*Journal of Chromatography*, 114 (1975) 15–21


CHROM. 8409

# STUDIES ON LIGAND-EXCHANGE CHROMATOGRAPHY

# V. GAS CHROMATOGRAPHIC SEPARATION OF LOWER ALIPHATIC AMINES BY LIGAND EXCHANGE

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(First received March 3rd, 1975; revised manuscript received April 29th, 1975)

## SUMMARY

In the ligand-exchange gas chromatographic separation of lower aliphatic amines developed, an inorganic cation exchanger (zirconium phosphate) in the $Cu^{2+}$ form is used as the stationary phase, and the mobile phase is nitrogen gas containing ammonia and water vapour. By studying the effects of the structures of the amines, the cation of the stationary phase and the composition of the mobile phase on the retention values, it has been found that the amine–stationary phase interaction is correlated with the stability of the complexes formed and with steric effects on the amine–metal ion reaction. The concentration of ammonia in the mobile phase also plays an important part in determining retention times and peak resolution.

## INTRODUCTION

Ligand-exchange reactions have been widely used in liquid chromatography; the separations of homologues or isomers of many polar compounds, such as amines and related compounds[1–4], carboxylic acids[5,6], amino acids and their racemates[7–9] and ribonucleic acids[10] were achieved with great success. The application of such reactions in gas chromatography, however, has not hitherto been studied.

The purpose of the present study was to confirm the utility of ligand-exchange gas chromatography as a separation technique: attempts were made to establish conditions suitable for separating lower aliphatic amines, which are known to be difficult to separate by gas–liquid partition chromatography because of peak tailing caused by adsorption effects of the support. Recently, gas–solid adsorption chromatography[11] or the use of water vapour or the vapour of an organic solvent as the mobile phase[12,13] has been shown to be effective in reducing peak tailing.

In the present study, an inorganic cation exchanger in the metal-ion form was used as stationary phase, with ammonia and/or water vapour as mobile phase.

## EXPERIMENTAL

*Reagents and column packings*

All the amines were of reagent grade and were used as 5% aqueous solutions without further purification; test mixtures were prepared so as to contain 5% of each amine. Nitrogen was used as carrier for the gaseous ligands, and ZP-1 zirconium phosphate crystals (Bio-Rad Labs., Richmond, Calif., U.S.A.) as cation exchanger. The ZP-1 crystals were sieved to 60–80 mesh, and, before use, were treated with a 10% aqueous solution of $CuCl_2$ or $ZnCl_2$ to convert them into the $Cu^{2+}$ or $Zn^{2+}$ form and then with concentrated aqueous ammonia to form their ammonia complexes. The air-dried packing material was placed in a spiral glass column (1 m × 4 mm I.D.) and then conditioned for 6 h at 60° by passing a constant flow of nitrogen containing ammonia gas and water vapour through the column.

*Equipment and working conditions*

A Hitachi gas chromatograph (model O-23) equipped with a flame ionization detector was used. Ammonia and water vapour were fed into the column by passing nitrogen through a container of concentrated aqueous ammonia maintained at constant temperature (between 30 and 40°) and sweeping away the generated vapour. The container was placed in a thermostat, and the concentration of ammonia in the carrier gas was controlled by changing the temperature of the thermostat. Sample solutions, 0.4 $\mu$l, were injected directly into the top of the column, and the carrier gas flow-rate was measured by using a soap-film flow-meter. The concentration of ammonia in the carrier gas was determined by absorbing it in hydrochloric acid for a fixed period (usually 3 min) and then titrating the excess of hydrochloric acid with sodium hydroxide solution (methyl red as indicator).

## RESULTS AND DISCUSSION

*Effect of ammonia concentration*

Fig. 1 shows a typical chromatogram of a mixture of trimethyl-, triethyl-, dimethyl-, and diethyl-amine on a column in $Cu^{2+}$ form, together with the experimental conditions; the retention times of these and other amines at different concentrations of ammonia are listed in Table I.

