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BEHAVIOUR OF DIHYDROXYNAPHTHALENES IN A REVERSED-PHASE CHROMATOGRAPHIC SYSTEM

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SUMMARY

The influence of the organic modifier of the mobile phase, the ionic strength, the control of pH by different procedures, and the presence of tetrabutylammonium ion on the separation of seven isomeric dihydroxynaphthalenes and two quinones derived from them was studied by using silica gel modified chemically with octadecyls as a stationary phase.

Substantial differences in the retention and in the selectivity of the separation with a change of the organic modifier or with a change in its concentration are caused by the contribution of the stationary phase to the retention of the solutes under study. Anomalous broadening of peaks of 2,3-dihydroxynaphthalenes in acetate buffer and in solutions of sulphuric acid was ascribed to the formation of complexes with heavy metals deposited in the stationary phase.

INTRODUCTION

2,3-Dihydroxynaphthalene (2,3-DHN) is noted for its ability to form complexes with inorganic anions of some heavy metals or metalloids[1–3].

Certain anomalies in the retention behaviour and the peak shapes of 2,3-DHN and its complexes were observed in preliminary attempts to separate complexes of oxo anions of metals with 2,3-DHN by reversed-phase liquid chromatography. In order to be able to define the reasons for these anomalies and to prevent them, the chromatographic behaviour of 2,3-DHN was studied under the conditions used for the separation of its complexes. The behaviour of 2,3-DHN was compared with the behaviour of its structural isomers possessing weaker complex-forming abilities (1,2-DHN) or not forming complexes at all, and with that of two naphthoquinones (NCH), which do not have any hydroxyl groups in their molecules.

EXPERIMENTAL

A laboratory arrangement, the main parts of which were a Varian 8500 pulseless pump (Varian, U.S.A.) and an LCD 254 photometric detector (Laboratory Instruments, Prague, Czechoslovakia), working at a wavelength of 254 nm, was used.

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A Knauer refractometer, type 2025/50 (Knauer, F.R.G.), was used to measure column dead volumes with the aid of ^2H_2O injection[4]. A septum injector and stainless-steel columns with 100 × 4 mm I.D. beds were of our own design.

Silasorb 300 ODS Silica gel (Lachema, Brno, Czechoslovakia) with chemically bonded octadecyl groups and 10 μm particle diameter served as a stationary phase. The viscosity variant of the high-pressure filtration technique, with cyclohexanol as a suspension liquid, was used to pack the columns. In the studies of anomalous broadening of 2,3-DHN one column was washed with 170 ml of an aqueous solution of the disodium salt of ethylenediaminotetra-acetic acid at a concentration of 1 m*M*.

Chemicals used for the preparation of the mobile phases (Lachema) were of analytical grade, except for acetonitrile (Laborchemie, Apolda, G.D.R.). Tetrabutylammonium hydroxide (TBAOH) was used as an ion-pairing agent. The pH of its aqueous solution at a concentration of 2 m*M* was adjusted with sulphuric acid or by the addition of buffers and was measured prior to mixing with an organic moderator. This value is also stated as the pH of the resulting mobile phase[5,6].

2,3-DHN (Loba-Chemie, Vienna, Austria) was purified prior to use until it appeared as chromatographically pure when up to 20 μl of its saturated solution in acetonitrile were injected. The other dihydroxynaphthalenes and quinones, obtained from different sources, were used without any preliminary purification. Their 5 m*M* solutions in acetonitrile were injected in volumes of up to 1 μl.

Elution volumes were read at peak maxima regardless of peak symmetry. Peak asymmetry is expressed in terms of the ratios of the rear and the front peak widths measured at one tenth of the peak height. Efficiencies and retention data were calculated from the position of peak maxima and peak widths.

RESULTS AND DISCUSSION

Influence of the organic modifier

When complexes are separated, modifiers show variable ability to compete with ligands or water in complex formation. Hence the best modifier for the separation of the ligand itself is not necessarily suitable for the separation of its complexes. The retentions of 2,3-DHN and its isomers were therefore investigated with the use of four mobile phase modifiers. Acetonitrile, belonging to the VIb group according to Snyder's classification[7], should exert the least influence of all of these modifiers on 2,3-DHN complexes with anions of heavy metals. Similarly acetone (VIa group) should not influence chromatographed complexes significantly; however, it can be expected to affect the selectivity of the separation. The strongest influence on the complexes under separation ought to be produced by methanol, owing to its marked affinity for water. Methanol belongs to selectivity group II, as does 2-propanol.

