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LIGAND-EXCHANGE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF ALIPHATIC AMINES

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SUMMARY

The separation and retention behaviour of some aliphatic amines in ligand-exchange liquid chromatography has been studied using copper(II)-modified bonded silica gels as the stationary phase. Hexane containing ammonia and methanol as the mobile phase showed good efficiency for separating sample amines. Ammonia has been found to be very effective for improving both the separation and the peak shape. Sample selectivity and the effect of additives in the mobile phase are discussed.

INTRODUCTION

Ligand-exchange chromatography (LEC) has become one of the most popular techniques in high-performance liquid chromatography (HPLC). The advantage of LEC is that sample selectivity observed in LEC is quite different from that observed in conventional partition or adsorption chromatography: retention of samples in LEC is based on the formation of metal-ligand complexes. To date, homologues or isomers of nitrogen, oxygen, and sulphur compounds have successfully been separated by LEC^{1-2,6}. However, since LEC has generally been directed towards the practical use and application, the fundamental investigation of LEC is still insufficient even for basic compounds such as aliphatic amines.

Although several techniques have been proposed for HPLC separation or determination of aliphatic amines^{2,7-3,5}, most of them need derivatization or other additional treatment of samples.

In the present study, an LEC system similar to the one that gave satisfactory results for the separation of dialkyl sulphides^{3,6} was applied to separate aliphatic amines by LEC without pretreatment of samples and, at the same time, to obtain their basic retention characteristics.

There are three important factors in LEC: (1) the mobile phase and the competitive ligand involved; (2) the ligand species in the stationary phase; (3) the metal ion species used in the stationary phase. Usually, aqueous or hydro-organic mobile phases are used in LEC, but these require the addition of metal ion or metal-ligand

complexes to avoid column instability, which causes contamination of effluents and complicates the retention mechanisms. Preliminary study showed that only weak retention and poor selectivity were obtained for aliphatic amines by the use of an aqueous copper(II) solution as the mobile phase and many types of chemically bonded silica gel as the stationary phase. To obtain the retention characteristics of free, not ionized, amines and to simplify the mechanisms, non-aqueous solvents were exclusively used as the mobile phase.

The type of stationary phase ligand is also of importance; bonded ligand must bind the metal ions tightly so that they cannot leak from the column. Because earlier studies^{5,19,23,37} had shown the effectiveness of bonded ethylenediamine (N2) or iminodiacetate (IDA) for the separation of aromatic amines, these two types of stationary phase were employed. Bonded diethylenetriamine (N3) phase was also prepared to compare the retention characteristics and stability.

Metal ion species used in the stationary phase should be selected based on the affinity with sample molecules. Since the copper(II) ion is known to form stable complexes with amines, it was exclusively used in the present study.

EXPERIMENTAL

Liquid chromatograph

The HPLC system consisted of a Waters 6000A solvent delivery pump (Waters Assoc., Milford, MA, U.S.A.), a Rheodyne 7125 loop injector (Rheodyne, Berkeley, CA, U.S.A.) with a 25- μ l sample loop, and a Jasco UVIDEC type II variable-wavelength UV detector (Jasco, Tokyo, Japan). The flow-rate was 1.0 ml/min. UV detection was performed at 225 nm. Retention times were measured at room temperature.

Chemicals and samples

All chemicals were of the highest grade available and purchased from various suppliers. HPLC-grade silica gel used was Cosmosil SSL (Nakarai Chemicals, Kyoto, Japan) of mean diameter 5 μ m, mean pore size 11 nm and mean surface area 330 m²/g. Silylating agents, 3-triethoxysilylpropylamine, N-(3-trimethoxysilylpropyl)ethylenediamine, N-(3-trimethoxysilylpropyl)diethylenetriamine, and hexamethyldisilazane (Tokyo Kasei, Tokyo, Japan) were distilled prior to use. LC-grade hexane and other solvents were used as received. Dry ammonia solution was prepared by introducing ammonia-equilibrated nitrogen gas (7%; Taiyo Sanso, Kanagawa, Japan) into methanol.