As was expected from studies of ligand-exchange liquid chromatography[5,14], the retention times of the amines varied with change in concentration of ammonia in the carrier gas.

Table I also shows that, irrespective of the concentration of ammonia in the carrier gas, the retention time increases in the order tertiary, secondary, primary amine, and in each class with increase in the carbon number. This order differs from those that have been observed on partition columns, such as a liquid paraffin or Lubrol MO (a polystyrene oxide) column[15], where the elution order is approximately that of the boiling points. Evidently steric interference exerts a major effect on amine–metal interactions in ligand-exchange gas chromatography.

The concentration of ammonia in the carrier gas also affects the peak resolution; the results obtained for a mixture of trimethyl-, triethyl-, dimethyl-, and diethyl-

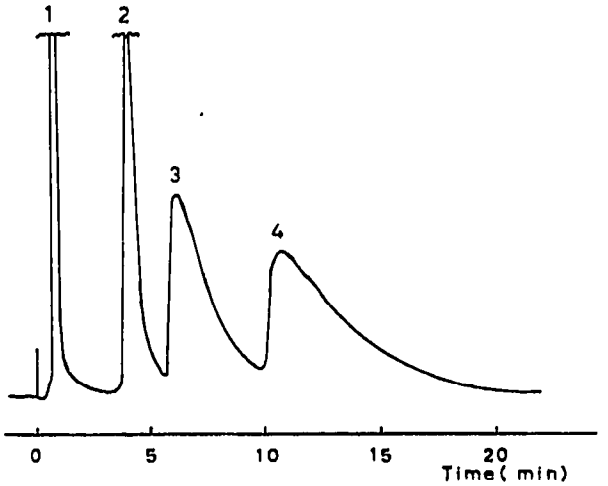


Fig. 1. Separation of trimethylamine (1), triethylamine (2), dimethylamine (3) and diethylamine (4) on a column of ZP-1 ($Cu^{2+}$). Column dimensions: 1 m × 4 mm I.D.; temperature: 60°; flow-rate: 20 ml/min; ammonia concentration in carrier gas 9.0 μmoles/ml.

TABLE I

EFFECT OF THE CONCENTRATION OF AMMONIA IN CARRIER GAS ON RETENTION TIMES OF AMINES

Column: ZP-1 ($Cu^{2+}$), 1 m × 4 mm I.D.; column temperature, 60°; flow-rate, 20 ml/min.

| *Amine* | *Retention time (min)* | |
|---|---|---|
| | *14.8 μmoles $NH_3$/ml* | *21.5 μmoles $NH_3$/ml* |
| Trimethyl | 0.5 | 0.4 |
| Triethyl | 2.4 | 1.6 |
| Dimethyl | 3.4 | 1.8 |
| Diethyl | 5.7 | 2.6 |
| Di-isopropyl | 6.4 | 3.0 |
| Ethyl | 8.3 | 4.3 |
| Isopropyl | 11.2 | 5.0 |
| *n*-Propyl | 18.4 | 9.0 |
| *sec.*-Butyl | 22.4 | 9.4 |
| Isobutyl | 30.1 | 12.9 |
| *n*-Butyl | 60.2 | 25.0 |

amine are shown in Table II. Generally, lower concentrations of ammonia gave better results at a flow-rate of 20 or 30 ml/min.

*Effect of metallic form*

In Table III are listed the retention times of trimethyl-, triethyl-, dimethyl-, and diethyl-amine, and the peak resolutions between triethyl- and dimethyl-, and between dimethyl- and diethyl-amine, obtained on columns in the $Cu^{2+}$ and $Zn^{2+}$ forms under different sets of chromatographic conditions. It is obvious that, under the conditions used, the retention time of each amine on a $Cu^{2+}$-form column is always longer than that on a $Zn^{2+}$-form column.

