Journal of Chromatography, 371 (1986) 269–282
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 943

MOBILE PHASE EFFECTS ON THE SEPARATIONS OF SUBSTITUTED ANILINES WITH A β-CYCLODEXTRIN-BONDED COLUMN

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SUMMARY

The capacity factors of several substituted anilines were measured on a β-cyclodextrin (β-CD)-bonded column with mobile phases varying from the classical, normal-phase condition, *e.g.* *n*-heptane–2-propanol, to the classical, reversed-phase condition, *e.g.* water–2-propanol. Different modifiers, such as acetonitrile, methanol, and tetrahydrofuran and a Partisil PXS-ODS column were also used for comparison. In general, it was found that the β-CD column, having the ability to form inclusion complexes with certain substrates is better and more selective in the reversed-phase separation of many aromatic compounds than the Partisil PXS-ODS column. The normal-phase separation on β-CD was even more efficient, owing to the presence of large number of hydroxy groups on the surface of β-CD molecules and the more rapid mass transfer in the column. In the present case, alcohols were found to be better modifiers than aprotic solvents. The effects of organic solvents on both normal- and reversed-phase separations are also discussed. The minima observed for the log k' *vs.* percent organic solvent plots for a number of substituted anilines are reasoned to originate from solute–solvent competition, which interacts with the stationary phase, as well as from the "relative solubility" of solutes in the stationary and mobile phases.

INTRODUCTION

Recent research advances in the field of high-performance liquid chromatography (HPLC) have led to new insight concerning the separation mechanisms, particularly, at the molecular level. Spectroscopic techniques such as NMR, IR, luminescence spectroscopy[1] and several others[2] have now been widely used in the study of stationary phases and of the cooperative effects between the stationary and mobile phases that account for several experimental observations such as column selectivities. Moreover, the sorption–desorption kinetics of an ion-pairing system with octadecylsilica packing materials has now been measured directly by a pressure-jump relaxation kinetics method[3], which opens yet another approach to the examination of liquid chromatographic systems.

It is now generally agreed that both physical effects, *e.g.* column efficiency and capacity, and chemical effects, *e.g.* mobile and stationary phase compositions, are

0021-9673/86/$03.50

significant in the achievement of more efficient and selective separations. With respect to physical effects, well-packed, small-particle (3–10 μm) columns, operated to give solute capacity factor values (k') of about 1–10 provide excellent resolution[4]. Methods of selecting columns for reversed-phase HPLC on the basis of column and packing configuration are now well documented[5,6]. On the other hand, the chemical effects are still not well understood and predictable, despite of all the above and related studies.

Although factors such as temperature, pH, and secondary chemical equilibria are among the important chemical effects in HPLC, the mobile and stationary phase compositions are probably those most frequently considered for optimization of selectivity in liquid chromatography[7]. In particular, for a tailor-made stationary-phase material, the mobile phase composition becomes the primary factor in the various strategies for separation optimization.

The mobile phase effects for reversed-phase HPLC (RPLC) separations have been the research subject of many groups[8,9]. While hydrophobic phenomena are generally associated with non-polar solute selectivity, it is also recognized that selectivity based on polar group differences of solute molecules can be very significant in RPLC[10,11]. In the case of normal-phase separations, a recently modified displacement theory is particularly useful as a guide to an understanding of mobile phase effects[12]. However, when a high concentration of polar eluent is used, the retention may be determined by specific solute–stationary and mobile phase interactions[13,14]. One application of this concept is illustrated by the optimization of enantiomeric resolution by mobile-phase variation on a chiral stationary phase, derived from *R*-N-(3,5-dinitrobenzoyl)phenylglycine silica packings[15].

Recently we have been interested in the separations on β-cyclodextrin (β-CD)-bonded columns. The β-CD column is tailor-made to achieve specific separation selectivities, and it is packed with 5-μm silica material, covalently bonded with β-CD molecules[16]. The β-CD molecule is toroidal-shaped and consists of seven glucose units, linked in a cyclic fashion, thus providing a hydrophobic cavity and a hydrophilic exterior with hydroxyl groups. Guest molecules of proper size, such as naphthalene, can form strong β-CD inclusion complexes in a relatively hydrophilic environment. Separation, therefore, occurs when inclusion complexes of different stability are formed. If the eluite molecules are chiral, enantiomer discrimination can be observed due to the chirality of the β-CD molecules[17,18]. On the other hand, molecules that do not form strong β-CD inclusion complexes —whether this is due to their unfavorable size or the mobile-phase conditions— can still be separated by either normal-phase or reversed-phase chromatography[19–21].

