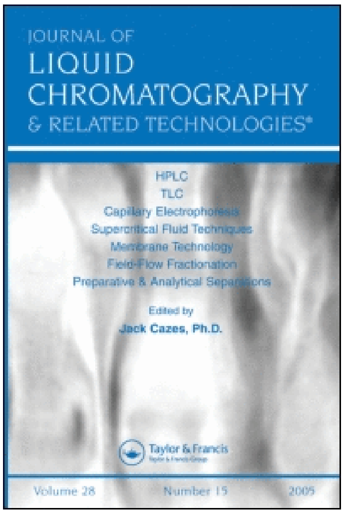

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Liquid Chromatographic Behavior of Nitrogen Compounds

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LIQUID CHROMATOGRAPHIC BEHAVIOR OF NITROGEN COMPOUNDS

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ABSTRACT

Chromatographic behavior of nitrogen compounds differed from others. The column efficiency was poor for the compounds and sometimes solutes were not eluted out from a column. Therefore, the elution volume of alkylamines, anilines, pyridines, pyrazines, quinolines and aminopolyaromatic hydrocarbons was measured on a methacrylate gel and octadecyl bonded silica gels in pH controlled acetonitrile/water mixtures. The solvent effect on the dissociation constant differs from that obtained for aromatic acids. The values in acetonitrile/water mixtures are smaller than those obtained in 100% water. The linear relation between log P and log k' values is obtained in eluents of pH 7 where the retention of these compounds is maximized. Some hydrophobic fragmental constants are proposed from this result. Prediction of retention time of these compounds from their log P values can be done in the individual groups on octadecyl bonded silica gels in pH controlled acetonitrile/water mixtures.

INTRODUCTION

Many nitrogen compounds are suspected as carcinogenic substances due to the adsorption on tissues and the biological activity The analysis of nitrogen compounds has been done with several techniques in different fields [1 - 6]. Gas and liquid chromatographies are very suitable methods to analyze complex mixtures; however, the chromatography of these compounds is not satisfied due to poor column efficiency. Some approaches have been developed to improve the chromatographic analysis.

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Precolumn derivatization has been used for gas and liquid chromatographies [7 - 13]. A ligand exchange method has also been applied to both gas and liquid chromatographies [14,15]. Ion-pair liquid chromatography has often been used to reduce the tailing of peaks [16,17]. Furthermore, the several compounds have been selectively analyzed on a cation exchange resin [18].
In this paper, the elution volume of 66 nitrogen compounds was measured by reversed phase mode liquid chromatography to develop a qualitative analysis method of these compounds. The pH effect of the eluent and the hydrophobicity of the compounds are discussed.

EXPERIMENTAL

The detail of the instrumentation was previously described [19]. The improvement of the precision was done by using an on-line degasser (ERMA model ERC-3310, ERMA Optical Works, Tokyo, Japan) and an integrated recorder (Shimadzu CRA-1, Maryland). Most of the chemicals were supplied from Chem Service (West Chester, Pa) and Aldrich Chemical Co. (Milwaukee, IL). The packings used were a polymethacrylate gel (TSK LS 140 P5) which was kindly given by Dr. T. Hashimoto (Toyo Soda Mfc. Com., Tokyo) and two octadecyl bonded silica gels (Hypersil ODS and Hitachi 3056).

RESULT AND DISCUSSION

The pH effect was measured on the methacrylate gel in 0.01M sodium phosphate buffer with 50% acetonitrile at 30 °C. The elution volumes of several compounds are graphically shown in Fig. 1 (A-F).

