PEG and 4-chloromethyl styrene or condensation of aryl-substituted PEG with acrylic acid. The authors achieved good separation of oligomers up to an average EO number of 10, whereas oligomer mixtures containing an average of 20 EO units show substantially lower separation, and those up to 40 EO units are not separated at all.

The work of Alexander et al. [80] provided evidence that separation of NIS on RP materials increases with decreasing surface coverage with alkylsilyl substituents. As observed by the same authors in NP-HPLC (Section 2.2.1), addition of m*M* amounts of sodium iodide substantially decreases retention, and ethoxymers previously merging with the baseline can be recognized after addition of salt to the mobile phase. However, signal resolution decreases by this treatment and often only one large peak is observed, and thus, "fine-tuning" experi-

ments are necessary to achieve at least partial oligomer separation.

By comparison of either retention behavior or signal resolution on both a weakly hydrophobic vinyl alcohol copolymer and an octadecylsilyl (ODS) silica gel matrix, Noguchi et al. [47] provided evidence that the mechanism of retention is primarily governed by hydrophobic interactions of both the alkyl or arylalkyl substituents and the dimethylene bridges of the EO units. The authors observed that the concentration of acetonitrile required to yield similar retention was about 15–20% higher on the ODS packing compared with the polymer gel, and retention of NIS on the latter stationary phase increases with decreasing surface density of hydroxyl groups. Furthermore, peak resolution is much better on the polymer gel than on the ODS material. This observation can be explained in the way that significant hydrophobic interactions between the hydrophilic polymer gel and the NIS involves only their unpolar arylalkyl groups and that the longer EO chains simply serve to interfere sterically with this interaction. The combination of both effects results in later elution of NIS with a smaller number of EO units and satisfactory separation. In contrast, the hydrophobic interaction between the ODS matrix and the NIS seems to include both the arylalkyl groups and the ethylene bridges of the polyethoxylate chains, the interaction with the former ones being stronger for the oligomers with smaller numbers (n) of EO units, whereas interaction with the polyethoxylate backbone is far stronger at a higher degree of ethoxylation. As a consequence, a "cancelling effect" occurs and results in poorer resolution between NIS with smaller n values compared with the hydrophilic polymer gel. It may thus be a reasonable assumption that both effects work against each other and, as a consequence, peak resolution of the whole range of oligomers decreases compared with that obtained on the polymer gel. Moreover, the authors discussed a size exclusion mechanism when high amounts of organic solvents (>60%) are used. This effect was convincingly demonstrated by the choice of 2-propanol, a solvent having an unpolar alkyl moiety and thus capable of a more effective inhibition of hydrophobic interactions between the NIS and the hydrophilic polymer gel. However, in contrast to the findings of Melander et al. [82], no inversion of the elution order of OPEOs as a function of the acetonitrile content in the mobile phase was observed by the authors [47].

Desbène et al. [111] studied separation of 2,4-dinitrobenzoyl (DNB) derivatives of NIS in the isocratic mode on either typical RP matrices (C_{18}, C_8, C_6, C_4, C_2, phenyl) as well as on so-called bonded phases (CN, diol) under typical RP conditions, using aqueous organic solvents prepared from different modifiers (methanol, acetonitrile, acetone, THF, 2-propanol, dioxan). The best results were obtained on a C_{18} matrix using acetonitrile, THF or acetone as solvent additive, whereas methanol yielded only separation with respect to the type of the fatty alcohol chain. Compared with the C_{18} matrix, separation efficiency on the other RP materials of either intermediate hydrophobicity or even substantial polarity, such as C_8, C_6, C_4, C_2 and phenyl materials was markedly lower, despite the use of "good" solvents successfully tested with the C_{18} stationary phase. Furthermore, different peak clusters attributable to substantial structural heterogeneity within the side chains of the hydrophobic moieties (e.g. $C_{12}H_{25}$–O–, $C_{18}H_{37}$–O– etc.) were observed on C_6, C_8 and C_{18} stationary phases. In contrast to these findings, methanol, dioxan and 2-propanol are not applicable for separation of NIS according to the number of EO units, whereas the polar bonded phases CN and diol showed neither separation with respect to the number of EO units nor to the structure of the alkyl chains.

After derivatization of decyl and lauryl ethoxylates with 1-anthroylnitrile Kudoh et al. [92] observed that the alkyl ethoxylate 1-anthroyl ester oligomers can be well separated from each other by isocratic elution with acetonitrile–water on a C_{18} matrix, but OPEOs and NPEOs revealed relatively poor separation with respect to the number of EO units. The inverse is true on a NP phase system (Section 2.2.1), which impressively demonstrates the complementary use of both alternatives.

The small amounts of NIS often encountered in "enhanced oil recovery" prompted Desbène et al. [112] to achieve separation of the corresponding DNB-derivatives and subsequent electrochemical detection. For this purpose the authors used a C_{18} column and isocratic acetonitrile–water and THF–water eluent systems containing mM amounts of

sodium or ammonium perchlorate as the conducting salt.

Almost baseline separation of ethoxylates up to an EO number of more than 30 and their ω-carboxyl derivatives containing an α-C_{12} or C_{14} aliphatic chain, was accomplished by Mengerink et al. [113] on a C_{18} matrix with a gradient of 0.1% aqueous acetic acid and acetonitrile.

Escott and Mortimer [75] applied gradient HPLC on a C_{18} column in series with a C_8 matrix with acetonitrile–water mixtures for separation of methoxy-PEG as well as PEG acrylates and achieved separation of oligomers with M_r up to 2500 as shown in Fig. 8 and Fig. 9. Traces of sodium azide (5 μg/l) were added to the aqueous phase in order to compensate for the baseline drift at the low wavelength of 190 nm used for detection (Section 3.4).

In contrast to Okada [114], who only achieved separation of NIS according to the nature of the aliphatic fatty alcohol chain on a polystyrene matrix cross-linked with divinylbenzene (DVB–PS materials), Brossard et al. [76] effected at least partial separation of alkyl ethoxylates according to the number of EO units using gradient elution with aqueous acetonitrile on the same column type.

Rapid separation of OPEOs by isocratic elution with mixtures of methanol and water containing 0.02 g/l ammonium acetate for baseline stabilization on a C_1 material was reported by Wang and Fingas et al. [115]. This technique allows separation of samples with complex ethoxylate distributions and oligomers consisting of more than 40 different EO units could be detected as depicted in Fig. 10. Furthermore, the authors found that best resolution of EO units was achieved when the samples have been dissolved in water or mobile phase, whereas in contrast, methanol as the solvent yielded poorly separated peaks. Moreover, it is interesting than unlike the elution range of NIS under "pure" RP conditions (e.g. C_{18}, C_8, C_2 stationary phases), a complete reversal of elution order takes place on a C_1 matrix and oligomers are eluted in the range of increasing number of EO units, whereas on a C_2 stationary phase, the normal elution behavior of RP systems was observed [110,111]. The "unusual" retention on the C_1 phase may be explained by the substantial increase of its polarity with respect to the more hydrophobic materials and thus revealing marked similarities with bare silica gel. Perhaps this material marks the "transition point" between typical RP-LC and NP adsorption chromatography.

Schröder [40] reported a gradient system consist-

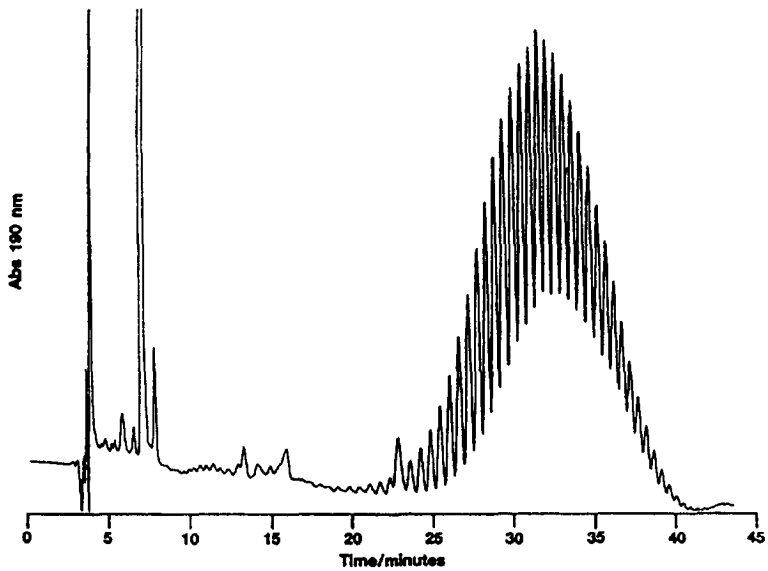

Fig. 8. HPLC analysis of methoxy PEG methacrylate with an average molecular mass of 2000 Da. The analysis conditions are given in Fig. 3 and Table 1. (From Ref. [75], with permission).

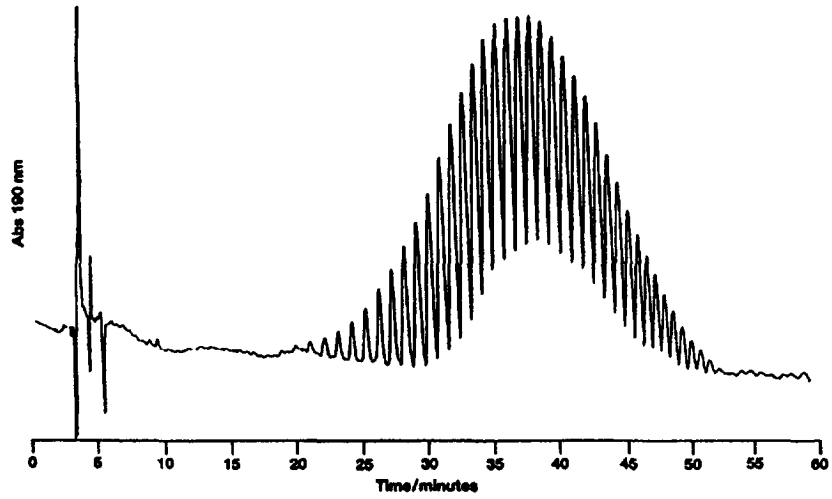

Fig. 9. HPLC analysis of methoxy PEG with an average molecular mass of 2000 Da, carried out on an ODS column coupled with a 300 Å octyl column at 60°C, and with a detector sensitivity of 0.2 AUFS. (From Ref. [75], with permission).

ing of acetonitrile (solvent A) and water–methanol (80:20) (solvent B) mixtures and a C_{18} stationary phase for separation of alkylethoxylate NIS from their acidic (carboxyl-terminated) and de-alkylated metabolites (i.e. the corresponding α-, ω-dihydroxy-ethoxylates) in sewage treatment plant effluents.

An isocratic system of water–tetrahydrofuran mobile phases and a C_{18} column matrix was used by Evans et al. [116] for separation of alkylpolyethoxylates with respect to either length of the alkyl chain or number of ethoxylate units in samples of sewage treatment plant influents and effluents.

Jandera and Urbánek [117] performed extensive investigations on separation of NPEOs and their O-sulfated derivatives, which may serve as model components for degradation of the NPEO starting materials in a biological environment (e.g. in "in-vivo" degradation studies). The authors applied isocratic elution conditions with 2-propanol–water mobile phases containing cetyltrimethylammonium bromide (CTAB) as the ion pairing agent in order to improve retention of the NPEO sulfates on a C_{18} column. Whereas in pure 2-propanol–water eluent systems lacking any ion-pairing agent the NPEO sulfates leave the column unretained as a single peak, addition of CTAB caused either significant retention or separation into a multitude of individual components according to the number of EO units. In contrast to the NPEO sulfates, CTAB yielded only minor influences on the retention behavior of NPEOs, but nevertheless, retention was substantially decreased with increasing concentrations of CTAB and thus reveals a marked "salt effect" similar to that already described by Alexander et al. in the case of iodide ions [80].

Lemr et al. [118] described an optimized HPLC assay of 1-naphtyl isocyanate derivatives of linear fatty alcohol ethoxylates by isocratic elution with acetonitrile–water mobile phases on a C_{18} column. They observed inversion of the "normal" elution order of alkylated polyethoxylates with decreasing number of EO units when extremely high fractions φ of organic modifier were applied. They found that at $\varphi=0.948$ all ethoxylate homologues merged into one peak, and at values of $\varphi>0.948$ elution of ethoxymers takes place in the order of increasing number of EO units. This behavior resembles the conditions typically found in LCCC, which are characterized by "co-elution" of homologues and separation only according to the nature of the functional endgroups (Section 2.5). Comparable effects, where elution order changes by a concomitant change of eluent composition, and presumably attributable to alterations in the conformation of the polyether backbone, have been reported by Melander et al. [82] and Okada [83] (Section 2.1).

Sun et al. [119] obtained excellent separation of about 30 different oligomers of NPEO on an amino-

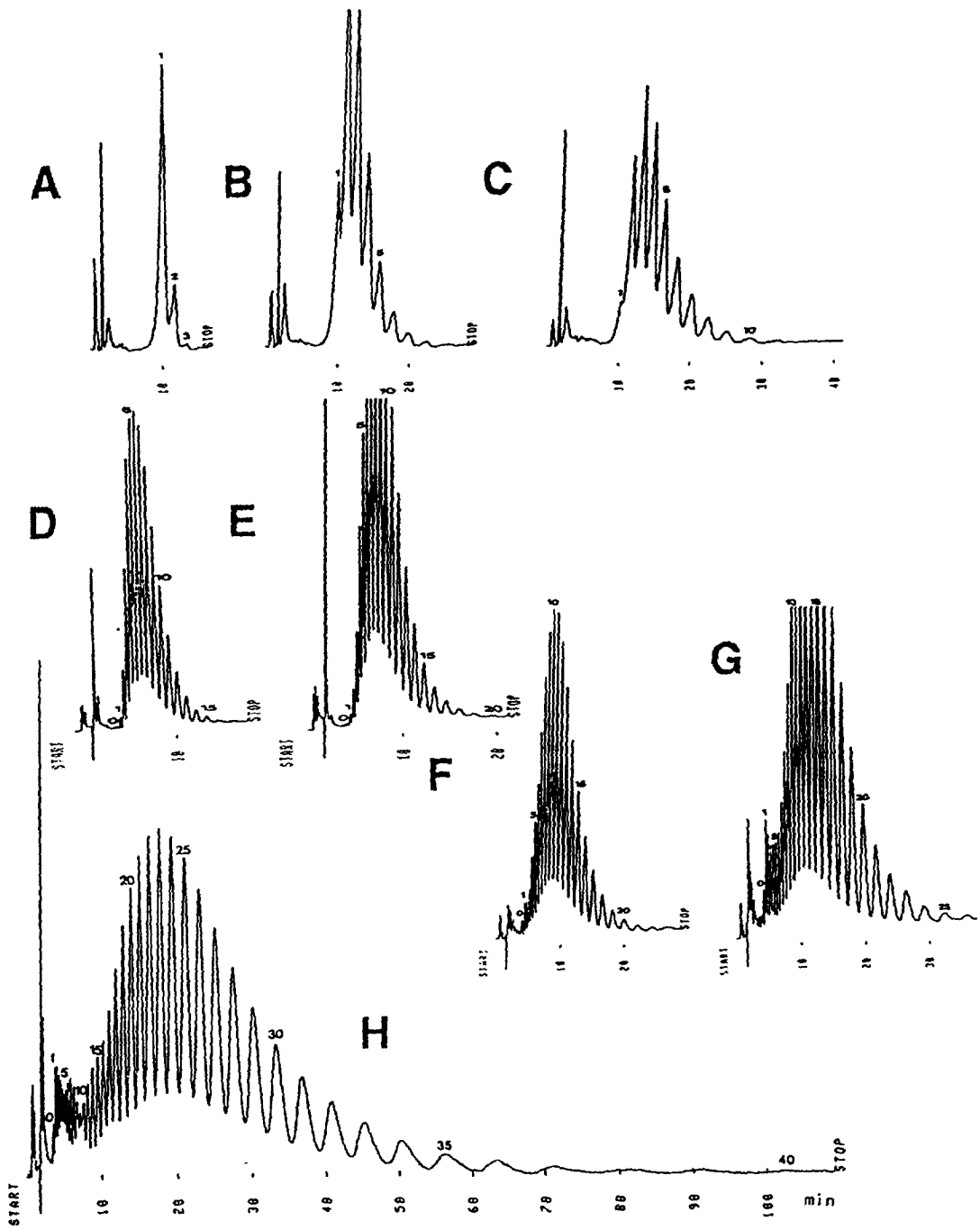

Fig. 10. HPLC of non-ionic surfactants of polyethoxylated octylphenol. (A) Triton X-15 (0.02 mg/ml); (B) Triton X-35 (0.1 mg/ml); (C) Triton X-45 (0.1 mg/ml); (D) Triton X-114 (0.2 mg/ml); (E) Triton X-100 (0.2 mg/ml); (F) Triton X-102 (0.5 mg/ml); (G) Triton X-165 (0.8 mg/ml); (H) POE (30) octylphenol (1.0 mg/ml). Conditions: CSC–C1 TMS column; temperature, 22±1°C; mobile phase, methanol–water [(A)–(C) 53:47; (D)–(H) 60:40]; elution mode, isocratic; flow-rate, 1.0 ml/min; UV detection at 225 nm. The numbers assigned to the individual peaks represent the number of EO units in the oligomers; 0 represents the parent *tert.*-octylphenol. The integrator attenuation was set at 3, 4 or 5 according to the intensities of the peaks. (From Ref. [115], with permission).

propyl column when using gradient chromatography with acetonitrile–water mixtures.

Meissner et al. [120] used "off-line" pre-column derivatization of C_{12}–C_{18} fatty alcohol ethoxylates with FMOC-Cl, followed by separation of the corresponding derivatives with a gradient of acetonitrile and water and subsequent fluorimetric detection for measurement of NIS at the trace level in environmental samples.

Ye et al. [79] separated OPEOs isocratically with an acetonitrile–water mobile phase on both a C_{18} and a C_8 column matrix. With a commercially available OPEO sample they observed two well-resolved groups of peaks on a C_{18} stationary phase, that one eluting at lower retention times presumably being attributable to more polar sample constituents with lower M_r. As expected, peak resolution of this latter peak group decreased significantly by use of the C_8 column exhibiting substantially lower hydrophobicity compared with a C_{18} matrix.

Separation according to the chain length of fatty alcohol ethoxylates, but lacking resolution of individual peaks attributable to the number of EO units, which up to now is the preponderantly used alternative in reversed-phase chromatography of polyether-like NIS, is described by several authors [76,90,94–96,104,105,108,114,121–127] and is referred to below in more detail.

Methanol–water gradients and a C_{18} matrix were used by Otsuki and Shiraishi [121] for determination of OPEOs, NPEOs and dodecylphenyl ethoxylates at trace levels in water. Kudoh et al. [122,123] performed separation of ethoxylates of different alkyl chain length and alkylated copolymers of EO and propylene oxide (PO) on a porous polymer gel [122] and further separated mono- and dilauryl ethoxylates from the PEG starting material on a C_{18} material [123]. In both cases, mixtures of acetone and water were used isocratically for sample elution. Baseline separation of lauryl, cetyl and stearyl ethoxylates was achieved isocratically by Kudoh [124] with acetone–water mobile phases on C_{18} matrices, whereas in contrast, separation with respect to the number of EO units is favored by methanol, acetonitrile and tetrahydrofuran as the organic modifiers.

Isocratic conditions with methanol–water mobile phases on a C_8 matrix were applied to the separation of C_8, C_{10}, C_{12}, C_{14} and C_{16} ethoxylates by Bogatzki and Lippmann [90] and to NIS of environmental samples by Ahel and Giger [94,95] and Marcomini and Giger [96].

