Journal of Chromatography, 241 (1982) 295–304
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,737

LIGAND-EXCHANGE GAS CHROMATOGRAPHIC SEPARATION OF ANILINE BASES

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(Received September 25th, 1981)

SUMMARY

Chromosorb G AW DMCS, coated with manganese(II) stearate, was used as column packing in ligand-exchange gas chromatography, and found to be more suitable for the separation of aniline bases than zirconium phosphate microcrystalline gels in the manganese(II) form, because the former has a lower adsorptivity and gives more symmetrical sharp peaks than the latter. From the comparison of retention data of aniline bases obtained on the manganese(II) stearate column with those obtained on the zirconium phosphate column, the retention order was found to be affected not only by the gas-phase basicity and the molecular configuration of the sample compound, but also by the surface structure of the metal support.

INTRODUCTION

Ligand-exchange liquid chromatography is now a well established method for separation of complex-forming organic compounds. It has, however, some drawbacks such as metal leakage from the stationary phase and the difficulty in adjusting the metal concentration in the stationary phase. In gas chromatography (GC), the former cannot occur and the latter can easily be overcome by impregnating or coating the support with a suitable amount of metal salt, instead of using a stationary phase bonded with a limited amount of chelating moiety.

Recent studies on ligand-exchange GC using zirconium phosphate microcrystalline gels as a metal support have shown that the use of a metal capable of forming a labile complex with the sample as well as with the mobile phase ligand is effective in improving the peak shape and reducing peak tailing. However, the peak tailing due to physical adsorption on the matrices of metal supports could not be eliminated completely, although the technique of gradient elution was useful in reducing the tailing of later peaks[1]. This means that better peak resolutions would not be obtained unless metal supports having more inert surface are used.

In the present study, Chromosorb G AW DMCS, coated with manganese(II) stearate, was selected as a stationary phase, and comparison was made between the retention behaviour of a number of aniline bases on this column and that on a

0021-9673/82/0000–0000/$02.75

zirconium phosphate column in the Mn^{2+} form.

Gas chromatographic separation of aniline bases has been achieved by many workers in partition[2–12] or adsorption[13,14] modes, but to our knowledge has never been tried in a ligand-exchange mode.

EXPERIMENTAL

Reagents

The aniline bases used in the present study are listed in Table I. They were of the highest purity available and were used without further purification as 1% solutions in *n*-hexane. Test solutions were prepared so as to contain 1% of each component.

Manganese(II) stearate was purified by recrystallization from benzene. Chromosorb G AW DMCS (80–100 mesh), used as a support of manganese(II) stearate, was obtained from Johns-Manville (Denver, CO, U.S.A.). Zirconium phosphate microcrystalline gel, Bio-Rad ZP-1, also used as a metal support, was purchased from Bio-Rad Labs. (Richmond, CA, U.S.A.).

Karl Fischer reagent, Mitsubishi SS (titre 3.0 mg water per ml), and the absorbing solvent, Mitsubishi ME (a mixture of dry methanol and ethylene glycol), were purchased from Mitsubishi Chemical Industries (Tokyo, Japan).

Column preparation

Manganese(II) stearate column. Chromosorb G AW DMCS was coated with 3% (w/w) manganese(II) stearate in the following way. A solution of manganese stearate in refluxing benzene was cooled to room temperature and then added to Chromosorb G AW DMCS. The mixture was stirred gently for 10 min, and the solvent was evaporated slowly under reduced pressure by use of a rotary evaporator, to leave the packing material. After rinsing with *n*-hexane and drying in air at room temperature, the packing was equilibrated with ammonia vapour for 24 h in a dish in a desiccator containing 14% ammonia at the bottom. After air-drying, the packing was sieved to 80–100 mesh and was placed in a glass spiral column (3 m × 4 mm I.D.) by suction. The packed column was conditioned at 80°C for 24 h by use of nitrogen containing ammonia and water vapour as carrier gas at a flow-rate of 15 ml/min. The detector was then connected and the conditioning was continued until the baseline was stable.

Zirconium phosphate column. Microcrystalline gel as received was treated with a 20% aqueous solution of manganese nitrate to convert its ionic form into the manganese(II) form. It was washed with distilled water until the filtrate was free from manganese, and then air-dried. Equilibration of the gel with ammonia vapour, packing of the gel into a column and conditioning of the column were performed as described above.