Earlier, we have published a paper concerning the liquid chromatographic retention behavior of organometallic compounds and ligands, which are benzene or aniline derivatives, with amine-, octadecylsilica (ODS)- and β-CD-bonded columns[19]. Of particular interest is that the reversed-phase selectivity exhibited by the β-CD column is quite different from that of the ODS column. In order to understand the mobile phase effects in more detail, we have carried out studies of the retentions of 17 aniline derivatives on a β-CD column with mobile phases varying from the classical, normal-phase condition, *e.g.* *n*-heptane–2-propanol, to the classical, reversed-phase condition, *e.g.* water–2-propanol. Different modifiers, such as acetonitrile, methanol, and tetrahydrofuran and a different column, *i.e.* a Partisil PXS-ODS

column, were also used for comparison. It should be noted that Partisil PXS-ODS was chosen because this column has a 50% ODS coverage with residual silanol groups, which is considered to be structurally similar to that of the β-CD column, except that Partisil PXS-ODS does not have the well defined cavities of β-CD molecules.

EXPERIMENTAL

Apparatus

A Beckman (Berkeley, CA, U.S.A.) Model 332 gradient liquid chromatography system was used. This system was equipped with two Altex (Beckman) Model 110A pumps, a Model 210 sample injector valve, and a Model 420 system controller. A Micromeritics (Norcross, GA, U.S.A.) Model 786 variable-wavelength detector (200–600 nm) and a Waters (Milford, MA, U.S.A.) Model 440 adsorbance detector (254 nm) with a Houston Instrument (Houston, TX, U.S.A.) Omniscribe Model D5000 recorder were also applied. Pressure-Lok series C-160 (Precision Sampling Corp., Baton Rouge, LA, U.S.A.) 25-μl syringes were employed for injection.

Columns

Two columns were used in this work: (1) a β-cyclodextrin-bonded (β-CD) column (Advanced Separation Technologies, Whippany, NJ, U.S.A.), 5-μm particle size and 25 cm × 4.6 mm I.D., and (2) a Partisil PXS-ODS column (Whatman, Clifton, NJ, U.S.A.), 5 μm, 25 cm × 4.6 mm I.D.

Reagents

Several positional isomers of substituted anilines from Aldrich (Milwaukee, WI, U.S.A.) and others were used for the study. The substituents include chloro (Cl), methoxy (OCH_3), methyl (CH_3), and nitro (NO_2) groups. All other chemicals were of reagent grade and were obtained from various sources.

Water of high quality (18 MΩ) was produced by passing boiled, deionized water through a Milli-Q reagent water system (Millipore, Milford, MA, U.S.A.). Other HPLC-grade solvents were obtained from Fisher (Fairlawn, NJ, U.S.A.).

Chromatographic procedures

Before the separation experiments, the columns were pre-equilibrated, using the mobile phase. After pre-equilibrium was achieved, a flow-rate of 1 ml/min was set for the chromatographic process.

For each separation, solute mixtures were dissolved in the eluent. The concentration of each solute was *ca.* 1–2 mg/ml. A 3-μl sample of the solute mixture was injected into the system. A back-pressure of less than 1000 p.s.i. was usually observed in the course of the normal-phase separation experiments, and 3000 p.s.i. pressure in reversed-phase experiments. All data points were established by averaging more than three reproducible separations. Published methods were used for the determination of t_0 values for the β-CD column[17] as well as the Partisil PXS-ODS column[22].

TABLE I

CAPACITY FACTORS (k') OF SEVERAL SUBSTITUTED ANILINES ON A β-CD COLUMN

The relative errors are within 1%. Heptane–2-propanol, 2-propanol–water and water–methanol mobile-phase systems.

Samples	*% 2-Propanol in heptane*						*100% 2-propanol*	*% 2-Propanol in water*							*100% water*	*% Methanol in water*						
	10	*15*	*20*	*40*	*60*	*80*		*80*	*60*	*40*	*30*	*20*	*10*	*5*		*5*	*10*	*20*	*40*	*60*	*80*	*100*
Aniline	2.0	1.65	1.27	0.75	0.53	0.43	0.30	0.05	0.04	0.3	0.48	0.58	0.70	0.94	2.54	1.72	1.37	0.98	0.50	0.23	0.14	0.11
o-Nitroaniline	3.29	2.19	1.61	0.65	0.42	0.30	0.22	0.03	0.03	0.24	0.67	1.23	2.23	3.46	10.0	4.41	3.48	2.34	0.77	0.33	0.12	0.10
m-Nitroaniline	6.94	4.56	3.30	1.23	0.71	0.47	0.31	0.03	0.01	0.20	0.59	0.81	1.45	1.92	5.11	3.19	2.57	1.64	0.57	0.24	0.13	0.11
p-Nitroaniline	15.6	11.06	6.73	1.84	0.92	0.53	0.34	0.05	0.04	0.34	0.72	1.49	3.16	4.70	13.1	9.09	8.10	5.80	2.17	0.60	0.28	0.16
p-Methylaniline	–	1.49	1.21	0.72	0.48	0.40	0.33	0.03	0.01	0.34	0.56	0.71	1.21	1.73		3.57	2.74	1.83	0.75	0.31	0.14	0.13
p-Methoxyaniline	–	3.50	2.78	1.53	0.95	0.71	0.53	0.08	0.06	0.31	0.34	0.48	0.84	1.23	–	–	2.16	1.26	0.46	0.21	0.13	0.12
p-Chloroaniline	–	2.30	1.71	0.84	0.52	0.43	0.35	0.03	0.03	0.35	0.67	1.29	2.26	3.09	8.55	–	5.05	3.19	1.22	0.42	0.17	0.11
N,N-Dimethylaniline	–	0.15	0.14	0.11	0.10	0.10	0.10	0.09	0.10	0.15	0.74	1.61	2.74	4.87	–	–	6.49	3.57	1.41	0.46	0.14	0.08