Separation of OPEOs and NPEOs from linear alkylsulfonate surfactants (LAS) in sludges, soils and sediments was accomplished by Marcomini et al. [125,126] on C_8- and C_{18}-matrices with gradients of 2-propanol, acetonitrile and water containing 20 m*M* of sodium perchlorate as ion-pairing agent to improve either separation or peak shape of LAS and thus effecting complete separation from NIS without interferences. In order to effect complete separation of LAS from alkylphenol polyethoxylates and their carboxylic biotransformation products, Marcomini et al. [126] tested various eluent systems of either methanol or acetonitrile as organic modifiers containing tetrabutyl ammonium dihydrogen phosphate or sodium perchlorate as ion-pairing agents and trifluoro acetic acid (TFA) as mobile phase additive on a C_{18} matrix. Best results were obtained with acetonitrile–water gradients containing sodium perchlorate and TFA.

NPEO isomers with differently branched lateral nonyl chains were base-line separated by Zhou et al. [104] using a C_{18} matrix and isocratic elution with water–THF–hexane.

A polymer column containing a DVB–PS material was used by Okada et al. [114] for isocratic separation of C_{12}, C_{16} and C_{18} alkyl ethoxylates with acetonitrile–methanol mixtures.

Brossard et al. [76] separated C_{10}, C_{14} and C_{18} ethoxylates ($n=6$) from each other and from the starting material PEG on a C_{18} matrix with methanol–water mixtures.

Nitschke and Huber [105] achieved excellent separation of the phenylisocyanate derivatives of C_8–C_{14} as well as C_{16}, C_{18} and C_{20} polyethoxylates with respect to the length of the alkyl chain on a C_{18} matrix with methanol–water gradients.

Martin [108] applied a C_{18} column and an isocratic system of methanol and water for separation of C_{10}, C_{12} and C_{14} polyethoxylates and Crescenci et al. [127] used methanol–water gradients containing 0.1 m*M* TFA and a C_8 column for separation of NPEOs and C_{12}, C_{13}, C_{14}, C_{15}, C_{16} and C_{18} polyethoxylates ($n=1$–20).

2.2.3. Ion-exchange chromatography (IEC)

Although polyether-like surfactants[2] lack any group susceptible to classical ion-exchange, IEC separation of the DNB derivatives of C_{12}, C_{16} and C_{18} polyethoxylates according to the number of EO units was performed by Okada [114] and Okada and Usui [128] on ion-exchange columns. Okada [114] used a TSK-gel IC-Cation-SW matrix for separation of more than 30 different oligomers. The non-existence of ionizable sites on the polyether backbone was overcome by admixture of potassium salt to the mobile phase and, presumably due to the ability of the K^+-ion to effect a cyclic five-membered complex structure with 1,2-dioxoethylene structural segments, sufficient "binding" onto the surface of the ion-exchange resin was achieved. As the retention time increases exponentially with increasing number of EO units, gradient elution has to be applied for complete release of ethoxylates. The authors found that the latter technique, with increasing concentrations of potassium chloride in methanol, yielded either excellent separation with respect to the EO number or performance of the analysis in a reasonable time scale.

Although not being non-ionic surfactants, IEC of polyethers containing hydroxyl groups at their ends, will be treated here. Okada and Usui [128] applied an aminopropyl column for separation of DNB-derivatized polyoxyethylenes. The primary retention mechanism consists in complexation of PEG with the ammonium sites of the stationary phase being protonated by use of a slightly acidic mobile phase system, from which it is competitively displaced by use of K^+-ions in the mobile phase. The authors observed that the choice of the appropriate counter-anion markedly effects retention and peak resolution, with perchlorate being the best suited among the species tested. As a consequence, elution was performed with a gradient of potassium perchlorate in methanol. Moreover, the authors obtained separation in terms of both hydrophobic moiety and EO number. Separation of DNB-PEG by IEC on a NH_2-matrix is shown in Fig. 11.

No work has been published hitherto concerning ion-exchange separation of PPG, which like PEG, also contains a 1,2-dioxoethylene bridge. However, the reduced flexibility of the PPG chain due to the additional methyl substituent may prevent formation of a suitable conformation for optimum complexation with potassium, whereas PBG will not be a good substrate for IEC presumably due to the high flexibility of the 1,4-oxotetramethylene structural unit, and thus adaption of a suitable conformation yielding a five-membered complex, will be achievable only with difficulty.

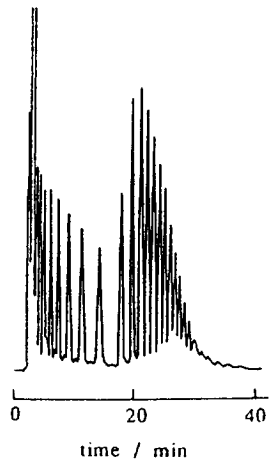

Fig. 11. Separation of POE oligomers contained in POE(20)S. Conditions as in Fig. 2. (From Ref. [128], with permission).

2.3. Chromatography of native (underivatized) polyethylene glycols (PEGs)

2.3.1. General considerations

Although native PEGs represent a minor proportion of polyether derivatives for either industrial or scientific use, there are still sufficient applications, which require exact knowledge of molecular heterogeneity, e.g. when the free hydroxyl groups of PEGs are to be reacted with appropriate agents during the synthesis of intermediates.

Due to the lack of a nonpolar anchor group, the physico-chemical properties differ markedly from those of, e.g., NIS carrying long fatty alcohol chains at one hydroxyl terminus. These differences will of course be more significant the higher the mass ratio of hydrophobic to hydrophilic structural elements in

[2]The corresponding carboxyl terminated derivatives as the products of a biodegradation process have not been considered.

the backbone for a given polyether derivative. For this reason, it is obvious that more or less substantial changes in the chromatographic behavior are to be expected between PEG-based NIS and their underivatized polyether parent components. Furthermore, sufficient separation of individual ethoxymers facilitates calculation of the number-average (M_n) and the weight-average (M_w) molecular mass and the polydispersity index (M_w/M_n), as proposed by Trathnigg et al. [51].

Native PEGs represent a rather difficult class of substrates for either RP-HPLC or NP-HPLC due to their high polarity, which often causes too strong retention on polar stationary phases [76], such as silica gel, diol, CN and aminopropy materials, necessitating the use of strong solvents like methanol, ethanol or 2-propanol, which however elute the whole amount of oligomers as one common peak without individual signal resolution (see Section 2.1 for NIS). In contrast, very often only weak solute–matrix interactions are observed on hydrophobic matrices, such as C_{18} and C_8 matrices. Nevertheless, excellent resolution of PEG oligomers far exceeding M_r 1500 were reported by a number of authors and Section 2.3.2 Section 2.3.3 will give a brief survey of promising separation systems.

2.3.2. Reversed-phase liquid chromatography with aqueous organic solvents

Murphy et al. [44] applied gradient HPLC with methanol–water after prior conversion of the PEGs to their dibenzoates to distinguish the different oligomers, but only PEG 400 shows substantial peak resolution, whereas the whole amount of PEG 1500 and PEG 4000 oligomers merged into one common peak.

Gradient elution of PEGs, markedly differing in M_r distribution, with acetonitrile and water was successfully applied on a C_{18} column by Melander et al. [82].

Escott and Mortimer [75] also used gradient HPLC for chromatography of native PEGs on two C_{18} columns in series with acetonitrile–water mixtures the aqueous phase containing 5 μg/l of sodium azide (Section 2.2.2). The authors reported successful separation of a wide variety of PEGs up to M_r 5000 when using a temperature range from RT to about 80°C. The separation pattern of PEG 2000 by use of the tandem C_{18} column technique is shown in Fig. 12.

Gradient elution of various PEG mixtures with aqueous mixtures of acetonitrile containing 1 mM phosphoric acid and 5 ppm of nitric acid on a C_8

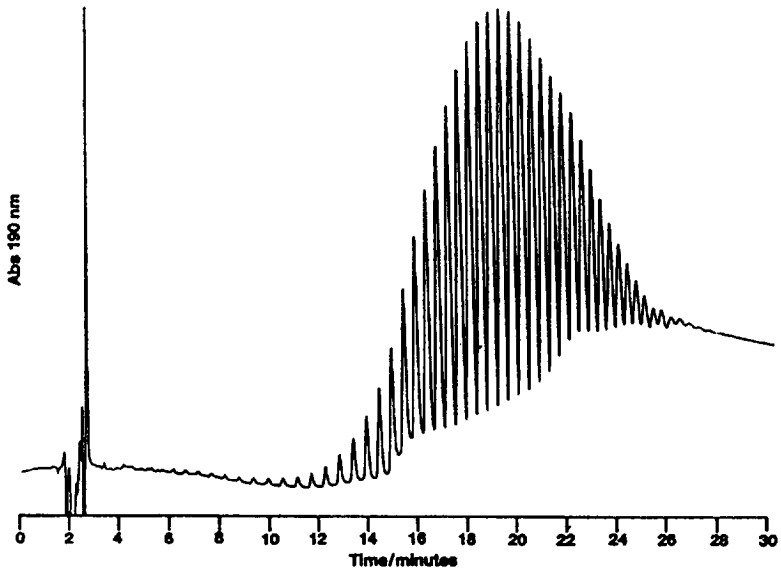

Fig. 12. HPLC analysis of PEG with an average molecular mass of 2000 Da, carried out on two ODS columns at 60°C and with a detector sensitivity of 0.2 AUFS. (From Ref. [75], with permission).

column was reported by van der Wal and Snyder [129], and baseline separation could be achieved in most cases. A convex gradient profile with gradually decreasing steepness of $\Delta S/\Delta t$ (S≡percentage of "good" organic modifier) was used in order to achieve sufficient separation of late-eluting oligomers.

Bergmann and Möller [130] effected separation of PEG oligomers by gradient HPLC with mixtures of acetonitrile and water, the latter containing 4 ppm of nitric acid and 60 ppm of phosphoric acid. Addition of trace amounts of both acids was performed in order to achieve measurement of signal responses at wavelengths below 200 nm Section 3.4.

Lai et al. [131] used an isocratic acetonitrile–water system and a phenyl-bonded stationary phase for separation of PEG 400 oligomers.

Barka and Hoffmann [132] investigated PEGs with a degree of polymerization of 1–110 by gradient elution with acetonitrile–water mixtures on a C_8 matrix.

Jandera [81] subjected Carbowax 200 to gradient chromatography with 2-propanol in water and observed nine peaks, which however, are more or less distorted presumably due to problems in detection at the low wavelength of 200 nm used for signal monitoring.

A size exclusion mechanism was postulated by Noguchi et al. [47] on a (hydrophilic) vinyl alcohol copolymer even with water as the mobile phase, whereas the same authors reported that retention increases with increasing M_r on a C_{18} matrix, which is in accordance with typical RP chromatography.

PEG 600 was determined in human urine on a C_{18} column and methanol–water mobile phases after derivatization with benzoyl chloride and pyridine as the catalyst by Kinahan and Smyth [3]. Isocratic elution yielded only one peak, whereas the gradient technique effected at least partial resolution of 13 oligomers.

Baseline separation of more than 10 oligomers of PEG 400 was achieved by Brossard et al. [76] with a gradient of acetonitrile and water on a PS–DVB matrix and Oliva et al. [5] resolved up to eight PEG 400 oligomers in urine samples on a C_{18} stationary phase by isocratic elution with methanol–water.

Isocratic separation of PEG 300, PEG 600, PEG 400, PEG 1000, PEG 3000, PEG 550 monomethylether and PEG 500 dimethylether was effected by Trathnigg et al. [50,133] on a C_{18} column with methanol–water mobile phases. Despite satisfactory signal resolution in the lower M_r range, it is obvious that oligomers with higher M_r exhibit substantial peak tailing and more and more merge with the baseline as shown in Fig. 13 for PEG 400. Similarly, Meyer et al. [134] also used isocratic methanol–

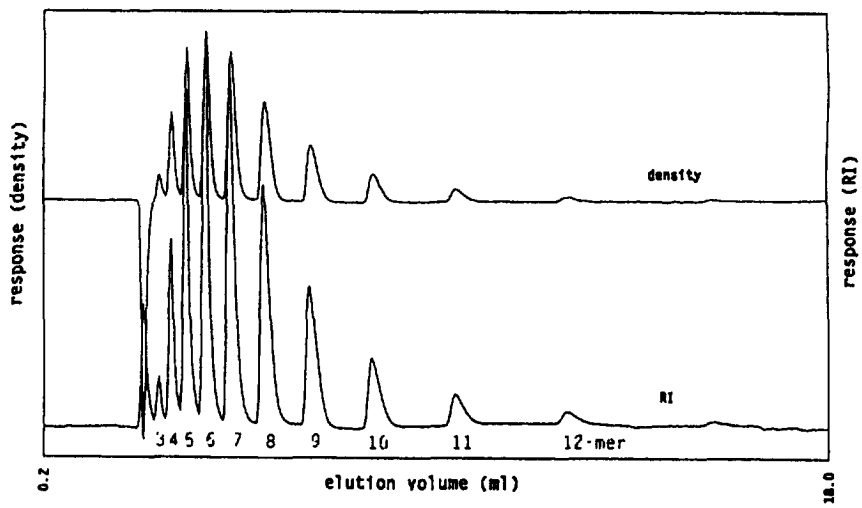

Fig. 13. Density and RI trace of a chromatogram of PEG 400 obtained in methanol–water (30:70) as mobile phase. (From Ref. [133], with permission).

water mixtures for separation of PEGs up to $M_r \cong 2000$, but as in the case of PEG 400 (see Fig. 13), peak broadening with increasing M_r impairs sensitive detection of higher oligomers as shown in Fig. 14. Thus, the use of gradient conditions will be indispensable in overcoming this drawback.

Gradients of acetonitrile (solvent A) and water–methanol (80:20) (solvent B) mixtures as mentioned in Section 2.2.2 also proved to be suitable for separation of PEG oligomers as shown by Schröder [40].

Rissler et al. [135] investigated chromatographic separation of native polyethers and their DNB-derivatives with a wide range of hydrophobicity, including PEG 1000, PPG 1200 and PBG 1000, with either methanol–water or acetonitrile–water gradients on C_1, C_{phenyl}, C_4, C_8 and C_{18} stationary phases. However, due to the comparative purpose of the study also involving much more hydrophobic samples than PEG, such as PPG and PBG, selection of "common" chromatographic conditions was required, where retention was mainly dominated by the latter solutes leading to a substantial loss of signal resolution of PEG 1000. Nevertheless, the oligomeric character of the PEG 1000 sample was still recognizable when the underivatized samples were subjected to these HPLC conditions. In contrast, peak resolution vanished completely after derivatization with DNB-Cl, regardless if methanol or acetonitrile were used as the modifier, except when a C_1 matrix and methanol–water gradients were applied. However, when a more shallow gradient profile was used, substantial improvement of PEG 1000 separation (more than 20 oligomers can be seen with both organic modifiers) and nearly baseline resolution of PEG 600 was achieved on a C_{18} matrix [136]. Similar elution profiles were observed for PEG 200 and PEG 300 yielding baseline separation with methanol as well as acetone and acetonitrile as organic modifier. The elution profiles of PEG 200, PEG 300, PEG 600 and PEG 1000 on a C_{18} stationary phase with acetonitrile as organic modifier are shown in Fig. 15.

Satisfactory resolution of PEG 200, PEG 300, PEG 400, PEG 600, PEG 900 and PEG 1000 was performed by Ruddy and Hadzija [137] in the isocratic mode with methanol–water mixtures on a base-deactivated C_8 column.

A rapid and highly efficient gradient RP-HPLC method for routine analysis of PEGs in water and thus suitable for measurement of samples, which had been subjected to anaerobic digestion, was developed by Moldovan et al. [138]. The authors used different mixtures of acetonitrile in water each containing 1 m*M* of phosphoric acid and a C_{18} column matrix, and separation of up to 50 peaks was effected with a sample containing a mixture of equal amounts of PEG 200, PEG 300, PEG 400, PEG 600, PEG 1000 and PEG 1500.

Despite not belonging to the class of native

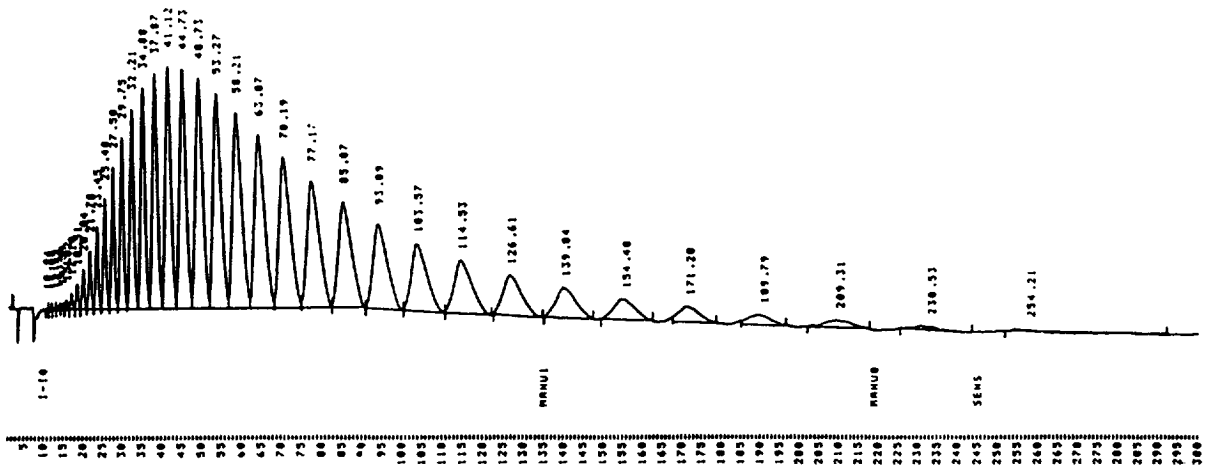

Fig. 14. Chromatogram for PEG 2000.(From Ref. [134], with permission).

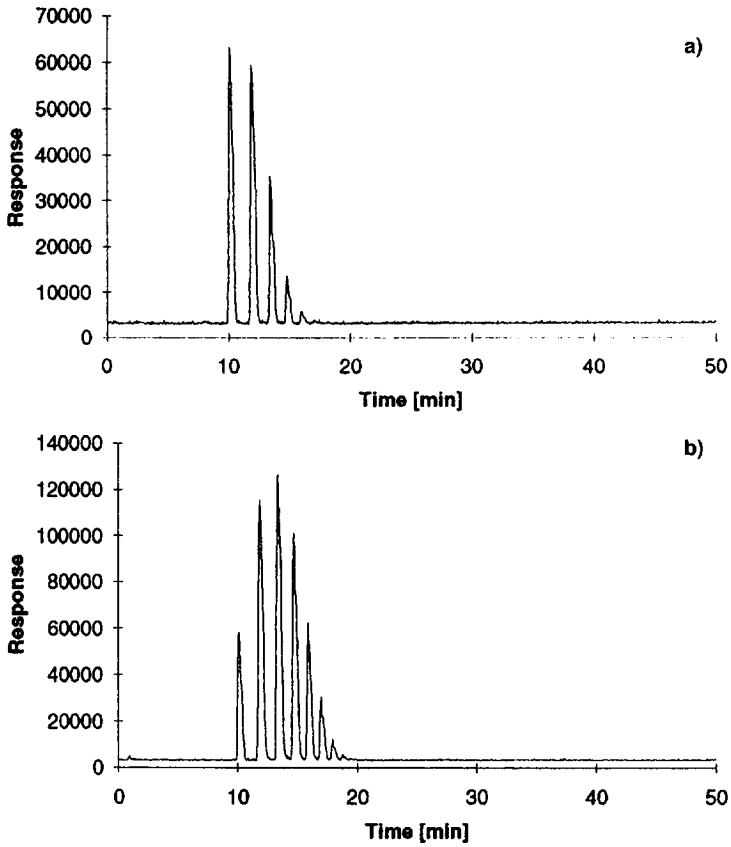

Fig. 15. HPLC-chromatograms of (a) PEG 200 (b) PEG 300 (c) PEG 600 (d) PEG 1000 on a C_{18} column with acetonitrile as organic modifier. (From Ref. [136], with permission).