Apparatus and procedure

A 0-23 Hitachi gas chromatograph (Hitachi, Tokyo, Japan), equipped with a hydrogen flame ionization detector, was used isothermally in single-column mode. The injection port and the detector were kept at the same temperature as the column bath.

Nitrogen containing ammonia and water vapour, employed as the carrier gas, was obtained by passing nitrogen over the surface of aqueous ammonia, and was fed into the column through the injection port. The container of aqueous ammonia was placed in a thermostat and was kept at a constant temperature.

The concentration of ammonia and/or water vapour in the carrier gas was controlled by changing either the concentration of ammonia solution in the container or the temperature of the thermostat. The concentration of ammonia in the carrier gas was measured by absorbing the gas at the column outlet in a standard solution of hydrochloric acid for 3 min and titrating the excess of acid with sodium hydroxide. Water in the carrier gas was determined by absorbing the gas in absolute methanol containing glacial acetic acid for 30 min, and titrating the solution with Karl Fischer reagent. The apparatus used for titration was a Kyoto Electric Manufacturing MK-S titrator (Kyoto, Japan), the end-point being detected by the dead-stop method.

Sample solution (usually 0.4 μl) was injected into the top of the column using a 10-μl syringe. The column hold-up volume was measured from the retention time of methane and the flow-rate of the carrier gas.

RESULTS AND DISCUSSION

Adsorption on zirconium phosphate matrices

To examine the adsorption on zirconium phosphate, some N-monoalkyl-substituted anilines were chromatographed on NH_4^+ or Mn^{2+} columns packed with microcrystalline zirconium phosphate gels with nitrogen gas containing ammonia and water vapour as mobile phase. As can be seen from Fig. 1, the NH_4^+ gave larger

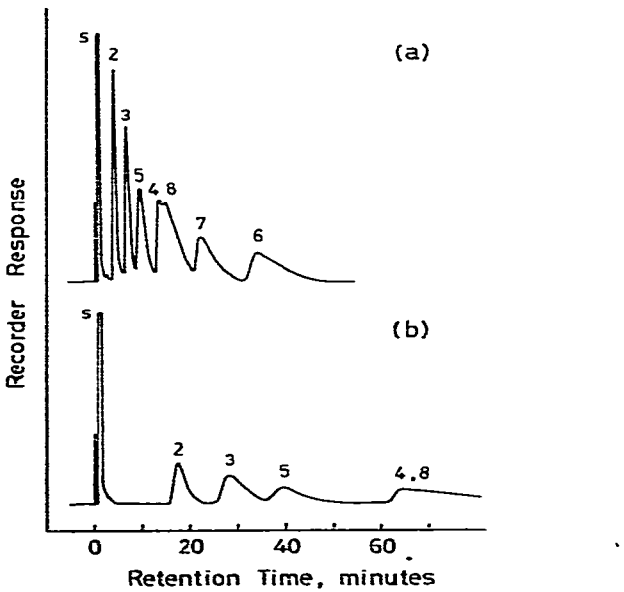

Fig. 1. Chromatograms of a mixture of N-monoalkyl-substituted anilines on zirconium phosphate (60–80 mesh) in Mn^{2+} form (a) and NH_4^+ form (b) at 80°C. Column: 3 m × 4 mm I.D. Flow-rate: 20.0 ml/min. Ammonia concentration (μmol/ml) in mobile phase; 0.70 (a); 0.73 (b). Water concentration (μmol/ml) in mobile phase: 0.09 (a); 0.10 (b). Peak identification as in Table I (s = solvent).

retention times and severer peak tailing than the Mn^{2+} form, under the same chromatographic conditions. This means that zirconium phosphate matrices exhibit a very high adsorptivity, since the retention of aniline bases on the NH_4^+ form cannot be based on complex formation between the sample and NH_4^+.

The adsorption on zirconium phosphate matrices can be explained in terms of hydrogen bond formation between the oxygen atom in zirconium phosphate and the amino hydrogen atom in the aniline bases. On the contrary, the retention of aniline bases on the Mn^{2+} form may be attributed primarily to complex formation; the adsorption due to hydrogen bonding is decreased on this column, because the active surface of the zirconium phosphate matrix is covered partially with bulky octahedral manganese ammine complexes formed with ammonia in the mobile phase.