The selective interactions of solutes in the mobile phase are decisive for the separation of solutes in typical reversed-phase systems according to current theories. Hence under these circumstances the variations in retention caused by changes in the mobile phase polarity can be described by the following relationship

$$\frac{k_2}{k_1} = 10^{(P_2 - P_1)/2} \qquad (1)$$

where P_1 and P_2 are polarity parameters of mobile phases 1 and 2 in which capacity factors k_1 and k_2, respectively, were measured for the given solute[8]. For a binary mobile phase consisting of the aqueous solution of an organic solvent the polarity parameter[7] reads as follows

$$P = P'_w \cdot \varphi_w + P'_0 \cdot \varphi_0 \tag{2}$$

where P'_w and P'_0 are the tabulated polarity parameters[8,9] of pure water and the organic component, respectively, and φ_w and φ_0 their volume fractions in the mobile phase.

It follows from eqns. 1 and 2 that, with a change in the ratio of water to organic solvent,

$$\log k_2/k_1 = \frac{1}{2}(\varphi_{w,2} - \varphi_{w,1}) \cdot (P'_w - P'_0) \tag{3}$$

If the change in retention results from the change of organic modifier in spite of the fact that its volume fraction, φ_0, in the mobile phase remains constant, then

$$\log k_2/k_1 = \frac{1}{2}\varphi_0(P'_{0,2} - P'_{0,1}) \tag{4}$$

where $P'_{0,1}$ and $P'_{0,2}$ are the polarity indices of the pure organic solvents 1 and 2, respectively.

When investigating the influence of methanol, 2-propanol, acetonitrile and acetone on the retentions of selected solutes, it was found for the elution from the ODS column that the capacity factors of solutes, the relative retentions and the elution sequence depend both on the organic modifier used and on its concentration in the mobile phase (Table I). However, these variations do not correspond to the relative elution strengths of the solvents used as reported for reversed-phase systems[10]. Also, the polarity parameters of acetonitrile and 2-propanol, calculated according to eqn. 3 from the changes in the solute retentions measured following a change in the concentration of acetonitrile or 2-propanol in the mobile phase from 20 to 30% (Table II), agree with the tabulated values (Table III) only exceptionally. Poor mutual agreement and poor agreement with theoretical values is also found for the data calculated according to eqn. 4 from the changes in retentions of tested solutes caused by the change in the organic solvent provided that its ratio to water, 3:7, maintains constant. It makes no difference whether acetonitrile or 2-propanol is selected as a reference modifier.

The values of polarity parameters (Table II), calculated for various solutes from two different sets of input data, correspond to one another for 1,5-DHN only. Moreover, an acceptable agreement with the tabulated data was found only for the measurements with 30% methanol and 30% acetone, respectively, in the mobile phase. Relatively, the best agreement of the values calculated for various solutes with the tabulated data was obtained when the mobile phase was moderated with methanol. However, even in this instance the deviations found for various solutes and various reference organic modifiers are mostly so significant that they cannot be

TABLE I

INFLUENCE OF THE ORGANIC MODIFIER AND ITS CONCENTRATION ON RETENTIONS RELATIVE TO 1,2-DHN

	Acetonitrile (%)			*Methanol (30%)**	*2-Propanol (%)*		*Acetone (30%)**
	*30**	*20***	*30***		*20**	*30**	
1,2-DHN	(2.47)***	(5.01)	(2.51)	(4.26)	(1.58)	(0.79)	(1.85)
1,3-DHN	1.43	2.19	1.35	3.19	5.07	2.97	1.81
1,4-DHN	2.26	2.59	2.20	3.09	4.72	3.34	2.99
2,3-DHN	1.63	2.43	1.55	2.80	5.48	3.43	4.36
1,5-DHN	0.99	1.11	1.00	1.23	1.98	1.56	2.11
1,7-DHN	1.19	1.75	1.14	2.74	3.83	2.23	3.21
2,7-DHN	0.83	1.13	0.80	1.42	2.23	1.30	2.18
1,2-NCH	0.98	1.01	1.00	0.98	1.06	1.03	0.98
1,4-NCH	2.17	2.61	2.12	3.17	4.43	3.64	2.94

* Volume percentage of the organic modifier mixed with water; pH adjusted to pH 3 with sulphuric acid.

** Volume percentage of the modifier mixed with water; pH adjusted to pH 5.3 with phosphate buffer at a concentration of 33 m*M*.

*** Numbers in parentheses indicate reference values of capacity factors.

ascribed to errors of measurement. The greatest deviations from the theoretical values were found when the mobile phase was moderated with 2-propanol. For four of nine selected solutes even lower polarity parameters were calculated from the experimental data than have been reported for aliphatic hydrocarbons[13,14].

