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Comparison of various stationary phases for normal-phase high-performance liquid chromatography of ethoxylated alkylphenols

PAVEL JANDERA*
Department of Analytical Chemistry, Institute of Chemical Technology, Nám. Legií 565, 532 10 Pardubice (Czechoslovakia)
JOSEF URBÁNEK
SPOLCHEM Ústí nad Labem, Chemical Plant Děčín XXXII Boletice n. Labem (Czechoslovakia)
and
BOŘIVOJ PROKEŠ and JAROSLAV CHURÁČEK
Department of Analytical Chemistry, Institute of Chemical Technology, Nám. Legií 565, 532 10 Pardubice (Czechoslovakia)
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SUMMARY

Unmodified silica and chemically bonded diol, nitrile and amino phases were tested as column packings for normal-phase liquid chromatographic separations of ethoxylated alkylphenols in mobile phases consisting of aliphatic alcohol(s) and a non-polar hydrocarbon. The performance of unmodified silica is improved when ethanol is used instead of propanol in binary mobile phases or when ethanol–propanol–aliphatic hydrocarbon ternary mobile phases are applied.

Diol and nitrile bonded phases show regular retention behaviour only in mobile phases with a low content of propanol, where 6–8 ethoxymers can be resolved in a reasonable time. In propanol-rich mobile phases, a mixed retention mechanism causes a non-linear increase in log k' with increasing number of oxyethylene units, which hinders the prediction of retention by calculation and deteriorates the separation of the individual oligomers in propanol–*n*-alkane mobile phases.

The amino-bonded stationary phase shows regular retention behaviour even for higher ethoxymers in mobile phases containing high proportions of propanol in *n*-alkane, so that the capacity factors can be predicted by calculation. The amino-bonded phase offers better separation possibilities for the individual ethoxymers than the other stationary phases tested, in both the isocratic and gradient modes of elution.

INTRODUCTION

Ethoxylated alcohols and alkylphenols are important non-ionic surfactants with widespread industrial and domestic applications. Consequently, there is considerable

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interest not only in the determination of these materials, but also in the characterization of the distribution of the individual ethoxymers in both industrial and environmental samples. Although gas and thin-layer chromatography have been applied for this purpose, the applications of high-performance liquid chromatography (HPLC) have acquired major attention recently because of the simplicity of the analytical procedure and its straightforward applicability to the separation of higher ethoxymers.

Steric exclusion chromatography usually requires recycling and long analysis times[1], so that interaction modes of liquid chromatography are more suitable in practice.

To improve the possibilities of UV detection, ethoxylated alcohols can be separated after derivatization with 3,5-dinitrobenzoyl chloride in either reversed-phase[2] or normal-phase[3] systems, or as anthroyl derivatives in reversed-phase systems[4]. Underivatized condensates of ethylene oxide with alcohols or fatty acids may be separated using refractometric[5] or mass spectrometric[6] detection. Normal-phase gradient-elution chromatography on a bonded amino phase[6] and isocratic partition chromatography on a bonded diol phase using a mobile phase consisting of *n*-hexane, 2-propanol, water and acetic acid[5] have been employed for this purpose.

Ethoxylated alkylphenols can be partially separated in reversed-phase chromatographic systems using octadecyl- or octylsilica columns[7–11], but the quality of the separation depends on the type of alkyl group and on the mobile phase components and the individual ethoxymeres may be eluted in order of either decreasing or increasing number of oxyethylene units[7–9]. In normal-phase systems, silica gel and mobile phases consisting of an *n*-alkane and propanol, ethanol or acetonitrile have been employed for the separation of ethoxylated alkylphenols[10,12,13]. Nitrile-[4,12–14], diol-[13] and amino-bonded phases[6,13,15–21] have been employed more often than unmodified silica, but the mobile phases used usually consisted of water, acetic acid or other very strongly polar compounds in addition to the *n*-alkane and another organic solvent of medium polarity, which makes these systems more difficult to understand; obviously these are dynamically induced partition rather than adsorption systems. Ethoxylated alkylphenols are readily detected by UV detectors but fluorimetric[18] or mass spectrometric[8] detection systems are also occasionally used.

Desbène *et al.*[3] compared the performances of diol-, amino- and nitrile-bonded phases for the separation of 3,5-dinitrobenzoyl esters of ethoxylated long-chain aliphatic alcohols, but no systematic comparison of stationary phases for the separation of underivatized ethoxylated alkylphenols has been reported.

This work is a continuation of previous investigations of selectivity in various oligomeric series in reversed-phase and normal-phase systems[13]. The main aim was to compare the chromatographic behaviour of underivatized ethoxylated nonylphenols on unmodified silica gel and chemically bonded diol-, nitrile- and amino-bonded stationary phases in mobile phases consisting of an *n*-alkane and lower aliphatic alcohols and to find the optimum separation conditions. Further, the suitability of the individual normal-phase systems for the prediction of retention was tested. This aspect is considered to be important for two reasons: (a) this prediction may be useful for identifying the peaks in chromatograms of more complex samples, which may contain not only the pure oligomers but also isomers, adducts, additives, and other compounds; and (b) irregular band spacing in the chromatogram may affect

unfavourably the results of quantification of the oligomer distribution and the prediction of retention is important for optimization of the separation process so as to obtain regularly spaced symmetrical peaks of the individual oligomers in as short a separation time as possible.

Therefore, chromatographic systems that yield regular and predictible spacing of the peaks of the individual oligomers in the chromatogram are to be preferred to other systems. For this reason, we compared the individual chromatographic systems from the point of view of regular band spacing, predictability of retention and reliability of the quantitative results.

As has been shown earlier, the dependence of the capacity factors, k', of the individual oligomers on the number of oligomeric units, n, and on the concentration, φ, of the more polar component in a binary mobile phase can often be described by[22]

$$\log k' = a_0 - m_0 \log \varphi + n\,(a_1 - m_1 \log \varphi) \quad (1)$$

where a_0, m_0, a_1 and m_1 are constants related to the adsorption energy and adsorbed area of the repeat structural (oligomeric) unit and of the structural residue (end-groups)[13,22].

EXPERIMENTAL

Some experiments were performed using an M6000A pump, a U6K injector and an M440 UV detector operated at 254 nm (all from Waters Assoc., Milford, MA, U.S.A.), connected to a TZ 4241 line recorder and a CI 100 computing integrator (both from Laboratory Instrument Works, Prague, Czechoslovakia). An HP 1090M liquid chromatograph equipped with a UV diode-array detector, operated at 230 nm, an automatic sample injector, a 3DR solvent delivery system, a thermostated column compartment, a Series 79994A workstation and an HP 2225 Think-Jet printer (Hewlett-Packard, Avondale, PA, U.S.A.) was used for other experiments.

Stainless-steel columns (300 × 3.8, 4.2 or 4.4 mm I.D.) were packed in the laboratory with the spherical materials Silasorb 600 SPH (silica gel), 5 μm, Silasorb SPH Amine and Silasorb SPH Nitrile, 7.5 μm (all from Lachema, Brno, Czechoslovakia), using a high-pressure slurry packing technique. A Silasorb Diol (7.5 μm) stainless-steel column (250 × 4 mm I.D.) was obtained pre-packed from Lachema.

Oligomeric ethoxylated nonylphenols with various declared average stoichiometric ratios of oxyethylene units to nonylphenol were obtained from Servo (Delden, The Netherlands) under the commercial names Serdox NNP 1.5, Serdox NNP 4, Serdox NNP 8, Serdox NNP 12 and Serdox NNP 20. The samples were dissolved in the mobile phase in appropriate concentrations to yield good UV detector responses.

1-Propanol, 2-propanol, ethanol and *n*-heptane used as mobile phase components were all of spectroscopic or analytical-reagent grade from Lachema. The solvents were kept dried over molecular sieves S5A (Lachema) and were filtered using a Millipore non-aqueous 0.45-μm filter before use. The mobile phases were either pre-mixed in the required volume ratios and de-gassed by ultrasonication prior to use in the Waters Assoc. equipment, or were prepared directly in the HP 1090M instrument by mixing the pure solvents and de-gassed during operation by continuous stripping with helium.

Column dead volumes, V_M, were determined using refractometric detection with an R401 differential refractometer (Waters Assoc.) and *n*-heptane as the dead volume marker. The retention volumes, V_R, of the individual ethoxymers in the samples tested were measured at different mobile phase compositions. The capacity factors, k', were calculated from the mean V_R value of three repeated experiments under given conditions ($k' = V_R/V_M - 1$). Linear regression analysis was used to calculate the constants a_0, m_0, a_1 and m_1 in eqn. 1, necessary for predictive calculations of k'.