The above rationalization is supported by the finding that in the absence of mobile phase ligands such as ammonia and/or water none of the aniline bases tested could be eluted from the column, whether in the NH_4^+ form or in the Mn^{2+} form, within a reasonable time.

The peak tailing observed in Fig. 1a suggests that the adsorption due to hydrogen bonding still remains even on the Mn^{2+} form. Although not indicated in Fig. 1, some samples showed different elution orders between the NH_4^+ and Mn^{2+} forms. Thus, chloro- and fluoroanilines were eluted in the order *o*- < *m*- < *p*-isomers on the NH_4^+ form, and *o*- < *p*- < *m*-isomers on the Mn^{2+} form. This variation may be ascribed to the difference in retention mechanism on the two forms.

Effect of ammonia concentration on retention on zirconium phosphate

The adjusted retention times, t'_R, of some N,N-dialkyl-substituted anilines and xylidines measured on a column of zirconium phosphate in the Mn^{2+} form are shown in Fig. 2 as a function of the ammonia concentration in the mobile phase. The water

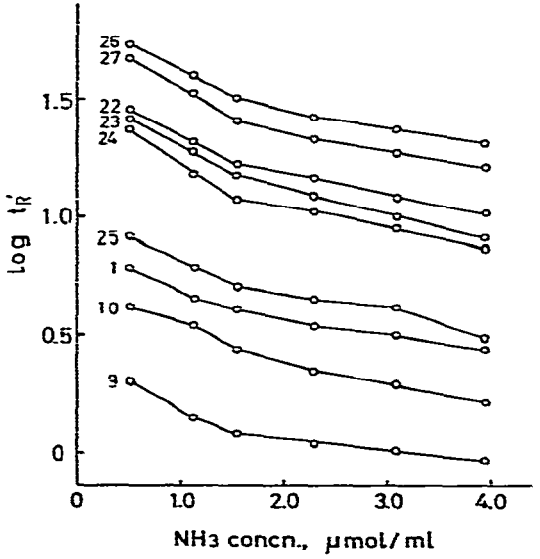

Fig. 2. Effect of ammonia concentration in the mobile phase on log t'_R of some N,N-dialkyl-substituted anilines and xylidines. Column: 3 m × 4 mm I.D. Packings: zirconium phosphate (60–80 mesh), Mn^{2+} form. Column temperature: 80°C. Flow-rate: 15.5 ml/min. Line identification as in Table I.

concentration was kept in the range 0.82–0.97 μM/ml throughout the measurement. Fig. 2 shows an approximately linear relationship between log adjusted retention time (t'_R) and the ammonia concentration, with a small but distinct change of the slope at an ammonia concentration of about 1.5 μM/ml. It also shows that the elution order as well as the peak resolution do not vary with the ammonia concentration, because the slope, *i.e.*, the decrease of t'_R with a unit increase of ammonia concentration, is nearly equal for all samples examined.

These findings suggest that the retention of aniline bases on a zirconium phosphate column (Mn^{2+}) is governed by the concentration of mobile phase ligands, and that the elution order and the peak resolution of aniline bases depend on their inherent nature.

TABLE I

ANILINE BASES USED AND THEIR ADJUSTED RETENTION TIMES (MIN) ON CHROMOSORB G AW DMCS WITH AND WITHOUT MANGANESE(II) STEARATE AT 75°C

Column: 3 m × 4 mm I.D. Flow-rate: 10.0 ml/min.