These curves differed from those theoretically expected. The maximum retention decreased at high pH. The retention of aliphatic amines was dramatically reduced at higher pH. This may be due to a formation of the salt form of their amines. Therefore, pH where the capacity ratio was a half of sum of maximum and minimum capacity ratios was defined as the pKa. The measured dissociation constants (pKa (II) in Table I) with values less than 7 were related to the reference values (pKa (I)) and the following equation was obtained.

$$pKa\ (II) = a \cdot pKa\ (I) + b$$

The constants a and b were 0.632 and 0.659, respectively. The correlation coefficient was 0.952 (n=19).
The values of pKa (II) measured in 50% acetonitrile were 1.04 unit lower than those in 100% water. This result was the reverse of that obtained for aromatic acids whose pKa values were 1.10 unit higher in 50% acetonitrile than those in 100% water [20]. The pKa (II) higher than 7 had no relation with pKa (I). The pKa of several compounds could not be measured in this system due to the necessity of eluents of very low pH or their very small capacity ratios.
Further study, the discussion of the relation between the logarithm of capacity ratios and the log P values, was done in eluents of pH 8 on octadecyl bonded silica gels. Capacity ratios obtained

Table I Dissociation constant of nitrogen compounds

		pKa(I)	(Temp.°C)*	pKa(II)**				pKa(I)	(Temp.°C)*	pKa(II)**	
1	n-Propylamine	10.708	(20)	-		31	3,4-Dichloroaniline	-		3.05	
2	Isobutylamine	-		-		32	4-Bromoaniline	3.58	(25)	2.96	
3	n-Butylamine	10.77	(20)	6.47	10.65	33	2-Nitroaniline	-		2.94	
4	n-Hexylamine	-		6.63	11.03	34	3-Nitroaniline	2.466	(25)	-	
5	Cyclohexylamine	10.66	(24)	6.53	10.38	35	4-Nitroaniline	1.0	(25)	-	
6	Diallylamine	-		6.11	8.48	36	Pyridine	5.25	(25)	3.90	
7	Diisopropylamine	10.96	(28.5)	6.45	10.15	37	2-Aminopyridine	6.82	(20)	-	
8	Dibutylamine	-		6.50	10.37	38	4-Aminopyridine	9.114	(25)	6.03	8.89
9	Diisobutylamine	10.91	(21)	6.40	9.80	39	2-Methylpyridiene	5.97	(20)	-	
10	Dicyclohexylamine	-		6.23	10.75	40	3-Methylpyridine	5.68	(20)	-	
11	Triethanolamine	-		5.95	7.29	41	4-Methylpyridine	6.02	(20)	4.19	
12	Triethylamine	-		6.29	10.15	42	4-tert. Butylpyridine	-		4.29	
13	Tributylamine	11.01	(18)	6.70	9.72	43	2,4-Dimethylpyridine	6.99	(25)	-	
14	Benzylamine	9.33	(25)	6.11	9.11	44	Pyrazine	0.65	(27)	-	
15	Dibenzylamine	-		5.81	8.23	45	2-Methylpyrazine	1.45	(27)	-	
16	Aniline	4.63	(25)	3.66		46	Quinoline	4.90	(20)	3.81	
17	N-Methylaniline	-		3.75		47	8-Hydroxyquinoline	5.017 8.812	(20) (25)	4.11	11.12
18	N-Ethylaniline	5.12	(24)	3.92		48	2-Methylquinoline	5.83	(20)	4.21	
19	N-Butylaniline	-		3.78		49	4-Methylquinoline	5.67	(20)	4.05	
20	N,N-Dimethylaniline	5.15	(25)	3.84		50	8-Methylquinoline	-		3.71	
21	N,N-Diethylaniline	6.61	(22)	5.01		51	5-Aminoindan	-		4.09	
22	2-Methylaniline	4.44	(25)	3.45		52	1-Aminoindan	9.21	(22.5)	6.14	9.40
23	3-Methylaniline	4.73	(25)	3.68		53	5-Aminoindole	-		4.98	
24	4-Methylaniline	5.08	(25)	4.01		54	1-Aminonaphthalene	3.92	(25)	3.01	
25	2,4-Dimethylaniline	-		3.82		55	2-Aminonaphthalene	4.16	(25)	3.27	
26	4-Methoxyaniline	5.34	(25)	4.18		56	1-Aminoanthracene	-		2.95	
27	2,4-Diethoxyaniline	-		3.00		57	1-Aminopyrene	-		2.90	
28	2-Chloroaniline	2.65	(25)	-							
29	3-Chloroaniline	3.46	(25)	2.73							
30	4-Chloroaniline	4.15	(25)	3.04							

*1 obtained from Handbook of Chemistry and Physics, 61st edition, CRC Press (1980). ** These values were grafically obtained based on the result of pH effect measured on the polymethacrylate gel in 0.01M sodium phosphate buffer with 50% acetonitrile at 30°C.