RESULTS AND DISCUSSION

Seventeen substituted aniline derivatives were studied. These include most *o*-, *m*-, and *p*-isomers of toluidine, chloroaniline, anisole, and nitroaniline, as well as aniline, N-methylaniline, N,N-dimethylaniline, 2,5-dimethylaniline, 2,6-dimethylaniline, and 2-ethylaniline.

β-Cyclodextrin-bonded column

Heptane–methanol and 2-propanol–water mobile phase systems. The capacity factors for a group of 8 selected substituted aniline derivatives are listed in Table I as a function of mobile phase composition. The mobile phase composition varies from that in the classical, normal-phase mode, *i.e.* 2-propanol–heptane, through an intermediate condition, *i.e.* pure 2-propanol, to that in a reversed-phase mode, *i.e.* 2-propanol–water. If the normal-phase data, *e.g.* 2-propanol–heptane (1:9), are compared with the set published previously when an amine-bonded column was used[19], it is found the elution sequences of these 8 compounds are similar except that in the case of the β-CD column *o*-nitroaniline is eluted earlier than *p*-chloroaniline. In view of the large number of NH and OH functional groups on the respective amine and β-CD columns, it is not surprising to observe the similarity in their retention order.

When nitroaniline isomers are considered, it is observed that the retention order is *o*- < *m*- < *p*- in the normal-phase mode. In the reversed-phase mode, the same trend is observed when the water content is low, *i.e.* below 40%. On the other hand, when the water content is above 40%, the retention order changes to *m*- < *o*- < *p*-. This type of change in elution order with mobile phase composition was also seen with nitrophenol isomers[20]. Plots of log *k'* values *vs.* mobile phase compositions for a few aniline isomers are shown in Fig. 1. It is interesting to see that

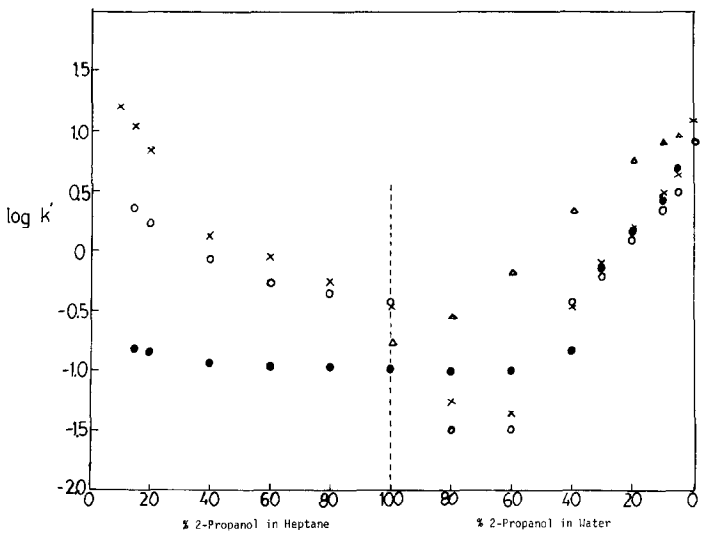

Fig. 1. Plots of log *k'* values *vs.* mobile phase compositions for *p*-nitroaniline, *p*-chloroniline, and N,N-dimethylaniline on a β-CD column. Heptane–2-propanol–water mobile phase systems. (○) *p*-Chloroaniline; (×) *p*-nitroaniline; (●) N,N-dimethylaniline; (△) *p*-nitroaniline in methanol–water mobile phase systems.

the decrease in log k' with increasing mobile phase polarity extends into the reversed-phase region, *i.e.* minima are observed when the water content is about 40%. Moreover, when N,N-dimethylaniline is examined, *i.e.* a compound that, for steric reasons, cannot form reasonable hydrogen bonds with the stationary phase, no change in log k' values is seen until the water content of 2-propanol is high (no single minimum is observed). If methanol is used as the organic solvent in the reversed-phase mode, a common behavior is observed, *i.e.* the retention time increases with increasing water content (Fig. 1).