For mass spectrometric identification of the individual ethoxymers in fractions from the liquid chromatograph, a Varian-MAT 44s quadrupole mass spectrometer (Varian, Bremen, F.R.G.) was employed, using chemical ionization with methane. Mass spectrometric measurements were performed at the Institute for Synthetic Resins and Lacquers, Pardubice, Czechoslovakia.

RESULTS AND DISCUSSION

Identification of individual ethoxymers

As pure etoxymer standards were not available and the commercial samples were mixtures of various oligomers the identification of the individual oligomers was based on mass spectrometry of the fractions separated by HPLC. For this purpose, Serdox NNP 1.5 was chosen, as it contains only low oligomers in relatively high concentrations (declared stoichiometric ratio of ethylene oxide to nonylphenol = 1.5:1). The individual ethoxymers in the sample were completely separated on the column packed with Silasorb SPH Amine in 1-propanol–*n*-heptane (2.5:97.5). A 150-μl volume of a 5% solution of Serdox NNP 1.5 was separated into three main fractions (A, B and C, Fig. 1), the individual fractions from two repeated runs were collected and their mass spectra measured. These spectra are shown in Fig. 2. Fractions A and B, corresponding to the first two chromatographic peaks, show peaks of the molecular ion at $m/z = 264$,

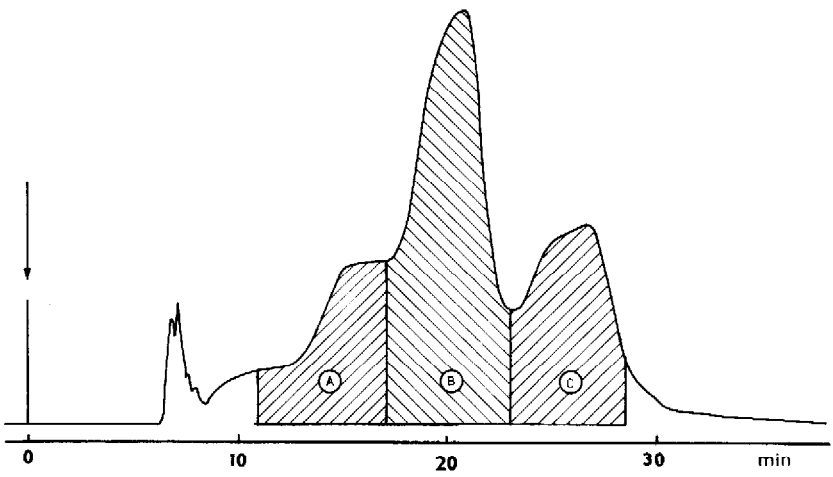

Fig. 1. Semi-preparative separation of 150 μl of a 5% sample of Serdox NNP 1.5 on a Silasorb SPH Amine (7.5 μm) column (300 × 4.2 mm I.D.) with 1-propanol–*n*-heptane (2.5:97.5) mobile phase. Instrument, Waters Assoc.; UV detection at 254 nm. Fractions A, B and C correspond to the three dominant peaks in the chromatogram.

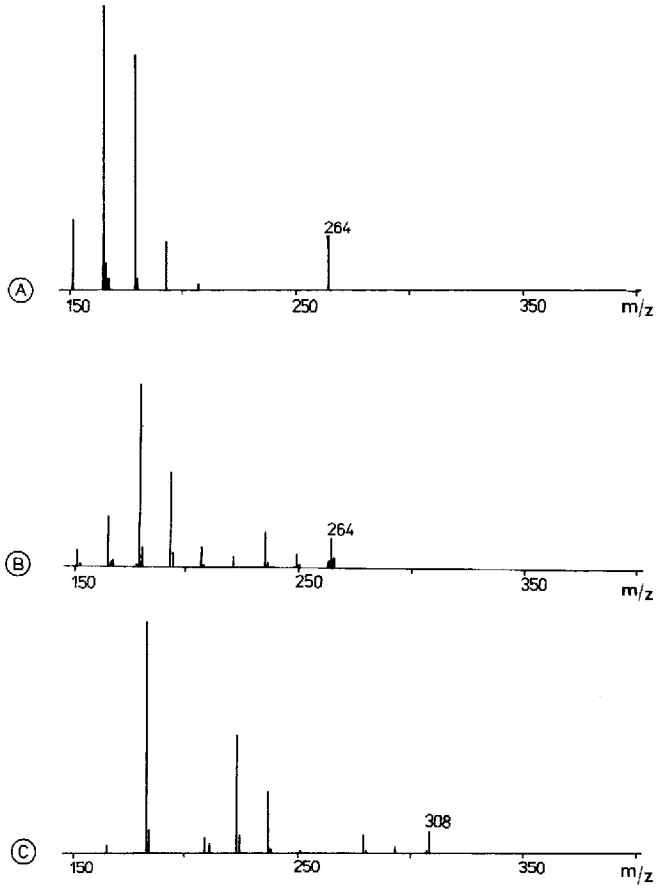

Fig. 2. Mass spectra of fractions A, B, C in Fig. 1. Conditions are given under Experimental.

which corresponds to nonylphenol with one oxyethylene unit, and the third peak (fraction C) shows the peak of the molecular ion at $m/z = 308$, which represents the molecular mass of nonylphenol with two oxyethylene units. In fraction B, the spectrum is dominated by a fragment at $m/z = 179$, which is probably formed by the loss of a hexyl radical ($\Delta m/z = 85$) from the molecular ion. The fragment peak at $m/z = 233$ in the mass spectrum of fraction C corresponds to the same loss. In the mass spectrum of fraction A, the fragment peak at $m/z = 165$ is more intense than at $m/z = 179$. The former peak probably corresponds to the loss of a heptyl radical from the molecular ion.

From this it can be concluded that the first peak in Fig. 1 (fraction A) probably corresponds to $C_7H_{15}CH(CH_3)C_6H_4$-*p*-OCH_2Ch_2OH (I), the second peak (fraction B) to $C_6H_{13}C(CH_3)_2C_6H_4$-*p*-OCH_2CH_2OH (II) and the third peak (fraction C) to $C_6H_{13}C(CH_3)_2C_6H_4$-*p*-$O(CH_2CH_2O)_2H$ (III).

From Fig. 1 it can be seen that isomer II is present in the sample at a *ca.* five times higher concentration than isomer I. In some of the experiments the chromatograms

TABLE I

EXPERIMENTAL RETENTION VOLUMES (V_R, cm^3) AND CAPACITY FACTORS [k'(e)] OF THE INDIVIDUAL OLIGOMERS WITH n OLIGOMERIC UNITS ON SILASORB 600 SPH, 5 μm (COLUMN 300 × 3.8 mm I.D.), IN MOBILE PHASES CONTAINING VARIOUS CONCENTRATIONS [φ, % (v/v) · 10^{-2}] OF 1-PROPANOL IN n-HEPTANE

Sample: Serdox NNP 4. V_M = column dead volume (cm^3); k'(c) are the capacity factors predicted by calculation using the equation $\log k' = -1.47 + 0.35n - (1.00 + 0.07n) \log \varphi$.

n	$\varphi = 1$			$\varphi = 0.9$			$\varphi = 0.8$			$\varphi = 0.6$			$\varphi = 0.5$		
	V_R	$k'(e)$	$k'(c)$	V_R	$k'(e)$	$k'(c)$	V_R	$k'(e)$	$k'(c)$	V_R	$k'(e)$	$k'(c)$	V_R	$k'(e)$	$k'(c)$
2	3.22	0.15	0.17	3.29	0.19	0.19	3.38	0.21	0.22	3.58	0.32	0.30	3.76	0.35	0.37
3	3.83	0.37	0.38	4.15	0.50	0.43	4.38	0.57	0.50	4.75	0.76	0.71	5.31	0.90	0.89
4	5.06	0.80	0.85	5.56	1.02	0.97	6.28	1.25	1.13	7.09	1.62	1.64	8.34	1.99	2.06
5	7.75	1.77	1.91	8.88	2.22	2.20	10.74	2.86	2.58	12.62	3.67	3.80	16.20	4.81	4.86
6	14.29	4.10	4.26	17.13	5.22	4.95	21.82	6.84	5.86	26.52	8.81	8.81			11.41
V_M		2.80			2.75			2.78			2.70			2.79	

showed a slight indication of the separation of isomeric peaks of higher ethoxymers, possibly of type I, but in most instances the isomeric peaks were not apparent and therefore they are not considered in further discusson.

From a comparison of the elution pattern of the Serdox NNP 1.5 sample with chromatograms for other samples with higher nominal degrees of etoxylation, the peaks of the individual ethoxymers were readily identified.