Table II log P and capacity ratios of nitrogen compounds

				k'(I)*4 (% acetonitrile)							k'(II)*5		k'(III)*6
	logP(I)*1	logP(II)*2	logP(III)*3	70	60	50	40	30	20	10	50	30	50
Isopropylamine	0.21	-		-	-	-	-	-	-	-	-	-	-
n-Propylamine	0.33	-	0.48	-	-	-	-	-	-	-	-	-	-
Isobutylamine	0.74	-	0.73, 0.88	-	-	-	-	-	-	-	-	-	-
n-Butylamine	0.86	-	0.68-1.02	-	-	-	-	-	-	-	6.25	-	2.27
n-Hexylamine	1.92	-	1.98-2.15	-	-	-	-	-	-	-	11.38	-	3.42
Cyclohexylamine	1.46	-	1.49	-	-	-	-	-	-	-	9.36	-	2.83
Hexadecylamine	7.22	-		-	-	-	-	-	-	-	-	-	-
Diallylamine	1.10	-	1.11	-	-	-	-	-	-	-	-	-	2.17
Diisopropylamine	1.45	-	1.46-1.73	-	-	-	-	-	-	-	7.41	-	2.20
Dibutylamine	2.76	-	2.68, 2.83	-	-	-	-	-	-	-	12.48	-	3.90
Diisobutylamine	2.51	-		-	-	-	-	-	-	-	12.70	-	3.28
Dicyclohexylamine	3.94	-		-	-	-	-	-	-	-	20.01	-	1.66
Triethanolamine	-3.45	-		-	-	-	-	-	-	-	0.55	-	0.53
Triethylamine	1.54	-	1.15-1.45	-	-	-	-	-	-	-	10.28	-	2.00
Tributylamine	4.72	-		-	-	-	-	-	-	-	-	-	6.59
Benzylamine	0.99	-	1.09	-	-	-	-	-	-	-	8.91	-	3.93
Dibenzylamine	3.01	-		-	-	-	-	-	-	-	20.24	-	7.54
Aniline	1.03	1.06	0.85-0.98	0.53	0.67	0.91	1.35	2.09	3.51	6.98	1.35	3.37	1.42
N-Methylaniline	1.62	1.92	1.66, 1.82	0.87	1.23	1.91	3.29	6.04	11.74	-	2.98	10.15	2.17
N-Ethylaniline	2.15	2.40	2.26	1.15	1.71	2.90	5.46	11.22	24.56	-	4.61	19.41	2.65
N-Butylaniline	3.21	3.78	3.58	2.15	3.75	7.72	19.06	-	-	-	12.83	-	4.66
N,N-Dimethylaniline	2.28	2.82	2.31	1.50	2.26	3.89	7.53	16.29	-	-	6.45	28.34	2.63
N,N-Diethylaniline	3.34	4.08	2.29-3.31	2.71	4.71	9.71	24.13	-	-	-	16.69	-	4.10
2-Methylaniline	1.54	1.50	1.29-1.40	0.67	0.89	1.31	2.09	3.67	7.25	16.96	19.70	5.84	1.61
3-Methylaniline	1.54	1.51	1.40, 1.43	0.70	0.87	1.30	2.12	3.85	7.82	18.79	2.04	6.03	1.68
4-Methylaniline	1.54	1.49	1.39, 1.41	0.67	0.87	1.27	2.07	3.77	7.69	18.79	1.94	5.92	1.51
2,4-Dimethylaniline	1.98	1.94		0.86	1.18	1.86	3.25	6.61	15.79	-	2.89	10.77	1.76
4-Methoxyaniline	1.10	0.76	0.78, 0.95	0.44	0.49	0.69	0.99	1.59	2.95	7.06	0.98	2.34	0.99
2,4-Diethoxyaniline	2.17	2.19		0.92	1.32	2.22	4.24	9.61	27.19	-	3.34	15.20	1.67
2-Chloroaniline	1.76	1.95	1.90, 1.92	0.84	1.18	1.90	3.41	6.76	14.32	-	2.89	10.84	2.98
3-Chloroaniline	1.76	1.87	1.88, 1.90	0.76	1.06	1.73	3.15	6.50	14.79	-	2.57	10.23	3.06
4-Chloroaniline	1.76	1.79	1.83	0.74	1.01	1.60	2.84	5.82	13.33	-	2.39	9.23	2.71
2,5-Dichloroaniline	2.42	2.77		1.25	1.94	3.57	7.67	19.52	-	-	5.67	32.88	5.78
3,4-Dichloroaniline	2.42	2.45	2.69	1.01	1.52	2.72	5.69	14.55	-	-	4.19	23.62	4.72

