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## SEPARATION OF AROMATIC NITROGEN COMPOUNDS INTO COMPOUND-CLASS TYPES USING NORMAL-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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### SUMMARY

Normal-phase high-performance liquid chromatography (HPLC) was used for the separation of aromatic nitrogen model compounds known to be present in solvent refined coal liquids. Three commercially available stationary phases, silica, cyanosilica, and amino-silica, were investigated with model compounds and various *n*-heptane-2-propanol mobile phases. Only the amino-silica stationary phase with *n*-heptane-2-propanol (85:15) as mobile phase was able to separate the aromatic nitrogen containing model compounds into three main compound-class groups.

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### INTRODUCTION

There are many reasons for the isolation and characterization of nitrogen compounds in fossil fuel and environmental samples. Several of these samples contain very toxic, carcinogenic and mutagenic compounds<sup>1-12</sup>. It is necessary to identify and to quantify these compounds to help in the assessment of the health hazards involved in working with coal-derived liquids and any possible effects they may have on the environment<sup>2</sup>. It has been reported that certain heteroatom species decrease the efficiency of catalytic processes and contribute to the instability of stored fuels<sup>5</sup>. The types and amounts of specific compound-classes in coal-derived samples are important so the appropriate engineering conditions for optimum coal conversion can be achieved<sup>1</sup>.

Recently, special attention has been shown towards nitrogen heterocycles in coal-derived liquids because of their carcinogenic activity<sup>6-9</sup>. It has been reported that the nitrogen content in coal-derived oils is a great deal higher than in petroleum samples<sup>9,10</sup>.

The separation of petroleum and synthetic fuels into compound-classes has been reported by several researchers<sup>2,13-16</sup>. Additionally, specific group type separations for the isolation of nitrogen containing aromatic compounds has also been reported<sup>4,6,7,9,15,17-20</sup>. Techniques for the further separation of aromatic nitrogen containing compounds into group or class types has also been discussed<sup>20-23</sup>. Amateis and Taylor<sup>24</sup> have used Fourier transform infrared spectroscopy coupled with high-

performance liquid chromatography (HPLC) to identify basic nitrogen containing compounds in coal-derived samples.

Burchill *et al.*<sup>20</sup> successfully separated the nitrogen compounds present in an anthracene oil into two classes, neutral and basic, by using an OPN/Porasil C chromatographic system. With this method, three mobile phases were used to effect a separation of the anthracene oil into a hydrocarbon class fraction, a neutral nitrogen compound-class fraction, and a basic nitrogen compound-class fraction. Further separation of the two nitrogen compound-classes was accomplished with gas chromatography-mass spectrometry.

Colin *et al.*<sup>21</sup> reported that the separation of azaarenes with a LiChrosorb NH<sub>2</sub> column and mobile phases consisting of *n*-heptane-chloroform was on the basis of shielded and free electron pairs of the nitrogen atom. Those azaarenes with a shielded electron pair on the nitrogen eluted much more rapidly than those azaarenes with a free or unshielded electron pair on the nitrogen atom. In later work, Schmitter *et al.*<sup>25</sup> identified triaromatic nitrogen compounds and determined the location of the nitrogen atom in petroleum triaromatic azaarenes with ultraviolet spectroscopy and reversed-phase HPLC.

Green and co-workers<sup>22,23</sup> used plain silica and added carboxylic acid and amine modifiers to the mobile phases for the separation of acidic and basic components present in coal-derived liquids. They were able to effect a group type separation based on the acid-base properties of the solutes. With the addition of a carboxylic acid to the mobile phase, the retention of basic type materials was greatly enhanced and acidic type materials eluted somewhat more quickly. It was found for amine containing mobile phases that the best separation occurred with samples that were already prefractionated.

Reported here is a method for the separation of aromatic nitrogen containing model compounds into three main compound-class types. The compound-class types are basic, non-basic, and amino types. The separation into compound-class types is relatively rapid and simple compared to earlier methods. The approach involves the use of one normal-phase chromatographic system in the isocratic mode. The method developed for model compounds does not require prefractionation of the model compounds.