(underivatized) PEGs, the investigations of Leister et al. [139] performed with methoxy-, amino- and carboxy-substituted samples of average $M_r \cong 3400$ and 5000, and their *p*-nitrophenyl esters and semicarbazides, will be inserted. The authors used a cyanopropyl matrix and acetonitrile–aqueous sodium perchlorate gradients, but the different PEG derivatives eluted as broad peaks. The latter observation is at least partially caused by the low wavelength of 192 nm used for signal monitoring. Nevertheless, the chromatographic patterns are different for the different samples and thus imply a substantial participation of the individual functional groups on separation characteristics, although they contribute only about 1% to the M_r value.

Finally, polythioethylene glycol derivatives exhibit similar chromatographic patterns when subjected to RP-HPLC, as evaluated by Doster and Zentner [140].

2.3.3. Normal-phase liquid chromatography with aqueous organic solvents[3]

The observations of Alexander et al. [80] that peak resolution in RP-HPLC of NPEOs increases with decreasing carbon content of the RP column matrix, prompted Rissler et al. [136] to attempt separation of underivatized PEGs on bare silica gel with mobile phases generally used in RP systems. This rather novel chromatographic alternative will now be reported in more detail.

PEG 200, PEG 300, PEG 600 and PEG 1000 were subjected to gradient chromatography on a Si-80

[3]Recently, separation of alkylphenol ethoxylate non-ionic surfactants on bare silica gel with aqueous acetonitrile eluents and designated as "pseudo reversed-phase" chromatography was reported by Ibrahim and Wheals (J. Chromatogr. A, 731 (1996) 171).

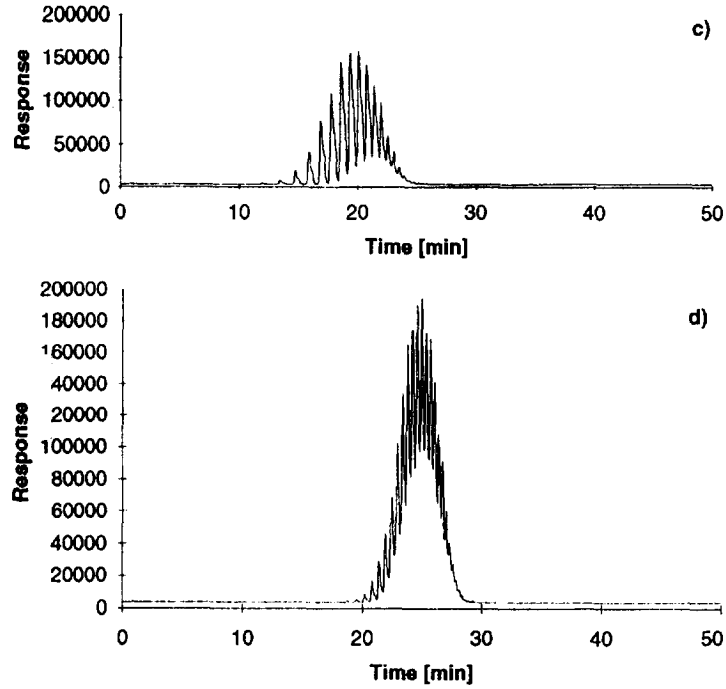

Fig. 15. (*continued*)

column with aqueous solutions of methanol, acetone and acetonitrile as organic modifier. For comparative purpose the PEGs were chromatographed on a C_{18} column (Section 2.3.2). Although the gradient used for the C_{18} stationary phase was less steep and elution was started with 0% acetonitrile, peak resolution on the Si-80 matrix was significantly better, despite the use of a much steeper gradient shape and an initial concentration of 10% acetonitrile. Furthermore, when acetone and acetonitrile were used as modifier, resolution of oligomers was better than with methanol, regardless if bare silica or its octadecylsilyl derivative were used as column materials. Application of bare silica gel and water-soluble organic solvents, normally applied in typical RP-LC, is not a widely used alternative, although some authors have reported separation of polar and even basic solutes with this technique [141–145]. However, it must be taken into account that according to Nahum and Horvàth [146] and Bij et al. [147], reversed-phase chromatography can also be achieved on "naked" silica by use of excessive water in the mobile phase due to its extensive silanol-coating effect. Despite this "coating" of silanol groups, the observations of Alexander et al. [80] nevertheless imply that the separation characteristics of polyethers carrying a hydrophobic alkyl or arylalkyl substituent are mainly governed by the polar superficial layer of free matrix silanols rather than by interactions with ether-like oxygens of the polysiloxane backbone, which in turn, may be responsible for distinct "modulating" effects during the separation process. This view is further corroborated by the observation that a better accessibility of solute molecules to polysiloxane oxygens, as it should be the case with "short-chain" substituted silica gels, such as C_8, C_4, C_{phenyl} and C_1 matrices, did not result in improvement of peak resolution R_s of PEG 1000 oligomers [135]. Furthermore, R_s seems to be preponderantly influenced by interactions between the polyether backbone of the PEGs and the hydrophilic stationary phase and only to a small extent by those between the silica matrix and the hydroxy endgroups of the solute. This is supported by an almost identical

chromatographic pattern after conversion of the PEGs to their diacetates. In conclusion, normal-phase chromatography with solvents used in RPLC opens an effective alternative tool to achieve highly efficient separation of polar polyethers, such as PEGs. The elution profiles of PEG 200, PEG 300, PEG 600 and PEG 1000 on a bare silica gel column with acetonitrile as the organic modifier is shown in Fig. 16.

2.4. Chromatography of native (underivatized) polypropylene and polybutylene glycols

2.4.1. Reversed-phase liquid chromatography of polypropylene glycols (PPGs)

As expected and evidenced by LC, PPGs are much more hydrophobic components when compared with PEGs. Perhaps this substantially increased hydrophobicity may at least partially originate from con-

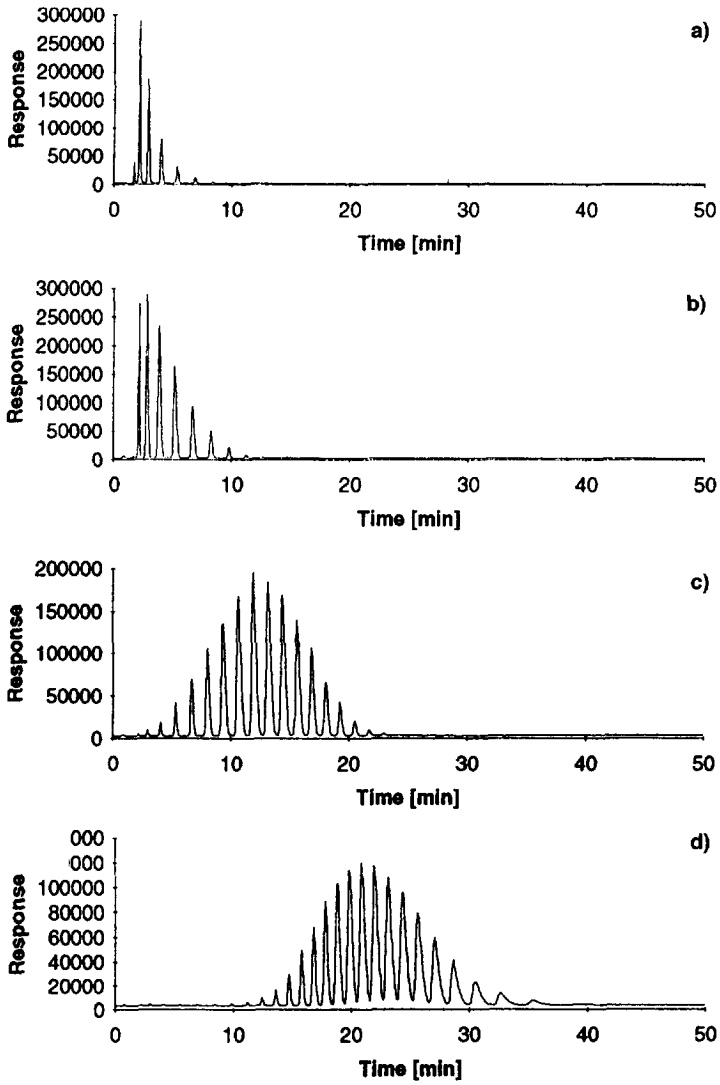

Fig. 16. a–d: HPLC-chromatogrammes of (a) PEG 200 (b) PEG 300 (c) PEG 600 (d) PEG 1000 on a Si 80 column with acetonitrile as organic modifier. (From Ref. [136], with permission).

formational changes attributable to the C_1 "side chain", which compels the polyether to adapt a more unpolar structure by a shielding effect on the polar ether oxygens. Thus, retention will be mainly governed by the hydrophobic C_3 backbone. Due to the lack of work in this area, this section is treated in more detail.

Bergmann and Möller [130] used gradient HPLC with acetonitrile and water the latter containing trace amounts of nitric acid (Section 3.4) for separation of PPG 400 on a C_{18} material and, as expected, a much larger amount of organic modifier as compared with PEGs had to be applied for sample elution. In a similar way, Martin [108] applied a gradient of methanol and water and a C_{18} column. Furthermore, the latter author achieved separation of PEG–PPG copolymers by use of a similar gradient HPLC system as used for separation of PPG oligomers [108].

On the other hand, Trathnigg et al. [133] were compelled to use an isocratic system of methanol and water and a C_{18} column for chromatography of PPG 425 and PPG 1000 oligomers due to signal monitoring by refractive index (RI) and density detection. However, signals of the higher M_r oligomers become progressively broader and increasingly merge with the baseline as revealed for PPG 1000 in Fig. 17. For this reason, complete recovery from the column matrix remains a problem.

A very efficient gradient HPLC system for PPG was developed by Rissler et al. [135]. The authors subjected PPG 1200 as the model component in the underivatized form and its DNB derivative to separation on different silica gel based column materials widely differing in the length of the alkylsilyl chains (e.g., C_{18}, C_8, C_4, C_{phenyl}, C_1). The study revealed that a compromise between an optimum "recovery" of oligomers and corresponding resolution of underivatized oligomers is achievable on a matrix of intermediate hydrophobicity, such as a C_4 column, which also yields quantitative "recovery" of the whole amount of PPG-1200 oligomers. On the other hand, on less polar materials, such as C_8 and, in particular, C_{18} phases peaks of oligomers with higher M_r become increasingly broad and finally merge with the baseline. Fig. 18 shows the elution profiles of native PPG 1200 on C_{18}, C_8, C_4, C_{phenyl} and C_1 stationary phases. Despite the expected marked increase in retention of the PPG-1200 DNB derivative with respect to the native sample and attributable to the hydrophobic endgroups, R_s decreases, regardless of the column material used. Optimum R_s was obtained on a C_{18} matrix, despite the fact that quantitative "recovery" of the whole entity of oligomers requires a less hydrophobic C_8 stationary phase. The results reveal strong hydrophobic solute–stationary phase interactions, in particular, when C_8 and C_{18} materials are used, which however, are

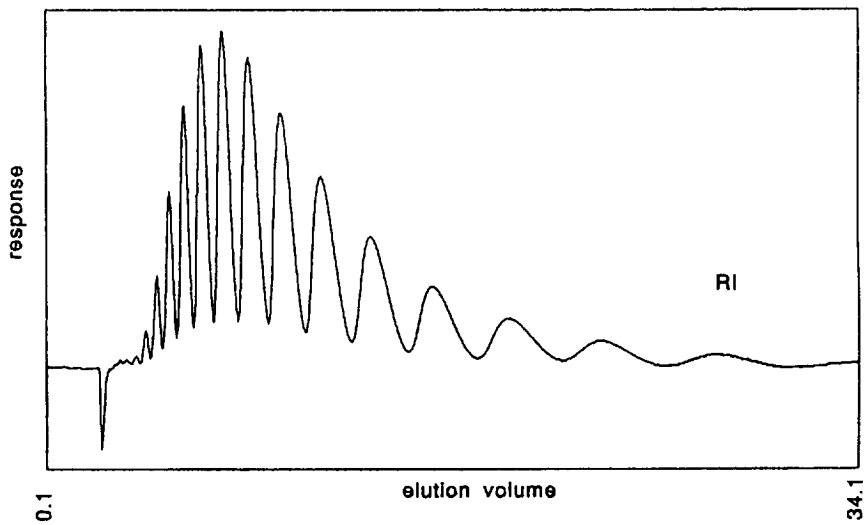

Fig. 17. RI trace of a chromatogram of PPG 1000 obtained in methanol–water (80:20) as mobile phase. (From Ref. [133], with permission).

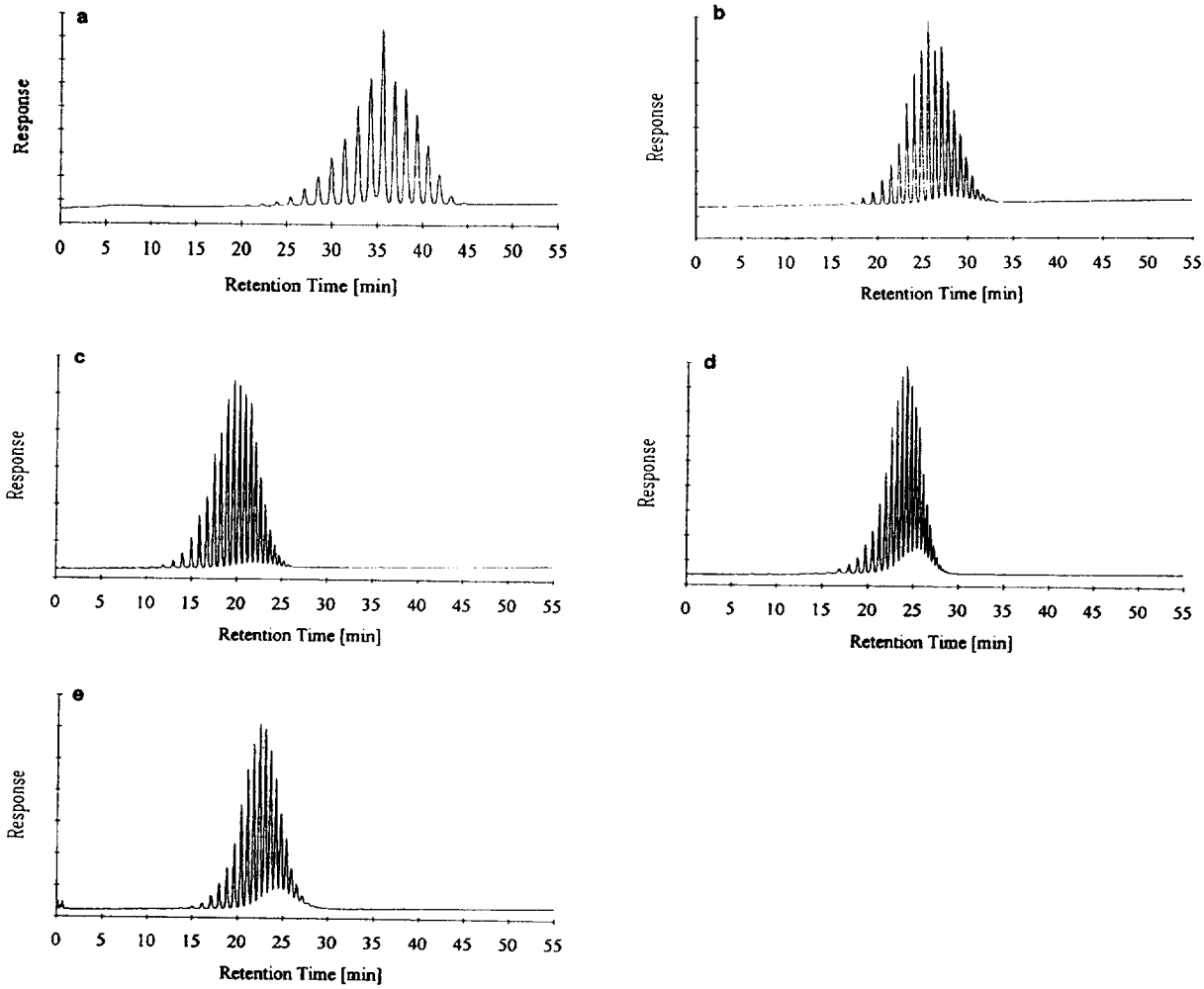

Fig. 18. Chromatograms with PPG-1200 and acetonitrile as organic solvent. (a) C_{18}; (b) C_8; (c) C_4; (d) C_{Phenyl}; (e) C_1. (From Ref. [135], with permission).

markedly attenuated on less hydrophobic column matrices (e.g., C_4, C_{phenyl}, C_1) due to the substantially smaller interactive surface area exposed to the unpolar structural segments of PPG. In a further study, Rissler et al. [148] examined the influence of different organic modifiers (e.g., acetonitrile, methanol, ethanol and 2-propanol) on retention of native PPG-1200 on a C_{18} matrix and found a decrease in retention in the order methanol>acetonitrile> ethanol>2-propanol. Peak resolution of PPG-1200 oligomers decreases in the order acetonitrile> methanol>ethanol>2-propanol. The decrease of retention within the class of the alcoholic modifiers from methanol to 2-propanol is in accordance with the expected increase in lipophilicity in the same direction and thus, displacement of PPG-1200 oligomers from the hydrophobic column matrix is much more effective with 2-propanol compared with methanol. The potency of 2-propanol on oligomer resolution is also markedly lower than with methanol, because it is a too strong solvent giving "compression" of high-M_r oligomers into one broad and unresolved signal and thus exerts a "levelling" effect.

When the M_r values exceed 2000 and more polar RP matrices, such as C_4, C_{phenyl} and C_1 stationary

phases and ethanol or 2-propanol as organic modifiers are used, which are necessary to effect complete elution, separation into individual oligomers becomes increasingly difficult and in most cases more or less broad and unresolved peaks are obtained (Rissler, unpublished results). This observation leads to the assumption that RP-LC will be more or less superimposed by a size exclusion mechanism, as already proposed by Noguchi et al. [47] for higher PEG oligomers with 2-propanol on a polymer gel. Nevertheless, retention times of peak maxima of PPG samples widely differing in M_r are sufficiently different from each other and thus allow an assignment to an individual type of PPG.

A special class of PPGs, which in the strongest sense do not directly belong to this family, but nevertheless derive from it, consists of the amino-terminated PPGs, the so-called Jeffamines as already mentioned in Section 1. Rissler [149] has investigated PPG derivatives of this type in the M_r range of 400 to approx. 5000 by HPLC after derivatization of the free primary amine groups with a mixture of pyridine and acetic acid anhydride with either acetonitrile or methanol as organic modifier on C_{18}, C_8, C_6, C_4 and C_1 stationary phases and evaporative light-scattering detection (ELSD). Due to their strong basic character the underivatized samples show extensive interactions with the column matrix, regardless if typical RP columns (see above) or so-called bonded-phase materials (CN, NH_2, diol) were used. These presumptive silanophilic interactions [146,149–155] prevent their elution from the column with pure aqueous organic solvents lacking any buffer components or ion-pairing reagents, as evidenced by LC–MS investigations of the column effluent from different column materials [149]. It should be emphasized that the choice of pure aqueous organic mixtures, which do not contain non-volatile buffer components, is a prerequisite for the use of ELSD treated in Section 3.6. Furthermore, it was found that in accordance with earlier investigations [135,148] concerning separation of native PPGs, acetonitrile was superior to methanol with respect to its potency to discriminate between different PPG amide oligomers. However, recovery remains incomplete on the RP matrices when the M_r range exceeds 2000, except on a C_1 material. In this case methanol proved to be the more efficient organic solvent and complete recovery was achieved on all tested stationary phases. Best results were obtained on a C_4-matrix with either acetonitrile and methanol, the latter being the solvent of choice for high M_r samples and despite the co-elution of oligomers with M_r>2000, different samples can still be discriminated by different retention times. The superiority of methanol over acetonitrile with respect to elution of high-M_r PPG amides was ascribed to a better solvation of the polypropylene glycol backbone by hydrogen bonding between the ether oxygens and the hydroxyl groups of the protic modifier, which is treated in more detail in Section 2.4.2. Fig. 19 reveals the elution profiles of Jeffamines D-230, D-400, T-403, D-2000 and T-5000 on a C_4-matrix with methanol as organic modifier. Silanophilic solute–matrix interactions of underivatized samples can also be overcome by addition of small amounts of trifluoroacetic acid (TFA) to the mobile phase, which is compatible with ELSD, but nevertheless resolution of oligomers is much lower as compared with the amide derivatives due to substantial peak broadening, especially for lower-M_r oligomers [149].