Since methanol is smaller in size and more polar than 2-propanol, and because the β-CD molecules are rather rigid, in contrast to ODS-bonded phases that may change conformation with changes in mobile phase composition, the above observations may be explained by a combination of two effects: (1) the relative stability of inclusion complex formation between the β-CD molecule and the solute or mobile phase modifier, and (2) the competition between the β-CD molecule and the mobile phase for solutes. The latter is equivalent to the concept of "relative solubility" of solutes in the stationary as well as mobile phases. The substituted aniline that has the greatest "relative solubility" in the mobile phase will be the least retarded molecule. Of course, the better solubility must result from considerations of both hydrophilic and hydrophobic parts of the molecule. On the other hand, the stability of the inclusion complex between the β-CD molecule and methanol is certainly weaker than that of the β-CD–2-propanol complex.

In the normal-phase condition, both heptane and 2-propanol form strong inclusion complexes with β-CD molecules, and this precludes the inclusion of the anilines due to unfavorable mass action. Thus hydrogen-bonding, dipole–dipole, and London dispersion interactions are significant in determining separation selectivities. When neat 2-propanol is used as the mobile phase, inclusion of anilines is still not likely, and the hydrogen-bonding effect may still be operative. As water is introduced into the mobile phase, the possibility of β-CD–aniline inclusion complex formation increases, but the probability of aniline hydrogen-bonding with β-CD also gradually diminishes. (CD compounds are known to have moderate solubilities in water.) The hydrogen bonding effect may have some weight at low water content until it becomes completely ineffective at high water content, when only the inclusion process and the solute solubility in the mobile phase are important in determining the separation selectivity.

Chromatograms of 17 anilines were obtained in both normal-phase (Fig. 2a) and reversed-phase (Fig. 2b) modes. The normal-phase chromatogram shows satisfactory separations of 16 compounds. In contrast, only 11 peaks can be obtained in the reversed-phase chromatogram. The peaks in the reversed-phase chromatogram are also slightly broader, indicating the relatively slower mass transfer due to the inclusion process[23].

Comparison of the elution orders of the 17 compounds in both normal- and reversed-phase separations indicates that only a few compounds (*e.g.* N,N-dimethylaniline) obey the common rule that compounds eluted more rapidly in normal-phase chromatography should be eluted more slowly in reversed-phase chromatography. The most obvious violator is *p*-nitroaniline. It is eluted very slowly in both modes. The reason why *p*-nitroaniline is eluted very slowly in reversed-phase chromatography is attributed to its strong inclusion complex formation with the β-CD mole-

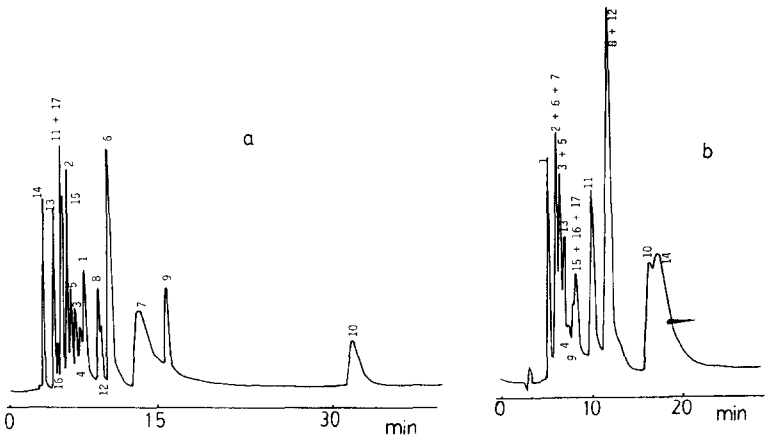

Fig. 2. Chromatograms of substituted anilines on a β-cyclodextrin with various mobile-phases conditions. (a) 2-Propanol–heptane (15:85); (b) water–2-propanol (95:5). Flow-rate = 1 ml/min, detection at 254 nm. Peak identification: 1 = aniline; 2 = *o*-methylaniline; 3 = *m*-methylaniline; 4 = *p*-methylaniline; 5 = *o*-methoxyaniline; 6 = *m*-methoxyaniline; 7 = *p*-methyoxyaniline; 8 = *o*-nitroaniline; 9 = *m*-nitroaniline; 10 = *p*-nitroaniline; 11 = *o*-chloroaniline; 12 = *p*-chloroaniline; 13 = N-methylaniline; 4 = N,N-dimethylaniline; 15 = 2,5-dimethylaniline; 16 = 2,6-dimethylaniline; 17 = *o*-ethylaniline.

cule, consistent with the fact that most of the *p*-isomers of disubstituted benzenes form stronger inclusion complexes with β-CD molecules than their corresponding *o*- and *m*-analogues[24]. The choice of mobile phase compositions for best separations in both modes requires that either the inclusion phenomena (reversed-phase) or the normal-phase interactions, *i.e.* hydrogen bonding, dipole interactions, etc. are maximized.