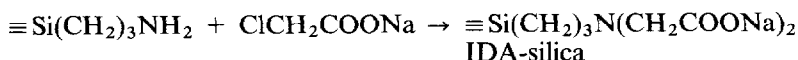
Sample amines were used without further purification. Each sample was diluted with hexane (1% volume to volume), and 1 μ l of the solution was injected. The column dead volume was determined from the retention volume of methanolic potassium nitrite solution.

Preparation of modified silica gels

Aminated silica gels were prepared by standard methods: treatment of silica gel, dried at 140°C *in vacuo*, with a 5% boiling solution of 3-triethoxysilylpropylamine, N-(3-trimethoxysilylpropyl)ethylenediamine, or N-(3-trimethoxysilylpropyl)diethylenetriamine in toluene gave mono-, di- (N2-), or triamine-bonded (N3-) silica, respectively:

silica gel + $(\text{RO})_3\text{Si}(\text{CH}_2)_3(\text{NHCH}_2\text{CH}_2)_n\text{NH}_2 \rightarrow \equiv\text{Si}(\text{CH}_2)_3(\text{NHCH}_2\text{CH}_2)_n\text{NH}_2$
 $n = 0$: monoamine silica ($\text{R} = \text{CH}_3\text{CH}_2-$), $n = 1$: diamine silica ($\text{R} = \text{CH}_3-$; N2-silica), $n = 2$: triamine silica ($\text{R} = \text{CH}_3-$; N3-silica).

IDA-bonded silica was prepared by the treatment of monoamine silica with a 0.1 M sodium monochloroacetate solution (pH 7.5, phosphate buffer) at 60°C. End-capping of silicas was performed by treatment of these silicas with a 5% solution of hexamethyldisilazane in toluene under reflux. Modified silicas were washed and dried *in vacuo*.



Columns

Modified silica gels were packed in stainless-steel columns by the balanced viscosity slurry technique at *ca.* 450 kg/cm². The slurry solvent was methanol–2-propanol–carbon tetrachloride–toluene–cyclohexanol (5:5:18:1.2:10.8, v/v), and the pumping solvent was methanol. Copper(II) ion was loaded on columns by pumping a 0.01 M methanolic solution of copper(II) nitrate, and the remaining metal solution in the column was replaced with hexane before conditioning. Each column was conditioned with mobile phase solvent for 5 h before measurements.

Mobile phase

LC solvents were filtered through a membrane filter before mixing. Pure or mixed solvents were degassed by applying ultrasonication under vacuum before ammonia was introduced. Ammonia-containing solvents were prepared by adding an appropriate volume of ammonia-saturated methanol to degassed hexane or to a readily mixed solvent. The concentration of ammonia was determined titrimetrically; a measured volume of ammonia-containing solvents was added to standard hydrochloric acid, and the excess hydrochloric acid was backtitrated with a standard sodium hydroxide solution.

Determination of copper(II) ion

A study of the uptake of copper(II) was carried out by the method previously described³⁶. Leakage of copper(II) from columns was determined similarly; copper(II) in the effluent, collected during a fixed time, was extracted with 1 N hydrochloric acid and determined photometrically according to the literature method³⁸.

RESULTS AND DISCUSSION

Characterization of bonded phases

The results of CH elemental analyses and copper(II) uptake measurement are summarized in Table I. The values of carbon content were obtained by analysing the phases before endcapping. It is of interest that the values obtained from elemental analyses and from copper(II) uptake are nearly the same for N2- and IDA-silicas, whereas the values for N3-silica are rather different. This means that N2- and IDA-silicas form 1:1 complexes with copper(II) but that N3-silica partially forms polynuclear complexes. This effect is probably due to the differences in the chelating nature of the three bonded phases.

TABLE I
CHARACTERIZATION OF STATIONARY PHASES

<i>Bonded phase</i>	<i>Carbon content (%)</i>	<i>Bonded ligand (10^{-6} mol/m²)</i>	<i>Copper(II) uptake (10^{-6} mol/m²)</i>
N2-silica	9.75	5.89	5.97
IDA-silica	5.72	2.34	2.01
N3-silica	10.99	4.89	7.10

Table I also shows that the multistep synthesis employed for preparing IDA-bonded phase is not suitable for obtaining a high metal capacity²³. N2- or N3-silica stationary phase may be preferable if IDA-silica phase does not offer much higher selectivity or efficiency than they do.