Comparison of retention behaviour on silica gel in binary and ternary mobile phases

The experimental retention volumes and capacity factors in mobile phases containing various concentrations of 1-propanol in *n*-heptane are given in Table I. The logarithms of the capacity factors increases regularly both with increasing number of oligomeric units, n, and with decreasing logarithm of the concentration of propanol in the mobile phase, φ, so that the eqn. 1 can be used to describe the retention of lower oligomers (up to five oxyethylene units). The capacity factors of the oligomer with six oxyethylene units show significant positive deviations from the values predicted by calculation using eqn. 1 in mobile phases containing 80–90% of propanol. A relatively high value of the constant $a_1 = 0.35$ and low value of the constant $m_1 = 0.07$ in eqn. 1 in propanol–heptane mobile phases mean a very high separation selectivity between neighbouring oligomers, so that it is not possible to separate more than four or five oligomers in a single run within a reasonable separation time under isocratic conditions.

In mobile phases consisting of ethanol and *n*-heptane, eqn. 1 can be used to describe appropriately the retention of the first seven oligomers in the ethoxylated nonylphenol series, higher oligomers showing negative deviations from the capacity factors predicted by calculation (Table II). The constants a_1 and m_1 in eqn. 1 are lower in ethanol–*n*-hexane mobile phases than in mobile phases containing propanol, which has been attributed to the higher polarity (solvent strength) of ethanol with respect to

TABLE II

AS TABLE I, FOR MOBILE PHASES CONTAINING VARIOUS CONCENTRATIONS [φ, % (v/v) · 10^{-2}] OF ETHANOL IN *n*-HEPTANE

k'(c) are the capacity factors predicted by calculation using the equation $\log k' = -2.46 + 0.29n - (3.28 - 0.27n) \log \varphi$.

n	$\varphi = 0.3$			$\varphi = 0.4$			$\varphi = 0.5$			$\varphi = 0.6$		
	V_R	$k'(e)$	$k'(c)$	V_R	$k'(e)$	$k'(c)$	V_R	$k'(e)$	$k'(c)$	V_R	$k'(e)$	$k'(c)$
1	3.22	0.26	0.25	2.89	0.08	0.11	2.78	0.03	0.03			0.02
2	3.38	0.32	0.36	3.10	0.15	0.16	2.90	0.08	0.09	2.88	0.05	0.05
3	3.83	0.50	0.50	3.38	0.26	0.25	3.15	0.17	0.14	3.05	0.12	0.09
4	4.57	0.79	0.71	3.84	0.43	0.38	3.43	0.28	0.23	3.25	0.18	0.15
5	4.94	0.93	1.00	4.17	0.56	0.57	3.76	0.40	0.37	3.54	0.29	0.26
6	5.96	1.33	1.41	4.96	0.85	0.87	4.33	0.61	0.60	4.03	0.47	0.44
7	7.47	1.92	1.98	5.99	1.23	1.33	5.15	0.92	0.98	4.77	0.74	0.76
8	9.31	2.64	2.79	7.60	1.84	2.02	6.26	1.33	1.57	5.76	1.10	1.28
9	12.12	3.75	3.39	9.92	2.70	3.08	7.93	1.96	2.55	7.08	1.59	2.18
V_M		2.55			2.68			2.68			2.73	

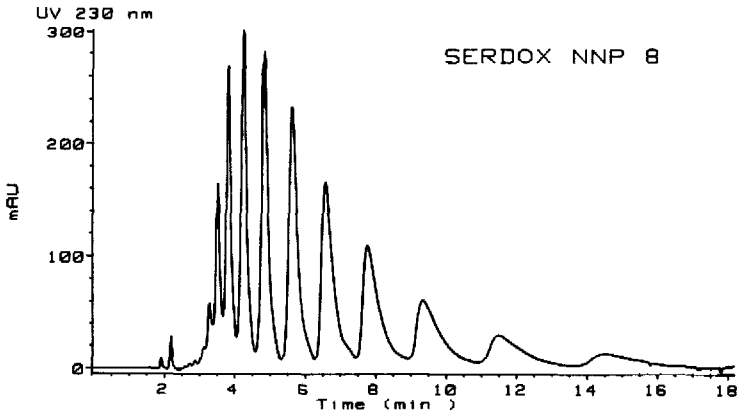

Fig. 3. Isocratic separation of Serdox NNP 8 on a Silasorb 600 SPH, 5 μm (silica gel) column (300 × 3.8 mm I.D.) with ethanol–*n*-heptane (30:70) mobile phase. Flow-rate, 1 cm^3/min; detection, UV (230 nm); instrument, HP 1090M; sample volume, 5 μl.

propanol[13]. Consequently, both the absolute retention and selectivity are lower in ethanol–*n*-heptane mobile phases, which in this instance favours isocratic separation of the individual oligomers in a shorter time than in propanol–*n*-heptane mobile phases. Fig. 3 shows the chromatographic separation of Serdox NNP 8 (with eight nominal oxyethylene units) on Silasorb 600 SPH, using ethanol–*n*-heptane (30:70) as the mobile phase. These conditions allow the first twelve oligomers to be separated in about 16 min.

In ternary mobile phases containing ethanol and 1-propanol in *n*-heptane, the chromatographic behaviour of ethoxylated nonylphenols is approximately between those in ethanol–*n*-heptane and propanol–*n*-heptane binary mobile phases[13]. This means that the selectivity of the separation of neighbouring oligomers is lower than in 1-propanol–*n*-heptane but higher than in ethanol–*n*-heptane mobile phases. Table III

TABLE III

AS TABLE I, FOR MOBILE PHASES CONTAINING VARIOUS CONCENTRATIONS OF 1-PROPANOL (φ_P) AND ETHANOL (φ_E) IN *n*-HEPTANE [φ_E, φ_P IN % (v/v) · 10^{-2}; $\varphi_E : \varphi_P$ = 1:1).

k'(c) are the capacity factors predicted by calculation using the equation $\log k' = -1.97 + 0.31n - (1.83 - 0.11n) \log (\varphi_E + \varphi_P)$.

n	$\varphi_E = \varphi_P = 0.5$			$\varphi_E = \varphi_P = 0.166$			$\varphi_E = \varphi_P = 0.125$		
	V_R	$k'(e)$	$k'(c)$	V_R	$k'(e)$	$k'(c)$	V_R	$k'(e)$	$k'(c)$
2			0.04			0.26			0.42
3	3.10	0.09	0.09	4.00	0.50	0.47	4.74	0.71	0.73
4	3.34	0.17	0.19	4.91	0.84	0.86	5.97	1.15	1.28
5	3.96	0.38	0.38	6.50	1.44	1.55	8.42	2.03	2.24
6	5.06	0.77	0.78	9.49	2.56	2.81	13.17	3.74	3.93
7	7.10	1.49	1.58	15.30	4.75	5.08	22.34	7.06	6.89
V_M		2.85			2.66			2.77	

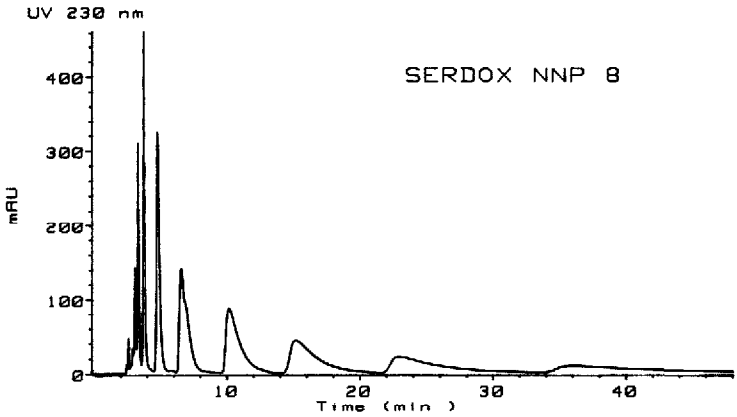

Fig. 4. Isocratic separation of Serdox NNP 8 on a Silasorb 600 SPH column with ethanol–1-propanol–*n*-heptane (1:1:3) mobile phase. Other conditions as in Fig. 3.

shows that eqn. 1 may be adequately used to describe the dependence of the retention ($\log k'$) of the first seven oligomers on the number of oligomeric units and on the sum of the concentrations of ethanol and propanol. Fig. 4 shows the separation of the first ten oligomers in ethanol–1-propanol–*n*-heptane (20:20:60) mobile phase, which takes about 45 min. Using a ternary gradient with simultaneously increasing concentration of ethanol and 1-propanol at a constant ratio of ethanol and 1-propanol concentrations (3:1), it was possible to achieve the separation of fourteen oligomers in 30 min (Fig. 5). The separation achieved under these conditions compares favourably with that using a binary gradient of increasing concentration of ethanol in *n*-heptane (Fig. 6). Hence the ratio of the concentrations of ethanol and 1-propanol in the mobile phase may be used with advantage to control the retention and the separation selectivity.