Heptane–tetrahydrofuran mobile phase system. The normal-phase systems consisting of heptane and tetrahydrofuran (THF) were also studied. The polarity parameter of THF (4.0) is similar to that of 2-propanol (3.9) but THF has a lower solvent strength parameter (0.57) than 2-propanol (0.82)[4]. The data listed in Table II, concerning log k' values of several substituted anilines with various heptane–THF combinations show that the 20% THF data are close to those of 10% 2-propanol. This is consistent with the argument that THF is indeed a weaker solvent.

A normal-phase retention behavior is also observed for heptane–THF mobile phases, *i.e.* the log k' value decreases as THF concentration in heptane is increased. A chromatogram of 17 substituted anilines with THF–heptane (1:4) as the mobile phase is shown in Fig. 3. The elution order is quite similar to that in Fig. 2a.

Water–acetonitrile mobile phase system. When acetonitrile replaces 2-propanol in the reversed-phase system, it is seen that the trend for the log k' values of all compounds is similar to that observed when 2-propanol is used, although subtle differences are indeed noticable due to differences in solvent properties (Table III). Again, it is found that both *m*- and *p*-nitroaniline have log k' minima at 80% aq. acetonitrile, qualitatively consistent with those observed previously when 2-propanol–water mixtures were used (Fig. 4). The chromatogram for the separation of 17 substituted aniline by acetonitrile–water (1:9) under optimized, isocratic conditions is shown in Fig. 5, which is different from that obtained when 2-propanol was

TABLE II

CAPACITY FACTORS (k') OF SEVERAL SUBSTITUTED ANILINES ON A β-CD COLUMN

The relative errors are within 1%. Tetrahydrofuran–heptane mobile phase systems.

Samples	*% THF in heptane*				
	20	*40*	*60*	*80*	*100*
Aniline	1.70	0.69	0.32	0.20	0.10
o-Nitroaniline	2.64	0.92	0.37	0.23	0.11
m-Nitroaniline	4.60	1.33	0.45	0.23	0.11
p-Nitroaniline	11.5	2.81	0.94	0.46	0.20
p-Methylaniline	1.77	0.68	0.31	0.18	0.09
p-Methoxyaniline	4.67	1.37	0.58	0.34	0.17
p-Chloroaniline	2.61	0.87	0.39	0.20	0.09
N,N-Dimethylaniline	0.28	0.15	0.09	0.06	0.06

used. Nevertheless, the elution order is similar, if positional isomers are compared for each compound.

Partisil PXS-ODS column

Heptane–2-propanol–water mobile phase system. The capacity factor values of 8 selected aniline derivatives at various mobile-phase compositions are listed in Table IV. Except for the nitroaniline isomers, the elution order for the remaining 5 com-

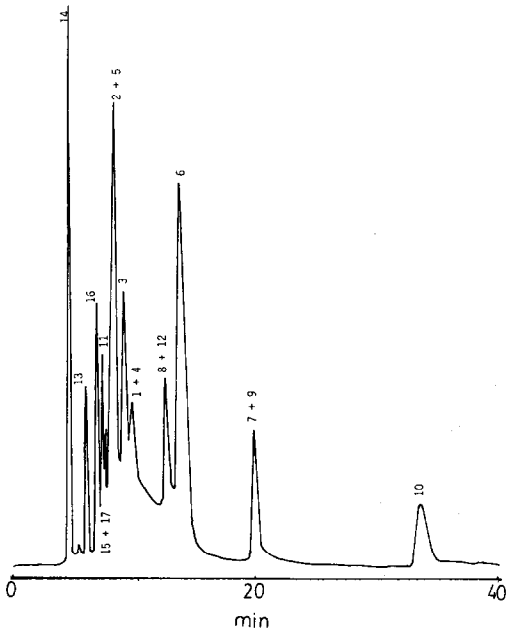

Fig. 3. Chromatogram of substituted anilines on a β-CD column with THF–heptane (1:4). Other conditions and peak identifications are the same as Fig. 2.

TABLE III

CAPACITY FACTORS (k') OF SEVERAL SUBSTITUTED ANILINES ON A β-CD COLUMN

Acetonitrile–water mobile phase systems. The relative errors are within 1%.