## EXPERIMENTAL

### *High-performance liquid chromatograph*

The liquid chromatograph used was a Waters Model ALC/GPC 244 equipped with a Model 6000A pump, a UK6 injector, a Model 480 variable-wavelength ultraviolet detector set at 254 nm, and a 10-mV strip chart recorder.

### *Columns*

The columns used were 30 cm × 3.9 mm I.D., prepacked and obtained from Waters Assoc. (Milford, MA, U.S.A.). The column packings were  $\mu$ Bondapak NH<sub>2</sub>,  $\mu$ Bondapak CN (normal phase), and  $\mu$ Porasil. The  $\mu$ Bondapak NH<sub>2</sub> and  $\mu$ Bondapak CN columns consisted of propylamine groups and cyanopropyl groups, respectively, chemically bonded to Waters  $\mu$ Porasil (10  $\mu$ m porous silica). The  $\mu$ Porasil column was packed with Waters  $\mu$ Porasil (10  $\mu$ m porous silica).

### Reagents

MCB-brand HPLC-grade *n*-heptane was obtained from VWR Scientific, (Denver, CO, U.S.A.) and HPLC-grade 2-propanol was obtained from Fisher (Fair Lawn, NJ, U.S.A.). These solvents were prefiltered through a Millipore Type FH 0.5- $\mu$ m filter. The aromatic nitrogen containing standards were obtained from commercially available sources and were purified when necessary.

### Chromatographic systems

(i) Normal phase,  $\mu$ Bondapak NH<sub>2</sub> with *n*-heptane–2-propanol (85:15) as mobile phase at a flow-rate of 1 ml/min. (ii) Normal phase,  $\mu$ Bondapak CN with *n*-heptane–2-propanol (85:15) as mobile phase at a flow-rate of 1 ml/min. (iii) Normal phase,  $\mu$ Porasil with *n*-heptane–2-propanol (95:5) as mobile phase at a flow-rate of 1 ml/min.

### Aromatic nitrogen containing standards

Solutions of 1 mg/ml of the aromatic nitrogen containing standards were prepared in methanol. The retention volumes of the aromatic nitrogen containing standards were determined by injecting 5  $\mu$ l of the standard solutions onto the chromatographic systems listed above. The capacity factor ( $k'$ ) was calculated by  $k' = (V_R - V_m)/V_m$ , where  $V_R$  (ml) is the measured retention volume and  $V_m$  (ml) is the column void volume. The values of  $V_m$  for the above chromatographic systems (i–iii) were obtained by injecting toluene. The  $V_m$  values were: (i) 2.95 ml, (ii) 3.08 ml, and (iii) 3.05 ml, respectively.

## RESULTS AND DISCUSSION

### Chromatographic data

In the investigation of retention characteristics of 31 model aromatic nitrogen compounds, it was discovered that the nitrogen compounds could be separated into three major compound-classes through the use of a normal-phase HPLC system. With an amino-silica column and a *n*-heptane–2-propanol mobile phase, the aromatic nitrogen containing compounds could be separated into a basic class (*e.g.*, acridine types), a non-basic class (*e.g.*, carbazole types), and an amino class (*e.g.*, 1-aminoanthracene types).

Table I presents the retention data for the aromatic nitrogen containing model compounds obtained from each of the normal-phase chromatographic systems studied. Additional retention data were collected for the 31 model compounds using *n*-heptane–2-propanol mobile phases ranging in composition from 99:1 to 60:40 with the three stationary phases. Only those mobile phases which provided the most useful separations are presented. A wide variety of aromatic nitrogen containing compounds was chosen to approximate the types of compounds present in fossil fuels and environmental samples. Table II gives the structures of the model compounds used in this investigation. Many studies of aromatic nitrogen containing compounds to date have focused on one particular class of nitrogen compounds<sup>19,21</sup>. As mentioned earlier, this work focused on the separation of the aromatic nitrogen containing compounds into major compound-classes using normal-phase chromatographic systems.