Leakage of copper(II) from copper(II)-modified N2- (N2-Cu) and N3-bonded phase (N3-Cu) was determined to evaluate the column stability. The amounts of copper(II) leaked from columns of 200 × 4 mm I.D. in 1 h were $2.33 \cdot 10^{-7}$ mol and $3.48 \cdot 10^{-8}$ mol for N2-Cu and N3-Cu columns, respectively, when hexane containing 3% methanol and 3.0 mM ammonia was used as the mobile phase at a flow-rate of 1.0 ml/min. Although a larger amount of copper(II) was retained in the N3-Cu column than in the N2-Cu column, as indicated by the data in Table I, leakage of copper(II) from the N3 column was much less, reflecting the difference in stability of bonded-ligand-copper(II) complexes. Accordingly, when the N3-Cu column was used, there was no need to reload copper(II) on the column until *ca.* 20 l of mobile phase had passed through.

Endcapping of silicas has been found to be of importance for improving peak shapes. The effect of endcapping was more marked for primary amines than for secondary or tertiary amines, *i.e.* separation of some primary amines can be achieved only on copper(II)-modified endcapped silicas, whereas separation of secondary or tertiary amines can be obtained on either endcapped or non-endcapped copper(II)-modified silicas.

Effect of type of mobile phase

Sample amines were strongly retained and could not be eluted from copper(II)-modified columns when pure hexane or methanol-containing hexane was used as the mobile phase, even though the samples showed weak retention on copper(II)-unmodified columns with these solvents. At a higher concentration of methanol, some secondary or tertiary amines carrying longer alkyl chains such as di-*n*-octylamine, di-*n*-hexylamine, and tri-*n*-butylamine were eluted from the copper(II)-modified columns at rather long retention times, but all the other amines were not eluted. Addition of 1-butanol, which allows much higher concentration of methanol in hexane, led to the elution of all secondary and tertiary amines examined. Some retention data obtained from the N2-Cu silica stationary phase with various mobile phases are summarized in Table II. Although the hexane-methanol-1-butanol system showed good selectivity for some secondary or tertiary amines, it did not have enough efficiency for copper(II)-modified columns; effective separation could not be achieved owing to broad and tailed peaks.

TABLE II

CAPACITY FACTORS OF SOME ALIPHATIC AMINES ON N₂-Cu COLUMN WITH THREE TYPES OF MOBILE PHASE SYSTEM

Amine	<i>k'</i>	Mobile phase		
		Hexane containing methanol-butanol (10%, 4%)*	Hexane containing methanol-ammonia (4%, 2 mM)*	Acetonitrile containing methanol-ammonia (5%, 3 mM)**
		<i>n</i> -Propylamine	—***	9.71
Isopropylamine	—	5.34	3.05	
<i>n</i> -Butylamine	—	7.54	7.54	
<i>tert.</i> -Butylamine	—	3.55	1.28	
<i>n</i> -Octylamine	—	3.46	6.94	
Di- <i>n</i> -propylamine	13.00	1.36	2.73	
Diisopropylamine	14.22	1.43	1.42	
Di- <i>n</i> -butylamine	9.98	1.18	2.25	
Diisobutylamine	9.55	0.84	1.00	
Di- <i>n</i> -octylamine	2.66	0.45	0.89	

* Column, 200 × 4 mm I.D.; flow-rate, 1.0 ml/min.

** Column, 250 × 4.6 mm I.D.; flow-rate, 0.7 ml/min.

*** — = Not eluted.

The use of ammonia as the competitive ligand in hexane-methanol solvent system has been found to be more effective for improving peak shapes and for controlling retention times, indicating that ammonia is a more suitable ligand for LEC of amines than alcoholic solvents. The effect of ammonia on retention will be discussed later.