Samples	*% Acetonitrile in water*					
	10	*20*	*40*	*60*	*80*	*100*
Aniline	1.11	0.72	0.29	0.12	0.10	0.33
o-Nitroaniline	2.01	0.88	0.20	0.05	0.03	0.24
m-Nitroaniline	1.55	0.73	0.19	0.03	0.01	0.25
p-Nitroaniline	4.26	1.60	0.33	0.07	0.05	0.28
p-Methylaniline	1.98	1.02	0.32	0.10	0.08	0.37
p-Methoxyaniline	1.72	0.68	0.27	0.14	0.13	0.43
p-Chloroaniline	3.07	1.35	0.33	0.06	0.05	0.30
N,N-Dimethylaniline	3.84	1.70	0.39	0.09	0.06	0.28

pounds is similar to that observed with the β-CD column. However, all nitroaniline isomers are much less retained. For reasons unknown, *p*-methoxyaniline is always eluted last.

Plots of log k' values *vs.* % organic solvent are shown in Fig. 6. They give patterns similar to those obtained for the β-CD column. The log k' value decreases in the normal-phase mode as the organic solvent concentration is increased. Except for N,N-dimentylaniline, it keeps decreasing until 20% water is added to 2-propanol.

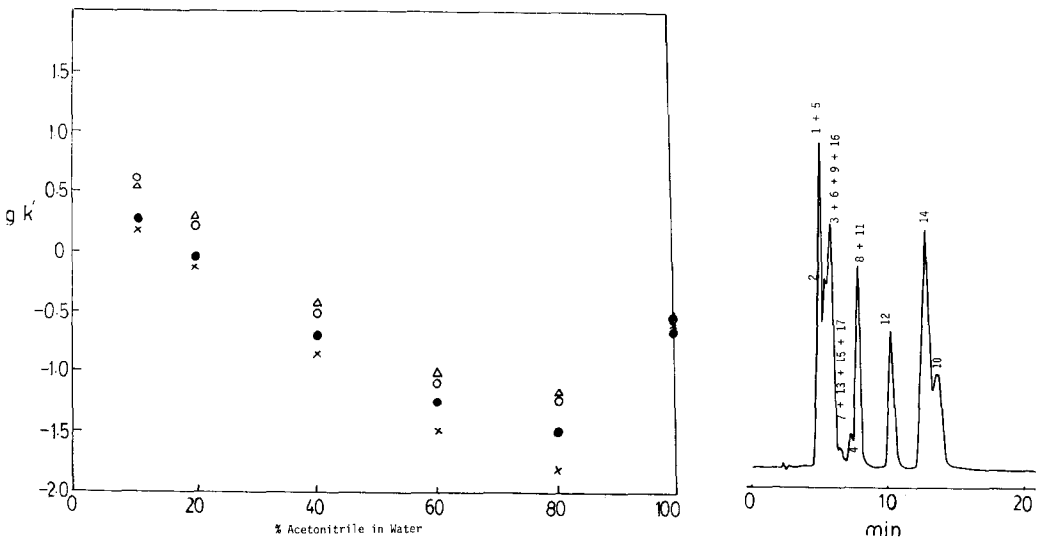

Fig. 4. Plots of log k' values *vs.* mobile phase composition for nitroaniline isomers and N,N'-dimethylaniline using a β-CD column. Acetonitrile–water mobile phase system. (●) *o*-Nitroaniline; (×) *m*-nitroaniline; (○) p-nitroaniline; (△) N,N-dimethylaniline.

Fig. 5. Chromatogram of substituted anilines on a β-CD column with acetonitrile–water (1:9). Other conditions and peak identifications are the same as Fig. 2.

TABLE IV

CAPACITY FACTORS (k') OF SEVERAL SUBSTITUTED ANILINES ON A PARTISIL PXS-ODS COLUMN

Heptane–2-propanol and 2-propanol–water mobile phase systems. The relative errors are within 1%.

Samples	*% 2-Propanol in heptane*					*100% 2-Propanol*	*% 2-Propanol in water*				
	10	*20*	*40*	*60*	*80*		*80*	*60*	*40*	*20*	*10*
Aniline	2.49	1.58	0.92	0.53	0.40	0.28	0.28	0.28	0.87	1.58	2.81
o-Nitroaniline	0.86	0.43	0.14	0.11	0.10	0.09	0.07	0.27	1.34	2.32	5.18
m-Nitroaniline	2.52	1.57	0.45	0.23	0.21	0.13	0.13	0.21	0.72	1.63	3.35
p-Nitroaniline	4.38	2.62	0.92	0.49	0.22	0.13	0.12	0.18	0.61	1.40	3.15
p-Methylaniline	2.79	1.91	1.32	0.69	0.52	0.40	0.40	0.40	2.04	5.28	8.07
p-Methoxyaniline	6.18	4.25	2.69	1.10	1.04	0.88	0.41	0.53	0.93	2.91	3.48
p-Chloroaniline	3.15	1.82	0.95	0.49	0.40	0.28	0.21	0.26	1.71	4.45	7.37
N,N-Dimethylaniline	0.22	0.19	0.17	0.16	0.16	0.15	0.36	0.60	2.48	4.65	7.63

The reversed-phase mode trend is then observed, *i.e.* the capacity factor increases as the water content of the mobile phase increases. This phenomenon can again be tentatively explained by using the "relative solubility" concept, discussed previously.