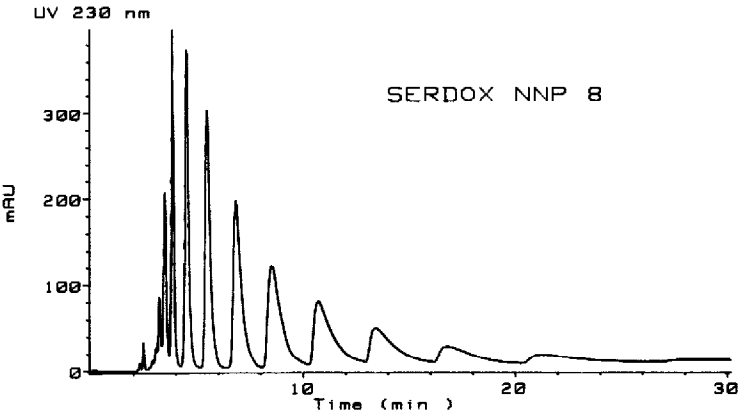

Fig. 5. Gradient elution separation of Serdox NNP 8 on a Silasorb 600 SPH column using a linear ternary gradient from ethanol–1-propanol–*n*-heptane (24:8:68) to ethanol–1-propanol (75:25). Other conditions as in Fig. 3.

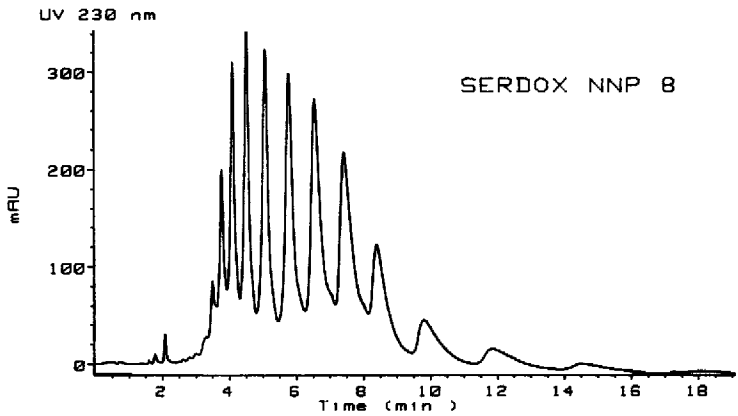

Fig. 6. Gradient elution separation of Serdox NNP 8 on a Silasorb 600 SPH column using a linear gradient from ethanol–*n*-heptane (25:75) to ethanol–*n*-heptane (85:15) in 30 min. Other conditions as in Fig. 3.

Retention behaviour on chemically bonded polar phases in organic mobile phases

The nitrile phase is the least polar of the chemically bonded phases tested. In mobile phases containing less then 25–30% of 2-propanol in *n*-heptane, the retention of the individual ethoxylated nonylphenols on Silasorb SPH Nitrile is adequately described by eqn. 1, *i.e.*, log k' increases regularly with increasing number of oligomeric units, n, and with decreasing logarithm of propanol concentration, φ (Table IV).

The elution behaviour of ethoxylated nonylphenols in mobile phases with a higher content of propanol is different (Table V). The decrease in log k' with increasing log φ of 1-propanol is almost linear and negative slopes of these plots increase with increasing number of oligomeric units, as expected according to eqn.

TABLE IV

EXPERIMENTAL RETENTION VOLUMES (V_R, cm^3) AND CAPACITY FACTORS [k'(e)] OF THE INDIVIDUAL OLIGOMERS WITH n OLIGOMERIC UNITS ON SILASORB SPH NITRILE, 7.5 μm (COLUMN 300 × 4.4 mm I.D.), IN MOBILE PHASES CONTAINING VARIOUS CONCENTRATIONS [φ, % (v/v) · 10^{-2}] OF 2-PROPANOL IN *n*-HEPTANE

Sample: Serdox NNP 4. V_M = column dead volume (cm^3); k'(c) are the capacity factors predicted by calculation using the equation $\log k' = -1.81 + 0.06n - (0.94 + 0.14n) \log \varphi$.

n	φ = 0.25			φ = 0.20			φ = 0.14		
	V_R	k'(e)	k'(c)	V_R	k'(e)	k'(c)	V_R	k'(e)	k'(c)
1	4.40	0.07	0.08	4.52	0.10	0.10	4.75	0.14	0.15
2	4.53	0.10	0.11	4.71	0.14	0.14	5.10	0.22	0.22
3	4.74	0.15	0.15	4.97	0.21	0.21	5.58	0.34	0.34
4	4.96	0.21	0.21	5.29	0.28	0.30	6.15	0.48	0.51
5	5.26	0.28	0.30	5.75	0.40	0.43	7.14	0.72	0.78
6	5.66	0.37	0.42	6.44	0.57	0.62	8.69	1.09	1.17
7	6.14	0.49	0.58	7.56	0.84	0.89	11.14	1.68	1.78
V_M		4.11			4.11			4.15	

TABLE V

AS TABLE IV, FOR MOBILE PHASES CONTAINING VARIOUS CONCENTRATIONS [φ, % (v/v) · 10^{-2}] OF 1-PROPANOL IN *n*-HEPTANE

Column dead volume (V_M = 3.45 cm^3).

n	$\varphi = 0.5$		$\varphi = 0.6$		$\varphi = 0.7$		$\varphi = 0.8$	
	V_R	$k'(e)$	V_R	$k'(e)$	V_R	$k'(e)$	V_R	$k'(e)$
1	4.09	0.19	4.08	0.18	–	–	–	–
2	4.27	0.24	4.22	0.22	4.19	0.21	4.18	0.21
3	5.34	0.55	5.26	0.52	5.05	0.46	4.96	0.44
4	10.12	1.93	9.18	1.66	8.11	1.35	7.54	1.19
5	16.92	3.90	14.32	3.15	11.64	2.37	10.29	1.98
6	25.73	6.46	21.26	5.16	15.87	3.60	13.06	2.78
7	33.57	8.73	25.75	6.46	18.40	4.33	14.66	3.25
8			29.76	7.63	20.53	4.95	15.97	3.63
9			33.22	8.63	22.38	5.49	17.03	3.94
10			37.15	9.77	24.45	6.09	18.22	4.28
11			42.59	11.35	27.40	6.94	20.17	4.85
12					32.00	8.28	23.10	5.70
13					38.74	10.23	27.47	6.96
14					48.35	13.01	33.42	8.69
15							41.21	10.96

1 (Fig. 7), but the plots of log k' *versus* *n* are non-linear and show a sigmoidal shape (Fig. 8). The increase in log k' per oligomeric unit is most significant in between two and five oxyethylene units; this increase is approximately six times less for the oligomers with more than six oxyethylene units, but for oligomers with more than ten oxyethylene units the increment in log k' per oligomeric unit again increases. The behaviour has not been observed either in mobile phases with a lower content of propanol or in chromatographic systems with unmodified silica Silasorb 600 SPH, and is not easy to explain. The increment in log k' is equivalent to a free energy of transfer from the mobile to the stationary phase per oligomeric unit and the retention behaviour observed with the nitrile stationary phase in propanol-rich mobile phases suggests a change in conformation of the oxyethylene chains depending on the chain length of the oligomer. This conformation change may be connected with solvation of the oxyethylene chains by propanol molecules and with steric possibilities for contact between the oxyethylene units and active sorption centres on the surface of the bonded phase. The different retention behaviour in mobile phases with low and high contents of propanol indicates that a possible mixed partition–adsorption mechanism may play an important role in propanol-rich mobile phases. This may possibly explain the lower capacity factors in 2-propanol–*n*-heptane (25:75) than in 1-propanol–*n*-heptane (50:50) (Tables IV and V). As a practical consequence, isocratic (Fig. 9) or gradient-elution (Fig. 10) separations of ethoxylated nonylphenols in propanol-rich mobile phases yield irregularly spaced peaks with poor separation of the first oligomers.