The polarity of various selected moderators at the elution of the tested series

TABLE II

POLARITY PARAMETERS OF ACETONITRILE, 2-PROPANOL, METHANOL AND ACETONE CALCULATED FROM THE CHANGES IN RETENTIONS

	Acetonitrile		*2-Propanol*		*Methanol*		*Acetone*	
	*20–30**	*IS***	*20–30**	*AC****	*AC****	*IS***	*AC****	*IS***
1,2-DHN	4.2	7.5	4.2	0.9	5.8	9.0	3.4	6.7
1,3-DHN	−0.1	0.7	−0.5	−1.3	3.8	4.6	−0.2	0.5
1,4-DHN	2.8	3.6	1.2	0.6	5.3	5.8	2.8	3.3
2,3-DHN	0.2	1.3	0.1	−1.0	3.3	4.4	2.2	3.3
1,5-DHN	3.3	3.3	1.2	1.3	5.5	5.5	4.7	4.6
1,7-DHN	0.5	0.9	−0.6	−1.0	4.5	4.9	2.5	2.9
2,7-DHN	1.1	1.6	−0.4	−0.9	4.3	4.7	3.1	3.5
1,2-NCH	4.1	7.1	3.9	0.9	5.7	8.7	3.3	6.2
1,4-NCH	2.4	4.3	2.5	0.6	5.1	7.0	2.4	4.3

* Values calculated according to eqn. 3 with the change in concentration of the organic component in the mobile phase from 20 to 30%.

** The values calculated according to eqn. 4 with 2-propanol selected as a reference solvent. The values calculated for particular solutes according to eqn. 3 presented in this table were taken as input data.

*** The values calculated according to eqn. 4 with acetonitrile selected as a reference solvent. The values calculated for particular solutes according to eqn. 3 were taken as input data.

TABLE III

POLARITY PARAMETERS OF THE USED SOLVENTS

Tabulated values of P', x_e, x_d and x_n were taken from ref. 14.

	P'	x_e	x_d	x_n	$P' x_e$	$P' x_d$	$P' x_n$
Water	10.2	0.37	0.37	0.25	3.77	3.77	2.55
Methanol	5.1	0.48	0.22	0.31	2.45	1.12	1.58
2-Propanol	3.9	0.55	0.19	0.27	2.15	0.74	1.05
Acetonitrile	5.8	0.31	0.27	0.42	1.80	1.57	2.44
Acetone	5.1	0.35	0.23	0.42	1.78	1.17	2.14

of dihydroxynaphthalenes thus cannot be characterized by a single quantitative value. Its magnitude depends both on the eluted compound and on the selection of the reference solvent. When the experimental data are processed according to eqn. 3, water is the reference solvent. From the measurements performed with a constant volume fraction of the organic component in the mobile phase, the practical relative polarity of the tested moderators can be derived only by using eqn. 4 unambiguously. In the present case, the relative polarity decreases for most of the solutes in the following sequence: methanol, acetonitrile, acetone, 2-propanol. Neither the sequence of polarities of organic modifiers nor numerical differences in their polarity parameters, calculated for various solutes, depend on the numerical value of the polarity parameter of the reference solvent (Table II). Hence tabulated values[8,9] can also be used as input values for the determination of relative polarities.

The contribution of the stationary phase to the retention of solutes is the only possible explanation for the difference between the predicted values of polarity parameters of the tested solvents (Table III), and the values found experimentally (Table II). All the facts mentioned above suggest a significant role for the stationary phase in the retention of dihydroxynaphthalenes and their quinones in the chromatographic system used. A comparison of the measured retentions and their changes

TABLE IV

ANTICIPATED RELATIVE CHANGES IN RETENTIONS CALCULATED ACCORDING TO EQN. 1 FROM THE POLARITY CHARACTERISTICS OF THE COMPONENTS OF THE MOBILE PHASES PRESENTED IN TABLE III

Data under $\Delta P'$ indicate anticipated relative changes in retentions if selective interactions of all types occur in the mobile phase simultaneously. Data in the other columns indicate anticipated retention changes if selective interactions of various types with the components of the mobile phase occur: proton accepting ($P'x_e$), proton donating ($P'x_d$) and strong dipole ($P'x_n$) interactions[12].

	$\Delta P'$	$\Delta P'x_e$	$\Delta P'x_d$	$\Delta P'x_n$
Water–methanol (70:30)	1.000	1.000	1.000	1.000
Water–2-propanol (70:30)	0.661	0.902	0.871	0.813
Water–acetonitrile (70:30)	1.274	0.804	1.161	1.303
Water–acetone (70:30)	1.000	0.804	1.012	1.175
Water–2-propanol (80:20)	1.380	1.096	1.245	0.966
Water–acetonitrile (80:20)	2.113	1.012	1.495	1.318
100% water	5.821	1.585	2.483	1.365