TABLE I

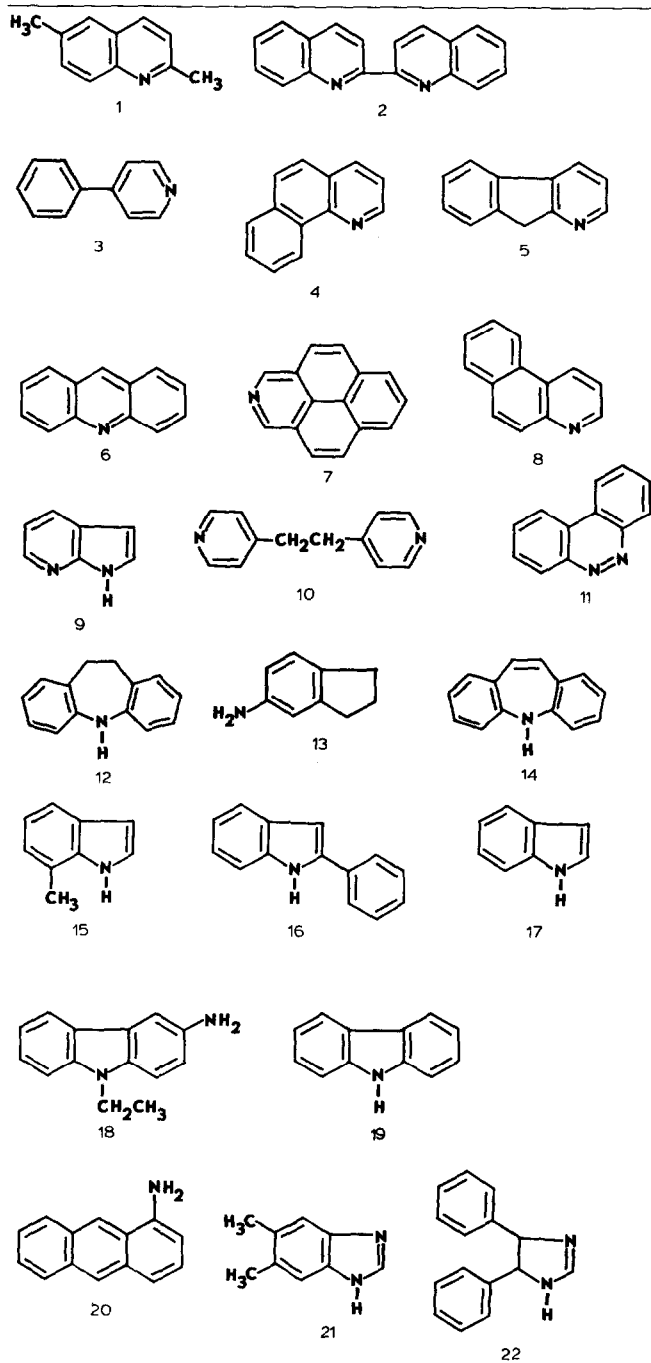
## CAPACITY FACTORS FOR MODEL COMPOUNDS FROM THREE CHROMATOGRAPHIC SYSTEMS

Mobile phases used for the three chromatographic systems:  $\mu$ Bondapak  $NH_2$ : *n*-heptane-2-propanol (85:15);  $\mu$ Bondapak CN: *n*-heptane-2-propanol (85:15);  $\mu$ Porasil: *n*-heptane-2-propanol (95:5).