2.4.2. Reversed-phase liquid chromatography of polybutylene glycols (PBGs)

Little chromatographic work has hitherto been published with respect to PBGs and, for this reason, as with the PPGs, the special structure-inherent problems are treated more extensively. PBGs are much more hydrophobic than either PEGs or PPGs due to the tetramethylene bridge between the ether oxygens and, therefore, some chromatographic peculiarities are observed.

In a comparative study including polyethers largely differing in hydrophobicity (e.g. PEG 1000, PPG 1200, PBG 1000) Rissler et al. [135] attempted to optimize the HPLC of PBGs. The authors observed that only a minor amount of PBG 1000 oligomers was eluted from a C_{18} column with acetonitrile as organic modifier, but the number of oligomers leaving the column increased significantly when separation was performed at 60°C. By use of a less hydrophobic C_8 matrix much more oligomers were eluted from the column compared with the C_{18} stationary phase, although quantitative "recovery" of the whole amount of oligomers was not achieved, and only when the C_8 matrix was operated at 60°C

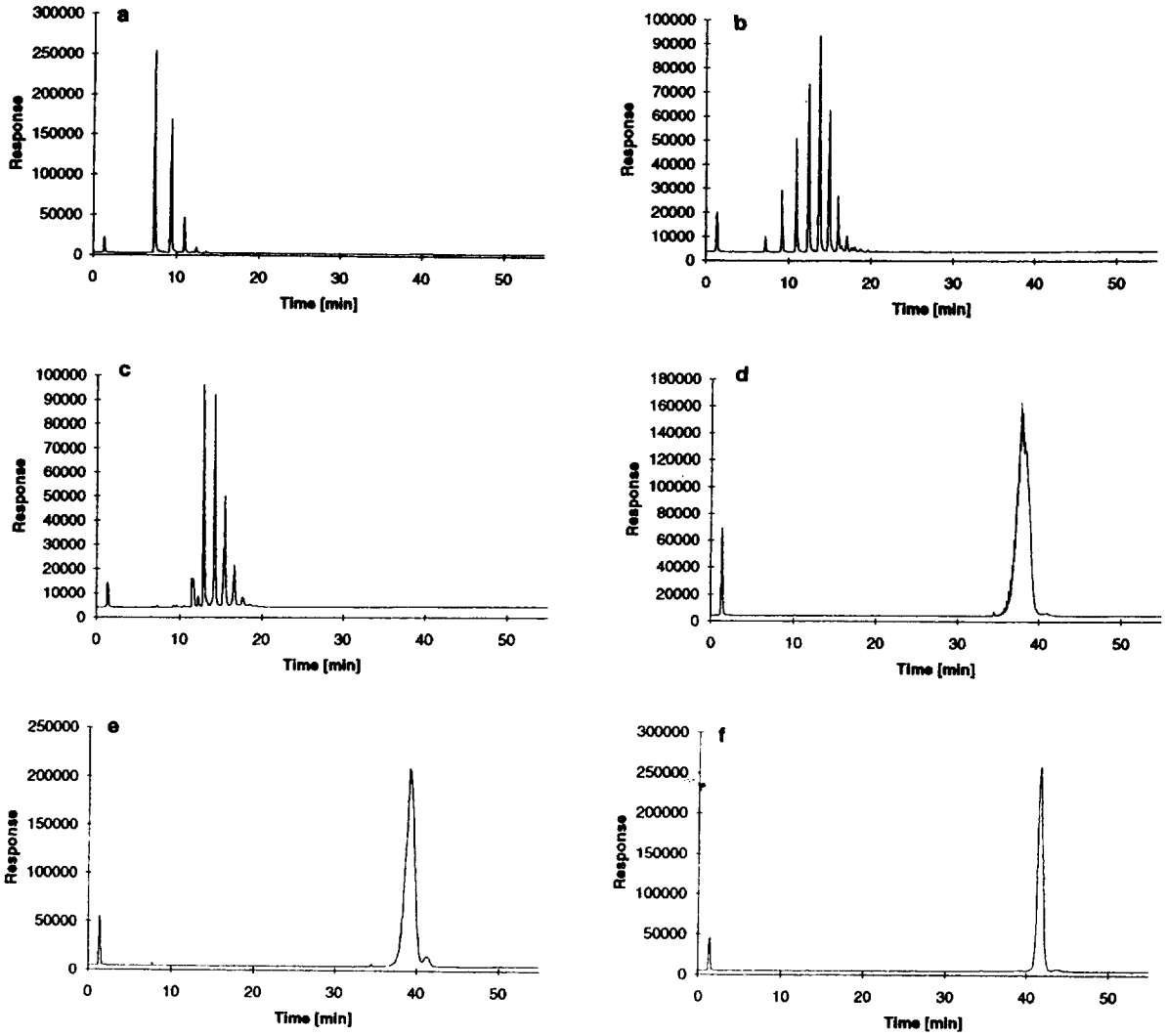

Fig. 19. Chromatograms of (a) Jeffamine D 230, (b) Jeffamine D 400, (c) Jeffamine T 403, (d) Jeffamine D 2000, (e) Jeffamine T 3000 and (f) Jeffamine T 5000 on a C_4 column with methanol as organic modifier. (From Ref. [149], with permission).

was complete "release" of all oligomers observed. In contrast to these findings, acetonitrile effected complete elution of PBG oligomers at room temperature (RT) on C_4, C_{phenyl} and C_1 phases. The elution profiles of native PBG 1000 on C_{18}, C_8, C_6, C_4 and C_1 stationary phases with acetonitrile as organic modifier at RT are depicted in Fig. 20.

When the aprotic solvent was replaced by methanol, approximately twice the number of PBG oligomers were eluted from a C_{18} column and complete elution took place at RT on a C_8 matrix, but unfortunately the higher-M_r oligomers showed unsatisfactory peak resolution and a concomitant tendency to merge into one broad peak. In order to give a reasonable explanation of the chromatographic observations, the authors discussed a "solubility effect", taking into account either the obvious increase of acetonitrile's elution potency at 60°C or the possible formation of hydrogen bonding between methanol and ether oxygens. In the first case a rise in temperature will cause a solubility shift of higher-M_r oligomers away from the network of the extremely

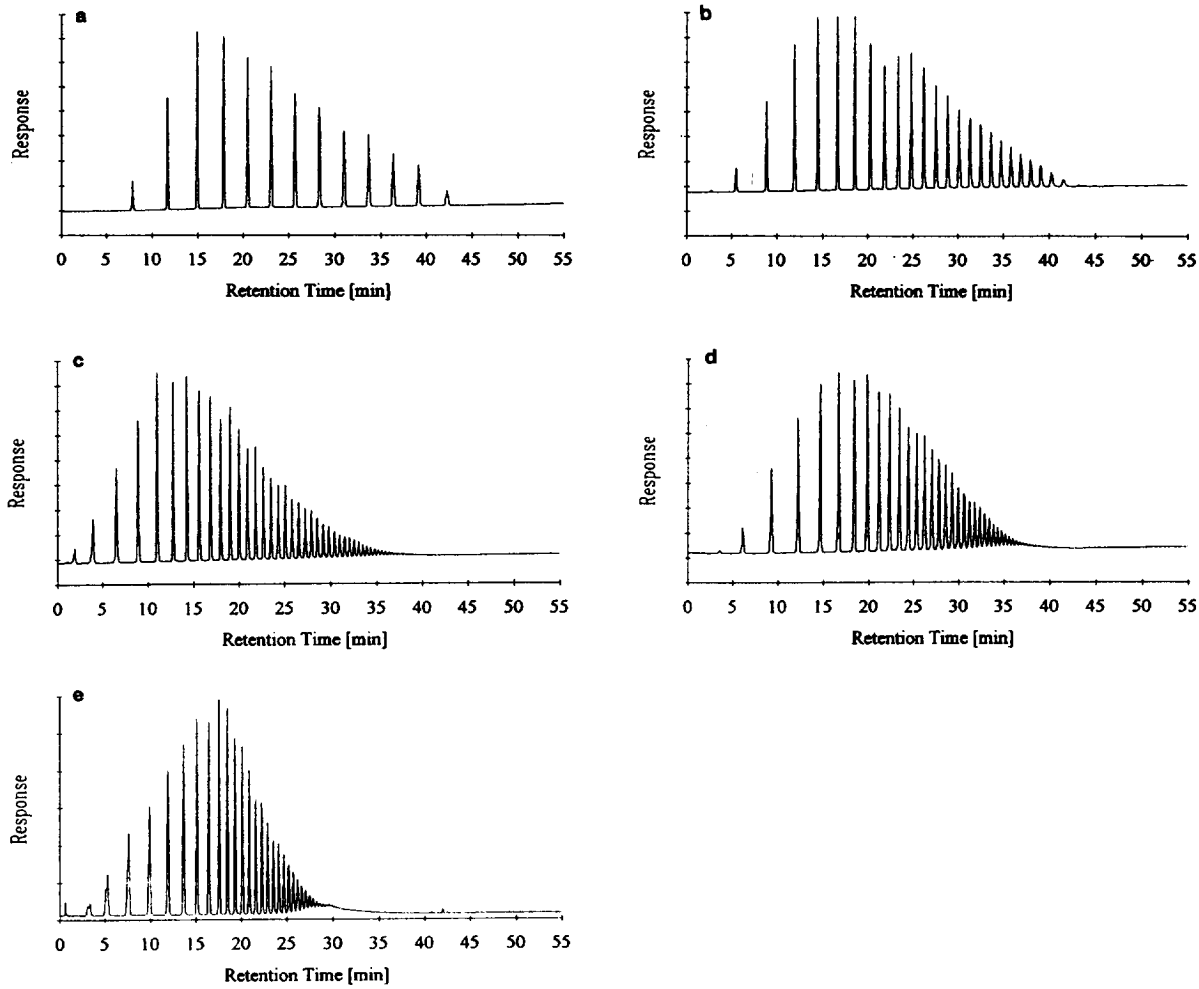

Fig. 20. Chromatograms with PBG-1000 and acetonitrile as organic solvent. (a) C_{18}; (b) C_8; (c) C_4; (d) C_{Phenyl}; (e) C_1. (From Ref. [135], with permission).

hydrophobic C_{18} stationary phase into the aprotic modifier, whereas in the latter case the better solubility of higher-M_r oligomers in the protic modifier, presumably mediated via hydrogen bonding interactions, will effect complete elution even at RT. No temperature effect was observed with methanol as organic solvent and, therefore, clearly supports the presumptive solubility increase by formation of hydrogen bonding, which can not be further improved by a rise in temperature. It is assumed that the elution power of acetonitrile did not suffice for efficient solute displacement from the dense layer of strongly hydrophobic C_{18} substituents of the column matrix. In particular for high-M_r oligomers, a greater interactive surface is exposed to the stationary phase. However in contrast, the aprotic modifier should be more effective when silica gels substituted with much shorter alkyl or even aryl groups are used. Indeed, complete "release" of the whole entity of PBG oligomers was accomplished on C_4, C_{phenyl} and C_1 materials.

As in the case of PPG 1200, the corresponding PBG 1000 DNB derrivatives exhibit substantial increase in retention. When compared with the native samples, the higher the M_r of the oligomers the lower the difference in retention compared with

corresponding peaks of underivatized PBG, whereas low-M_r oligomers exhibit much more retention with respect to oligomers with identical degree of polymerization in native PBG 1000. The same effect was seen after transesterification of 4-hydroxybenzoic acid methylester with PBG 1000 yielding the corresponding α,ω-di(4-hydroxybenzoyl) derivative (Rissler, unpublished results). The cause of this relative signal compression of high-M_r oligomers may presumably consist in an obvious decrease of hydrophobicity with increasing M_r. Perhaps this may be partially attributable to conformational effects. For this reason, the increase in hydrophobicity after derivatization with DNB-Cl will be the lower the higher the M_r of the PBG oligomers, whereas in contrast, the more polar low-M_r oligomers are much more effected by the hydrophobic "increment" of the DNB residues. Fig. 21 exhibits the elution profiles of PBG 1000 derivatized with DNB-Cl obtained at RT on C_{18}, C_8, C_6, C_4 and C_1 stationary phases with acetonitrile as organic modifier.

In another study, Rissler et al. [148] investigated the influence of the four organic modifiers acetonitrile, methanol, ethanol and 2-propanol on retention

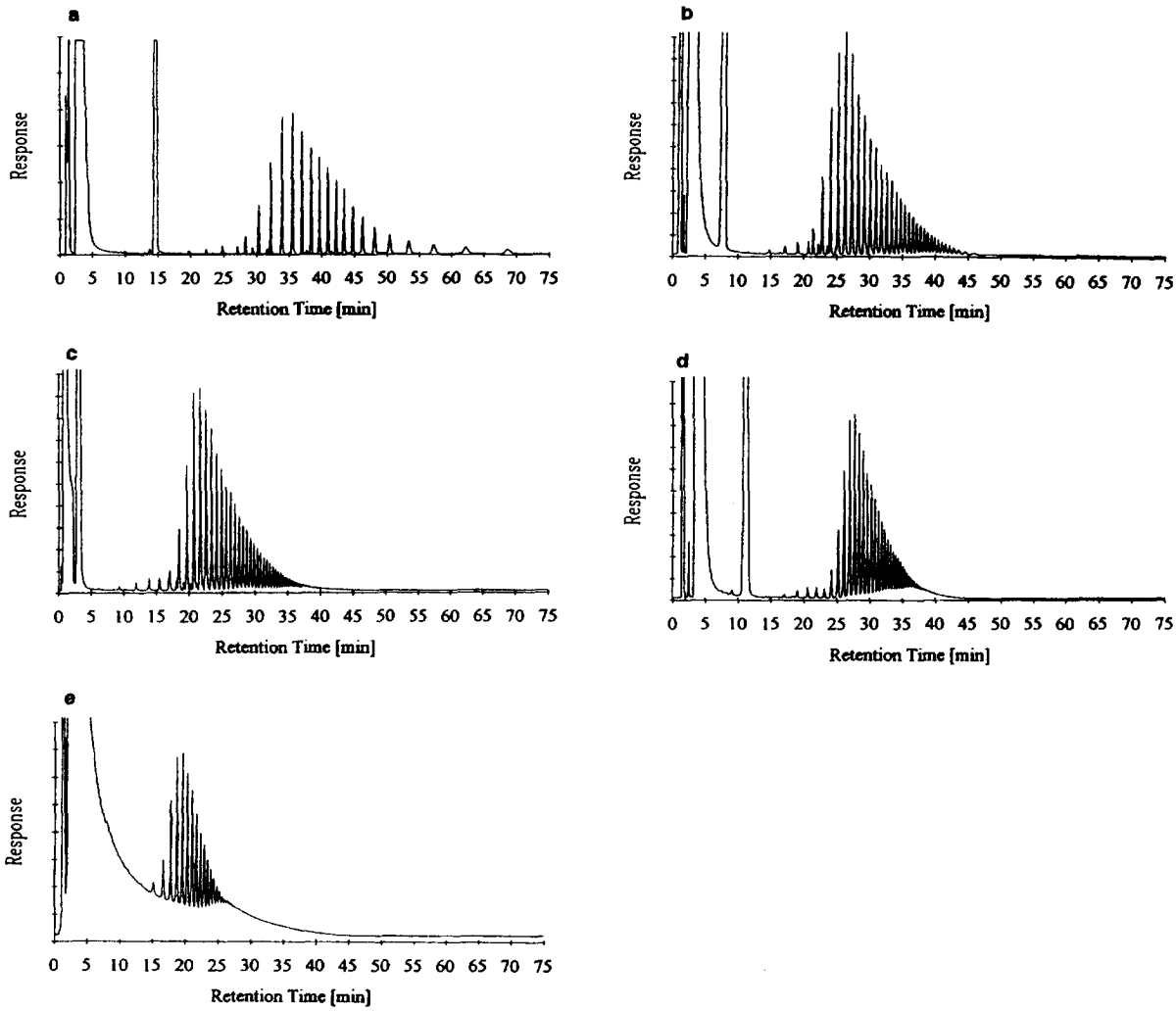

Fig. 21. Chromatograms with PBG-1000 after derivatization with DNBCl and acetonitrile as organic solvent. (a) C_{18}; (b) C_8; (c) C_4; (d) C_{Phenyl}; (e) C_1. (From Ref. [135], with permission).

and resolution characteristics of PBG 1000 on a C_{18} stationary phase. Whereas neither acetonitrile nor methanol effected complete elution of all oligomers, quantitative recovery was achieved with ethanol and 2-propanol. Although it may be a reasonable assumption that methanol will be equally effective in the formation of hydrogen bonding compared with ethanol and 2-propanol, the latter solvents show superior elution potencies. The authors hypothesized an additional solvation effect of the hydrophobic tetramethylene backbone of the PBGs by the more lipophilic alcohols ethanol and 2-propanol, which may be crucial for high-M_r oligomers. Thus, a synergy of both hydrogen bonding and "tetramethylene backbone solvation" was postulated by the authors, which should be responsible for complete elution of the whole amount of sample constituents. Nevertheless, this synergy invokes strong eluent characteristics unfortunately resulting in only poor resolution of high-M_r PBG oligomers. However, at least a partial superimposition of a size exclusion effect, as discussed in Section 2.4.1 by Noguchi et al. [47], cannot be ruled out. The influence of the different modifiers on the elution profiles of PBG 1000 on a C_{18} matrix is shown in Fig. 22.