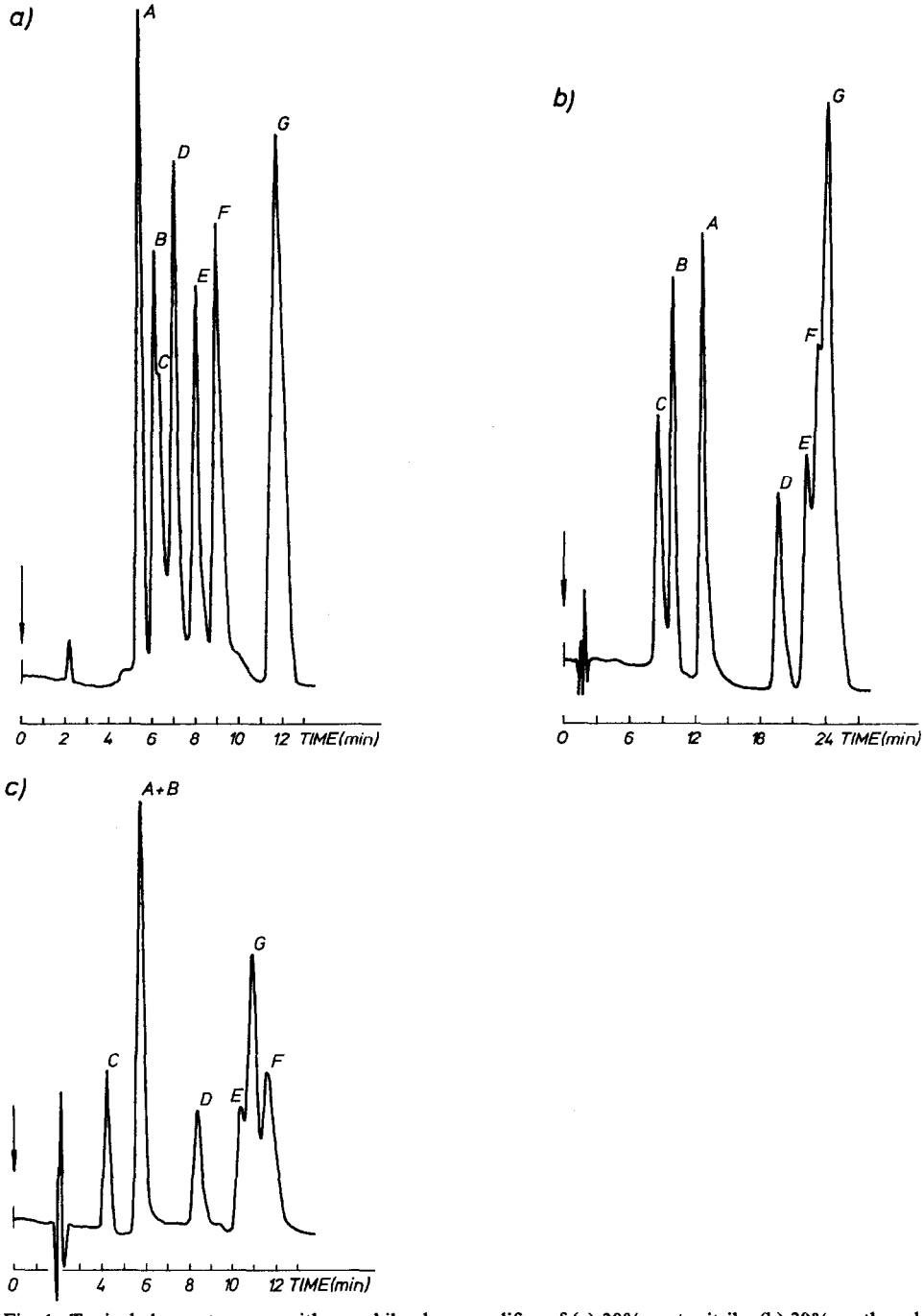

Fig. 1. Typical chromatograms with a mobile phase modifier of (a) 30% acetonitrile, (b) 30% methanol and (c) 25% 2-propanol. The pH was adjusted with H_2SO_4 to 4.35; flow-rates, 0.8 ml/min in a and b and 0.5 ml/min in c. Peaks: A = 2,7-DHN; B = 1,5-DHN; C = 1,2-DHN; D = 1,7-DHN; E = 1,3-DHN; F = 2,3-DHN; G = 1,4-DHN.

(Table I) with the retention values expected theoretically (Table IV) supports this view. It follows from the marked dependence of the separation selectivity on the concentration or type of modifier (Fig. 1) that strong specific interactions of the solute with the stationary phase differ in character from the interactions in the mobile phase. All this, similarly to the observation of Bidlingmeyer *et al.*[11], suggests a significant role for residual silanols on the surface of the reversed-phase.

On the basis of the demonstrated influence of the tested organic modifiers on the selectivity of chromatographic systems, and with regard to probable influence of these compounds on the stability of 2,3-DHN complexes, acetonitrile was chosen as a mobile phase modifier in the following measurements. It was mixed with aqueous solutions of salts and compounds controlling acidity in the ratio 3:7 in order to obtain a suitable elution strength for the mobile phases.