Two representative chromatograms are shown in Fig. 7a and b, optimized under normal- and reversed-phase conditions, respectively. It is seen that the normal-phase chromatogram is far more selective. The overall elution order for all compounds is quite different from that on a β-CD column, indicating a vast difference between the two bonded phases, *i.e.* presence and absence of inclusion complex formation.

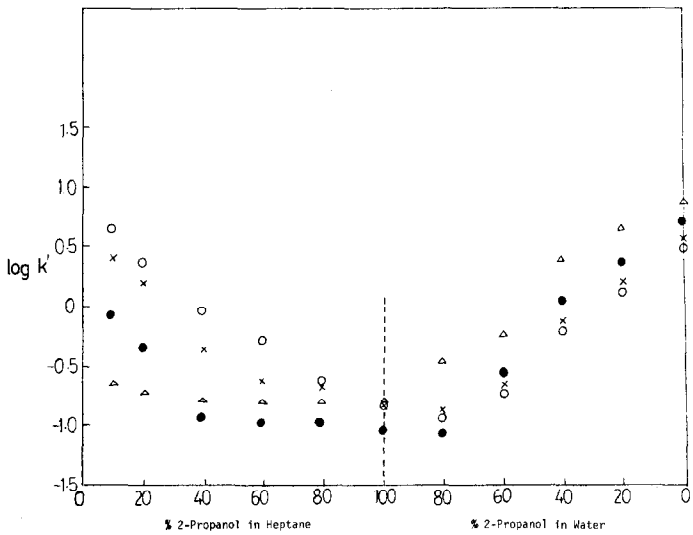

Fig. 6. Plots of log k' values *vs.* mobile phase composition for nitroaniline isomers and N,N'-dimethylaniline on a Partisil PXS-ODS column. Heptane–2-propanol–water mobile phase systems. (●) *o*-Nitroaniline; (×) *m*-nitroaniline; (○) *p*-nitroaniline; (△) N,N-dimethylaniline.

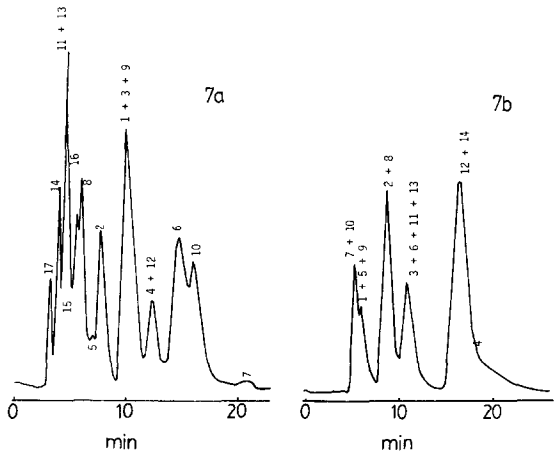

Fig. 7. Chromatograms of substituted anilines on a Partisil PXS-ODS column with different mobile phases. (a) 2-Propanol–heptane (1:9); (b) 2-propanol–water (1:4). Other conditions and peak identifications are the same as Fig. 2.

Heptane–THF mobile phase system. The values of capacity factors as a function of mobile phase composition are listed for some selected compounds in Table V. The common normal-phase trend is observed, *i.e.* k' values decrease as the organic solvent concentration increases. A solvent-specific effect is also observed, *e.g.* *p*-methoxyaniline is eluted faster than *p*-nitroaniline at 20% THF in heptane but slower at >40% THF in heptane. In Fig. 8 a chromatogram of the 17 compounds is shown which displays an elution order that is very different from that obtained when 2-propanol is used as the organic modifier.

Water–acetonitrile mobile phase system. Table VI lists the values of capacity factors for several aniline derivatives at various water–acetonitrile mobile phase compositions. It is observed that the elution order is quite different from that on a β-CD

TABLE V

CAPACITY FACTORS (k') OF SEVERAL SUBSTITUTED ANILINES ON A PARTISIL PXS-ODS COLUMN

Tetrahydrofuran–heptane mobile phase systems. The relative errors are within 1%.