As reported in Section 2.4.1, it becomes increasingly difficult to achieve satisfactory separation of PPGs exceeding a M_r of 2000. On the other hand, peak resolution increases with increasing hydrophobicity of the polyether backbone as seen with PBG 1000 [134]. This observation prompted Rissler and Fuchslueger [156] to exploit the separation capability of moderately hydrophobic stationary phases with respect to more hydrophobic polyethers, such as PBGs largely differing in M_r. For this reason, the authors subjected PBG 650, PBG 1000, PBG 2000 and PBG 3000 to RP-LC on a C_4 matrix with either acetonitrile or methanol as organic modifier. Excellent separation was achieved with acetonitrile for all four samples yielding 38 (PBG 650), 40 (PBG 1000), 47 (PBG 2000) and 51 (PBG 3000) clearly distinguishable oligomer peaks, most of which showed baseline separation. In contrast, methanol proved poor with respect to peak resolution. Only PBG 650 and PBG 1000 oligomers of low to medium M_r were sufficiently resolved, whereas in

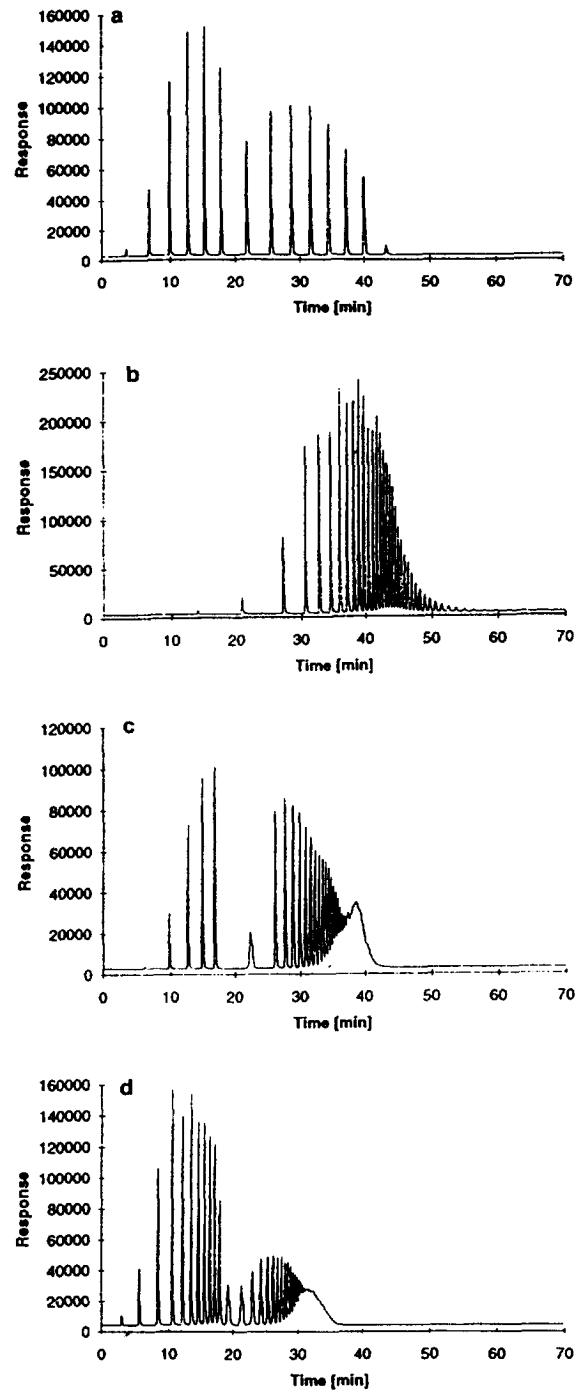

Fig. 22. HPLC of PBG 1000 on a C_{18} column with (a) acetonitrile, (b) methanol, (c) ethanol and (d) 2-propanol as the organic modifier. (From Ref. [148], with permission).

particular the high-M_r oligomers of PBG 2000 and PBG 3000 merge into a broad peak. In conclusion, peak resolution within a group of PBGs widely differing in M_r can be exploited in an optimum manner by use of moderately hydrophobic stationary phases, such as C_4 materials. In contrast, the PBG samples are too strongly retained on C_{18} and C_8 materials and cannot completely be eluted with acetonitrile, which on the other hand, proves to be the eluent of choice for low-M_r sample constituents. Unfortunately, protic modifiers (e.g., methanol, ethanol, 2-propanol) are less suited due to their poor discriminative potency for high-M_r oligomers. The capability of acetonitrile as organic modifier for peak resolution of PBG 650, PBG 1000, PBG 2000 and PBG 3000 on a C_4 material is demonstrated in Fig. 23.

2.5. Liquid chromatography under critical conditions (LCCC)

The so-called liquid chromatography under critical conditions (LCCC) is a typical method used in polymer analysis as the first step of "two-dimensional" chromatography. LCCC is applicable either in the normal or the reversed-phase mode and separates mixtures of polymer components according to their chemical composition. The peculiarity of this procedure is that it focuses on either distinct structural features within the molecular backbone (e.g. PEG, PPG, PBG) or different endgroups, such as alkyl, arylalkyl, alkanoyl and aroyl substituents of variable chain length. Depending on the separation conditions, all molecules possessing the same structural moiety (e.g. polymer backbone with identical repeat monomeric units) are merged into one peak and separation is only achieved according to the different functional endgroups. In a similar way oligomeric mixtures of heteropolymers can be separated according to the composition of monomer I and monomer II. At solvent composition I, separation according to the functionality type distribution or chemical composition of monomer I in the sample, regardless of the chain length of segments built up from monomer II and their distribution throughout the whole heteropolymer, is achieved the latter becoming chromatographically "invisible", whereas the reverse takes place at solvent composition II. In a following step, the different peaks containing components showing common structural sequences, but more or less different in M_r distribution are separated according to their molecular mass by SEC, which can be effected either "on-line" by use of a column-switching device and reconditioning of the mobile phase to SEC or "off-line" after direct isolation of components and re-injection into the SEC system. However, the restriction has to be made that in the strong sense LCCC can only be applied to block copolymers (e.g., two-block copolymers) or the backbone of grafted copolymers but, in contrast, is not valid for statistical copolymers.

Fundamental work on this highly efficient and promising separation technique has been published by Gorshkov et al. [157–160], Schulz et al. [161], Pasch et al. [162–166], and Skvortsov and Gorbunov [167]. Therefore, it is reasonable to present a short description of the principle of LCCC.

Isocratic retention for e.g., a homopolymer at a specific temperature can be divided into three different separation modes based on (i) an exclusion, (ii) a transition and a (iii) an adsorption mechanism. Depending on the composition of the used solvents and non-solvents[4], all three separation modes can be observed. In the size exclusion mode a strong eluent is used, which in the ideal case prevents any interactions between solute and stationary phase, thus effecting elution of sample constituents in the range of decreasing M_r. By increasing the percentage of non-solvent the solvent strength decreases and thus, retention increases. Therefore, the retention mechanism more and more changes from pure size exclusion to adsorption. When the percentage of non-solvent is further increased, the solvent strength gradually decreases and, as a consequence, interaction between solute and the column packing occurs, resulting in separation of molecules proportional to their M_r. The peculiarity of LCCC is that it

[4]The term solvent–non-solvent depends on the solute used. As an example of LC of polystyrenes on either NP or RP matrices methanol is a non-solvent, whereas in contrast it is a good solvent for polyether derivatives by use of either NPLC or RPLC.

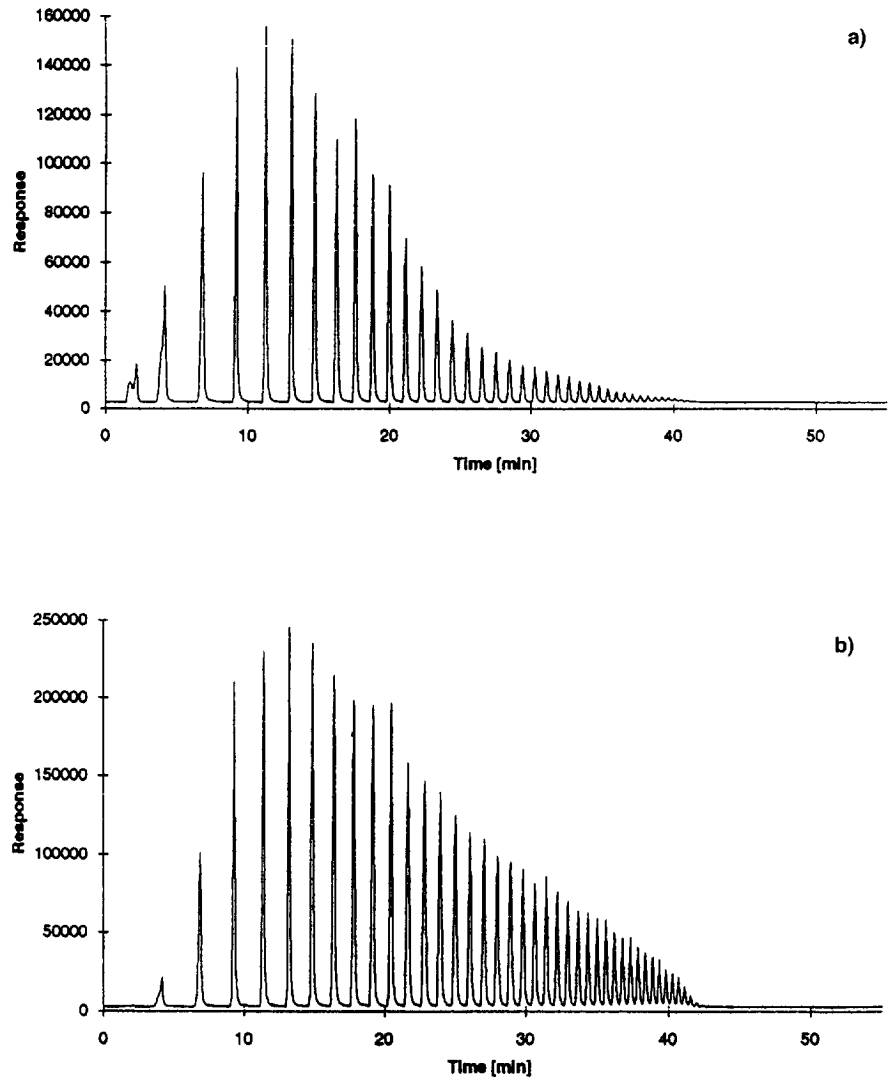

Fig. 23. Chromatograms of (a) PBG 650, (b) PBG 1000, (c) PBG 2000 (d) PBG 3000 on a C_4 column and acetonitrile as organic modifier. (From Ref. [156], with permission).

represents the transition point between size exclusion and adsorption. In other words, the critical point of adsorption is characterized by a complete compensation of the enthalpic and entropic terms of adsorption of a solute during its interaction with the stationary phase. If the polymers differ in molar mass, but not in chemical structure of repeat units, a non-solvent–solvent ratio can be found, at which the polymer molecules merge into a common peak, regardless of their M_r. The specific and "functionality-type" dependent non-solvent–solvent ratio is referred to as the critical solvent composition (CSC). The CSC depends on the temperature, the type of non-solvent–solvent mixture and the polymer. At the CSC separation is independent of M_r of the polymer molecules, which means that e.g., two oligomeric samples containing the same polymer backbone (i.e., consisting of identical repeat units) but having different endgroups yield only two signals in the chromatogram instead of the multitude of signals

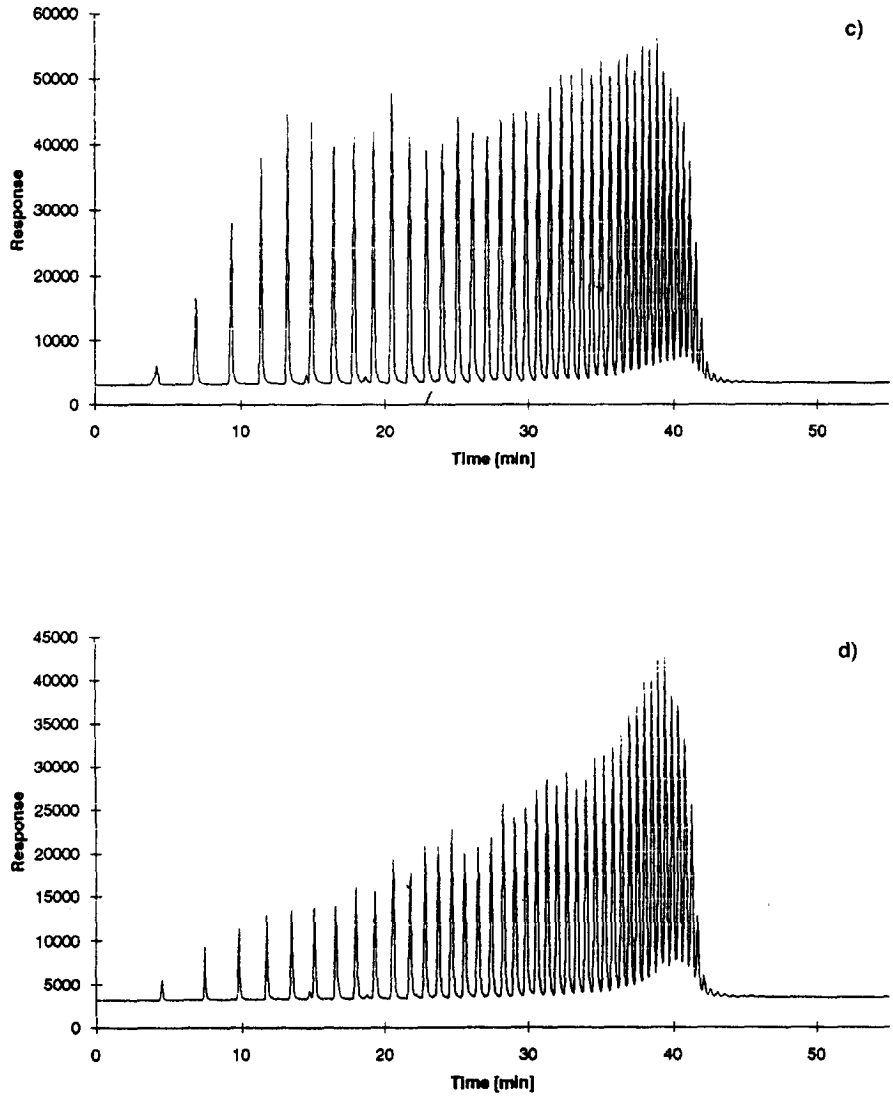

Fig. 23. (*continued*)

obtained by liquid adsorption chromatography (LAC). The same procedure can be applied to polymers having identical endgroups, but markedly differ in the chemistry of the repeat units. Nevertheless, it should be emphasized that the LCCC principle is only valid if the polymer coils are smaller than the largest pores of the stationary phase.

Despite its elegancy, the method suffers from the time-consuming procedure, which is necessary to determine the CSC point. For this reason, Cools et al. [168] have developed a new method allowing a relatively fast approximation of CSC conditions. The authors determined the retention volumes of different polymer standards starting isocratically with 100% of "good" solvent and then increased the amount of non-solvent. The curves (k' versus percentage of non-solvent) of all standards with different M_r were plotted in one figure, which is called the CSC plot. The intersection point of the individual curves relates to the non-solvent composition, at which all standards containing the same structural segments (e.g., repeat units) but showing quite different M_r dis-

tribution, elute simultaneously. The method offers the advantage that only a few solvent–non-solvent compositions are required for identification of the CSC point.

The published contributions with respect to LCCC of polyether derivatives are still relatively small, but a large increase is to be expected in the near future.

Gorshkov et al. [158] achieved separation of different trifunctional PPGs according to their functionality type distribution (FTD) by normal-phase chromatography with methyl ethyl ketone–ethyl acetate (7:93) on a bare silica gel matrix. Furthermore, Gorshkov et al. [160] separated PEGs, PPGs and block copolymers of both components according to FTD, which are invoked by terminal alkyl groups of different size and chemistry, and to structural inhomogeneity within the copolymers. Satisfactory information with respect to the oligomer distribution within the PPG block was obtained at critical conditions of PEG (42% acetonitrile). However in contrast, when attempting analysis of these block copolymers at critical conditions of PPG by reversed-phase HPLC in order to get insight into the oligomer distribution of the PEG block(s), the authors observed strong tailing of peaks, which strongly impaired complete information on the whole composition of the block copolymer. According to the authors, strong interactions between residual silanols on the column matrix and the polar PEG structural units are responsible for the undesired chromatographic behavior.

Separation of reaction products obtained by polymerization of 1,3,6-trioxocane (which represents a cyclic acetal between diethylene glycol and formaldehyde) with benzyl alcohol as the starter according to FTD and subsequent SEC of preparatively isolated fractions was described by Schulz et al. [161]. The repeating unit of the oligomer (polymer) is given by the structural segment $-(O-CH_2-O-CH_2-CH_2-O-CH_2-CH_2-)_n-$. On a C_{18} stationary phase the solvent composition at critical conditions with respect to the repeating trioxocane unit is a mixture of acetonitrile–water (49.5:50.5). Besides hydroxy-, benzyloxy-, and dibenzyloxy-terminated species, small amounts (approx. 1%) of cyclic oligomers were observed.

Pasch et al. [162] reported on two-dimensional separations of block copolymers of PEG and PPG with respect to the length of the PPG block with LCCC as the first dimension at critical conditions of PEG, followed by analysis of separated fractions concerning oligomer distribution of the PEG block by either SFC or SEC.

Trathnigg et al. [169] described separation of ethoxylated C_8 fatty alcohols under critical conditions in methanol–water mobile phases using a C_{18} matrix, and clear separation of the whole entity of ethoxymers, all merging into one common peak from small amounts of the starting material octanol and PEG as the by-product, was achieved. Furthermore, in a study on reproducibility and optimization of LCCC conditions Trathnigg et al. [170] subjected PEG, PPG and fatty alcohols to RPLC on either analytical or semi-preparative C_{18} columns after different times of use with methanol–water mixtures as mobile phases. The authors observed a wide variation of the methanol/water ratio when they established the CSC values for the different columns. In order to optimize chromatographic conditions for a block copolymer of PEG and PPG, the optimum will depend on the relative length of both blocks. In a copolymer with a rather short PEG block the more hydrophobic PPG structural segment determines the mobile phase composition, whereas if it is longer, the best composition must approximate to the critical point of adsorption.

Pasch and Zammert [165] subjected polyethoxylates carrying C_{10}, C_{12}, C_{13}, C_{13}/C_{15} alkyl substituents as well as OPEOs and NPEOs to separation conditions with acetonitrile–water mixtures at the critical point of adsorption of PEG. However, due to the marked hydrophobicity, even the C_{12} polyethoxylate exhibits a broad and largely tailing peak and the C_{15} analogue was not elutable at all within 60 min. As a consequence, a less hydrophobic C_8 matrix was chosen in order to achieve separation within a reasonable time scale. Their investigations further suggest that even in the "SEC region" separation according to chemical composition should be possible, as can be concluded from the differences in retention times at higher concentrations of acetonitrile. Although substantial "peak-splitting" into a multitude of oligomers was obtained, which thus is in contrast to "classical" LCCC, a substantial shift of oligomers differing in only one methylene group in the alkyl chain from each other, was observed.

This has been verified by subjection of the preparatively isolated individual peaks to matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) yielding proof of separation according to different functionality type distribution (FTD). Despite good signal resolution with respect to FTD, there are still some amounts of oligomers recognizable, which arise from a neighbouring FTD due to incomplete separation under the experimental conditions. Fig. 24 shows chromatograms of functional PEGs at the critical point of adsorption and a "critical diagram" of molar mass vs. retention time, and Fig. 25 reveals that under critical conditions separation is only achieved with respect to the endgroups, but not according to the number of EO units. Pasch and Rode [166] separated PEGs of different FTD near the critical point of adsorption, which still revealed distribution of individual oligomers, and measured the contents of collected fractions mass spectrometrically by MALDI-MS. In addition, they subjected a three-block copolymer of PEG and PPG to LCCC at the critical

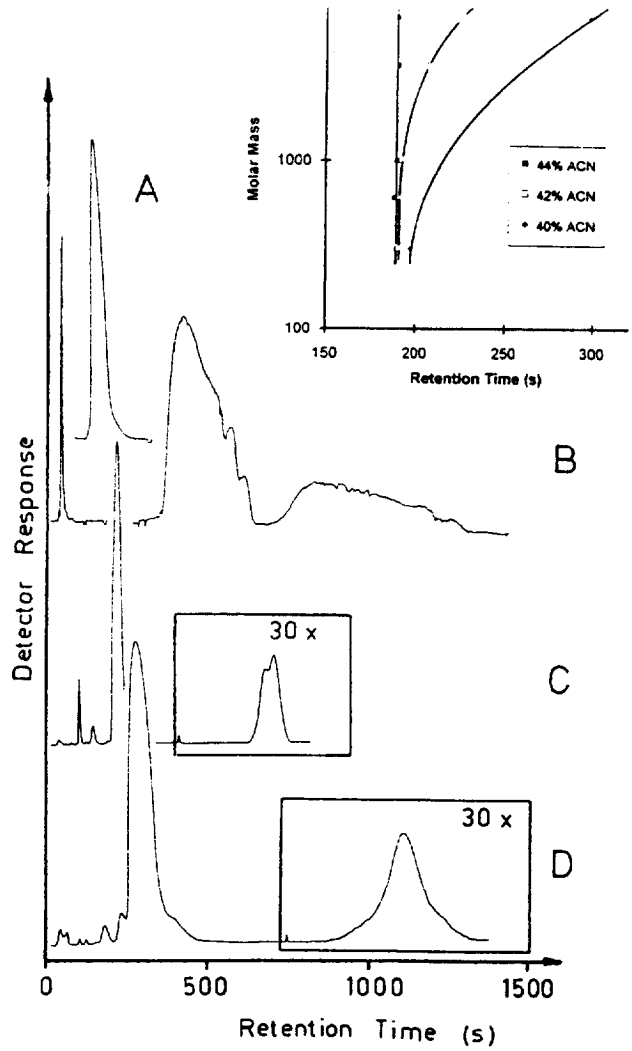

Fig. 24. Critical diagram molar mass vs. retention time of polyethylene glycol (insert upper right corner) and chromatograms of functional PEOs at the critical point of adsorption of PEO, stationary phase: Nucleosil RP-8, 60×4 mm I.D., solvent: acetonitrile–water (44:56) by volume, samples: C_{10}-PEO (A), C_{13}, C_{15}-PEO (B), Octylphenol-PEO (C), Nonylphenol-PEO (D). (From Ref. [165], with permission).