Influence of ionic strength

The influence of ionic strength on the magnitude of retentions and band broadening of dihydroxynaphthalenes was investigated only in the range of low ionic

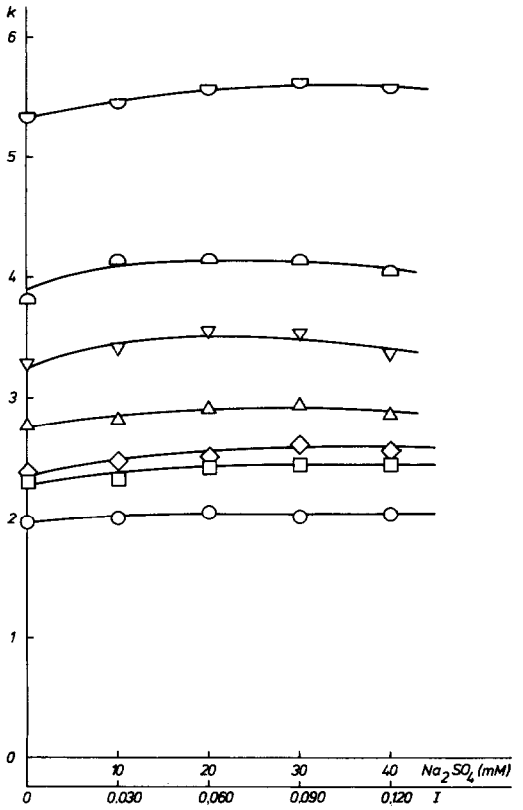

Fig. 2. Influence of the ionic strength of the mobile phase on the capacity factors of dihydroxynaphthalenes. Mobile phase, acetonitrile–distilled water (3:7); pH adjusted to 5 with H_2SO_4; flow-rate, 1 ml/min. Curves: ○ = 2,7-DHN; □ = 1,5-DHN; ◇ = 1,2-DHN; △ = 1,7-DHN; ▽ = 1,3-DHN; ⌓ = 2,3-DHN; ⊔ = 1,4-DHN.

TABLE V

INFLUENCE OF THE IONIC STRENGTH ON PEAK BROADENING OF SOME DIHYDROXYNAPHTHALENES, CHARACTERIZED BY THE NUMBER OF THEORETICAL PLATES, N, AND ON THEIR ASYMMETRY, A_s

	N					A_s				
	$I = 0.000$	$I = 0.030$	$I = 0.060$	$I = 0.090$	$I = 0.120$	$I = 0.000$	$I = 0.030$	$I = 0.060$	$I = 0.090$	$I = 0.120$
2,3-DHN	1900	1350	1000	870	840	3.30	4.90	5.33	5.56	6.30
1,7-DHN	3140	2880	2810	2820	3200	1.94	1.98	2.06	1.82	1.72
1,4-DHN	4000	4040	4304	4020	4050	1.49	1.93	1.80	1.62	1.90
1,4-NCH	4080	3910	4030	4270	4110	1.31	2.11	1.82	1.53	1.55

strength (low concentrations of strong electrolytes), which is topical[12] for the study of separations of inorganic anions or their complexes with 2,3-DHN. The ionic strength of the stock solution of the aqueous component of the mobile phase, the acidity of which was adjusted to pH 5 with sulphuric acid, was considered to be zero. The molarity of disodium sulphate, which was used to adjust the ionic strength of the mobile phase, indicated the salt concentration in the aqueous solution prior to mixing with acetonitrile.

In accordance with the general behaviour of non-electrolytes, an increase in ionic strength leads to an increase in the activity coefficients of dihydroxynaphthalenes in the mobile phase, which appears as an increase in their retentions. In the range under study, the influence of ionic strength on the retention values is slight (Fig. 2). The changes, occurring mainly at concentrations up to 20 m*M* of sodium sulphate, do not exceed 10% of the capacity factor measured in the mobile phase with zero ionic strength for any of the solutes.