TABLE II

EFFECT OF CONCENTRATION OF AMMONIA IN CARRIER GAS ON PEAK RESOLUTION ($R$)

$R_1$ denotes the resolution between triethylamine and dimethylamine, and $R_2$ that between dimethylamine and diethylamine. Column: ZP-1 ($Cu^{2+}$), 1 m × 4 mm I.D., operated at 60°.

| *Ammonia concn. in carrier gas, μmoles/ml* | *Flow-rate, ml/min* | $R_1$ | $R_2$ |
|---|---|---|---|
| 7.5 | 20 | 1.45 | 1.06 |
| 8.0 | 20 | 1.27 | 1.20 |
| 9.0 | 20 | 1.26 | 1.03 |
| 12.0 | 20 | 1.03 | 0.91 |
| 15.0 | 20 | 1.19 | 0.82 |
| 20.0 | 20 | 0.71 | 0.53 |
| 5.0 | 30 | 1.46 | 1.19 |
| 9.0 | 30 | 1.25 | 1.13 |
| 11.0 | 30 | 0.87 | 0.75 |

In the present study, it may reasonably be assumed that the retention time of the amine is determined by the ratio of the stability of the metal–amine complex to that of the metal–ammonia complex; the larger the ratio, the longer will be the retention time. Moreover, it is well-known that the $Cu^{2+}$–ammonia complex is more stable than the $Zn^{2+}$–ammonia complex[16]. The data in Table III, therefore, show that the $Cu^{2+}$–amine complexes are generally more stable than the $Zn^{2+}$–amine complexes under the conditions used.

TABLE III

EFFECT OF METALLIC FORM OF STATIONARY PHASE ON RETENTION TIME ($t_R$, min) AND PEAK RESOLUTION ($R$)

Column: ZP-1, 1 m × 4 mm I.D., operated at 60°.

| *Amine* | *$Cu^{2+}$-form column* | | *$Zn^{2+}$-form column* | | *Conditions* |
|---|---|---|---|---|---|
| | $t_R$ | $R$ | $t_R$ | $R$ | |
| Trimethyl | 0.7 | | 0.4 | | $NH_3$ concn. in carrier gas, 7.5 μmoles/ml; flow-rate, 20 ml/min |
| Triethyl | 4.0 | 1.45 | 1.3 | 1.40 | |
| Dimethyl | 7.2 | 1.06 | 3.2 | 1.03 | |
| Diethyl | 11.6 | | 5.8 | | |
| Trimethyl | 0.4 | | 0.2 | | $NH_3$ concn. in carrier gas, 8.8 μmoles/ml; flow-rate, 30 ml/min |
| Triethyl | 2.8 | 1.25 | 0.9 | 1.26 | |
| Dimethyl | 4.8 | 1.13 | 2.0 | 1.01 | |
| Diethyl | 8.4 | | 3.8 | | |

*Effect of physical adsorption*

In order to investigate the effect of physical adsorption by zirconium phosphate itself on the ligand-exchange chromatographic behaviour of the amines, retention times were measured on a column in the $NH_4^+$ form, which has no capability for co-ordination; the results are shown in Table IV. Comparison of these values with those in Table I reveals that, except for dimethyl- and ethyl-amine, the retention times of the amines are shorter on the $NH_4^+$-form column than on the $Cu^{2+}$- or $Zn^{2+}$-form columns; the reasons for the exceptional behaviour of these two amines are not clear

TABLE IV

RETENTION TIMES OF AMINES ON $NH_4^+$-FORM COLUMN

Column: 1 m × 4 mm I.D.; column temperature, 60°; flow-rate, 20 ml/min; ammonia concentration in the carrier gas, 15 μmoles/ml.