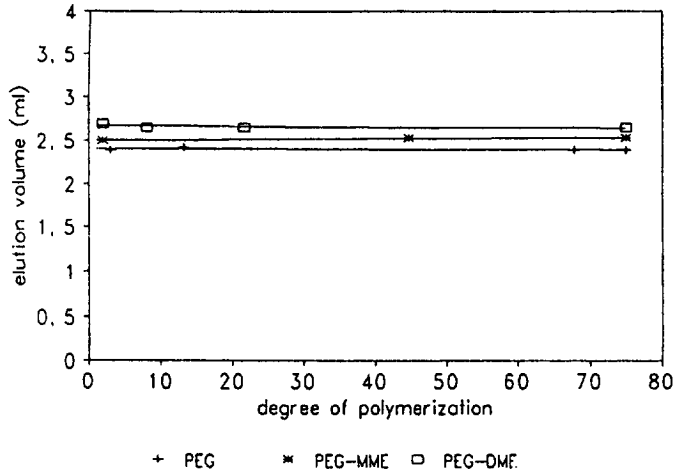

Fig. 25. Elution volumes of polyethylene glycol derivatives in methanol–water (80:20) (critical conditions for PEG) as a function of degree of polymerization. (From Ref. [133], with permission).

point of the PEG blocks. The degree of polymerization of the PPG block was then evaluated by comparison of peaks of a PPG reference sample and oligomer distribution within the PEG blocks of individual fractions was subsequently determined by MALDI-MS. Fig. 26 reveals separation of a PEG sample at the critical point of adsorption and analysis of fractions by MALDI-MS.

Desmaizières and Desbène [171] described two-dimensional analysis of fatty alcohol surfactants resulting from co-polymerization of EOs and POs. Separation of C_{16} and C_{18} fatty alcohol copolymers was accomplished with acetonitrile–water mixtures using C_2, C_8 and C_{18} stationary phases near "critical conditions" (65–35% of acetonitrile depending on the stationary phase used). The strong retention of C_{16} and C_{18} copolymers on strongly hydrophobic materials, such as e.g., a C_{18} matrix, which impairs quantitative determinations by substantial peak broadening could be markedly reduced by use of the more "polar" C_2 and C_8 matrices. Subsequently, separation according to the number of PO units was achieved with a 1:1:1 mixture of acetonitrile–methanol–water. Aqueous solutions of acetonitrile in water and bare silica gel was then applied for separation with respect to the number of EO units, as similarly described by Rissler et al. [136] in the case of underivatized PEGs. Structural confirmation was done "off-line" by physico-chemical measurement

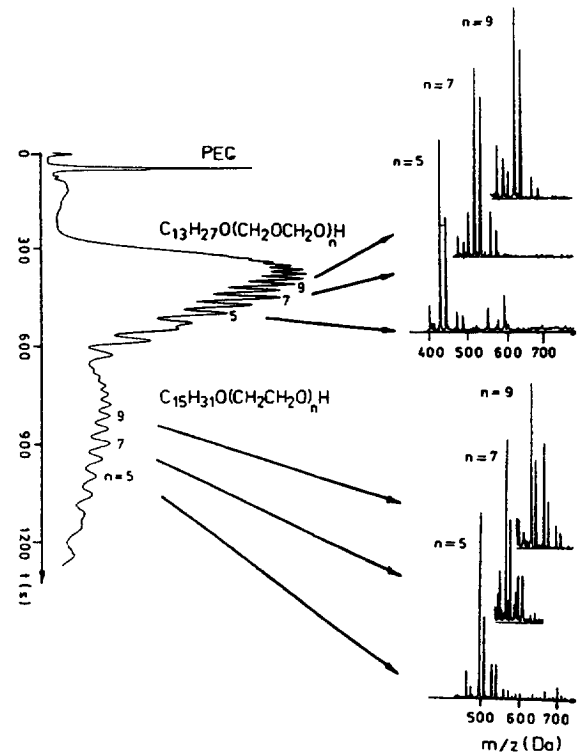

Fig. 26. Separation of a technical polyethylene oxide by liquid chromatography at the critical point of adsorption and analysis of fractions by MALDI-MS. Peak assignment indicates degree of polymerization (n). Column, Nucleosil 100 RP-18 (125×4 mm I.D.); eluent, acetonitrile–water (70:30, v/v). (From Ref. [166], with permission).

(^{1}H NMR, FAB-MS) of preparatively collected samples.

3. Detection of polyether derivatives

3.1. General considerations

For either detection or quantification of polyether derivatives, the following conditions should be met: (i) satisfactory separation of individual components, (ii) identification of ethoxylate peaks, (iii) knowledge of the response factors of the individual oligomers and (iv) sensitivity and reproducibility of detection.

For this reason, it is obvious that detection of samples like polyethers, which due to their manufacturing procedure, occur as complex mixtures, represents a great challenge to the analyst. In contrast to the quantification of pure components each giving one distinct signal, a multitude of signals with only small differences in retention times and, therefore, often more or less overlapping with adjacent peaks have to be considered. In order to identify a polyether sample unambiguously within a (complex) "real" sample exhibiting substantial differences in M_r distribution of oligomers, excellent separation of them has to be achieved. Therefore, the total signal response often composes of a large number of single contributions of the individual oligomers and the important question arises how to determine the detection sensitivity of a polyether, because minor signals are undetectable at very low concentrations and thus markedly impair identification of a sample according to its "fingerprint" pattern. Nevertheless, it will be a reasonable assumption to define the limit of detection as that concentration at which an unequivocal assignment to a reference sample can be done. On the other, hand it is preferable to obtain all oligomers packed into one common and sharp peak, which as a consequence, significantly lowers the detection limit. This in turn is of great importance for signal monitoring of e.g., NIS in environmental samples where the components are present at very low ppb levels. However, in a few cases it will be problematic to apply the latter procedure due to accidental co-elution of other sample constituents, whereas a multitude of clearly separated oligomers often still gives satisfactory analytical information.

As described in Section 2.5, the whole amount of oligomers containing different alkyl or arylalkyl endgroups can yet be merged into one peak under distinct conditions, mainly at the critical point of adsorption, i.e., by LCCC and thus, quantitative measurement of different NIS should be achievable when components of different chemical composition (e.g., containing different endgroups) are clearly separated. Nevertheless, despite the availability of an efficient experimental design as described by Cools et al. [168], evaluation of the "critical conditions" has to be performed as the prior step. This in turn raises the question whether direct quantification on the basis of the "resolved" or "partly resolved" oligomers by the "grouping of peaks" technique may be more convenient. Regardless whether the signals of all oligomers coincide or are individually separated, there still remains the question if all oligomers exhibit the same response factors on a molecular basis or not, which will markedly depend on the detection principle used. These questions and additional means to enhance the detector response of polyether derivatives after introduction of UV absorbing or fluorescent groups, are treated in more detail in Sections 3.2–3.8.

3.2. Derivatization

The wide concentration range of more than 3 orders of magnitude encountered in samples obtained from environmental sources, necessitates the use of a wide variety of detection methods. In most cases UV detection provides sufficient sensitivity for arylalkyl polyether derivatives, whereas fatty acid or fatty alcohol ethoxylates can be monitored by measurement of their refractive indexes (RI) or evaporative light scattering detection (ELSD) responses. Additionally, the latter sample type, lacking any inherent chromophor, can preferentially be derivatized with 3,5-dinitrobenzoyl chloride (DNB-Cl), the mostly used derivatization reagent for polyethers. According to the procedure of Nozawa and Ohnuma [109] both sample and DNB-Cl are dissolved in pyridine and heated to 65°C for 30 min. In general, the 3,5-dinitrobenzoyl (DNB) substituent gives sufficient detection sensitivity and is also amenable to electro-

chemical detection [112]. Additionally, phenylisocyanate [105], 1-naphthylisocyanate [118] and FMOC-Cl [120] present valuable alternatives for derivatization of alcoholic hydroxyl groups, the latter two reagents also being applicable as fluorotags. In those cases where RI, ELSD and UV responses are unsatisfactory, as e.g., in environmental analysis of river soil and sewage sludges from urban sewage plants, the inherent fluorescence of arylalkyl NIS can be exploited to achieve the desired sensitivity. In addition, fluorescent labelling of alkyl-substituted NIS and native polyethers can be effected with a multitude of appropriate reagents (see Section 2.2.2). Fluorogenic reagents have been excellently reviewed by Ohkura et al. [172], and Dou et al. [173] has extensively treated the use of reagents for subsequent electrochemical detection. No derivatization is required for MS detection directly coupled to LC (LC–MS), but nevertheless introduction of a chromophor is often feasible for providing an UV probe to facilitate peak identification.

3.3. Refractive index (RI) and density detection (DDS)

Although great efforts have been made to apply low wavelength UV detection to polyether samples lacking a chromophor, even in gradient HPLC (Section 3.4), signal monitoring based on "universal" detection is still widely used for the underivatized species. Therefore, detection by RI and DDS (density detection system), the latter method being developed by Trathnigg et al. [48,51,52,54–56,133,169,170], offer a means which is applicable to all components, regardless if they possess a chromophor or not and thus represent typical "universal" detection systems. However, both techniques show decreased sensitivity compared with signal monitoring by UV (approximately at least one order of magnitude lower) and are unsuitable for gradient HPLC due to large baseline deterioration as a result of the gradually changing RI and DDS signal. The latter disadvantage could be compensated when the reference cell of the detector is flushed with the same solvent composition as used for chromatography, but in practice it is very difficult to obtain a consistent gradient flow for either measuring or reference cell. For this reason, application of RI and DDS is generally restricted to isocratic HPLC. On the other hand, acetonitrile–water mobile phases show isorefractive behavior at 55°C, regardless of the acetonitrile–water ratio [174], thus making RI amenable to gradient HPLC, assuming that a temperature increase from RT to 55°C will not exert any detrimental effect on separation characteristics. Furthermore, RI is very sensitive towards even slight changes in temperature, necessitating thermostatic control of both column and measuring cell, whereas only minor effects on individual signal responses at, e.g., two substantially different temperatures are observed [43]. The magnitude of RI signal responses for a given sample is directly proportional to its concentration and, due to different refractive indexes, compounds differing in chemical structure show different RI responses. In these cases a calibration curve for each component in quantitative determinations is required.

In a multitude of isocratic HPLC separation experiments of polyether derivatives detection by means of either RI [2,4,5,43–52,54–56,81,83,90,98,113,122–124,133,134,137,169,170] or DDS [51,55,133,169,170] has been successfully applied. However, despite the abundant use of signal monitoring by RI measurements, questions arise as what precautions must be taken when RI responses are to be exploited for quantitative determinations. For this reason, the influences of different factors, such as molecular mass of oligomers or solvent effects on RI and DDS signal responses had to be investigated before the two universal techniques are to be used for quantitative analysis of polyethers. Additionally, to determine M_n, M_w and M_w/M_n (polydispersity index) values, which are fundamental parameters of polymer characterization, the exact molecular mass of the different oligomers had to be measured primarily. This can be done by both collection of individual "peaks" by semi-preparative HPLC and subsequent MS in the "off-line" mode (e.g., by MALDI-TOF-MS) or alternatively, without sample isolation in the "on-line" mode by LC–MS coupling techniques (see Section 3.8).

Within a homologous series of polymers, specific properties, such as partial specific volume, refractive index and refractive index increments, are dependent on the M_r attributable to the differential participation of the repeating monomer units and end-groups,

which was shown by Mori [43] and Trathnigg et al. [48,51,54–56].

Mori [43] investigated PEGs largely differing in M_r (PEG 200 to PEG 3400) by SEC. In addition, he determined the response factors of the individual oligomers of PEG 200 in THF and chloroform and observed either marked dependence on the M_r values or the solvent used for chromatography. In particular, when chloroform was used as the solvent, the low M_r oligomers are greatly underestimated and require large correction factors ranging from 6.15 ($n=1$) to 0.89 ($n=15$), whereas the differences are much lower in THF, yielding values ranging from 1.43 ($n=1$) to 0.96 ($n=15$). The large differences in RI between the individual oligomers of PEG 200 in THF and chloroform can be ascribed to the markedly differing density of both solvents. It is further evident, that the higher the M_r of sample constituents the lower are the differences in response factors between two oligomers differing in only one repeat unit, and the author observed only minor changes in response factors when the M_r of PEGs exceeded 1000 (approximately 1% in the range of 1000–10 000). Table 1 shows the dependence of response factors of PEGs ranging from M_r 106 ($n=2$) to 20 000 on the M_r in either THF or chloroform as the solvent. Response factors do not change significantly at M_r above 500.

Trathnigg et al. [48,51,54–56,169] subjected polyethers to either HPLC or SEC and calculated response factors from RI and DDS detection. As already investigated by Mori [43], the authors also found that within a homologous series response factors vary with M_r. However, it is evident that the M_r dependence of response factors has to be taken into account only for oligomers with $M_r<300$, whereas those exceeding this limit can be considered as constant. In Fig. 27 the response factors of PEGs as a function of $1/M_r$ are shown for an RI as well as a DDS system.

The large differences in response factors between low M_r (<300) oligomers is mainly caused by the substantial contribution of the endgroups to the specific refractivity relative to the repeating units, which however decrease with increasing M_r and the influence of the polymer backbone prevails.

Furthermore, "preferential solvation" as discussed by Trathnigg et al. [54] in SEC of PEGs with different endgroups may also exert an influence on signal responses of oligomers markedly differing in M_r, even when HPLC is used for separation. They observed substantial differences in response factors when, e.g., polyether derivatives are chromatographed in chloroform containing small amounts of ethanol as the stabilizer. In this case "preferential" solvation of the solute by the polar solvent "impuri-

Table 1
Refractive indices and response correction factors for polyethylene glycols[a]

M_r	RI, 25°C	Response correction factor	
		in THF	in chloroform
106 ($m=2$)	1.4455	1.655	8.912
150 ($m=3$)	1.4529	1.402	2.802
194 ($m=4$)	1.4563	1.310	2.134
238 ($m=5$)	1.4589	1.248	1.804
282 ($m=6$)	1.4597	1.230	1.722
326 ($m=7$)	1.4610	1.201	1.603
370 ($m=8$)	1.4619	1.183	1.530
414 ($m=9$)	1.4623	1.174	1.500
458 ($m=10$)	1.4630	1.160	1.450
500	1.4640	1.141	1.384
550	1.4653	1.117	1.306
600	1.4660	1.104	1.268
650	1.4664	1.096	1.247
700	1.4668	1.090	1.227
750	1.4670	1.086	1.217
800	1.4674	1.079	1.198
850	1.4676	1.076	1.188
900	1.4678	1.073	1.179
950	1.4680	1.069	1.170
1000	1.4682	1.1066	1.161
1100	1.4686	1.059	1.143
1200	1.4689	1.054	1.131
1300	1.4692	1.049	1.118
1400	1.4694	1.046	1.110
1500	1.4696	1.043	1.102
1700	1.4700	1.037	1.086
2000	1.470	1.030	1.071
2500	1.4708	1.024	1.056
3000	1.4710	1.021	1.048
3500	1.4713	1.016	1.038
4000	1.4715	1.013	1.031
5000	1.4718	1.009	1.020
6000	1.4720	1.006	1.013
7000	1.4721	1.004	1.010
8000	1.4722	1.002	1.007
20 000	1.4724	1.000	1.000

[a] Source: Ref. [43] Table III.

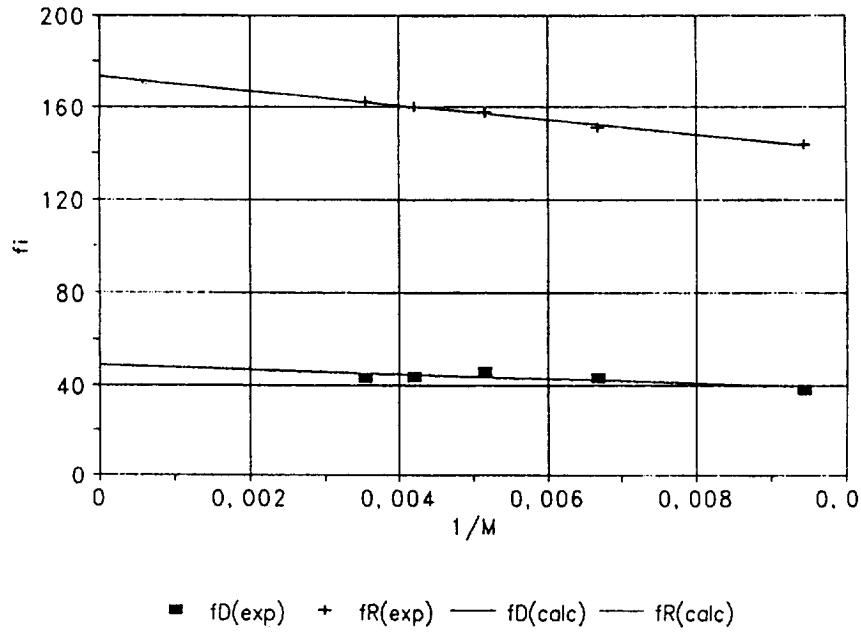

Fig. 27. Response factors of polyethylene glycols in methanol–water (40:60) at 25.0°C as a function of $1/M$. (From Ref. [51], with permission).

ty" occurs, which affects hydroxyl more than methoxyl end-groups, the latter showing a similar behavior as the polyoxyethylene backbone due to nearly identical chemical properties. This effect is quantifiable when a sample is either separated on a chromatographic column or directly injected into the detection system via a special by-pass device without prior separation. In the latter case the true response factors are observed, whereas prior separation yields the apparent response factors, because on a chromatographic column, the polymer peak will be separated from the zone of "dialyzed" solvent. For this reason, the measured area under the peak will include contributions from either polymer sample or preferentially adsorbed solvent.

Determination of response factors of individual polyether oligomers plays a crucial role for determination of M_n, M_w and M_w/M_n values, and Trathnigg et al. [51] emphasized that calculation of these fundamental parameters of polymer chemistry will provide much more reliable information when baseline separation of peaks has been achieved by use of high-resolution HPLC. However, this alternative means is restricted to low-to-medium molecular mass samples, which allow sufficient separation for species differing in only one repeat unit.

3.4. UV detection

Measurement of responses obtained from UV absorption is still the main method used for detection of polyether derivatives. NIS of the octyl- or nonylphenol type are directly amenable to UV detection due to the inherent chromophor, and alkyl-substituted or underivatized polyether species are easily derivatizable with an appropriate UV-absorbing moiety. Moreover, low wavelength UV detection ($\lambda \leq$ 200 nm) can also be used for the latter samples making derivatization unnecessary. However, measurement of samples at low wavelength ($\lambda \leq 200$ nm) is unspecific and more and more meets the conditions of universal detection, as already stated for the RI and DDS methods. Therefore, it is a concentration-dependent method and correction factors for oligomers markedly differing in M_r have to be considered. The precautions, which have to be taken into account when samples are to be measured at $\lambda < 210$ nm, will be treated in more detail below.

In contrast to RI and DDS detection, UV responses are, at first sight, independent on M_r when the measured signal exclusively involves the specific contributions from the aromatic moieties. This means that signal intensities in the wavelength range of approx. 210–280 nm reflect the molar distribution of oligomers. However, specific absorption of arylpolyethoxylates, which relates the measured extinction value to the unit weight, decreases with increasing number of EO units due to the reduced relative contribution of the aromatic chromophor to the molecule. As a consequence, response factors increase with increasing molecular mass. On the basis of the response factors determined for the low M_r members and considering the respective molecular masses, response factors can also be calculated for the higher oligomers and thus are applicable to quantitative determination of components widely differing in M_r distribution.