		Column			
		Coated with 3% manganese(II) stearate			*Not coated*
NH_3 concn. in mobile phase (μmol/ml)		1.20	4.69	–	1.14
H_2O concn. in mobile phase (μmol/ml)		0.13	0.15	–	0.11
No.	*Compound*				
1	Aniline	2.9	2.5	5.6	0.45
2	N-Methylaniline	4.0	3.5	6.3	0.40
3	N-Ethylaniline	6.2	5.4	9.4	0.54
4	N-*n*-Propylaniline	12.2	10.7	18.7	0.94
5	N-Isopropylaniline	6.7	5.8	10.3	0.58
6	N-*n*-Butylaniline	27.0	23.5	41.0	1.84
7	N-Isobutylaniline	16.7	15.1	25.8	1.23
8	N-*sec.*-Butylaniline	13.0	11.8	20.1	1.00
9	N,N-Dimethylaniline	4.5	3.8	6.8	0.37
10	N,N-Diethylaniline	11.3	9.8	17.3	0.83
11	N,N-Di-*n*-propylaniline	37.8	32.7	57.6	2.40
12	N,N-Di-*n*-butylaniline	–	–	–	7.80
13	*o*-Fluoroaniline	1.9	1.6	3.0	0.25
14	*m*-Fluoroaniline	3.5	3.1	6.2	0.48
15	*p*-Fluoroaniline	3.8	3.2	8.0	0.63
16	*o*-Chloroaniline	7.2	6.5	11.7	0.61
17	*m*-Chloroaniline	16.5	14.8	29.8	1.56
18	*p*-Chloroaniline	19.0	16.9	39.0	1.96
19	*o*-Toluidine	5.2	4.4	8.5	0.61
20	*m*-Toluidine	6.6	5.4	11.8	0.80
21	*p*-Toluidine	6.9	5.8	14.6	0.91
22	2,3-Xylidine	14.9	12.2	25.8	1.46
23	2,4-Xylidine	12.3	10.0	21.9	1.23
24	2,5-Xylidine	11.9	9.7	19.3	1.11
25	2,6-Xylidine	9.8	8.0	14.8	0.83
26	3,4-Xylidine	19.7	15.4	40.3	2.25
27	3,5-Xylidine	14.9	11.9	26.7	1.47

Retention behaviour on manganese(II) stearate-coated column

In an attempt to reduce the peak tailing due to physical adsorption on active metal support surfaces, use was made of Chromosorb G AW DMCS coated with manganese(II) stearate, instead of zirconium phosphate (Mn^{2+}). Table I lists the retention times of aniline bases, measured on columns of Chromosorb G AW DMCS coated with and without manganese(II) stearate, together with the chromatographic conditions.

As had been expected, the retention times on the non-coated column were very short and there was no pronounced difference among the samples, whereas those on the coated column increased without any change in the elution order with a decrease of the ammonia concentration in the mobile phase, as was the case on zirconium phosphate (Mn^{2+}). It is considered that the retention of aniline bases on a manganese(II) stearate-coated Chromosorb column is not based on partition, but on the interaction between the manganese and the amino group of the amines, since manganese(II) stearate (m.p. 112°C) acts as a solid at the column temperature of 75°C, at which the adjusted retention times were measured. A manganese(II) stearate-coated Chromosorb column differs from a zirconium phosphate column (Mn^{2+}) in the following way: all samples tested could be eluted, although with relatively long retention times, from a coated column even when neither ammonia nor water is present in the mobile phase. This may be explained by the smaller total amount of manganese as well as by the weaker interaction between the supports or matrices and the aniline bases on the coated column compared with the zirconium phosphate column.

It is to be noted, however, that the concentration of the mobile phase ligands, especially that of ammonia, greatly affects not only the retention time but also the peak resolution. Fig. 3 shows a poorer peak resolution and a severer peak tailing caused by the absence of ammonia and water vapour in the mobile phase.

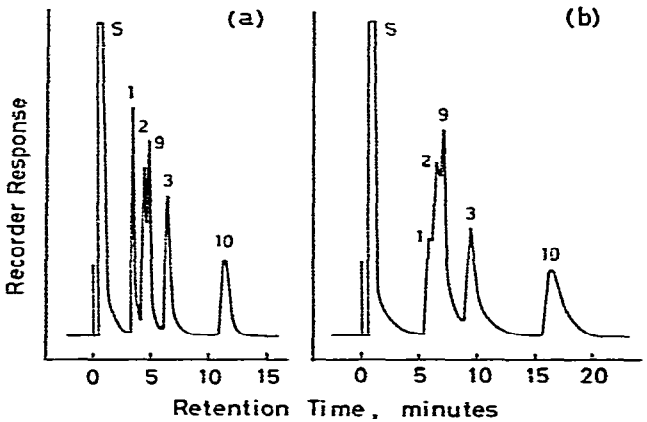

Fig. 3. Chromatograms of a mixture of some N-monoalkyl-substituted and N,N-dialkyl-substituted anilines at 75°C. Column: 3 m × 4 mm I.D. Packings: 3% manganese stearate coated on Chromosorb G AW DMCS (80–100 mesh). Flow-rate: 10.1 ml/min. Ammonia concentration (μmol/ml) in mobile phase: 1.20 (a); none (b). Water concentration (μmol/ml) in mobile phase: 0.13 (a); none (b). Peak identification as in Table I.