Reversed-phase solvents such as acetonitrile or methanol were also examined, but they could not elute the samples from copper(II)-modified columns when no competitive ligand was present. Although methanol and acetonitrile were found to behave as competitive ligands in LEC of sulphides³⁶, they play a less important role in the present system, reflecting their lower basicity than those of amines. Addition of ammonia in acetonitrile-methanol solvent system also gave good selectivity (Table II). It is of interest that the retention order of mono-, di- or tri-*n*-alkylamines was not affected by the change of the type of mobile phase systems, in spite of the expectation that the change in polarity of the mobile phase would change the retention order³⁶. On the contrary, differences in retention between isomers were relatively large compared with those observed in hexane-methanol systems. These findings reveal that the retention order of sample amines is dependent largely on the interaction between copper(II) ion in the stationary phase and the sample amines rather than on the polarity or strength of the mobile phase. The acetonitrile-methanol-ammonia system was not suitable for practical use, since this mobile phase system showed poor efficiency and required a high column inlet pressure owing to its high viscosity. In addition, it could easily elute copper(II) ion in the stationary phase, which led to column instability and to contamination of the effluent.

Sample selectivity

There was no significant difference in sample selectivity among the three bonded phases. Table III shows some retention values obtained on columns of copper-(II)-modified N2-, IDA- (IDA-Cu), and N3-silicas with hexane-methanol-ammonia as the mobile phase. The retention order of *n*-alkylamines followed the decreasing order of their carbon numbers, and a similar order was observed for di-*n*- or tri-*n*-alkylamines. These orders show that the retention of such homologues depends largely on the length of alkyl substituents, *i.e.* steric hindrance around the nitrogen atom of samples. Chromatograms of primary, secondary and tertiary amines are shown in Figs. 1, 2 and 3, respectively.

TABLE III
CAPACITY FACTORS OF AMINES ON THREE TYPES OF COLUMN

The mobile phase was hexane-methanol (97:3).

Amine	<i>k'</i>		
	Column		
	<i>N2</i> -Cu*, <i>NH</i> ₃ concn. 3.0 mM	<i>N3</i> -Cu**, <i>NH</i> ₃ concn. 4.4 mM	IDA-Cu*, <i>NH</i> ₃ concn. 5.5 mM
<i>n</i> -Propylamine	28.39	—***	—
Isopropylamine	26.82	28.70	25.96
<i>n</i> -Butylamine	21.68	25.86	21.43
<i>tert.</i> -Butylamine	19.61	23.91	18.02
<i>n</i> -Octylamine	9.39	13.48	11.64
Di- <i>n</i> -propylamine	3.66	4.88	4.02
Diisopropylamine	4.68	7.36	4.70
Di- <i>n</i> -butylamine	2.08	3.82	2.61
Diisobutylamine	0.82	1.43	1.14
Di- <i>n</i> -octylamine	0.26	0.61	0.59

* 200 × 4 mm I.D.

** 100 × 4 mm I.D.

*** — = Not eluted.

The elution order of isomers such as butyl- or pentylamines does not agree with the above explanation. The elution order did not follow the order of the degree of steric hindrance, probably because branching at the carbon atom that is adjacent to the nitrogen atom of an aliphatic amine increases the basicity of the nitrogen atom. The branching of the alkyl chain has, therefore, two opposing effects: the increased basicity will increase the retention time, and the increased steric hindrance around the nitrogen atom will decrease the retention time. The result of this complex phenomenon is illustrated in Figs. 4 and 5, where separation of isomers of butyl- and pentylamines are shown.

The peak of *tert.*-butylamine or *tert.*-pentylamine is narrow and symmetrical compared with those of the other isomers. This indicates that the branching of the