Samples	*% THF in heptane*				
	20	*40*	*60*	*80*	*100*
Aniline	3.42	1.31	0.64	0.41	0.31
o-Nitroaniline	1.92	0.66	0.34	0.22	0.16
m-Nitroaniline	5.65	1.50	0.61	0.36	0.24
p-Nitroaniline	10.07	2.24	0.84	0.44	0.27
p-Methylaniline	4.00	1.49	1.02	0.53	0.37
p-Methoxyaniline	6.77	2.95	1.61	0.88	0.62
p-Chloroaniline	2.19	1.46	0.96	0.50	0.33
N,N-Dimethylaniline	0.59	0.27	0.22	0.20	0.17

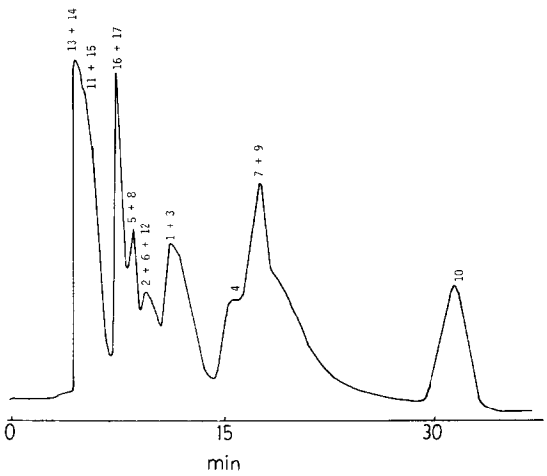

Fig. 8. Chromatogram of substituted anilines on a Partisil PXS-ODS column with THF–heptane (1:4). Other conditions and peak identifications are the same as Fig. 2.

column. The optimized chromatogram shown in Fig. 9 is also very poor in separation selectivity.

CONCLUSIONS

In this paper, we have shown through measurement of capacity factors of several substituted aniline derivatives on a β-CD column and an ODS column with various mobile-phase compositions, *i.e.* both normal- and reversed-phase modes, that mobile and stationary phase effects are quite significant in developing a satisfactory separation procedure. The β-CD column, being capable of forming inclusion complexes with certain substrates, is a better and more selective column for the reversed-

TABLE VI

CAPACITY FACTORS (k') OF SEVERAL SUBSTITUTED ANILINES ON A PARTISIL PXS-ODS COLUMN

Acetonitrile–water mobile phase systems. The relative errors are within 1%.

Sample	*% Acetonitrile in water*				
	20	*40*	*60*	*80*	*100*
Aniline	2.14	0.78	0.34	0.20	0.15
o-Nitroaniline	3.05	0.96	0.34	0.14	0.12
m-Nitroaniline	2.38	0.86	0.32	0.14	0.11
p-Nitroaniline	2.15	0.76	0.29	0.12	0.11
p-Methylaniline	3.09	1.22	0.43	0.30	0.20
p-Methoxyaniline	3.19	1.12	0.36	0.31	0.27
p-Chloroaniline	4.42	1.19	0.44	0.19	0.11
N,N-Dimethylaniline	8.02	2.14	0.63	0.34	0.16

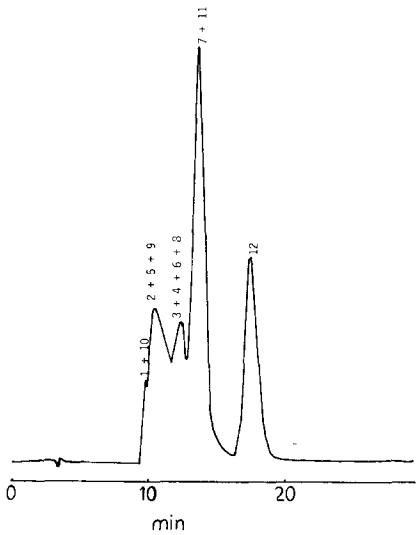

Fig. 9. Chromatogram of substituted anilines on a Partisil PXS-ODS column with acetonitrile–water (1:4). Other conditions and peak identifications are the same as Fig. 2.

phase separation of many aromatic compounds than the Partisil PXS-ODS column. On the other hand, with a large number of hydroxy groups on the surface of β-CD molecule, the β-CD column is also very selective when used for normal-phase chromatography. The latter is generally superior to reversed-phase chromatography for aniline derivatives. The choice of mobile phases is also quite obvious. Alcohol, *i.e.* 2-propanol, is better than tetrahydrofuran in the normal-phase mode and better than acetonitrile in the reversed-phase mode, presumably due to the dual H-bond acceptor and donor characteristics of alcohols.

ACKNOWLEDGEMENTS

Financial supports from the Robert A. Welch Foundation of Houston, TX (Grant No. AH877), the National Institute of Health-Minority Biomedical Research Study Program (Grant No. 5-SO6RR08012-15), the U.S. Department of Energy (Grant No. DE-FG05-84ER13292), and Texas Advanced Technologies Research Program are highly appreciated.

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