Compound number	Compound	$k'$		
		$NH_2$	CN	$\mu$ Porasil
1	2,6-Dimethylquinoline	0.12	0.20	0.79
2	2,2'-Biquinoline	0.15	0.28	0.048
3	4-Phenylpyridine	0.22	0.72	1.88
4	7,8-Benzoquinoline	0.22	0.22	0.16
5	4-Azafluorene	0.23	0.29	0.79
6	Acridine	0.27	0.28	0.44
7	1-Azapyrene	0.29	0.30	0.84
8	5,6-Benzoquinoline	0.30	0.42	1.17
9	7-Azaindole	0.36	0.15	0.86
10	1,2-Bis(4-pyridyl)ethane	0.49	2.67	0.19
11	Benzo[ <i>c</i> ]cinnoline	0.50	0.75	1.02
12	Iminodibenzyl	0.51	0.85	0.032
13	5-Aminoindan	0.52	0.41	1.81
14	Iminostilbene	0.77	0.72	0.13
15	7-Methylindole	1.18	0.78	0.36
16	2-Phenylindole	1.56	1.47	0.19
17	Indole	1.56	1.27	0.48
18	3-Amino-9-ethylcarbazole	1.63	1.69	3.71
19	Carbazole	1.64	1.30	0.32
20	1-Aminoanthracene	1.86	1.14	1.17
21	5,6-Dimethylbenzimidazole	1.88	1.06	15.2
22	4,5-Diphenylimidazole	1.92	0.61	10.4
23	13H-Dibenzo[ <i>a, i</i> ]carbazole	2.47	1.48	0.21
24	2-Phenylbenzimidazole	2.56	2.07	2.15
25	5-Aminoisoquinoline	2.75	2.60	21.4
26	4-Azabenzimidazole	3.56	1.61	45.6
27	3-Aminofluoranthene	3.93	1.97	1.40
28	5-Aminoquinoline	4.14	3.40	20.7
29	5-Aminoindazole	6.07	2.86	15.5
30	6-Aminoindazole	8.56	2.20	16.7
31	2,7-Diaminofluorene	9.52	5.97	11.6

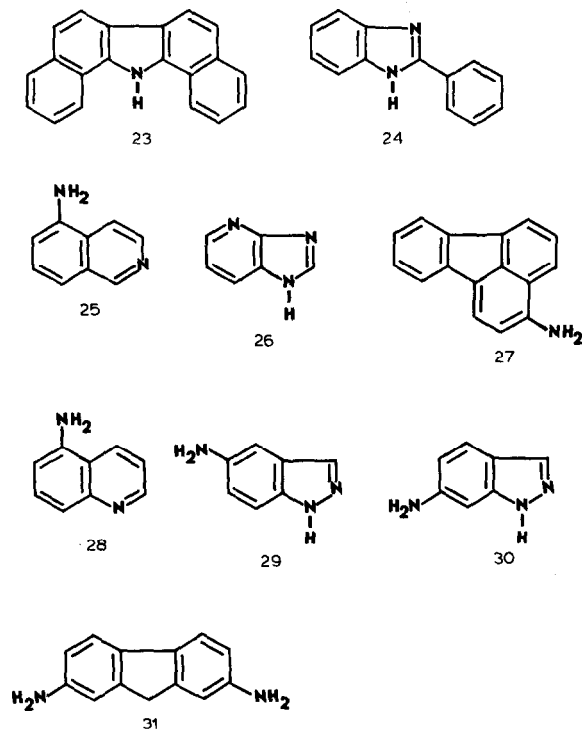
Due to the large amount of retention data that was obtained, a convenient method was sought for the presentation of the data. It was decided to display the data in the form of  $\log N$  vs.  $\log k'$  plots. This method of presentation also allowed for the relatively easy comparison of the data collected for the three chromatographic systems studied. Colin *et al.*<sup>21</sup> used the same types of plots in their discussion of the liquid chromatography of azaarenes. The three chromatographic systems studied were compared on the basis of the number of theoretical plates ( $N$ ) required to achieve the separation of two solutes consecutively eluted with a given resolution ( $R_s$ ). If  $k'_i$  and  $k'_j$  are the capacity factors for the two solutes  $i$  and  $j$  ( $i$  eluted first),