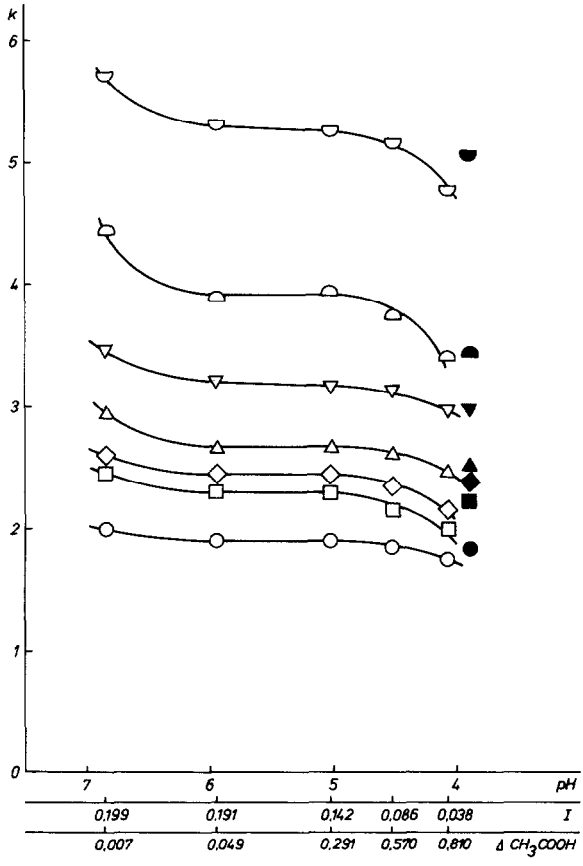

Fig. 3. Influence of the pH of the acetate buffer at a concentration of 0.2 *M* on the capacity factors of dihydroxynaphthalenes. *I* = Ionic strength of the buffer; ΔCH_3COOH = fraction of acetic acid in the mixture of acetic acid and sodium acetate; mobile phase, acetonitrile–acetate buffer (3:7). For the other conditions and identity of solutes see Fig. 2. The filled symbols represent the retentions measured with the mobile phase at pH 4.0 and the ionic strength adjusted to 0.199 by the addition of Na_2SO_4.

With the exception of 2,3-DHN, the ionic strength has no demonstrable effect on either broadening or symmetry of the peaks (Table V). The anomalous behaviour of 2,3-DHN is discussed below.

Influence of pH and pH regulator

The influence of the acidity of the mobile phase was studied in the pH range 7–3, within which the pH could be controlled with phosphate buffer at a concentration of 66.7 m*M*. In order to obtain pH $\leqslant$ 5, secondary phosphate was substituted in this buffer with phosphoric acid. The ionic strength of the buffer decreased from $I = 0.142$ at pH 6.93 to 0.066 at pH 5.05. On further decrease in pH it decreased only negligibly (ΔI less than -0.002). These changes in the ionic strength have a minimal effect on retentions (Fig. 2). Acetate buffer at a concentration of 0.2 *M* made it possible to obtain pH up to about 4. With the decrease in pH, the ionic strength decreased markedly and, simultaneously, the proportion of acetic acid in the buffer increased (Fig. 3). The ionic strength of the aqueous solution of sulphuric acid was 0.003 at pH 3.

When pH is controlled with strong inorganic electrolytes, retentions measured

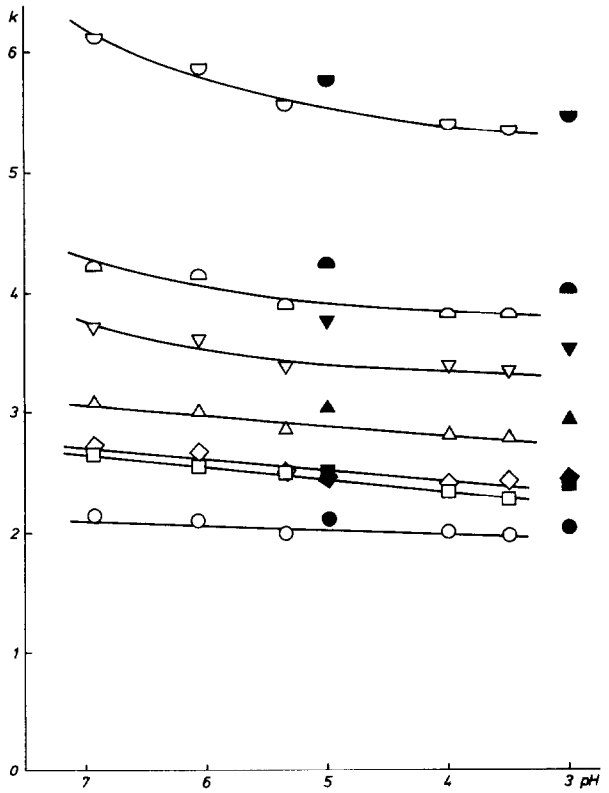

Fig. 4. Influence of the pH of the phosphate buffer at a concentration of 66.7 m*M* on the capacity factors of dihydroxynaphthalenes. Mobile phase, acetonitrile–phosphate buffer (3:7). For the other conditions and identity of solutes see Fig. 2. The filled symbols represent measurements with the mobile phase the pH of which was adjusted with H_2SO_4.

at the identical acidity of the mobile phase correlate well (Fig. 4). The increase in retention with pH cannot be explained by either changes in ionic strength of the mobile phase or dissociation of solutes (see below). Hence in accordance with the influence of the stationary phase on retentions, the reasons must be sought in the changes to the stationary phase caused by changes in pH.