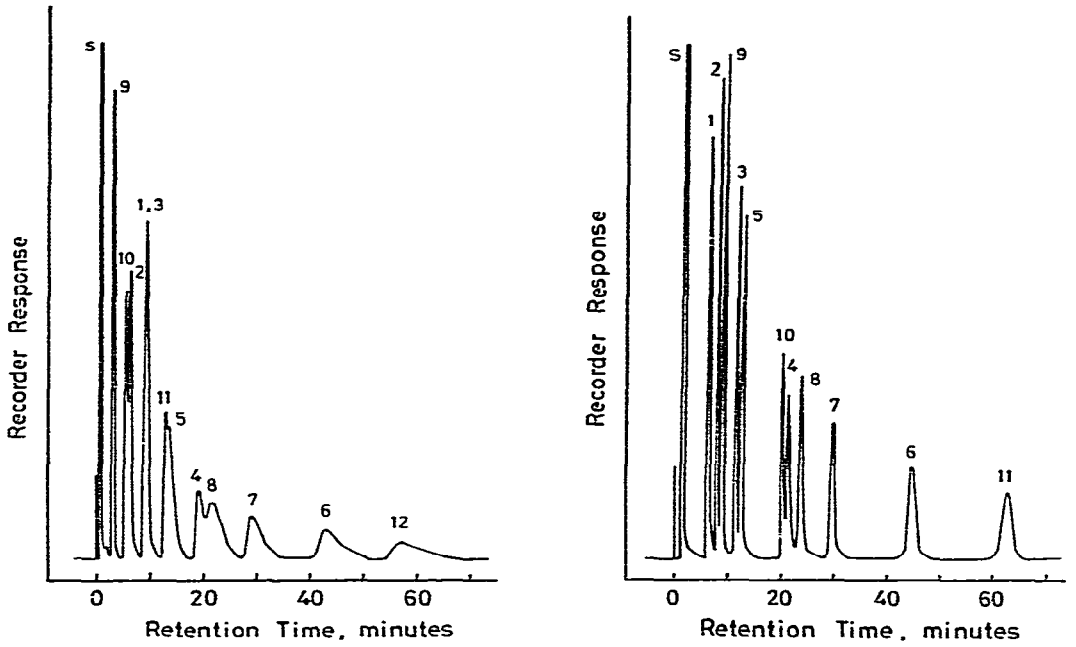

Fig. 4. Separation of N-alkyl-substituted anilines on zirconium phosphate (60–80 mesh), Mn^{2+} form at 90°C. Column: 3 m × 4 mm I.D. Flow-rate: 24.8 ml/min. Ammonia concentration in mobile phase: 3.07 μmol/ml. Water concentration in mobile phase: 0.20 μmol/ml. Peak identification as in Table I.

Fig. 5. Separation of N-alkyl-substituted anilines with 3% manganese stearate coated on Chromosorb G AW DMCS (80–100 mesh) at 80°C. Column: 3 m × 4 mm I.D. Flow-rate: 21.8 ml/min. Ammonia concentration in mobile phase: 2.89 μmol/ml. Water concentration in mobile phase: 0.13 μmol/ml. Peak identification as in Table I.

Comparison of column efficiency and selectivity

Chromatographic separation of aniline bases was performed by use of a manganese(II) stearate-coated Chromosorb column and a zirconium phosphate column (Mn^{2+}) under the most suitable conditions. The chromatograms obtained are shown in Figs. 4–7. Comparison of Fig. 5 with Fig. 4 and of Fig. 6b with Fig. 6a shows that the manganese stearate-coated Chromosorb column gives sharper and more symmetrical peaks, although there is still a slight peak tailing at a later peak (No. 26) in Fig. 6b. While the peak tailing on the zirconium phosphate column may be attributed to the adsorption on the zirconium phosphate matrix and/or to the slow rate of the ligand exchange reaction, only the latter can be responsible for the peak tailing on the manganese stearate-coated column. It may be concluded, therefore, that the column of manganese stearate coated on Chromosorb G AW DMCS is superior in efficiency to the column of zirconium phosphate (Mn^{2+}).