TABLE II  
MODEL COMPOUND STRUCTURES AND NUMBERS



(Continued on p. 132)

TABLE II (continued)



it can be shown that the number of theoretical plates required to separate  $i$  and  $j$  with a resolution  $R_s = 1$  is given by

$$N = \left[ \frac{1}{(0.25)(\alpha - 1) \frac{k'}{1 + k'}} \right]^2$$

This equation simply results from the rearrangement of the fundamental equation which defines  $R_s$  (ref. 26). In the above equation  $\alpha = k'_i/k'_j$  or  $k'_j/k'_i$  such that  $\alpha \geq 1$ , and  $k' = (k'_i + k'_j)/2$ .

Figs. 1-3 illustrate the various theoretical plates needed for the 31 model compounds with the different chromatographic systems. As illustrated in Figs. 1-3, the elution orders are not the same in the three systems. Additionally, the separations which require a very large number of theoretical plates are not the same. The data points in Figs. 1-3 represent the number of theoretical plates required to separate two consecutively eluted compounds with a resolution of one. For example, in Fig. 1 the first data point indicates the number of theoretical plates needed to separate compound 1 from compound 2.

For the graph of  $\log N$  vs.  $\log k'$  for the model compounds separated with the amino-silica and *n*-heptane-2-propanol (85:15) system, compound-class separation

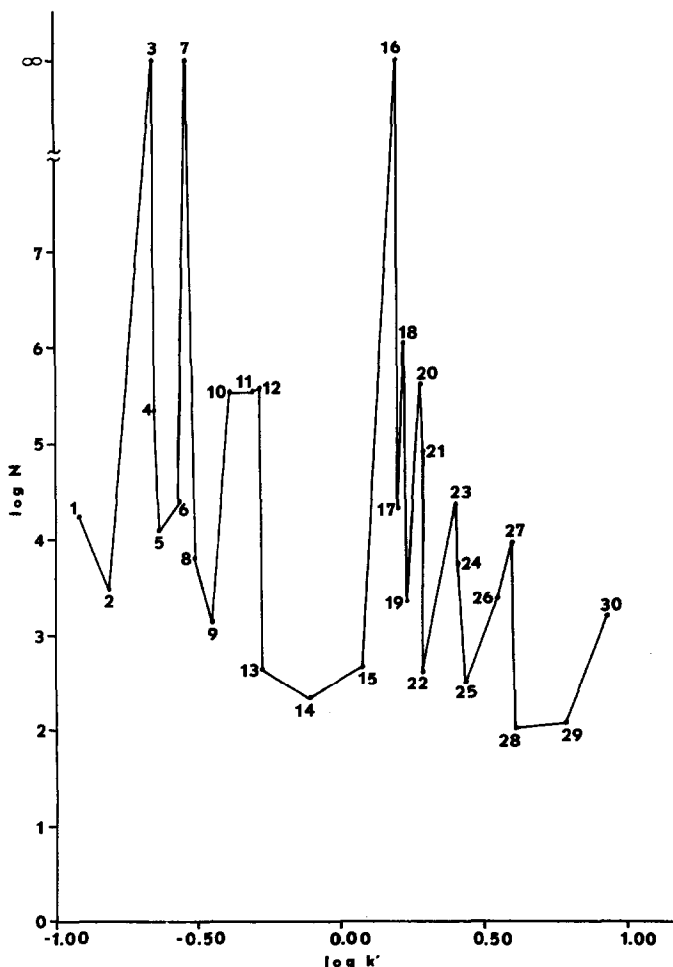


Fig. 1. Graph of  $\log N$  vs.  $\log k'$  for amino-silica/*n*-heptane-2-propanol (85:15) system.

was apparent (Fig. 1). Although some overlap between compound-classes was obtained, the separation into class types was quite distinct. The basic types had the smallest capacity factors, the non-basic types had intermediate values, and the amino types had the largest capacity factors.