The compounds of acetate buffer, particularly acetic acid, possess the character of an organic modifier with respect to water. At a concentration of 0.2 *M* they increase the proportion of organic compounds in the mobile phase by *ca.* 1.1% (calculated for acetic acid). Lower values of retentions, in comparison with phosphate buffer, and overall shape of the dependence (Fig. 3) can be explained by a combination of the simultaneous influence of three factors: (1) an increased proportion of organic components in the mobile phase; (2) changes in the stationary phase caused by changes in pH; (3) changes in the ionic strength of the acetate buffer associated with the changes in its pH.

Anomalous broadening of peaks of 2,3-DHN

Except for 2,3-DHN the zone broadening of solute peaks was small (Table V). Regardless of the organic modifier, the pH of the mobile phase and the compound used to control its acidity, typical efficiencies of the 100 × 4 mm I.D. bed ranged from 2500 to 3500 theoretical plates. Efficiencies even better than 4000 theoretical plates ($h \leqslant 2.5\ d_p$) were obtained for 1,4-DHN and 1,4-NCH. At the same time, the linear velocities of the mobile phase were always double the optimal linear velocity, and the peak width was not corrected for the broadening in the detector.

However, a marked dependence of the broadening and asymmetry of the peak on the mobile phase acidity and on the nature of the compound used to control pH was observed for the peaks of 2,3-DHN. The same dependences on the pH of the mobile phase were observed in preliminary attempts to separate quinaldine from its complexes[13] or molybdates and tungstates from free 2,3-DHN or in the chromatography of pyrocatechols.

The ability to form complexes with cations of heavy metals is a common feature of all of these compounds. The pH-dependent tailing and broadening of their peaks could therefore be explained by formation of complexes with heavy metal ions, particularly iron. Heavy metals can be deposited in the stationary phase as impurities from its preparation[14] or they can be trapped from dissolved products of the corrosion of metallic parts of the chromatograph[15,16] by ion exchange on residual surface silanols[17,18]. The effect of the compounds used to control the acidity on the broadening of the 2,3-DHN peak can be explained by the competition in the formation of complexes with metal cations deposited in the stationary phase. For instance, phosphate ions can form iron complexes relatively strongly even in acidic media, whereas acetate or sulphate ions cannot[19].

It follows from this theory that elimination of metal ions from the surface of the silica gel adsorbent, or their masking with a very strong complex-forming agent, should prevent the tailing of 2,3-DHN peaks. One column was therefore washed with EDTA, and smaller broadening of 2,3-DHN peak was obtained, even following elution with a mobile phase the pH of which was adjusted with sulphuric acid, than for elution with phosphate buffer from the unmodified column (Fig. 5). Similar significant effects were obtained in some other instances[13,20].

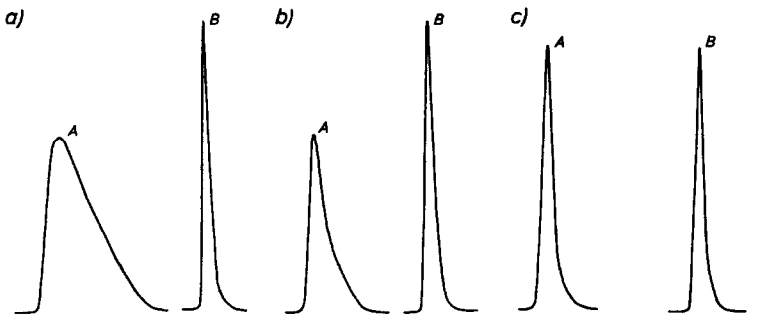

Fig. 5. Influence of the compound used to control pH and of column washing with EDTA solution on the shape of peaks of complex-forming 2,3-DHN and non-complexing 1,7-DHN. (a) The bed was not washed with EDTA solution; mobile phase, acetonitrile–distilled water (3:7) adjusted with H_2SO_4 to pH 6.7. (b) The bed was not washed with EDTA solution; mobile phase, acetonitrile–phosphate buffer (0.067 *M*) (3:7); pH 6.8. (c) The bed was washed with EDTA solution; mobile phase, see under (a). Peaks: A = 2,3-DHN; B = 1,7-DHN.

The dependence of the broadening of 2,3-DHN peaks on the ionic strength of the mobile phase can therefore be explained by the influence of the changes in the activity coefficient of 2,3-DHN in the mobile phase. This dependence reflects the formation of complexes of 2,3-DHN with heavy metal ions deposited in the stationary phase.

It is likely that the formation of such metal complexes is one of the reasons of the anomalous peak broadening or tailing. An earlier observation[21] that acetate buffer sometimes provides less efficient chromatographic systems than phosphate buffer supports this conclusion.