On the other hand, there is a difference in the elution order between the two kinds of columns. As can be seen from Fig. 5, N,N-dialkyl-substituted anilines are generally more strongly retained on the manganese stearate-coated Chromosorb column than on the zirconium phosphate column (Mn^{2+}). For example, N,N-di-*n*-propylaniline (11) is eluted from the coated column after, but from the zirconium

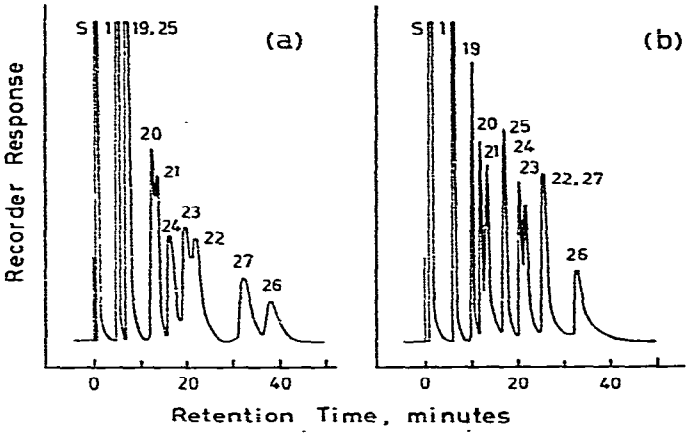

Fig. 6. Separation of aniline, toluidines and xylidines on (a) zirconium phosphate (60–80 mesh), Mn^{2+} form, and (b) manganese stearate coated on Chromosorb G AW DMCS (80–100 mesh). Chromatographic conditions for (a) and (b) as for Figs. 4 and 5, respectively. Peak identification as in Table I.

phosphate column before, N-monoalkyl-substituted anilines (5, 4, 8, 7, 6), the retention times of which are nearly the same on either column. Another example is N,N-di-*n*-butylaniline, which cannot be eluted within 150 min from the coated column under the conditions shown in Fig. 5.

It is to be noted that the same elution order is observed within the homologous series of aniline bases; the order is 2 < 3 < 5 < 4 < 8 < 7 < 6 for N-monoalkyl-substituted anilines and 9 < 10 < 11 < 12 for N,N-dialkyl-substituted anilines irrespective of the column. These orders agree with that of the carbon chain lengths of the alkyl groups in the homologues and are opposite to the order of bulkiness of the alkyl groups within isomers. The mechanism of retention thus seems to be similar on the two columns. The differences between the two columns in elution order, as shown in Figs. 4 and 5, is probably due to the difference in selectivities of the metal supports used.

It has been shown that in ligand-exchange chromatography the retention behaviour of sample compounds depends largely on their gas-phase basicity as well as on their molecular configuration; the smaller the gas-phase basicity of the aniline derivative and the bulkier the moiety around the amino nitrogen, the shorter is the retention time. Although very few gas-phase basicities of aniline derivatives are known, it can be expected from the data pertaining to aliphatic amines that the gas-phase basicity of alkyl-substituted anilines increases with an increase of the alkyl chain length as well as with the number of the alkyl substituents[15–17]. For aniline bases carrying isomeric alkyl groups, the gas-phase basicity is expected to follow the order *tert.*- > *sec.*- > *iso*- > *n*-alkyl-substituted anilines.

Experimentally, however, the elution order of N-propyl- and N-butylaniline isomers does not agree with the above expectation on either column. This is probably due to the steric hindrance to complex formation. Furthermore, aniline was eluted from the zirconium phosphate column (Mn^{2+}) after N,N-dimethyl-, N,N-diethyl- and N-methylanilines, although it had the shortest retention time on the manganese stearate-coated Chromosorb column as expected. Again, this may be explained in

terms of steric factors, since microcrystalline zirconium phosphate gels are of a smaller pore size than Chromosorb G. The above findings strongly suggest that the elution order of N-substituted anilines in ligand-exchange GC is determined by the balance of the effects of gas-phase basicity and steric bulk.

When a manganese stearate-coated Chromosorb column is used, the hydrophobic interaction, or the Van der Waals interaction, between the stearate group and the alkyl chain in the sample can also affect the sample retention. This is evidenced by the retention times of aliphatic and aromatic hydrocarbons, which have no coordination sites; their retention times increase with increasing carbon number. For example, the retention times of benzene, toluene, ethylbenzene and isopropylbenzene were 1.89, 2.19, 2.71 and 3.25 min, respectively, and those of methane, *n*-pentane, *n*-hexane, *n*-heptane and *n*-decane were 1.63, 1.72, 1.80, 1.96 and 4.64 min, respectively, under the conditions cited in Fig. 5.