4-Bromoaniline	1.96	1.96	2.05, 2.26	0.80	1.13	1.83	3.40	7.29	17.82	-	2.81	11.64	3.22
2-Nitroaniline	1.87	1.66	1.44-1.83	0.67	0.91	1.41	2.56	5.09	11.22	27.94	2.80	7.72	2.65
3-Nitroaniline	1.22	1.45	1.37	0.58	0.77	1.22	2.10	3.98	8.07	17.54	1.74	5.94	2.62
4-Nitroaniline	1.22	1.24	1.39	0.48	0.64	0.99	1.69	3.17	6.53	14.80	1.36	5.01	2.42
Pyridine	0.70	0.63	0.62-0.78	0.61	0.64	0.77	1.01	1.46	2.45	5.64	1.32	2.70	0.44
2-Aminopyridine	-0.33	-0.45	0.49, 0.58	0.28	0.22	0.24	0.28	0.38	0.59	1.28	0.35	0.65	0.27
3-Aminopyridine	-0.33	-0.12	0.11, 0.20	0.32	0.31	0.35	0.44	0.61	1.02	2.40	0.54	0.72	0.38
4-Aminopyridine	-0.33	-	0.26, 0.28	-	-	-	-	-	-	-	0.58	2.03	2.36
2-Methylpyridine	1.06	1.09	1.11	0.78	0.88	1.11	1.57	2.55	5.05	15.77	2.01	5.20	0.51
3-Methylpyridine	1.06	1.26	1.20, 1.24	0.86	0.98	1.31	1.84	3.17	6.34	18.92	2.35	7.07	0.50
4-Methylpyridine	1.06	1.25	1.22, 1.33	0.86	0.99	1.28	1.84	3.04	6.33	19.40	2.30	6.31	0.52
4-Ethylpyridine	1.59	1.82		1.12	1.40	1.98	3.15	6.04	15.44	-	3.51	12.52	0.70
4-tert.Butylpyridine	2.65	2.76		1.70	2.41	3.80	7.17	17.95	-	-	6.77	36.80	1.00
2,4-Dimethylpyridine	-	1.81		1.06	1.42	1.92	3.01	5.73	-	-	3.30	11.52	0.62
2,5-Dimethylpyridine	-	1.67		1.03	1.29	1.76	2.75	5.08	12.29	-	3.25	10.91	0.61
2,6-Dimethylpyridine	-	1.46	1.68	0.94	1.09	1.49	2.27	4.05	9.30	34.48	2.88	8.71	0.55
Pyrazine	-	-0.20	0.23	0.36	0.32	0.34	0.38	0.48	0.69	1.29	0.48	0.72	0.29
2-Methylpyrazine	-	0.08		0.44	0.41	0.45	0.53	0.73	1.16	2.75	0.68	1.23	0.31
2,5-Dimethylpyrazine	-	0.38		0.53	0.52	0.60	0.74	1.06	1.94	5.86	0.95	1.93	0.31
2,6-Dimethylpyrazine	-	0.36		0.52	0.52	0.52	0.72	1.03	1.86	5.51	0.89	1.62	0.30
Quinoline	1.98	1.82	2.02-2.06	0.93	1.14	1.64	2.68	5.40	14.58	-	2.72	9.49	1.14
8-Hydroxyquinoline	-	-	1.94-2.02	-	-	-	-	-	-	-	-	-	2.25
2-Methylquinoline	2.51	2.13	2.59	1.07	1.40	2.09	3.65	8.02	24.26	-	3.68	14.76	1.19
4-Methylquinoline	2.51	2.29		1.26	1.63	2.47	4.39	9.94	24.36	-	4.39	18.57	1.23
8-Methylquinoline	2.51	2.41	2.60	1.29	1.75	2.73	4.92	11.17	32.10	-	4.62	20.76	1.76
5-Aminoindan	2.27	2.12		0.95	1.31	2.11	3.86	8.46	22.16	-	3.28	13.84	2.27
1-Aminoindan	2.27	-		-	-	-	-	-	-	-	1.38	19.45	5.79
5-Aminoindole	1.36	0.69		0.36	0.38	0.54	0.78	1.26	2.32	5.34	0.73	1.80	1.37
1-Aminonaphthalene	2.18	2.12	2.22, 2.25	0.86	1.24	2.09	3.99	9.06	24.48	-	3.12	14.52	3.78
2-Aminonaphthalene	2.18	2.12	2.28	0.85	1.21	2.05	3.98	9.36	26.14	-	3.10	14.84	3.78
1-Aminoanthracene	3.35	3.25		1.54	2.53	5.05	12.52	42.18	-	-	8.23	56.34	8.72
1-Aminopyrene	4.00	3.69		2.01	3.41	7.11	18.96	-	-	-	12.12	73.78	12.65