| *Amine* | $t_R$ |
|---|---|
| Trimethyl | 0.4 |
| Triethyl | 0.8 |
| Dimethyl | 7.4 |
| Diethyl | 4.0 |
| Di-isopropyl | 2.4 |
| Ethyl | 14.7 |
| Isopropyl | 8.4 |
| *n*-Propyl | 14.0 |
| *sec.*-Butyl | 13.0 |
| Isobutyl | 14.5 |
| *n*-Butyl | 29.0 |

at present, and both compounds gave very broad peaks in comparison with the other amines or with those obtained on the $Cu^{2+}$- or $Zn^{2+}$-form of the ion exchanger. The elution order of the amines on the $NH_4^+$-form column is also different from that on the $Cu^{2+}$ or $Zn^{2+}$-form. Since a $Cu^{2+}$- or $Zn^{2+}$-form column has both co-ordination and adsorption sites, and an $NH_4^+$-form column has only adsorption sites, these results may be interpreted as suggesting that the retention of amines on either metal-form column is based essentially on co-ordination between the metal and the amines.

*Effect of water vapour*

In this study, the ammonia vapour used as mobile-phase ligand was obtained by warming concentrated aqueous ammonia and was introduced into the column with nitrogen carrier gas. Thus, the ammonia vapour is always accompanied by water vapour when it is introduced into the column. In order to determine the effect of water vapour on the elution behaviour of the amines, chromatography on a $Cu^{2+}$-form column was carried out with a mixture of nitrogen and ammonia as mobile phase; this gas was prepared, by mixing ammonia with nitrogen in a bomb, to contain 25% (v/v) of ammonia.

The chromatogram obtained is shown in Fig. 2, together with a chromatogram obtained in the usual manner for comparison. In spite of there being the same ammonia concentration in the carrier gas, the elution for an amine was much longer when the mixed gas was used. This shows that water vapour plays an important role in the ligand-exchange reaction, probably as an additional ligand.

However, when a mixture of nitrogen and water (or nitrogen alone) was used as mobile phase, none of the amines could be eluted. So far, the proposed elution system, in which both ammonia and water vapour are used as mobile-phase ligands, has been found to give the best results.

*Separation of test mixtures*

Some test mixtures were chromatographed by the proposed method to afford the chromatograms shown in Figs. 3–5. From the elution order of the primary-amine

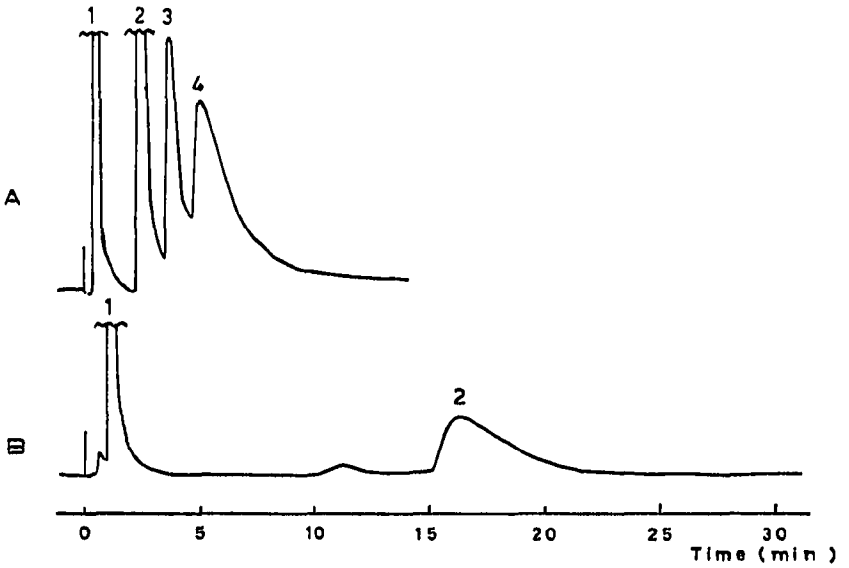


Fig. 2. Comparison of two methods of supplying the ammonia: A, usual manner; B, mixed-gas system. 1 = Trimethylamine; 2 = triethylamine; 3 = dimethylamine; 4 = diethylamine. Column conditions as for Fig. 1, but ammonia concentration in carrier gas 14.8 μmoles per ml (A) or 15.0 μmoles per ml (B).