The retention behaviour of ethoxylated nonylphenols on the bonded diol phase is very similar to that on the nitrile phase. In mobile phases containing less than 25%

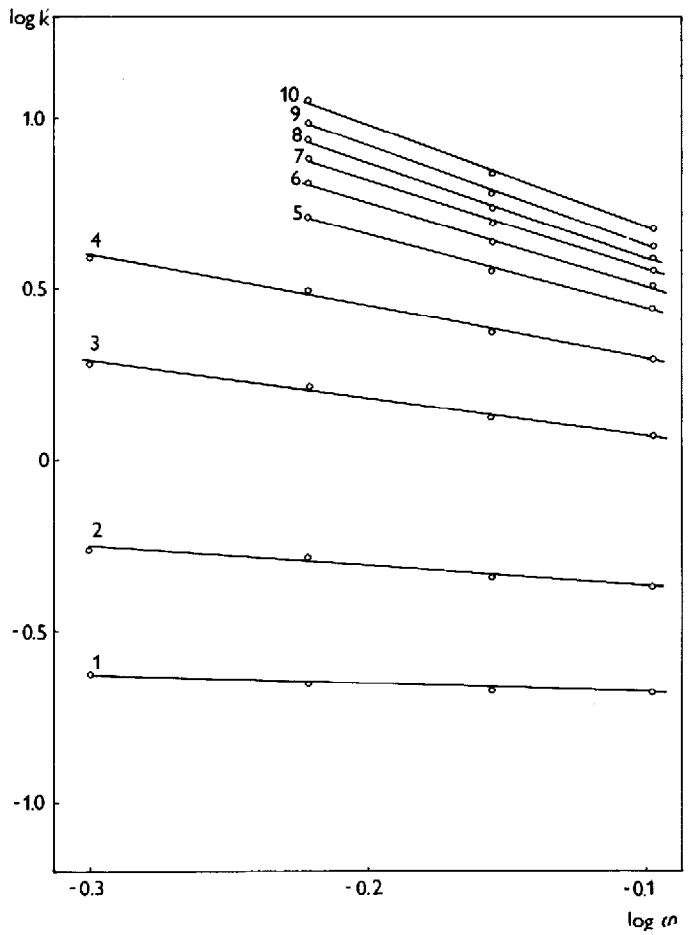

Fig. 7. Dependence of retention (k') of the individual ethoxymers in the sample of Serdox NNP 8 on the concentration [φ, % (v/v) · 10^{-2}] of 2-propanol in *n*-heptane as the mobile phase on a Silasorb SPH Nitrile, 7.5 μm, column (300 × 4.0 mm I.D.). The numbers on the lines are the numbers of oxethylene units in the ethoxymers.

1-propanol in heptane, the retention behaviour of the ethoxylated nonylphenols with up to eight oligomeric units is well described by eqn. 1 (Table VI). However, the capacity factors are greater in the mobile phase containing 40% than in that with only 20% of propanol (Table VI), and the plots of log k' *versus* the number of oligomeric units in propanol-rich mobile phases show an analogous sigmoidal shape to the corresponding dependences for Silasorb Nitrile (Fig. 11). Obviously, the reason for this behaviour is the same with the two stationary phases. Also, the spacing of the peaks of the individual oligomers is similar to that with the nitrile phase (Fig. 12).

The retention behaviour of ethoxylated nonylphenols on the bonded amino phase differs significantly from that on the other bonded phases tested. Eqn. 1 can describe adequately the retention of ethoxylated nonylphenols on Silasorb SPH Amine up to the seventh or eighth oligomer in mobile phases containing 20–75% of

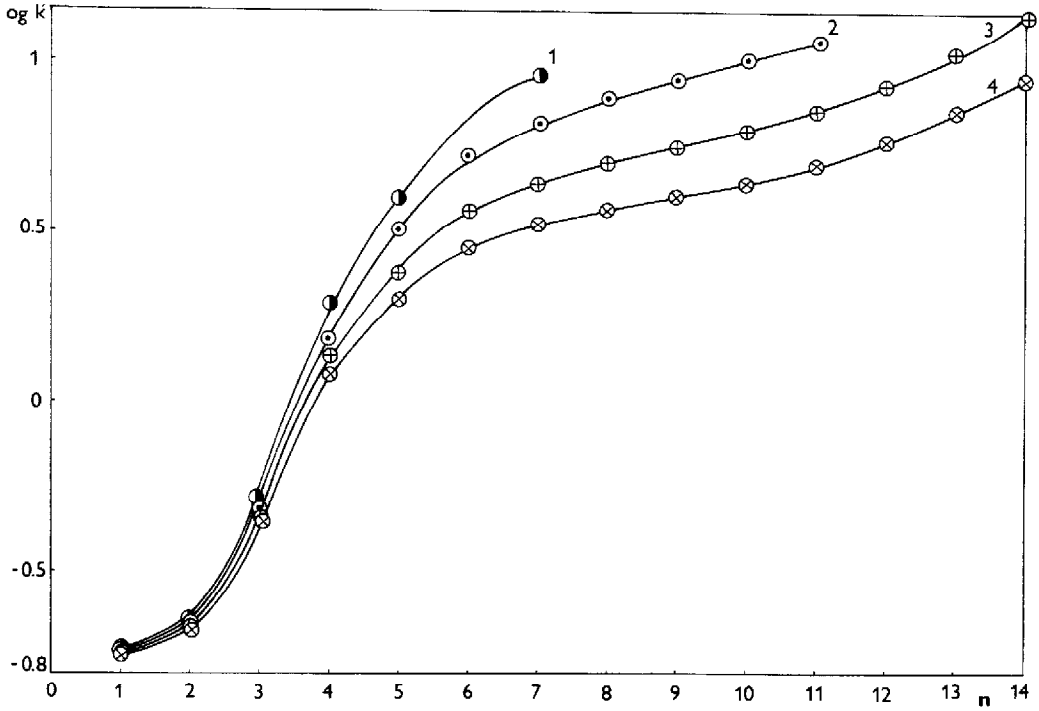

Fig. 8. Dependence of retention (k') of the individual ethoxymers in the sample of Serdox NNP 8 on the number of oxyethylene units, n. Column as in Fig. 7. Mobile phases with different ratios of 2-propanol to n-heptane: (1) 50:50; (2) 60:40; (3) 70:30; (4) 80:20.

2-propanol in n-heptane; positive deviations are observed with 10% propanol for the oligomers with $n \geqslant 5$ (Table VII). This "regular" dependence of k' on the number of oligomeric units and on the mobile phase composition is similar to that observed with unmodified silica Silasorb 600 SPH. Unmodified silica and the bonded amino phase

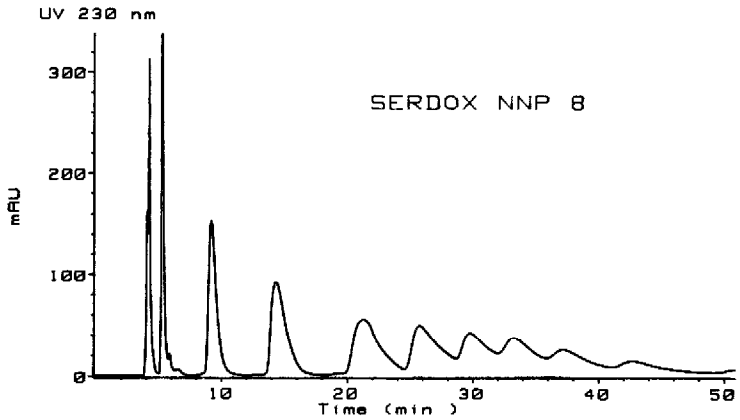

Fig. 9. Isocratic separation of Serdox NNP 8 on a Silasorb SPH Nitrile, 7.5 μm, column (300 × 4.4 mm I.D.) with 1-propanol–n-heptane (60:40) mobile phase. Other conditions as in Fig. 3.