Influence of onium ion

Complexes of oxo anions of metals (Mo, W, etc.) with 2,3-DHN are negatively charged. Moreover, 2,3-DHN is ionized weakly in aqueous media[22,23]. The influence of onium ion on the retention behaviour of dihydroxynaphthalenes was therefore

TABLE VI

INFLUENCE OF THE ADDITION OF TETRABUTYLAMMONIUM ION TO THE MOBILE PHASE ON THE CHANGES IN THE RETENTIONS RELATIVE TO 2,7-DHN

	Phosphate buffer	*H_2SO_4*	*Acetate buffer*
2,7-DHN	0	0	0
1,2-DHN	–	−0.09	−0.17
1,3-DHN	0.04	0.05	0.08
1,4-DHN	−0.26	−0.16	−0.39
2,3-DHN	0.02	0.04	0.08
1,5-DHN	0.01	−0.01	0.00
1,7-DHN	−0.02	0.02	−0.03
1,2-NCH	–	−0.09	−0.17
1,4-NCH	−0.26	−0.16	−0.39
Pyrocatechol	−0.01	−0.04	−0.04

studied using the tetrabutylammonium (TBA) cation. The acid–base reaction of a 2 mM aqueous solution of TBAOH was adjusted to pH 6.80 ± 0.05 with acetic buffer (ionic strength I = 0.119), phosphate (I = 0.113) or by titration with sulphuric acid (I = 0.006).

The influence of TBA on retentions was evaluated from the changes relative to 2,7-DHN (Table VI) which had the lowest capacity factor (Table I), minimally dependent on the presence of onium ion in the system. The varying influence of onium ion can be explained by the differences in ionic strength of the mobile phases. The retention changes found for the isomers with substituents on both rings range within experimental errors. The increase in retentions for the 1,3-isomer, which is systematically greater than for 2,3-DHN, confirms the partial dissociation of these compounds in neutral mobile phases with the origin of negative charge[22,23]. The decrease in retentions of other solutes, indicating the origin of positive charge, are considerably more marked, particularly for the 1,4-isomer. It is noteworthy that decreased retentions in the presence of onium ion were also found for quinones.

The presence of TBA in the mobile phase did not exert any noticeable effect on the peak shapes of solutes.

REFERENCES

1 L. Sommer, *Z. Anal. Chem.*, 187 (1962) 7.
2 L. Sommer, *Z. Anal. Chem.*, 187 (1962) 263.
3 R. N. Soni and M. Bartušek, *J. Nucl. Chem.*, 33 (1971) 2557.
4 H. Engelhardt, *Hochdruck Flüssigkeitschromatographie*, Springer, Berlin, 2nd ed., 1977, p. 137.
5 R. G. Bates, *Determination of pH. Theory and Practice*, Wiley-Interscience, New York, 1973.
6 B. A. Bidlingmeyer, *J. Chromatogr. Sci.*, 18 (1980) 525.
7 L. R. Snyder, *J. Chromatogr.*, 92 (1974) 223.
8 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley-Interscience, New York, 2nd ed., 1978, pp. 247–267.
9 L. R. Snyder, *J. Chromatogr. Sci.*, 16 (1978) 223.
10 L. R. Snyder, J. W. Dolan and J. R. Gant, *J. Chromatogr.*, 165 (1979) 3.
11 B. A. Bidlingmeyer, J. K. Del Rios and J. Korpi, *Anal. Chem.*, 54 (1982) 442.
12 I. Molnár, H. Knauer and D. Wilk, *J. Chromatogr.*, 201 (1980) 225.
13 M. Vrchlabský, M. Navrátilová and K. Ballschmiter, in preparation.
14 M. Verzele and C. Dewaele, *J. Chromatogr.*, 217 (1981) 399.
15 N. Haering and K. Ballschmiter, *Talanta*, 27 (1980) 873.
16 Y.-T. Strin and P. W. Carr, *Talenta*, 28 (1981) 411.
17 R. K. Iler, *The Chemistry of Silica*, Wiley-Interscience, New York, 1979, pp. 665–676.
18 K. K. Unger, *Porous Silica*, J. Chromatogr. Library, Vol. 16, Elsevier, Amsterdam, 1978, pp. 129–140.
19 L. G. Sillén, *Stability Constants of Metal Ion Complexes*, Special Publication No. 17, Chemical Society, London, 1964.
20 S. R. Hutchins, P. R. Haddad and S. Dilli, *J. Chromatogr.*, 252 (1982) 185.
21 W. R. Melander and C. Horváth, in C. Horváth (Editor), *High-Performance Liquid Chromatography, Advances and Perspectives*, Academic Press, New York, 1980, Vol. 2, p. 189.
22 L. G. Sillén and A. E. Martell, *Stability Constants of Metal Ion Complexes*, Supplement No. 1 to Special Publication No. 17, Special Publication No. 25, Chemical Society, London, 1971, p. 598.
23 M. Bartušek, *Coll. Czech. Chem. Commun.*, 32 (1967) 757; *J. Inorg. Nucl. Chem.*, 29 (1967) 1089.