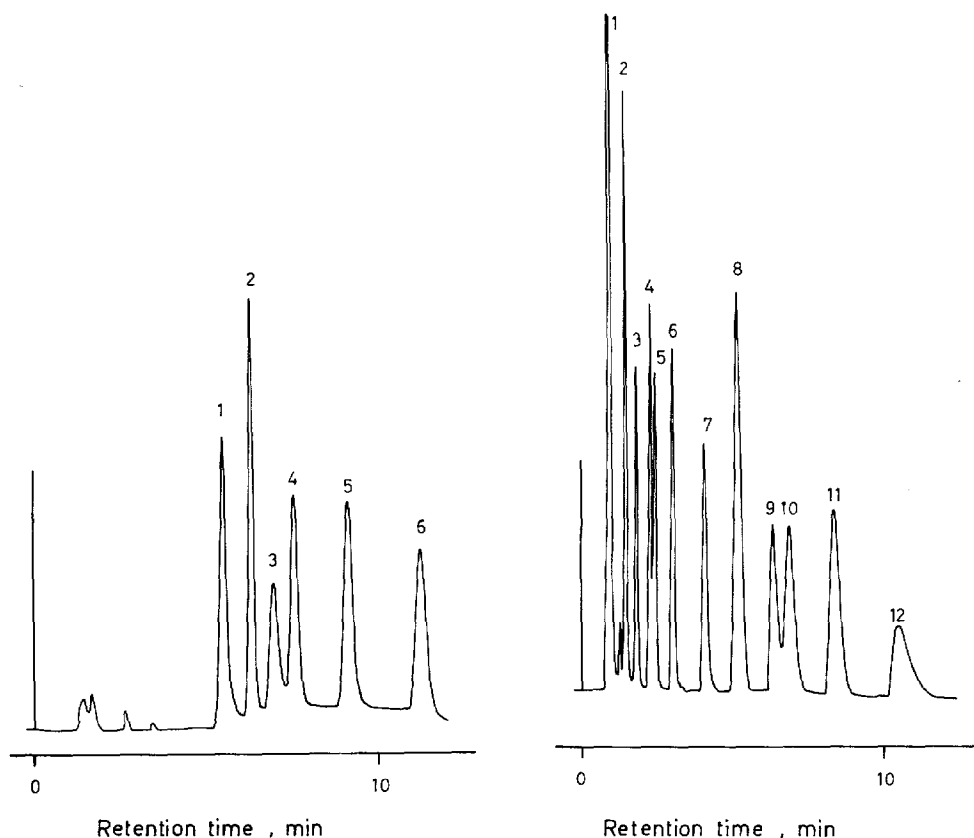


Fig. 1. Separation of *n*-alkylamines. Column, N3-Cu, 100 × 4.0 mm I.D.; two columns in series. Mobile phase, hexane-methanol (97:3) 3.3 mM NH₃; flow-rate, 1.0 ml/min. Samples: 1 = octylamine; 2 = heptylamine; 3 = hexylamine; 4 = pentylamine; 5 = butylamine; 6 = propylamine.

Fig. 2. Separation of secondary amines. Column, N3-Cu, 100 × 4.0 mm I.D.; mobile phase, hexane-methanol (97:3) 3.3 mM NH₃; flow-rate 1.0 ml/min. Samples: 1 = di-*n*-octylamine; 2 = di-*n*-hexylamine; 3 = diisobutylamine; 4 = di-*n*-pentylamine; 5 = diisopentylamine; 6 = di-*n*-butylamine; 7 = di-*n*-propylamine; 8 = diisopropylamine and N-ethyl-*n*-butylamine; 9 = N-ethyl-*n*-propylamine; 10 = N-ethyl-isopropylamine; 11 = N-ethyl-*tert*-butylamine; 12 = diethylamine.

alkyl group also affects the peak shape. The difference in peak width may be attributed to the difference in sorption rate, *i.e.*, the complex containing *tert*-butyl- or *tert*-pentylamine is so labile compared with those containing other isomeric amines, owing to the steric hindrance, that the sorption rate of this amine becomes more rapid.

Effect of mobile phase ligand on retention

Since the hexane-methanol-ammonia system gave good selectivity and efficiency as described above, the discussion below is based on the data obtained by this solvent system. Fig. 6 shows some plots of capacity factor (k') obtained on an N3-Cu column against the reciprocal of the concentration of ammonia in the mobile

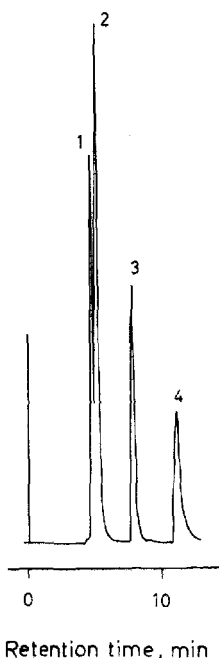


Fig. 3. Separation of tertiary amines. Column, N2-Cu, 250 × 4.6 mm I.D.; mobile phase, hexane-methanol (95:5) 3.0 mM NH₃; flow-rate, 0.7 ml/min. Samples: 1 = tri-*n*-butylamine; 2 = tri-*n*-propylamine; 3 = triethylamine; 4 = trimethylamine.