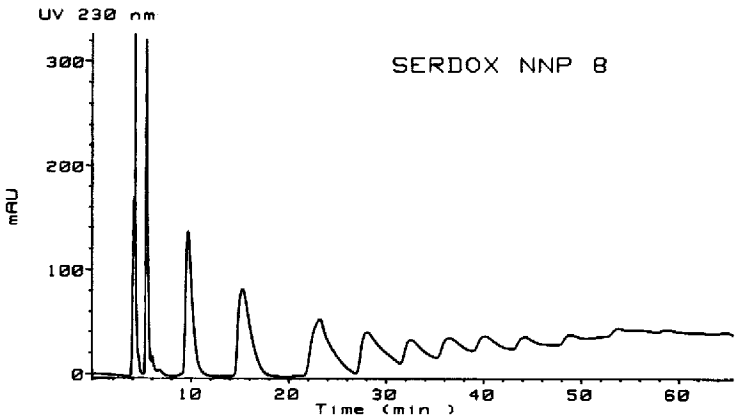

Fig. 10. Gradient elution separation of Serdox NNP 8 on a Silasorb SPH Nitrile column using two-step elution with 1-propanol–*n*-heptane (60:40) for 30 min, followed by a linear gradient to 1-propanol–*n*-heptane (90:10) in the following 30 min. Column as in Fig. 9; other conditions as in Fig. 3.

possess stronger polar adsorption centres than the nitrile and diol phases and the mixed retention mechanism and conformational change of the oxyethylene chain depending on the chain length probably do not occur, or are far less significant on the former two stationary phases. The spacing of the individual oligomers on the chromatograms obtained with the bonded amino phase is more regular than that with the nitrile- and diol-bonded phases and allows a better separation of the first

TABLE VI

EXPERIMENTAL RETENTION VOLUMES (V_R, cm^3) AND CAPACITY FACTORS [k'(e)] OF THE INDIVIDUAL OLIGOMERS WITH n OLIGOMERIC UNITS ON SILASORB DIOL, 7.5 μm (COLUMN, 250 × 4 mm I.D.), IN MOBILE PHASES CONTAINING VARIOUS CONCENTRATIONS [φ, % (v/v) · 10^{-2}] OF 1-PROPANOL IN *n*-HEPTANE

Sample: Serdox NNP 4. V_M = column dead volume (cm^3); k'(c) are the capacity factors predicted by calculation using the equation $\log k' = -1.60 + 0.15n - (0.58 + 0.03n) \log \varphi$.

n	$\varphi=0.2$			$\varphi=0.1$			$\varphi=0.05$			$\varphi=0.01$		
	V_R	$k'(e)$	$k'(c)$	V_R	$k'(e)$	$k'(c)$	V_R	$k'(e)$	$k'(c)$	V_R	$k'(e)$	$k'(c)$
1												
2	3.02	0.12	0.14	3.29	0.23	0.22	3.50	0.31	0.34	5.41	0.91	0.95
3	3.20	0.16	0.21	3.58	0.34	0.33	4.00	0.49	0.53	7.41	1.61	1.54
4	3.41	0.24	0.31	4.08	0.53	0.50	4.58	0.71	0.81	9.50	2.35	2.51
5	3.95	0.43	0.46	4.62	0.73	0.76	5.50	1.06	1.25	13.91	3.91	4.07
6	4.50	0.63	0.68	5.75	1.15	1.15	7.37	1.76	1.94	21.29	6.51	6.61
7	5.12	0.86	1.00	7.70	1.89	1.74						
8	6.75	1.45	1.49	9.45	2.54	2.63						
9												
10												
11												
12												
V_M		2.73			2.73			2.73			2.73	

oligomers, with relatively short elution times of the ethoxymers up to $n = 14$–18 (Fig. 13). If gradient elution is applied in this chromatographic system, approximately 25 oligomers can be separated in 1 h (Fig. 14). The separation achieved on Silasorb SPH Amine was better than that on the other column packings tested, which can be explained as follows. As the adsorption energy and activity of the adsorbent surface are lower on the amino-bonded phase than on silica gel, so are the partial constants a_1 and m_1 in eqn. 1, which determine the selectivity of separation between neighbouring oligomers. Hence, the rate of increase in the capacity factors with increasing number of oligomeric units is lower on the amino-bonded phase and a greater number of oligomers can be separated in reasonable time than on unmodified silica. The diol- and nitrile-bonded phases are similar to the amino bonded phase as far as the separation of lower oligomers is concerned, but unfortunately the possibilities of separating higher oligomers are impaired by the irregular retention behaviour discussed above. Previous workers using nitrile- or diol-bonded phases usually added water, acetic acid or methanol to the mobile phase to achieve a good separation, but such systems are more difficult to understand and describe because of the mixed separation mechanism (partition–adsorption). Although the separation efficiency in these systems is similar to those in non-aqueous systems employing amino-bonded phases, in our experience the latter chromatographic system increases the number of oligomers that can be separated or suppresses band tailing and makes it possible to achieve more reproducible quantitative results.

$\varphi = 1$		$\varphi = 0.8$		$\varphi = 0.7$		$\varphi = 0.6$		$\varphi = 0.4$	
V_R	$k'(e)$	V_R	$k'(e)$	V_R	$k'(e)$	V_R	$k'(e)$	V_R	$k'(e)$
		2.56	0.08	2.56	0.08	2.55	0.08	2.67	0.13
2.62	0.10	2.67	0.12	2.69	0.14	2.67	0.13	2.83	0.20
3.16	0.33	3.30	0.39	3.38	0.42	3.53	0.49	3.94	0.66
4.65	0.96	5.46	1.30	6.05	1.55	6.85	1.89	8.51	2.59
6.08	1.56	7.75	2.27	9.34	2.94	11.44	3.83	16.65	6.02
7.72	2.26	10.45	3.41	13.66	4.76	18.38	6.76	29.75	11.55
8.67	2.66	11.98	4.06	17.36	6.32	24.55	9.36		
9.50	3.01	13.34	4.63	20.43	7.62	29.77	11.56		
9.99	3.21	14.71	5.10	21.87	8.23	33.84	13.28		
10.51	3.44	15.99	5.75			38.69	15.32		
11.78	3.97	18.64	6.87			46.16	18.48		
13.83	4.84	23.20	8.79						
	2.37		2.37		2.37		2.37		2.37

TABLE VII

EXPERIMENTAL RETENTION VOLUMES (V_R, cm^3) AND CAPACITY FACTORS [k'(e)] OF THE INDIVIDUAL OLIGOMERS WITH n OLIGOMERIC UNITS ON SILASORB SPH AMINE, 7.5 μm (COLUMN, 300 × 4.2 mm I.D.), IN MOBILE PHASES CONTAINING VARIOUS CONCENTRATIONS [φ, % (v/v) · 10^{-2}] OF 2-PROPANOL IN n-HEPTANE

Sample: Serdox NNP 4. Column dead volume (V_M) = 3.50 cm^3. k'(c) are the capacity factors predicted by calculation using the equation $\log k' = -1.78 + 0.16n - (1.31 + 0.01n) \log \varphi$.

n	$\varphi = 0.735$			$\varphi = 0.5$			$\varphi = 0.4$			$\varphi = 0.3$			$\varphi = 0.2$			$\varphi = 0.1$		
	V_R	$k'(e)$	$k'(c)$	V_R	$k'(e)$	$k'(c)$	V_R	$k'(e)$	$k'(c)$	V_R	$k'(e)$	$k'(c)$	V_R	$k'(e)$	$k'(c)$	V_R	$k'(e)$	$k'(c)$
1										3.91	0.11	0.12	4.24	0.21	0.20	5.33	0.52	0.50
2	3.66	0.04	0.05	3.83	0.09	0.09	3.91	0.11	0.12	4.08	0.16	0.18	4.57	0.30	0.30	6.00	0.71	0.74
3	3.75	0.07	0.08	4.00	0.14	0.13	4.08	0.16	0.18	4.33	0.23	0.26	4.94	0.41	0.44	7.16	1.04	1.09
4	3.91	0.11	0.12	4.16	0.19	0.20	4.33	0.23	0.26	4.79	0.36	0.38	5.65	0.61	0.65	9.12	1.60	1.62
5	4.08	0.16	0.17	4.50	0.28	0.29	4.83	0.38	0.39	5.54	0.58	0.57	6.86	0.96	0.97	13.08	2.73	2.40
6	4.50	0.28	0.26	5.16	0.47	0.43	5.66	0.61	0.58	7.00	1.00	0.84	9.23	1.63	1.43	21.08	5.02	3.55
7	4.75	0.35	0.38	5.83	0.66	0.64	6.50	0.85	0.85	8.45	1.41	1.24	11.80	2.37	2.12	31.50	8.00	5.25
8	5.08	0.45	0.57	6.50	0.85	0.94	7.41	1.11	1.26	10.08	1.88	1.84	14.92	3.26	3.13			
9	5.41	0.54	0.84	7.25	1.07	1.39	8.45	1.41	1.87	12.04	2.44	2.72	18.87	4.39	4.63			