Toluidine and xylidine isomers showed similar retention behaviour as N-alkyl-substitited anilines. As Fig. 6 shows, toluidine isomers are eluted in the order of *o*- < *m*- < *p*-isomer from either column. This order is the opposite to that of their ionization potentials, which in turn are closely related to their proton affinities or basicities in the gas phase[18,19]. In contrast, the bulkiness effect is the dominant factor determining the elution order of xylidine isomers; 2,6-xylidine, whose nitrogen atom is completely shielded by the two adjacent methyl groups, has the shortest retention time, followed by 2,5-, 2,4-, 2,3-, 3,5- and 3,4-xylidine. This order is the same as that of the distance between the amino group and the nearest methyl group. The elution order of 3,4- and 3,5-xylidines is the opposite to that of their ionization potentials.

It is interesting to note that the pairs 24/23 and 22/27 showed better separation on a zirconium phosphate column than on a coated column, whereas the paris 23/22, 20/21 and 19/25 showed better separation on a coated column than on a zirconium phosphate column. Of additional interest is the behaviour of 2,6-xylidine on a zirconium phosphate column. It elutes before *m*- and *p*-toluidines because of its larger steric hindrance to complex formation on the microcrystalline structure, in spite of

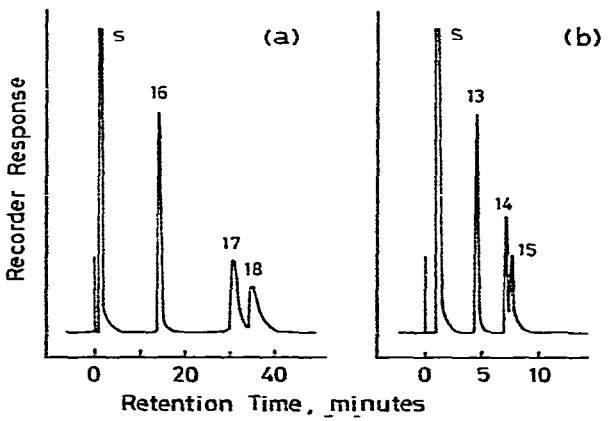

Fig. 7. Separation of chloroanilines (a) and fluoroanilines (b) with 3% manganese stearate coated on Chromosorb G AW DMCS (80–100 mesh) at 80°C. Chromatographic conditions for (a) as for Fig. 5, and for (b): column 3 m × 4 mm I.D.; flow-rate, 16.1 ml/min; ammonia concentration in mobile phase, 2.03 μmol/ml; water concentration in mobile phase, 0.15 μmol/ml. Peak identification as in Table I.

the expected larger gas-phase basicity than those of toluidines.

The separation of chloro- and fluoroaniline isomers was best achieved on a coated column as shown in Fig. 7. Fluoroanilines are retained lesser strongly than chloroanilines, with the same elution order, *o*- < *m*- < *p*-isomer. On a zirconium phosphate column the orders both changed to *o*- < *p*- < *m*-isomer and the peak resolution decreased. These orders do not agree with the one expected from the ionization potentials of chloroanilines, *i.e.*, *p*- < *o*- < *m*-isomer.

CONCLUSIONS

The work reported here has demonstrated that metal stearate-coated Chromosorb G AW DMCS is a much more useful stationary phase for the ligand-exchange GC separation of aniline bases than zirconium phosphate in a metal ion form; the latter fails to give sharp symmetrical peaks because of its high adsorptivity based on hydrogen bonding, whereas the former gives better peak shape and resolution because of its decreased adsorptivity caused by complex formation.

The elution order of samples has been found to depend on the surface structure of the supports or matrices of the metal, as well as on the complex-forming ability of the sample and the steric hindrance to complex formation, which in turn are closely related to the gas-phase basicity of the sample and its molecular configuration, respectively. It has also been suggested that not only complex formation but also hydrophobic interaction plays a role in determining the retention of aniline bases, when the metal stearate-coated stationary phase is used. This column cannot be used, however, in an adsorption chromatographic mode, because the peak resolution is markedly decreased in the absence of mobile phase ligands, although all the samples tested in this study could be eluted under such conditions.

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