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*1: log P(I) after Rekker from ref. 24; *2: log P(II) experimentally obtained from the result in 20-60% acetonitrile; *3: log P(III) from ref. 25; *4: experimental condition, column 15 cm x 4.6 mm i.d. packed with Hypersil ODS; eluent, 0.0025M sodium phosphate in 10-70% acetonitrile; column temperature, 30°C; void volume, 1.26 mL; *5: experimental condition, column 15 cm x 4.1 mm i.d. packed with Hitachi 3056; eluent, 0.01M sodium phosphate buffer in 50% acetonitrile at pH 8.0 (void volume, 0.86 mL) and 0.01M sodium phosphate buffer in 30% acetonitrile at pH 7.4 (void volume, 0.92 mL);column temperature, 30°C; *6: experimental condition, column, 25 cm x 4.1 mm i.d. packed with TSK LS 140P5; eluent,0.01M sodium phosphate buffer in 50% acetonitrile at pH 7.7;void volume, 2.0 mL;column temperature,30°C.

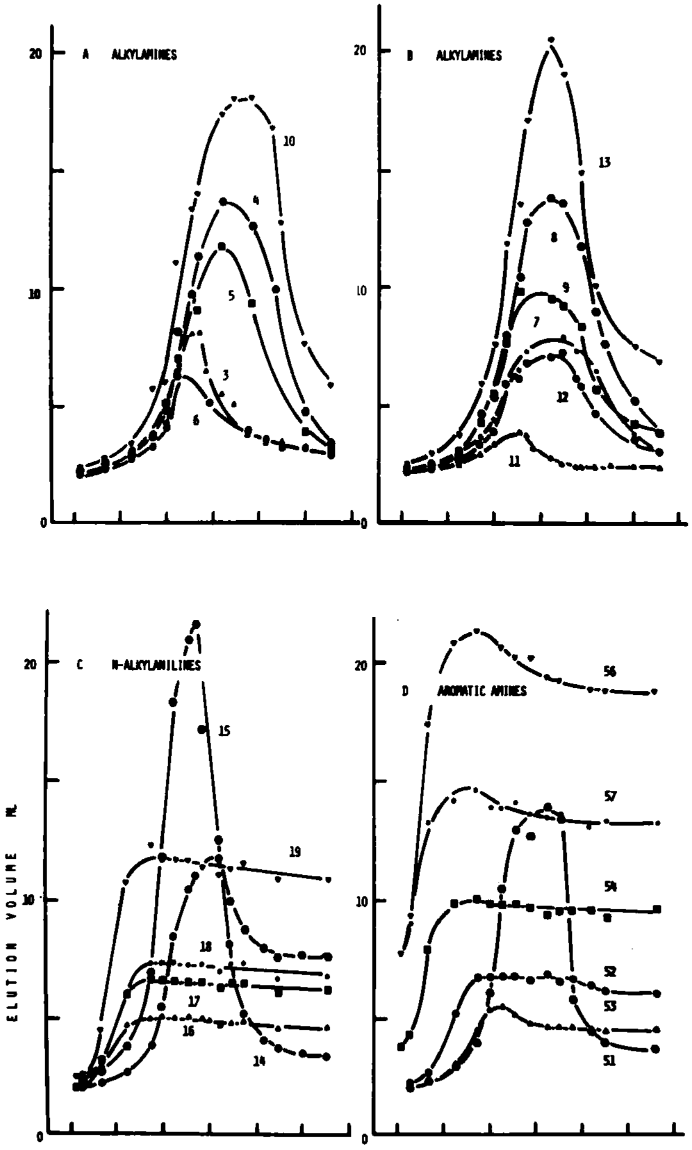

Fig. 1 (A-F) Elution volume of nitrogen compounds on a methacrylate gel

Experimental condition: column, 25 cm x 4.1 mm i.d., packed with TSK LS140 P5; eluent, 0.01M sodium phosphate buffer with 50% acetonitrile; column temperature, 30°C. Numbers beside curves are indicated in Table I. ⓐ not indicated by No. is 2,6-dimethylpyridine and the scale of 1-aminopyrene is a half.

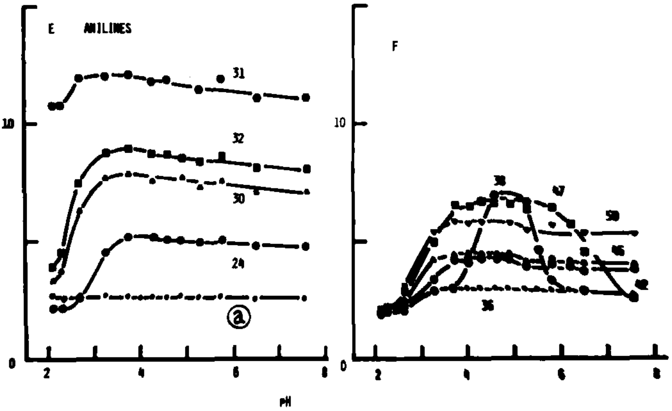