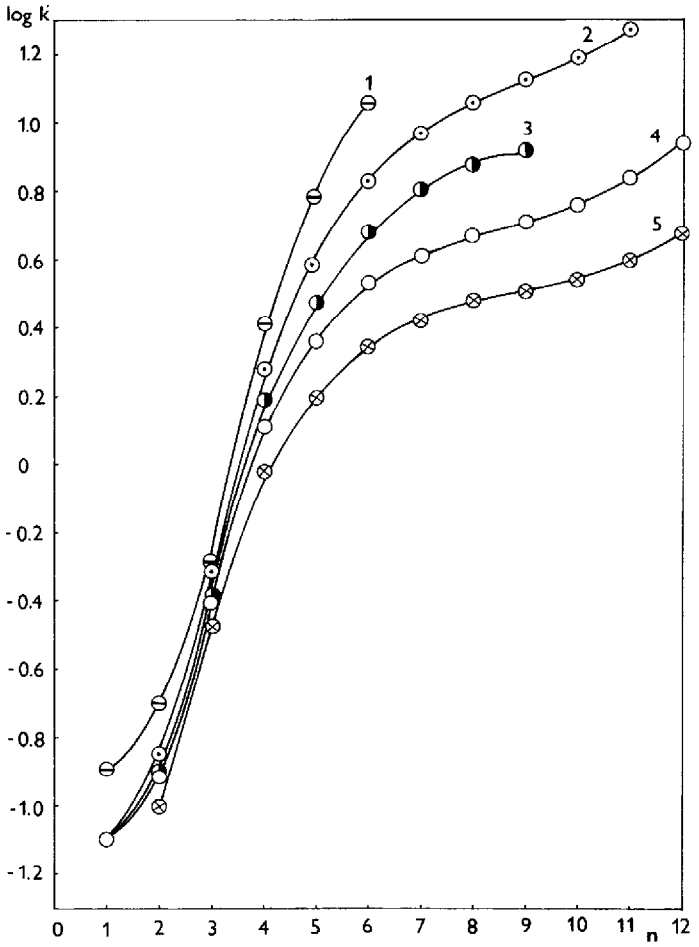

Fig. 11. Dependence of retention (k') of the individual ethoxymers in the sample of Serdox NNP 8 on the number of oxyethylene units, n, on a Silasorb SPH Diol, 7.5 μm, column (250 × 4 mm I.D.). Mobile phase with different ratios of 1-propanol to n-heptane: (1) 40:60; (2) 60:40; (3) 70:30; (4) 80:20; (5) 100:0.

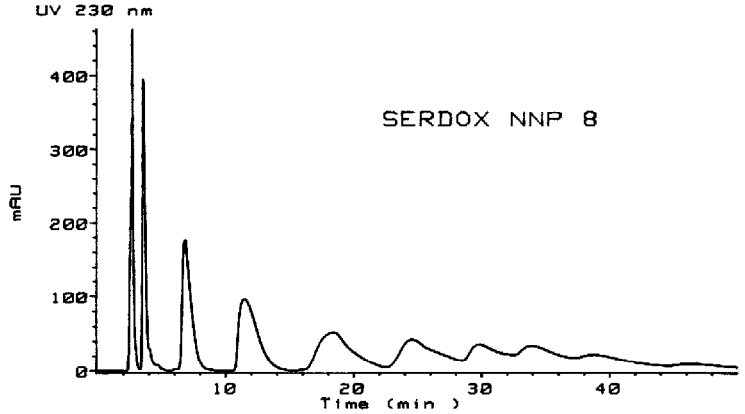

Fig. 12. Isocratic separation of Serdox NNP 8 on a Silasorb SPH Diol, 7.5 μm, column (250 × 4 mm I.D.) with 1-propanol–n-heptane (60:40). Other conditions as in Fig. 3.

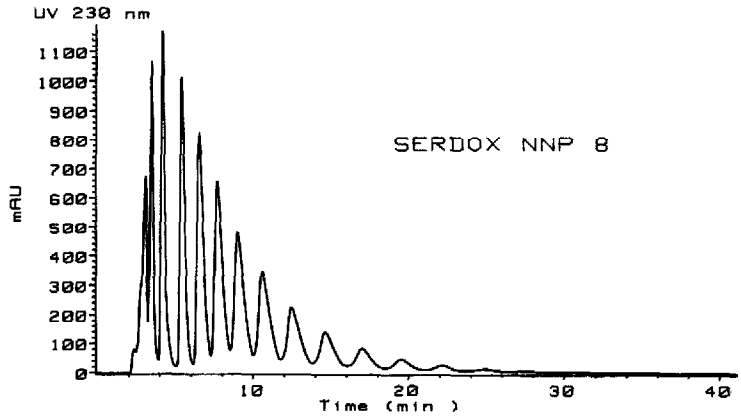

Fig. 13. Isocratic separation of Serdox NNP 8 on a Silasorb SPH Amine, 7.5 μm, column (300 × 4.2 mm I.D.) with 2-propanol–*n*-heptane (25:75). Other conditions as in Fig. 3.

Dependence of results of quantification on separation conditions

The results of the distribution of the individual ethoxymers in a given product determined by the evaluation of chromatographic data can be subject to a systematic error, depending on the separation conditions. Sources of errors in quantitative determinations are imperfect resolution of low ethoxymers and band tailing in some chromatographic systems, which may lead to errors in the integration of the peak areas and to underestimation of the concentrations of higher ethoxymers. The latter errors should increase with decreasing concentration of polar solvent(s) in the organic mobile phase, as the tailing usually increases with increasing elution volume in inadequately selected chromatographic systems.

Chromatographic systems with Silasorb Nitrile and Silasorb Diol as the stationary phases yield irregular spacing of the peaks of ethoxymers and only 8–12 ethoxymers could be resolved in a reasonable time. For this reason, these systems were

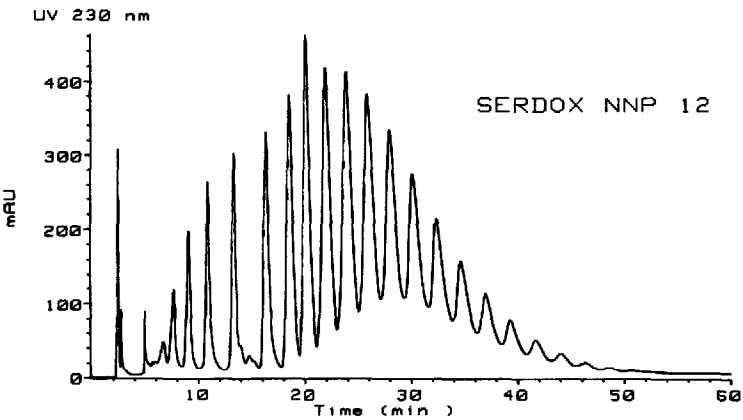

Fig. 14. Gradient elution separation of Serdox NNP 12 on a Silasorb SPH Amine column using linear gradient from 100% *n*-heptane to 2-propanol–*n*-heptane (90:10) in 60 min. Column as in Fig. 13; other conditions as in Fig. 3.

omitted from the quantification experiments. Systems with silica gel and propanol–heptane mobile phases were also omitted, as they were poorer in this respect than systems with ethanol–heptane or propanol–ethanol–heptane mobile phases.

As pure ethoxymer standards were not available, theoretical response factors were considered, based on a constant value of the molar absorption coefficients of ethoxylated nonylphenols, independent of the number of oxyethylene units. Ahel and Giger[20] found previously that such theoretical response factors were very close to the experimental values for ethoxylated alkylphenols. Serdox NNP 8, with eight nominal oxyethylene units, was used as the test sample in quantitation experiments and the response factors were related to the response factor of the oligomer with five oxyethylene units. The results of the quantitative evaluation of the ethoxymer distribution were expressed relative to the oligomer with eight oxyethylene units, rather than to the sum of all the ethoxymers in the sample, as in some experiments a significant proportion of higher ethoxymers could not be eluted. The results are given in Table VIII.

TABLE VIII

RESULTS OF THE DETERMINATION OF DISTRIBUTION OF THE INDIVIDUAL ETHOXYMERS IN SERDOX NNP 8 USING VARIOUS NORMAL-PHASE HPLC SYSTEMS

The results are expressed as mass % calculated from peak areas relative to the oligomer with eight oxyethylene units, considering the theoretical (stoichiometric) response factors, R, related to the oligomer with five oxyethylene units ($R_5 = 1$). Columns: (I) Silasorb 600 SPH, 5 μm, 300 × 3.8 mm I.D.; (II) Silasorb SPH Amine, 7.5 μm, 300 × 4.2 mm I.D. Mobile phases: (1) ethanol–*n*-heptane (70:30); (2) ethanol–*n*-heptane (30:70); (3) ethanol–1-propanol–*n*-heptane (20:20:60); (4) gradient from ethanol–1-propanol–*n*-heptane (8:24:68) to ethanol–1-propanol (75:25) in 30 min; (5) 2-propanol–*n*-heptane (25:75); (6) gradient from 0 to 90% 2-propanol in *n*-heptane in 60 min. n = Number of oxyethylene units in the individual ethoxymers.