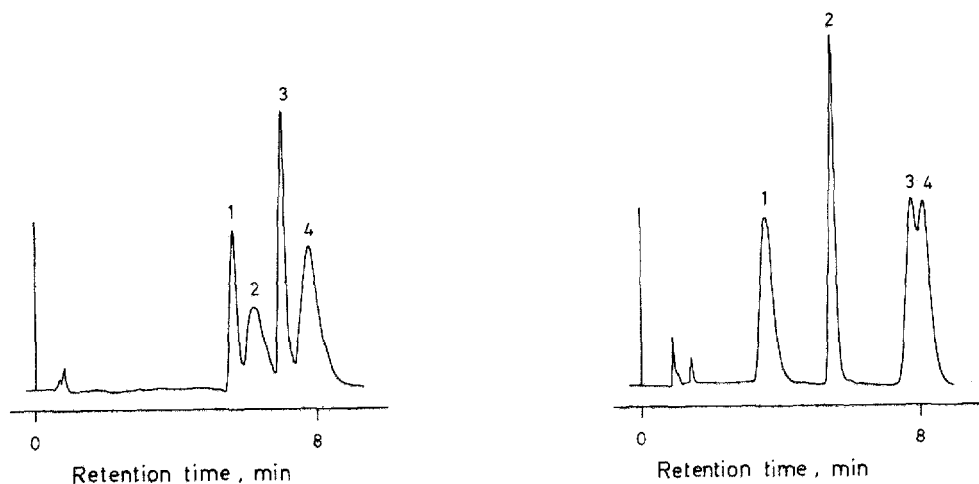


Fig. 4. Separation of butylamine isomers. Column, N3-Cu, 100 × 4.0 mm I.D.; two columns in series. Mobile phase, hexane-methanol (96:4) 3.3 mM NH₃; flow-rate, 1.0 ml/min. Samples: 1 = *sec.*-butylamine; 2 = isobutylamine; 3 = *tert.*-butylamine; 4 = *n*-butylamine.

Fig. 5. Separation of pentylamine isomers. Column, N3-Cu, 100 × 4.0 mm I.D.; mobile phase, hexane-methanol (98.5:1.5) 2.3 mM NH₃; flow-rate, 1.0 ml/min. Samples: 1 = neopentylamine; 2 = *tert.*-pentylamine; 3 = *n*-pentylamine; 4 = isopentylamine.