Fig. 1 (continued)

on Hypersil ODS in 10 to 70% acetonitrile with 0.0025M sodium phosphate dibasic are listed in Table I.

The values of log P (II) were obtained from the result of aniline, 3-methoxyaniline, 2,4-dimethylaniline, 2,4-diethoxyaniline, 3,4-dichloroaniline and 4-bromoaniline as the standard compounds are also listed in Table II.

Some hydrophobic fragmental constants not listed in Rekker's system are estimated from the above result. The proposed log P value for pyridinyl is 0.552 and that for disubstituted pyridine is 0.270. The log P for pyrazines is derived from the comparison of the result of pyridines and pyrazines. The log P values for mono- and di-substituted pyrazines are -0.455 and -1.026 respectively.

Prediction of retention volume of molecular form anilines (n = 19) in 35 and 55% acetonitrile/water with 0.0025M sodium phosphate dibasic was tried from log P (I) and log P (II) values. The equations used for the calculation were:

$$\log k' = y.\log P + m \qquad (1)$$

$$y = \sum_{i=0}^{i=n} C_i X_i \qquad (2)$$

The slope y of the equation (1) was derived from the equation (2) and i = 4. The slopes obtained in 20, 30, 40, 50 and 60% acetonitrile by using capacity ratios of the standard compounds were used to obtain the constant of equation (2) [21].