n	R	*Mass %*					
		I,1	*I,2*	*I,3*	*I,4*	*II,5*	*II,6*
1	0.6		1.8	1.9	1.6		1.4
2	0.7		6.8	8.8	6.8	2.7	6.8
3	0.8	27.9	22.2	25.2	22.4	38.0	21.2
4	0.9	49.2	44.9	48.5	44.9	47.4	42.7
5	1.0	66.7	64.3	67.3	64.4	68.0	62.3
6	1.1	81.5	81.0	85.9	80.1	80.2	80.1
7	1.2	91.4	94.4	97.9	93.5	93.5	92.5
8	1.3	100.0	100.0	100.0	100.0	100.0	100.0
9	1.4	98.7	95.4	82.9	100.7	98.2	100.4
10	1.5	88.8	81.2		84.0	86.8	90.3
11	1.6	71.9	57.7		57.1	70.2	73.3
12	1.7	52.1	29.8		38.8	52.6	55.6
13	1.8	27.4				36.9	38.5
14	1.9					24.2	24.6
15	2.0					14.8	14.6
16	2.1					8.6	8.1
17	2.2					4.6	4.4
18	2.3					2.0	2.4
19	2.4						1.2
20	2.5						0.7
21	2.6						0.4
22	2.7						0.2

On a silica gel column, the first three ethoxymers are eluted in a common peak with ethanol–*n*-heptane (70:30) as the mobile phase, but the peak areas of ethoxymers with $n = 4$–12 are in approximate agreement with the results achieved on Silasorb SPH Amine. With 30% ethanol, the peak areas of ethoxymers with up to nine oxyethylene units and in the ternary mobile phase ethanol–1-propanol–*n*-heptane (20:20:60) those for the oligomers with up to eight oxyethylene units agree approximately with the results achieved on the amine column, but the peak areas of higher ethoxymers are significantly lower on the silica gel column. Ternary gradient elution on Silasorb 600 SPH yields a distribution in approximate agreement with the results on the amine column for ethoxymers with up to ten oligomeric units. With the Silasorb Amine column, approximately identical results were obtained for the ethoxymers with 5–8

TABLE IX

MASS DISTRIBUTION OF THE INDIVIDUAL ETHOXYMERS IN SERDOX NNP 4, NNP 8, NNP 12 AND NNP 20 WITH VARIOUS STOICHIOMETRIC RATIOS OF ETHYLENE OXIDE TO NONYLPHENOL

Results are given as mass % in sample calculated from peak areas with stoichiometric response factors (Table VIII). Column, Silasorb SPH Amine, 7.5 μm, 300 × 4.2 mm I.D.; linear gradient from 0 to 90% 2-propanol in *n*-heptane in 60 min; flow-rate, 1.0 cm^3/min. n = Number of oxyethylene units in the individual ethoxymers.

n	*Mass %*			
	NNP 4	*NNP 8*	*NNP 12*	*NNP 20*
1	3.12	0.17	0.17	0.07
2	12.86	0.83	0.53	0.10
3	17.11	2.58	1.01	0.14
4	18.49	5.20	1.61	0.19
5	16.16	7.58	2.08	0.18
6	11.89	9.74	3.16	0.10
7	8.17	11.26	4.68	0.25
8	5.16	12.17	6.58	0.37
9	3.19	12.22	8.45	0.60
10	1.81	10.99	10.03	0.96
11	1.09	8.93	11.10	1.54
12	0.52	6.77	11.32	2.37
13	0.28	4.68	10.37	3.49
14	0.14	2.99	8.72	4.93
15		1.78	6.90	6.32
16		0.99	5.14	7.65
17		0.54	3.58	8.84
18		0.29	2.31	9.82
19		0.15	1.35	9.85
20		0.08	0.68	8.61
21		0.04	0.23	7.06
22		0.01		5.74
23				4.77
24				3.98
25				3.21
26				2.86
$\Sigma > 26$				6.00

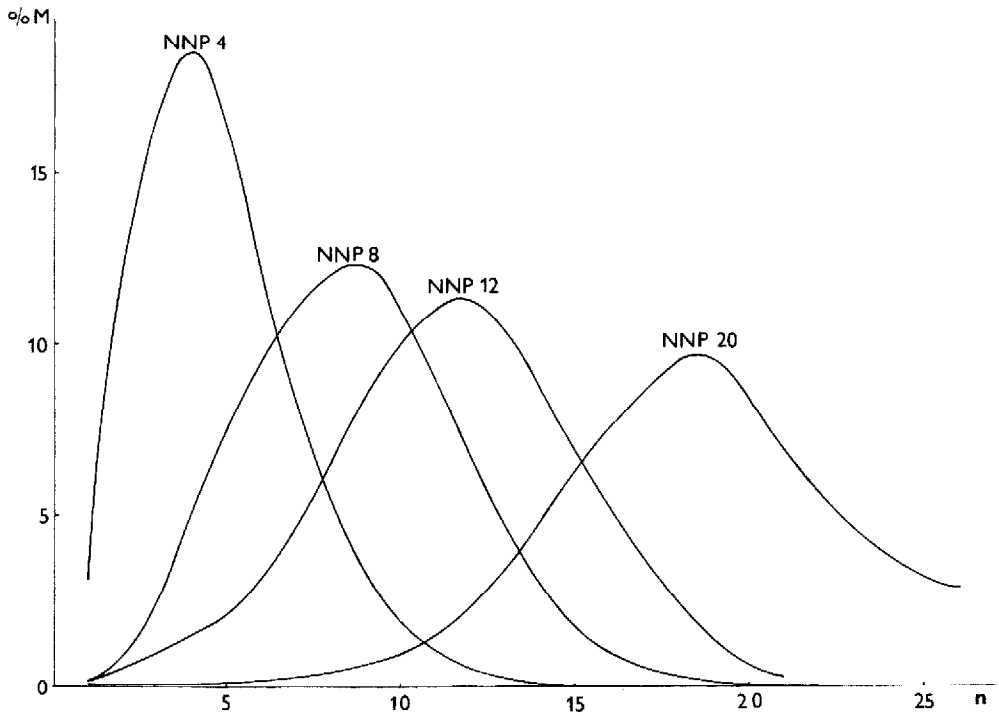

Fig. 15. Percentage mass distribution (%M) of the individual ethoxymers with n oxyethylene units in Serdox NNP 4, NNP 8, NNP 12 and NNP 20 determined with conditions as in Table IX.

oxyethylene units using either isocratic elution with 2-propanol–n-heptane (25:75) or gradient elution from 0 to 90% 2-propanol in n-heptane. The contents of the ethoxymers with $n>9$ were higher than those found with the Silasorb 600 SPH column, probably because of a considerably smaller peak tailing observed with the amino-bonded phase. With 25% propanol, the contents of the first three ethoxymers are not precise because of imperfect resolution of the individual compounds.

In conclusion, silica gel Silasorb 600 SPH can be used reliably only for the determination of the ethoxymer distribution up to $n = 10$, with an ethanol–propanol–n-heptane ternary gradient to obtain the best results with this stationary phase. If the distribution of the individual ethoxymers with 1 to 20–25 oxyethylene units is to be determined, an amino-bonded phase with a gradient of propanol in n-heptane offers the best results of all the systems tested. Table IX and Fig. 15 show the mass distributions of ethoxymers in samples with various stoichiometric ratios of ethylene oxide to nonylphenol (4, 8, 12 and 20:1).

CONCLUSIONS

In normal-phase HPLC with mobile phases consisting of dried aliphatic alcohols and n-alkanes, silica gel and an amino-bonded phase show regular retention behaviour, which can be predicted by calculation using the approach introduced

recently[22]. On the other hand non-linear log k' *versus* n plots are observed with diol- and nitrile-bonded phases using dried organic mobile phases, which indicates a mixed retention mechanism. Moreover, the peaks of the higher ethoxymers show significant tailing with the latter two bonded phases and with silica gel and only a limited number of ethoxymers can be resolved in a reasonable time. The results of integration depend on the mobile phase composition and, consequently, the diol- and nitrile-bonded phases are not suitable for the reliable determination of ethoxymer distributions using dried organic mobile phases. More or less successful separations of ethoxymers using the latter bonded phases have been reported recently using a strongly polar additive in the organic mobile phase, such as water [5,12], acetic acid[18] or 2-methoxyethanol[14]; however, a mixed retention mechanism controls the chromatographic behaviour in such systems, which makes it difficult to predict the elution volumes of ethoxymers in these systems and to compare different chromatographic systems.

Band tailing on silica gel columns in ethanol-containing mobile phases is less than in mobile phases containing propanol and ethoxymers with up to ten oligomeric units can be separated readily using gradient elution with ternary mobile phases consisting of ethanol, propanol and an *n*-alkane. The most reliable results can be achieved with the amino-bonded phase, where ethoxymers with up to 20–25 oxyethylene units can be separated in 1 h using gradient elution with an increasing concentration of propanol in an *n*-alkane, without the necessity to add water to the mobile phase, as has been suggested earlier[6,15,16,19,20].

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