Furthermore, gradient elution can still be performed within this wavelength range without severe baseline deterioration, at least in RP-HPLC. This however, is not true for NP-HPLC owing to the lower transparency of mixtures of pure organic solvents at wavelengths <250 nm, such as ethyl acetate, dichloromethane and chloroform compared with mixtures of aqueous organic solvents typically used in RP-HPLC. Thus, NP-HPLC signal responses are preferably measured in the wavelength range of 270–280 nm. When extreme sensitivity in RP-HPLC is required, as e.g., for measurement of environmental samples, signal monitoring at the lower limit of the usually applied wavelength range will invoke a dramatic drift of the baseline, thus impairing determination of trace amounts in the lower ppm and the whole ppb range. In these cases fluorescence detection (FD) will be the method of choice (Section 3.5).

Within the usual range of UV absorption a wide variety of wavelengths has been used by different research groups. In the case of polyether derivatives containing an aromatic moiety signal monitoring has been performed at 215 nm [45], 220 nm [89,91], 225 nm [96,101,115,125], 230 nm [65,81,102,103,117], 235 nm [99], 250 nm [128], 254 nm [44,65,87,102,103], 258 nm [65], 270 nm [86], 273 nm [82], 275 nm [80,104,109], 276 nm [88,97], 277 nm [94,95,125] and 280 nm [47,65,107,128]. UV responses of alkyl-substituted and native polyethers have been determined after incorporation of a benzoyl moiety at 254 nm [44] and 270 nm [3], and a 3,5-dinitrobenzoyl (DNB) residue at 254 nm [100,106,110,111,114,135]. The corresponding urethane derivatives obtained by reaction with phenylisocyanate [105] and 1-naphtylisocyanate [118] were measured at 240 and 291 nm, respectively. Despite restriction to wavelengths >250 nm in most cases, several authors reported detection of polyether derivatives by gradient NP-HPLC with hexane–ethanol at 220 nm [89], *n*-hexane–methanol–2-propanol [91] and 2-propanol–water in hexane–THF [101] at 225 nm, 2-propanol–*n*-heptane [81], hexane–2-propanol–water [102] and *n*-heptane–ethanol [103] at 230 nm. Native thiodiglycol oligomers could be detected at 210 nm [140], presumably due to the contribution of d-electrons in the sulfur shell to the UV response, thus making derivatization with a chromophor unnecessary.

The limit of detection for those polyether derivatives containing a chromophor is a very important aspect and, unfortunately, only minor or even no attention is paid to the question whether it is related to an individual oligomer or to the whole entity of oligomers. As mentioned in Section 3.1, the question arises if the limit of detection should be defined as that minimum concentration, which still allows an unambiguous assignment to the typical signal pattern of a reference polyether derivative as proposed by Rissler et al. [135]. The authors reported values for the DNB derivatives of PEG, PPG and PBG of about 0.5, 1 and 2 μg, and it should be expected that OPEOs, NPEOs and related compounds might reveal values in a similar range. Furthermore, the sensitivity of signal monitoring is at least one order of magnitude better compared with RI and DDS detection.

Special attention is addressed to the detection of polyether derivatives lacking a chromophor at wavelengths <210 nm, which is treated in more detail. From the reasons presented above, it will be evident that this alternative is restricted to RP-HPLC.

Either alkyl-substituted or native PEGs and PPGs have been detected after isocratic separation at 204 nm [171], 198 nm [171] and 190 nm [131], and gradient elution at 200 nm [81,82,171], 198 nm [171], 185 nm [129], 190 nm [75,113,138] and 192 nm [130,139]. As expected, no pronounced impair-

ment of baseline stability occurs when isocratic HPLC is applied, but the gradient technique has some peculiarities.

As evident from Mengerink et al. [113], a gradient of acetonitrile and water without compensation of the "UV mismatch" between initial and final gradient caused by the stronger absorption of the organic solvent vs. water, impairs detection of minor amounts of sample constituents due to a severe baseline drift. Different approaches have been used to overcome the problem. In a binary gradient of acetonitrile and water, Van der Wal and Snyder [129] added 5 ppm of nitric acid to the aqueous phase and Bergman and Möller [130] used an aqueous phase containing 4 ppm of nitric acid and 60 ppm of phosphoric acid in order to suppress the baseline drift. In a similar eluent system also consisting of acetonitrile in water Moldovan et al. [138] applied "spiking" of the component lean in acetonitrile with 3 ppm of nitric acid. Barka and Hoffmann [132] and Berry [175] added sodium azide to the water component until the aqueous phase reached the same absorption as the acetonitrile used. No additive to the acetonitrile–water mobile phase was required by Lai et al. [131] in the isocratic mode at 190 nm. Despite extensive compensation of the UV mismatch, some disturbances in the chromatograms occur at low wavelengths, such as a typical gradient defect termed "mid-gradient hump" by Berry [175], which is presumably caused by oligomeric contaminants. The latter author recommended some procedures to reduce the deleterious influences on chromatography. Solvent impurities, mainly responsible for the mid-gradient hump, should be removed by insertion of "elution conditioner columns", such as PS–DVB materials or C_{18} and C_8 "pre-columns" in the aqueous line before the mixing chamber. Mid-gradient humps caused by traces of amine impurities in acetonitrile can be eliminated when an "acetonitrile conditioner column" of acidic alumina is placed in the acetonitrile line. Additionally, the effect of solvent impurities can be greatly reduced using photo-oxidation of interferents by UV irradiation of the mobile phases. The influences of either mid-gradient hump or UV mismatch on the baseline profile is depicted in Fig. 28 and Fig. 29.

Van der Wal and Snyder [129] reported a limited gradient range of about 0–35% of acetonitrile and observed a marked mid-gradient hump when the percentage of organic modifier exceeds 40%. In accordance with Berry [175], the authors ascribed this effect to mobile phase impurities. Furthermore, the authors stated that at wavelengths below 200 nm the requirements of universal detection are met, as is also the case for signal monitoring by means of RI and DDS. They found that signal monitoring at 185

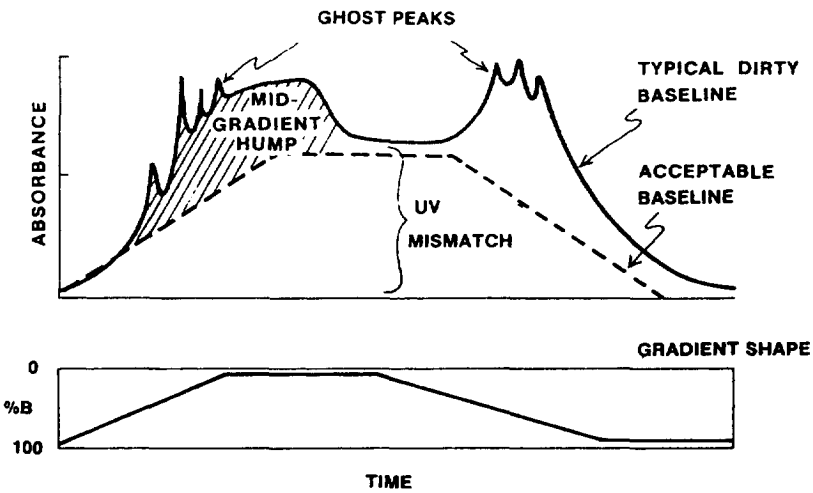

Fig. 28. Illustration of a typical "dirty" gradient baseline showing (1) the "mid-gradient hump" problem, (2) the problem of UV absorbance mismatch between initial and final eluents, and (3) the ghost peak problem. The "acceptable baseline" (dashed line) results if the mid-gradient hump and ghost peak problems are eliminated. (From Ref. [175], with permission).

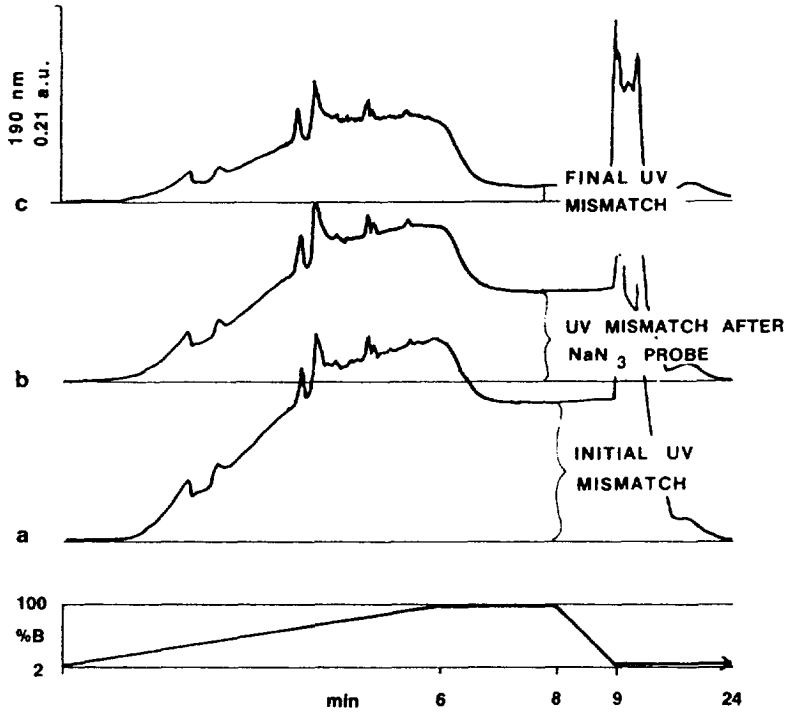

Fig. 29. Example of reducing the UV mismatch between the initial and final gradient eluents by adding an unretained component, 0.2% sodium azide, to the lower UV absorbing aqueous eluent. The 6-cm UV mismatch in (a) is reduced to 4 cm with a "probe" addition of 10 ml of sodium azide per liter of aqueous eluent in (b) and to 0.4 cm with a final addition of 20 ml/l in (c). The pH 2 aqueous uses 10 m*M* sodium phosphate buffer to acetonitrile gradient at 5 ml/min using a 25-cm Zorbax C_{18} column with no eluent conditioner columns. (From Ref. [175], with permission).

nm offers a convenient compromise between increasing baseline noise and maximum absorptivity for several compound classes and, even in the worst case (alcohols), detection at 185 nm is at least as sensitive as with an RI detector. Additionally, different sensitivities for a multitude of compounds are observed when the wavelength is varied over the region 185–200 nm, which further implies substantial selectivity for a variety of substance classes. According to Van der Wal and Snyder [129], detection limits of approx. 300 ng can be expected at 185 nm for alcoholic components and thus may also be found in a similar range with respect to polyether derivatives. In contrast to the results of Berry [175] and van der Wal and Snyder [129], Escott and Mortimer [75] did not observe a mid-gradient hump after addition of 5 μg/l of sodium azide to the aqueous phase of a binary solvent gradient. Unlike, the investigations of the latter authors, Desmaizières and Desbène [171] did not need any additive in order to compensate for an unacceptable "acetonitrile-invoked" baseline drift at 198 nm, although they applied the method for quantitative determinations. However, this goal could be only achieved by use of an extremely pure organic modifier.

Despite a lot of advantages of low wavelength detection, which makes the method amenable to signal monitoring of "non-chromophoric" compounds, the principal drawback is that the highest linear sample size[5] decreases significantly for three reasons: (i) absorption of sample increases, (ii) absorption of mobile phase increases and (iii) the detection system has a smaller linear range. Furthermore, due to its properties as an universal detector

[5]The highest linear sample size at a particular wavelength is defined as the amount of sample that causes a 10% lower value than expected from a plot (the response curve) of area counts vs. amount of sample injected [175].

and thus in accordance with RI and DDS detection, signal responses of low M_r oligomers may reveal some dependence on M_r, which should increase with increasing chain length of polyether derivatives, as observed in the same way for RI detection. However it appears that no investigations with special focus on this question have been hitherto undertaken.

It should be emphasized that detection at wavelengths below 200 will only be feasible when extremely pure solvents and solvent additives are available. Otherwise, alternative techniques, such as measurement of responses from UV and FD detection (Section 3.5) after introduction of appropriate chromotags and fluorotags or evaporative light scattering detection (Section 3.6) should be preferred.

3.5. Fluorescence detection (FD)

In general, this method is only used when UV detection yields inadequate sensitivity and thus is mainly restricted to the determination of "aromatic" polyether surfactants in environmental samples, where they very often occur down to the ppb range. Polyether derivatives lacking fluorophores can easily be reacted to the corresponding fluorescent derivatives by means of a wide variety of "labeling" reagents [160] (Section 3.2).

Owing to their inherent fluorescence, alkylphenol ethoxylates can be measured without derivatization. Although influences of the mobile phase composition on fluorescence yields are often observed, similar wavelengths for either excitation (λ_{ex}) or emission (λ_{em}) have been published, regardless if pure organic solvents in the NP mode or aqueous organic mixtures in the RP mode have been used. In this way OPEOs and NPEOs have been determined at λ_{ex}=225 nm and λ_{em}=295 nm [96,126], λ_{ex}=230 nm and λ_{em}=295 nm [96,125], λ_{ex}=230 nm and λ_{em}=302 nm [97] as well as λ_{ex}=277 nm and λ_{em}=298 nm [105]. Kudoh et al. [92] applied a substantially larger excitation wavelength of 280 nm for detection of NPEOs (λ_{em}=310 nm), whereas signal monitoring of fatty alcohol ethoxylate 1-anthroyl derivatives was performed at λ_{ex}=395 nm and λ_{em}=450 nm for determination of extremely low concentrations of NIS in environmental samples. The authors reported detection limits of 0.2 ppm for arylalkyl ethoxylates and 0.05 ppm for fatty alcohol ethoxylates. In addition, when quantitation of the 1-anthroyl-derivatives of lauryl alcohol ethoxylates on the one hand and NPEOs on the other hand by HPLC–FD is compared with the values obtained from the cobalt thiocyanate method, the former technique yielded concentrations 1–2 orders of magnitude lower and thus more matching with the expected concentration range. This observation underlines the feasibility of the HPLC–FD alternative, which is not as susceptible to interference as the non-chromatographic techniques.

The FD responses of isocyanate derivatives of fatty alcohol ethoxylates, which lack an inherent fluorophor was measured by Lemr et al. [118] at λ_{ex}=229 nm and λ_{em}=358 nm, whereas Meissner et al. [120] reacted the terminal hydroxyl group with FMOC-Cl followed by measurement of FD responses at λ_{ex}=260 nm and λ_{em}=310 nm.

Detection limits in the ppb range were also reported for OPEOs and NPEOs [125,126] and Holt et al. [97] determined individual oligomers of OPEOs down to 0.2 ng. Compared with UV detection, a gain in sensitivity of at least one order of magnitude is achieved with FD. In those rare cases, in which responses from "conventional" fluorescence are still insufficient for detection or quantitation of trace amounts of polyether derivatives, the "laser induced fluorescence" (LIF) technique may be applied. However, in order to exploit the full scale of this powerful alternative tool, the synthesis of appropriate reagents exhibiting maxima in λ_{ex} of about 488 nm (i.e., the $\lambda_{ex(max)}$ of the argon laser) should be intensified, such as 4-(*N*,*N*-dimethylaminosulfonyl)-7-(2-chloroformylpyrrolidin-1-yl)-2,1,3-benzodiazole introduced by Toyo'oka et al. [176] for derivatization of alcoholic compounds.

As for UV detection (Section 3.4), FD responses also reflect the distribution of polyether derivatives on a molar basis. However, when used for calculation of M_n, M_w and M_w/M_n values, which requires the absolute mass under each oligomer peak, it should be considered that the FD response per unit weight decreases with increasing chain length of the polyether derivative due to the relative decrease of the contribution of the aromatic moiety to the FD signal intensity. Thus, response factors have to be calculated in order to obtain reliable results. In general, it may be anticipated that the relative

amount of each aromatic residue to the FD response will be equal for all oligomers of the same polyether family. However, it cannot be completely excluded that "quenching effects" due to conformational effects, often observed in polymer chemistry, could impair the individual contribution to the FD response and thus yield more or less marked deviations from the "true" concentration in quantitative determinations. Perhaps effects like those play a substantial role, in particular after twofold derivatization of short-chain α,ω-dihydroxy polyethers, in which quenching effects are sometimes favored as a consequence of the proximity of both fluorophores.

3.6. Evaporative light-scattering detection (ELSD)

This technique is a typical universal detection method and is independent of the availability of chromophors in the molecule. It is a mass detector, which is sensitive towards non-volatile compounds and completely insensitive towards volatile components, such as the solvents typically used in HPLC. For this reason, gradient HPLC can be performed with every solvent combination without any baseline deterioration and thus represents the ideal method for gradient use. Moreover, ELSD is applicable to solvents, which cannot be used in HPLC–UV due to their inherent absorption in the usual UV wavelength range, such as acetone and methylethylketone.

Despite the immense advantages of ELSD over competing techniques, such as RI, DDS, UV and FD, the method is still rarely used for analysis of polyether derivatives, but a strong increase in application is expected when the advantages become more obvious to the analysts. Therefore, to encourage potential users to address the problem, the principle of the method will be explained in more detail.

Extensive experimental investigations of the various light scattering parameters on signal responses have been published by Charlesworth [177], Mourey and Oppenheimer [178], Oppenheimer and Mourey [179], Van der Meeren et al. [180] and Stolyhwo et al. [181–183] from the Guiochon group. An excellent overview of the methodological design was given by the latter authors [181–183] and Fig. 30 gives a schematic representation of an evaporative light scattering detector.

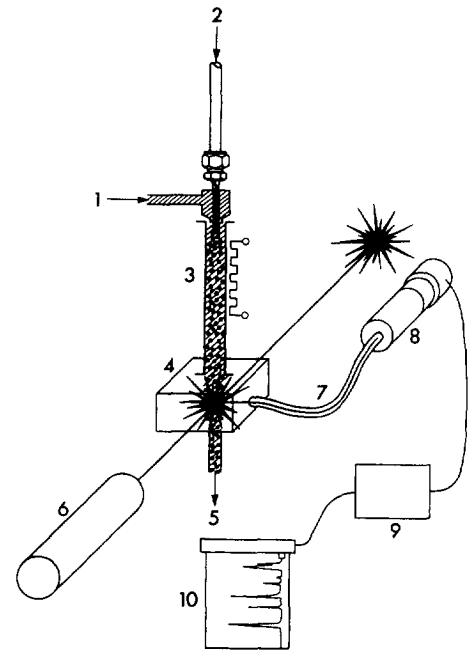

Fig. 30. Schematic of the evaporative light scattering detector. 1=carrier gas stream (constant temperature and flow-rate); 2=LC column effluent; 3=drift tube; 4=light scattering cell; 5=to water ejector; 6=He–Ne Laser (632.8 nm); 7=Optical fiber; 8=photomultiplier; 9=electrometer; 10=recorder. (From Ref. [183], with permission).

In the ELSD operation, the eluent from the HPLC column is introduced into the top of a heated evaporator tube, where it is nebulized by a stream of nitrogen. Droplets formed at the nebulizer pass through the heated tube. The solvent is vaporized and an aerosol is formed from the non-volatile solute particles contained in the eluent. The particles pass through a light path and the light scattered is detected at a fixed angle. The amount of scattered light is proportional to the sample concentration. The detector has many desirable features. It is inexpensive, stable and easy to operate. Most important, the detector is not subject to solvent interference and is insensitive to the chemical composition of detected species. It has been shown that detector linearity and detection limits are directly related to the size, shape and number of particles formed in the evaporator tube. Under fixed nebulization and evaporation conditions, the detector response is dependent on the density and refractive index of the aerosol particles.

For samples with similar densities and refractive indexes, the response is proportional to the mass of material present in each sample and independent of molecular mass. These characteristics allowed ELSD to be used as an universal detection method for polyether derivatives.

Subsequently, the most conspicuous properties of ELSD for analysis of mixtures of oligomeric components are presented more extensively.