The mean error between predicted and observed capacity ratios was 15% in 35% acetonitrile from log P (I) values. Use of log P(II) values reduced the error to 7.0%. The error in 55% acetonitrile was 14 and 4.9% from log P(I) and log P(II) values, respectively. The first approximation of retention volume of anilines in a given eluent can be done from log P but the error was larger than that of nonionic compounds [21] and phenols [19]. Furthermore, the

solvation for nitrogen containing aromatic rings differed from benzene rings due to the difference of the resonance; that is, the prediction of the retention time of anilines, pyridines, pyrazines and quinolines altogether was very difficult in this system. The retention volume was also measured on another octadecyl bonded silica gel (Hitachi 3056), and the capacity ratios are listed in Table I. The specific selectivity of the two packings was not found in the result in 50% acetonitrile of pH 8.
The capacity of Hitachi 3056 was 1.56 times higher than that of Hypersil ODS due to the difference of surface area. The pore diameter of Hitachi 3056 was 60 A and that of Hypersil was 110 A. The correlation coefficient of the capacity ratios on these packings was 0.9994 (n=45). The compounds having more than 10% difference in capacity ratio were 2-aminopyridine, 3-aminopyridine and 5-aminoindole. These compounds were weakly retained on Hitachi 3056 that may be well silanized.
Methacrylate gel was a selective packing for aromatic compounds due to the pi-pi interaction [22]. This tendency was also found for nitrogen compounds. N-alkylanilines, pyridine pyradines and quinolines were more retained on Hypersil ODS and other anilines were more done on the methacrylate gel. The correlation coefficient of the capacity ratios between on Hypersil ODS and on TSK LS 140 was 0.67 (n = 45).
The void volume, defined as the elution volume of non-retained compounds, was carefully measured in individual eluents. The elution volume of fructose, sodium nitrite, deuterium oxide, methanol and the components of the eluents were not constant. Therefore, the correlation between log P and log k' values of the standard compounds (aniline, 3-methylaniline, 2,4-dimethylaniline, 2,4-dimethylaniline, 2,4-diethoxyaniline, 3,4-dichloroaniline and 4-bromoaniline) was maximized by using authentic void volume. The maximum coefficient was 0.9940 in 70% - 0.9998 in 20% acetonitrile in water and the proposed void volume was changed from 0.40 mL in 70% acetonitrile to 3.25 mL in 20% acetonitrile. The coefficient obtained from the minimum elution volume of fructose (1.26 mL) was 0.9929 - 0.9988 in 70 - 20% acetonitrile. These values were similar to those obtained from the elution volume of fructose measured in the individual eluents. Elution volume of sodium nitrite showed a similar tendency to that of fructose but the coefficient was poor.
The similar approach was tested for other compounds together in 50% acetonitrile to study the utility of log P values. The maximum correlation coefficient (r = 0.9875, n = 45) was obtained from the void volume of 1.18 mL. The r was 0.9871 (n = 45) from the elution volume of fructose. For only anilines (n = 27), the maximum r was 0.9989 from void volume 0.96 mL and the r was 0.9977 from the elution volume of fructose. The slight change of the void volume did not make a significant difference in the correlation coefficient as observed for aromatic acids [23]. The elution volume of components of the eluents was not suitable to use as a void volume. The minimum elution volume of fructose was, therefore, selected as the void volume of this system.

CONCLUSION

Nitrogen compounds, especially aliphatic amines, may be directly adsorbed on octadecyl bonded silica gels because the peaks have tailing. Their chromatographic behavior with pH variation is not simple. These compounds are ionized at low pH and have the lowest capacity ratios, then take the maximum capacity ratios at pH 7 - 8 for their molecular form. That retention is, however, weakened at higher pH and may be due to the formation of their salt forms. The behavior of N-alkylanilines is similar to aliphatic amines. 4-Aminoaniline and 4-hydroxyquinoline may be irreversibly adsorbed on the octadecyl silica gels because the elution is not observed under the condition tried.

The prediction of the retention time from log P values has not been successfully done for nitrogen compounds altogether. Anilines, pyridines, pyrazines and quinolines show their individual chromatographic behavior due to the differences of the resonance of the heterocyclic rings. Estimation of retention volume in a given eluent can be done from the log P and the pKa values, but the standard compounds have to be carefully selected to characterize an octadecyl bonded silica gel column. The separation of these compounds is also possible on organic polymer gels in high pH eluent like separation of alkaloids.

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