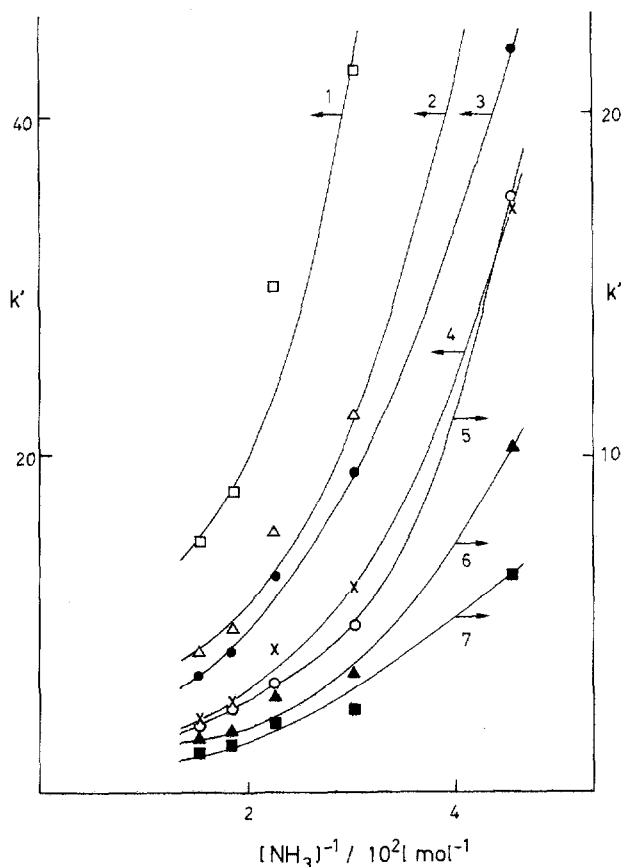


Fig. 6. Plot of k' vs. $[\text{NH}_3]^{-1}$. Column, N3-Cu, 100 × 4.0 mm I.D.; mobile phase; hexane-methanol (97:3); flow-rate, 1.0, ml/min. Samples: 1 = *n*-butylamine; 2 = *n*-heptylamine; 3 = *n*-octylamine; 4 = 1,1,3,3-tetramethylbutylamine; 5 = di-*n*-butylamine; 6 = di-*n*-pentylamine; 7 = di-*n*-hexylamine.

phase. The effect of ammonia on the retention was most marked for primary amines. The retention of tertiary amines was not much influenced by the change of the concentration of ammonia.

As discussed previously³⁶, a linear relationship exists between k' values and the reciprocal of the concentration of the competitive ligand in the mobile phase, if the retention is based on the ligand-exchange sorption mechanism and only one species is employed as competitive ligand. In the present system, however, such plots showed a quadratic dependence of the k' value on the reciprocal of the concentration of ammonia, when the methanol content was kept constant. A similar relationship was observed on both the N2-Cu and the IDA-Cu columns. This fact suggests that methanol also acts as a competitive ligand in a similar manner as was found in the LEC of dialkyl sulphides³⁶, though not so effectively as ammonia. The effect of methanol on the retention is shown in Table IV, indicating that the k' values decrease as the methanol content in the mobile phase increases, if the concentration of ammonia is kept constant. It should be noted that even if the copper ions in the N3-Cu

TABLE IV
EFFECT OF METHANOL CONTENT ON CAPACITY FACTORS

Column, N3-Cu, 100 × 4 mm I.D.; flow-rate, 1.0 ml/min.

Amine	k'			
	Methanol content (%) [*]			
	1.5	2.0	2.5	3.0
<i>n</i> -Propylamine	20.75	19.68	18.45	16.93
Isopropylamine	18.84	18.09	17.16	15.50
<i>n</i> -Butylamine	15.68	15.04	13.80	12.79
<i>tert.</i> -Butylamine	13.66	13.56	12.86	12.14
<i>n</i> -Octylamine	6.95	6.55	5.52	5.23
Di- <i>n</i> -propylamine	2.52	2.50	2.48	2.48
Diisopropylamine	2.75	2.73	2.71	2.70
Di- <i>n</i> -butylamine	1.73	1.71	1.70	1.70
Diisobutylamine	0.84	0.82	0.82	0.82
Di- <i>n</i> -octylamine	0.75	0.68	0.64	0.57

^{*} Volume fraction in hexane containing 2.3 mM NH₃.

stationary phase have partially formed polynuclear complexes with bonded ligands in the present system, the sample amines should be retained on such a stationary phase by the same ligand-exchange reaction as those occurring on a stationary phase containing only mononuclear complexes, because polynuclear complexes must also have bound one or more molecules of exchangeable mobile phase ligand, either ammonia or methanol.

Thus, the non-linear dependence of the k' values on the reciprocal of the concentration of ammonia may be attributed to the effect of methanol, acting as an additional competitive ligand. However, it is also probable that the increase in the solubility of sample amines in the mobile phase with increasing methanol content results in a decrease in the k' values. Obviously, much remains to be done for the satisfactory explanation of the quadratic dependence of k' values.

It has been established experimentally that the presence of ammonia in the mobile phase is indispensable for obtaining both narrow peaks and proper retention times, especially in the case of primary amines. The LEC system described here is expected to be practical for other samples also. The application of an LEC system employing such non-aqueous mobile phases will be presented later.

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