The light source used in ELSD emits nonpolarized light with a wavelength between 350 and 650 nm, the mean value generally lying at about 500 nm. It is important to know that the signal response derived from scattered light is a rather complex function of refractive index and absorption coefficients as well as density of the sample molecules and thus, responses may vary from compound to compound [178–181]. However, Brossard et al. [76] and Stolyhwo et al. [181,183] observed nearly identical concentration versus response curves for different kinds of PEGs and derivatives [76] as well as fatty acid methylesters [181], fatty acid methylesters, triglycerides and cholesterol [183], implying that signal intensity is largely independent of chemical composition and only depends on the concentration used, at least for similar classes of compounds. On the other hand, polar lipids, such as phospholipids, sphingomyelin and cardiolipin, show a fourfold increase in signal response [183], as treated below. Thus, within each class of chemically similar compounds quantitative analysis can be performed directly from determination of peak areas. Despite this advantage of ELSD in a lot of cases, it would be more convenient to use individual calibration curves for quantitative determinations. Hopia and Ollilainen [184] observed only a relatively small effect of the chemical structure on the ELSD response for mono-, di- and triolein. However, when applied to the quantification of PEG 1000 on the basis of a calibration curve obtained from trioleine standards, unsatisfactory results are observed for determination of the lowest concentration used, whereas measurement of the higher concentrations match more with the expected values. Although "universal calibration" often cannot be used for components differing too much in chemical structure, these examples demonstrate the unique properties of the ELSD method to quantify a multitude of samples, belonging to similar substance classes on the basis of a calibration curve obtained from a different but structurally related reference material.

Furthermore, results profoundly differ between compound classes, which on the one hand, are solid and, on the other hand, are liquid at the temperature of the drift tube. For example, fatty acid esters and triglycerides are viscous liquids, whereas phospholipids are solids. The latter compound series yields a four-fold increased detector response (see above) due to scattering on "condensed solids" compared with the "condensed liquids" in the former case. In an analogous manner, light is more strongly scattered by snow-flakes than by rain drops.

The following summary gives a more detailed compilation of the most important peculiarities of ELSD [177–183]:

1. The higher the flow-rates of either nebulizer gas or mobile phase the lower the signal response due to a decrease in droplet size.
2. The higher the evaporator temperature the lower the signal response of a given oligomer.
3. The higher the M_r of an oligomer the higher the evaporator temperature for achievement of an optimum signal response.
4. At small sample loads the signal response is mainly governed by the size of the scattering droplets, whereas at higher sample loads the response is predominantly determined by the concentration of the scattered droplets.
5. In general, detector responses are non-linear [177–183], but markedly depend on the experimental conditions used. At higher sample loads signal responses decrease with increasing flow-rate of the mobile phase and decreasing nebulizer gas pressure, whereas at small sample loads detection efficiency approximates 100%. The former effect is termed "detector saturation" [180] and implies that quantitative determinations should be performed in the "linear detector range" using small concentrations of solute.
6. The baseline is insensitive to changes in the

mobile phase as applied in gradient HPLC. In the case of aqueous organic solvents programming of the drift tube temperature may be recommendable and carried out in the way that temperature increases as the percentage of water increases.

7. Signal responses are extensively independent of M_r when the analytes consist of "condensed solids" (see above) but essentially depend on the sample size.
8. Signal responses change with mobile phase composition because the particle size is largely affected by mobile phase characteristics, such as density, viscosity and surface tension [76]. Thus differences in signal intensities will be observed, which are of great importance when a sample is separated by either NP-HPLC with pure organic solvents or RP-HPLC with aqueous organic mixtures. In this way the gradient technique will also invoke different responses due to the continuous change in eluent composition, but in practice the effects may be of minor significance.
9. Buffers cannot be used in ELSD, because the dissolved solid buffer ingredients behave in the same way as non-volatile solutes after loss of the surrounding solvent shell.
10. ELSD is characterized by low background noise, because the volatile solvent(s) are completely evaporated and thus do not provide scattering particles.

For chromatographic separations of NIS of the alkylethoxylate type, Mengerink et al. [113] investigated the influence of different nebulizer gases on signal intensities and found that at similar gas velocities the five-fold larger thermal conductivity of helium compared with air, nitrogen or carbon dioxide, permits a lower vaporization temperature and thus is the gas of choice for low volatility components. Helium is not only superior to other gases (nitrogen, air, carbon dioxide) with concern to its application to relatively volatile compounds, but also with respect to the intensity of signal responses, which e.g., is much larger than that obtained with carbon dioxide. Additionally, the signal-to-noise (S/N) ratio is better when helium is used instead of carbon dioxide. However, due to gas flow-rates of more than 2 l/min, the use of helium as the nebulizer gas is very expensive and thus prevents its application for daily routine investigations. The same authors [113] further propose that in general, non-volatile components should be measured at the lowest nebulizer gas flow and the highest temperature permitted by the analyte. When compared with UV and RI detection, the S/N ratio of ELSD proved to be markedly superior and detection limits of about 1 μg of sample have been achieved.

Bear [102] compared signal responses of NPEOs obtained by either UV absorption or ELSD and found that ELSD was as sensitive as UV detection, yielding detection limits in the nmol range. These results contrast with the findings of Rissler and al. [135], who measured a sensitivity of ELSD at least one order of magnitude lower for native polyethers than signal monitoring of the corresponding DNB derivatives by UV. The latter view is supported by Stolyhwo et al. [182], who reported that UV detection of dioctyl phthalate was about two orders of magnitude higher than ELSD. Although Bear [102] observed similar distribution profiles of oligomers by UV absorption and ELSD, the UV detector gave a higher response for low-M_r components ($n \leq 7$), which is ascribed to the "attenuation" of UV absorption by an increase in EO number, or in other words, by a relative decrease of the contribution from the aryl chromophore per unit mass at higher n values. However, as expected, the differences in relative signal responses decrease as n increases.

Desbène and Desmaizières [106] measured ELSD responses of alkylethoxylates but, in contrast with Mengerink et al. [113], an increased background noise was observed compared with UV detection.

Excellent results were obtained by Rissler et al. [135,136,148,149,156] for a wide variety of polyether derivatives including PEGs, PPGs, PBGs and PPG amides and Martin [108] for alkylethoxylates, PPGs and PEG–PPG copolymers. However, compared with measurement by UV, the detection limits were about one order of magnitude lower, lying in the μg range. For PEG 1000, PPG 1200 and PBG 1000 limits of detection were reported to be 5, 10 and 20 μg, respectively [135]. Derivatization of PPG amines (Jeffamines) with pyridine/acetanhydride

revealed an additional advantage of ELSD over UV detection [149]. Although the resulting PPG amides can be alternatively measured with high sensitivity at 210 nm, due to the presence of the amide chromophor as in the case of peptides and proteins, the excess of reagent has to be removed prior to HPLC–UV and thus, an additional sample preparation step is required. As evidenced for Jeffamines D-230, D-400 and T-403, the large and strongly tailing pyridine peak prevents signal monitoring of the lower M_r oligomers if excessive reagent has not been eliminated after derivatization. In contrast, ELSD "disregards" the pyridine due to its volatility and only the signal attributable to pyridinium acetate as a solid is visible. However, the latter does not impair detection of PPG amides because, as an extremely polar component, it leaves the column unretained.

Despite its properties as an universal detector, two major disadvantages of ELSD have to be taken into account. First, ELSD is not applicable to buffered mobile phases often required to reduce deleterious silanophilic interactions, and thus restrict its use to HPLC–ELSD of compounds lacking any substantial interactions with free silanols. On the other hand, this fact may encourage producers of stationary phases to prepare novel column materials, in which residual silanols are extensively shielded against interactions with solute molecules. The second drawback relates to different optimum temperatures of the evaporator for different compounds and thus, in particular, will give widely varying signal responses for oligomeric mixtures largely differing in M_r. Therefore, it is easily conceivable that at the optimum temperature of high-M_r oligomers low-M_r oligomers, which often are viscous liquids, may yield either smaller responses as expected or not be detected at all. The non-observation of a typical "gaussian-like" oligomer distribution in the case of acetylated Jeffamine D-230 [149] and low-M_r PEGs [136] may at first sight be attributable to the marked volatility of low-M_r oligomers. However, it should also be considered that low-M_r oligomers are extremely hygroscopic and thus lose their solvent shell only with difficulty. As a logical consequence, the extensively hydrated droplets are not suitable targets for ELSD and signal responses will be either very low or not detectable at all. Furthermore, response factors of fatty alcohol ethoxylates will also depend on the mass ratio of the hydrophobic alkyl group to the hydrophilic polyether backbone and thus, marked differences in surface properties of the formed droplets, i.e., surface tension and viscosity, are to be expected. This in turn markedly influences droplet size and, consequently, the signal response and thus may give rise to substantial differences in response factors between oligomers exhibiting a large difference in M_r distribution. Under these circumstances it is obvious, that both quantification of sample concentrations and determination of M_n, M_w and M_w/M_n values will be unsatisfactory and determination of response factors is required. The latter will be complicated by the fact that response factors will change with temperature, which makes comparison of results a very difficult task, except when a standardized temperature is used. Unfortunately, no investigations with special focus on these problems have hitherto been published, possibly due to their complexity.

Nevertheless, ELSD offers an efficient alternative tool for signal monitoring, which in most cases yields sufficient sensitivity and, as a consequence, is superior to RI and even UV detection, at least in the case of polyethers lacking any chromophor. Analysts should increasingly consider this fact by using this powerful alternative procedure.

3.7. Electrochemical detection (ED)

Although introduction of electro-active substituents ("electrophors") into "non-electro-active" compounds is gaining more and more importance, it seems of only minor importance, at least for detection of polyether derivatives, due to the availability of a multitude of competing techniques of similar efficiency. Thus, only one application will be presented.

Desbène et al. [112] reported ED of C_{16} and C_{18} ethoxylates with acetonitrile–water (3:2) and tetrabutylammonium perchlorate (TBAPC) as the supporting salt after prior derivatization with DNB-Cl. Due to the nitro groups in 3,5-position, signal monitoring was carried out at negative potential in either the direct current mode at −1 V versus Ag/AgCl or in the differential pulse (DP) mode at −0.8 V, the sensitivity being markedly increased by the

latter alternative. On the other hand, the electroactivity range can be extended to −2.2 V when a dropping mercury electrode is used in the presence of TBAPC and detection sensitivity is about 50 times higher compared with signal monitoring by UV. Furthermore, the linear dynamic range extended from 0.5 ppm to 100 ppm when polarographic detection is applied in the DP mode.

Due to the maintenance of constant buffer as well as supporting salt concentrations, ED is preferably used in combination with isocratic LC. Otherwise, significant baseline deterioration has to be taken into account, which impairs either sensitivity or quantitative determination. Nevertheless, gradient elution is applicable when the level of buffer and supporting salt present at the starting conditions does not change throughout sample elution, i.e., when both solvent components of a binary gradient contain identical concentrations of buffer and supporting salt. Another alternative would be admixture of a third solvent containing either buffer or conducting salt at a fixed ratio to solvents A and B, the latter ones being responsible for the gradient profile.

3.8. Mass spectrometric detection

Unfortunately, despite its unsurpassable capability to determine the exact molecular masses of each oligomer directly after the chromatographic run, "on-line" LC–MS techniques for polyether characterization are still rarely used. In contrast, direct mass spectrometric investigations lacking a prior separation step, such as FD-MS [28], FAB-MS [29], ES-MS [30], TOF-SIMS [31] and MALDI-FT-MS [32], seem to be preferred. On the other hand, mass spectrometric characterization of alkylphenol-ethoxylates, fatty alcohol ethoxylates and fatty acid ethoxylates using the FD-MS technique has been performed in the "off-line" mode by prior collection of individual oligomers during chromatography, removal of solvent and subsequent direct-inlet MS [103,121,123,124,171].

Off-line MALDI-MS of separated fractions obtained from LCCC was applied for characterization of PEGs by Pasch and Zammert [165], as well as for PEGs, PPGs and block copolymers of both components by Pasch and Rode [166]. Despite still being an "off-line" method, the eluates do not require prior evaporation in order to concentrate them and thus can subsequently be mixed with 2,4-dihydrobenzoic acid in water as the matrix.

A small survey of a few "on-line" LC–MS techniques will now be given briefly.

Levsen et al. [93] used a moving-belt LC–MS interface for detection of OPEOs and NPEOs and obtained mass spectra in either electron impact (EI) or chemical ionization (CI) mode.

Schröder [40] applied tandem mass spectrometric (MS–MS) detection coupled to a thermospray (TSP) interface, and thus was able to monitor the whole range of fragments derived from the breakdown of alkylpolyethoxylates at different cleavage sites. Collision-induced dissociation (CID) was used to generate the characteristic fragments from the individual oligomers. Furthermore, selected-ion monitoring (SIM) was exploited for quantitative sample measurements.

The TSP LC–MS technique was further exploited by Evans et al. [116] for quantification of either total concentrations of alkylpolyethoxylates in environmental samples ranging from 25 to 100 ppb or individual alkylethoxylate compounds, where concentrations as small as 3 ppb had been measured.

Crescenci et al. [127] performed LC–MS of NPEOs and alkyl ethoxylates widely varying in the chain length of the aliphatic moiety in the electrospray MS (ES-MS) mode and reported limits of detection of about 20 pg for individual oligomers.

Although surprisingly small attention has been paid to "on-line" LC–MS of polyether derivatives, it is expected that there will be increasing interest in the powerful technique in the near future. The method should provide an excellent means for solving fundamental problems of polymer chemistry, such as determination of masses of individual oligomers as well as their quantitative determination on the basis of, e.g., the peak heights. The latter results could be exploited thereafter for high accuracy measurement of typical parameters of polymer molecules, such as M_n, M_w and M_w/M_n values. In particular, the MALDI-MS technique will fulfil these requirements when being applicable in the "on-line" mode because, in contrast to ES-MS, oligomers with $M_r > 3000$ may be measured without fragmentation, thus yielding complete information with respect to either mass and FTD of polyether oligomers. En-

couraging preliminary results in this area have been published by Li et al. [185], Murray et al. [186] and Nagra and Li [187]. Nagra and Li [187] give a detailed report on application of "continuous-flow" MALDI-TOF-MS for analysis of large biomolecules. However, mass resolution requires some improvements before the system can be routinely used, as, e.g., for endgroup determinations in polymers, but these can be expected in the near future as stated by the latter authors [187].

One possible reason for the low number of applications to polyether derivatives may be in the high price for a mass spectrometer used in combination with LC.

4. Conclusions

It is evident from the data presented that different types of polyether, e.g., PEGs, PPGs and PBGs, and the different alkyl-, arylalkyl-, carboxyalkyl-substituted PEGs, require different separation conditions.

Polyethylene glycols carrying a long aliphatic or araliphatic chain at one end of the molecule, are preferably separated according to the number of EO units on bare silica gel or so-called bonded phases, such as CN-, diol- or NH_2-materials under normal-phase conditions using organic solvents, and elute in the order of increasing number of EO units. However, little or no discrimination can be made with respect to the chemical structure and chain length of the aliphatic or araliphatic moiety, which in contrast, can be achieved on alkyl-substituted silica gel phases of different length of the alkyl chain with aqueous organic solvents. The higher the number of EO units the better will be gradient elution, because high-M_r oligomers will increasingly merge with the baseline when isocratic conditions are used. Nevertheless, the high-M_r oligomers are increasingly adsorbed onto the polar stationary phases and often can only be "released" from them by use of very polar solvents, such as ethanol and 2-propanol, but unfortunately lacking any separation into individual oligomers as a consequence of their too strong eluotropic properties.

Alternatively, separation of non-ionic surfactants of the ethoxylate type can be performed by typical RP-HPLC, but it should be considered that either resolution is not as good as in NP-HPLC or that the different oligomers elute now in the order of decreasing number of EO units.

In contrast to alkyl- and arylalkyl-substituted PEGs, which are better resolved in NP-HPLC, RP-HPLC is to be preferred for the native analogues, due to their often too strong retention on bare silica gel or so-called bonded-phases, operated under normal-phase conditions. However, peak resolution often remains unsatisfactory, which can be ascribed to the rather polar character of the native PEGs, the oxyethylene groups obviously showing too weak interaction with hydrophobic stationary phases. Despite this apparent drawback, excellent separation is achieved by application of two C_{18} columns in series or, alternatively, by use of bare silica gel stationary phases with aqueous organic solvents.

Markedly higher retention as compared with PEGs is observed with PPGs and PBGs, because the latter polyethers are substantially more hydrophobic and thus display more pronounced solute-matrix interactions. Unfortunately, in the case of PBG these are so strong that complete elution of a variety of samples could neither be effected on a C_{18} nor a C_8 stationary phase, except when ethanol or 2-propanol are used as organic modifiers, and only a C_4 matrix with acetonitrile–water proved to be suitable for either quantitative "release" from the column or efficient separation of oligomers.

PPG amines are not "released" from the chromatographic column with pure aqueous organic eluents, presumably due to strong silanophilic interactions with the stationary phase, but the problem can be overcome by prior derivatization to the corresponding amides. The oligomers of native PEG, PPG, PBG and PPG amides elute in the order of increasing M_r.

Due to complex formation of the 1,2-dioxoethylene moiety with potassium ions, ion-exchange chromatography of PEG derivatives represents an additional highly efficient, but still rarely used technique, yielding separation according to increasing numbers of EO units. It is hoped that excellent oligomer resolution obtained by this technique will increase the potential use of IEC for analysis of PEGs in future.

Due to the fact that in an increasing number of applications "one-dimensional" chromatography is not sufficient for complete characterization of com-

plex mixtures including either samples with different endgroups or copolymers, LCCC gains more and more importance. This technique effects separation according to chemical composition in the first dimension, whereas characterization with respect to individual molecular heterogeneity is achievable by, e.g., "true" HPLC or SEC in the second.

UV detection is still the usual method for signal monitoring of polyether derivatives because, on the one hand, NIS of the ethoxylate type often contain an aromatic moiety and, on the other hand, both alkyl-substituted ethoxylates and native polyethers can be easily derivatized with a large number of available chromophoric agents. Furthermore, detection of native polyethers without a prior derivatization step can also be done at low wavelengths <200 nm, even when gradient HPLC is used. In this case the aqueous phase has to be adjusted to the absorption value of acetonitrile by addition of trace amounts of additives, such as nitric acid or sodium azide in order to compensate for the baseline drift of the organic solvent, which exhibits the stronger molar absorption with respect to water.

Fluorescence detection is required when the sensitivity of signal monitoring by UV is insufficient as, e.g., in determination of NIS in environmental samples.

In either UV or fluorescence detection, signal responses reflect the molar distribution of oligomers, but response factors for the individual oligomers have to be evaluated for subsequent determination of M_n, M_w and M_w/M_n values.

Refractive index and density detection are only applicable in isocratic HPLC and, as a consequence, are restricted to samples either containing a limited number of oligomers or showing a narrow M_r distribution. However, extensive work has been published with respect to the calculation of response factors of individual oligomers, which facilitates determination of M_n, M_w and M_w/M_n values. This can be exploited in HPLC when baseline separation of oligomers is effected. For this reason, further investigations are required in order to increase the feasibility of HPLC in combination with dual detection by, e.g., RI and DDS for calculation of M_r distribution parameters.

Despite being as sensitive as UV detection as evidenced by various authors, ELSD suffers from the main drawback that calculation of response factors for individual oligomers depends on a rather large number of variables and thus, prevents exact quantitative determination of the total amount of sample and values of M_n, M_w and M_w/M_n. However, the samples can be used in their native form without prior derivatization. Furthermore, ELSD is also insensitive to solvents and thus allows the use of eluent components showing marked interference with UV detection, such as ketones.

Unfortunately, the on-line LC–MS coupling technique, which represents the most promising alternative for quantification of samples or calculation of M_n, M_w and M_w/M_n values, has only found limited application. This may, at least partially, be attributable to the high prices of LC–MS interfaces, but nevertheless, extensive investigations using LC–MS techniques for exhaustive analysis of polyether derivatives are to be expected in the near future.

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