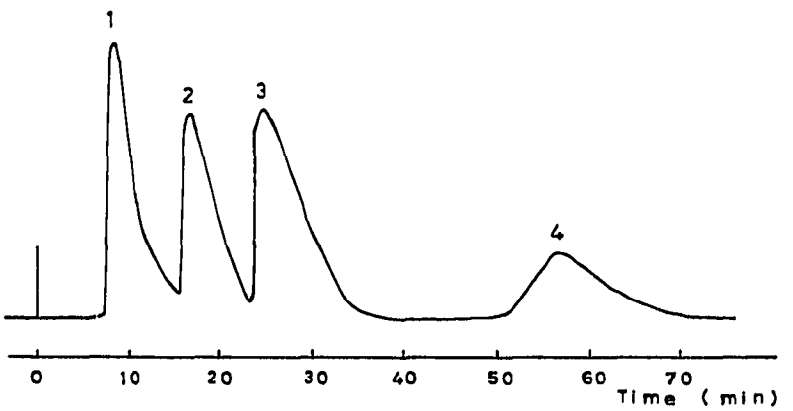


Fig. 3. Separation of ethylamine (1), *n*-propylamine (2), isobutylamine (3) and *n*-butylamine (4). Column conditions as for Fig. 1, but ammonia concentration in carrier gas 15.5 μmoles per ml.

isomers, it is evident that branched-chain amines are always eluted before straight-chain ones; in all instances, complete elimination of peak tailing was difficult, as the separation is based on a chemical reaction.

The present study has thus demonstrated the possibility of ligand-exchange gas chromatography as an analytical tool, and attempts are now being made to apply this technique to the separation of homologues or isomers: it is expected that the technique will be selective enough to permit separations not attainable by partition or adsorption chromatography.

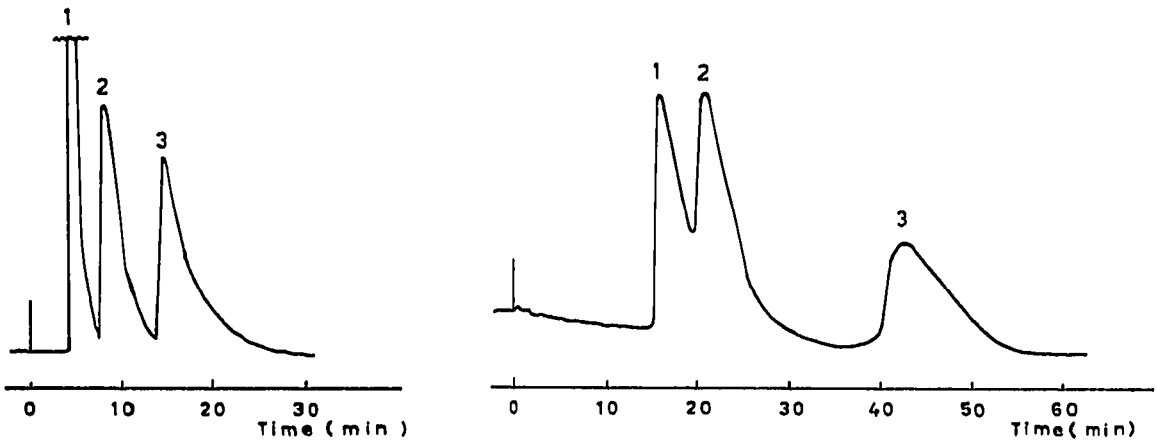


Fig. 4. Separation of di-isopropylamine (1), isopropylamine (2) and *n*-propylamine (3). Column conditions as for Fig. 1, but ammonia concentration. in carrier gas 18.0 μmoles per ml.

Fig. 5. Separation of *sec.*-butylamine (1), isobutylamine (2), and *n*-butylamine (3). Column conditions as for Fig. 1, but ammonia concentration in carrier gas 18.2 μmoles per ml.

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