Those standards which contained more than one type of nitrogen (multifunctional types), *e.g.* both a non-basic nitrogen and a basic nitrogen, or both an amino group and a basic nitrogen, were usually found in the  $\log k'$  range for the more strongly retained nitrogen class type for the amino-silica stationary phase. For example, a compound with both a basic nitrogen and a non-basic nitrogen would be found in the non-basic class type  $\log k'$  range. Those multifunctional type standards which eluted differently are considered as follows. As shown in Fig. 1, compound 9 (7-azaindole), which contains a basic nitrogen and a non-basic nitrogen, has a  $k'$  value of 0.36 and was found in the higher  $k'$  region where the basic type compounds

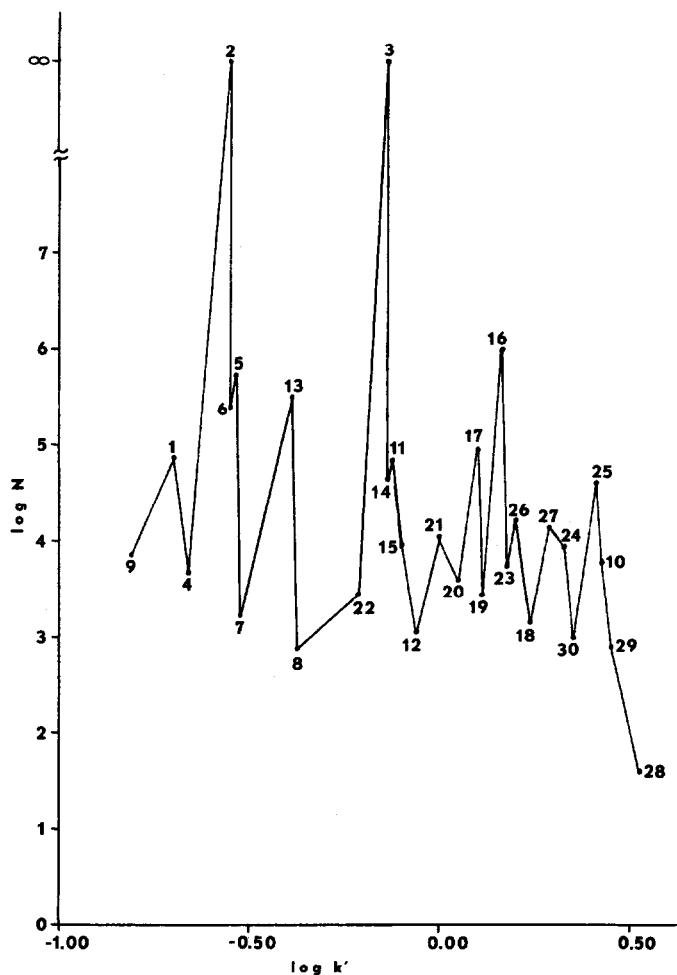


Fig. 2. Graph of  $\log N$  vs.  $\log k'$  for cyano-silica/*n*-heptane-2-propanol (85:15) system.

eluted. Compound 18 (3-amino-9-ethylcarbazole), which contains an amino group and a non-basic nitrogen and has a  $k'$  of 1.63, was found near the middle of the  $k'$  region for the non-basic type compounds. Compound 25 (5-aminoisoquinoline), which contains an amino group and a basic type nitrogen and has a  $k'$  of 2.75, was found at the upper limit of the  $k'$  region for the non-basic type compounds rather than in the  $k'$  region where the amino type compounds were found.

Three additional exceptions to the separation by class types are compounds 11, 13, and 20. As seen in Fig. 1 for the amino-silica stationary phase, compound 11 (benzo[*c*]cinnoline) has a  $k'$  value of 0.50 and was found early in the  $k'$  region for the non-basic type compounds. Most likely benzo[*c*]cinnoline is retained longer than would be expected because it contains a nitrogen-nitrogen double bond. There could be a relatively higher degree of hydrogen bonding interaction with the stationary phase because of this double bond and the close proximity of the nitrogen atoms in



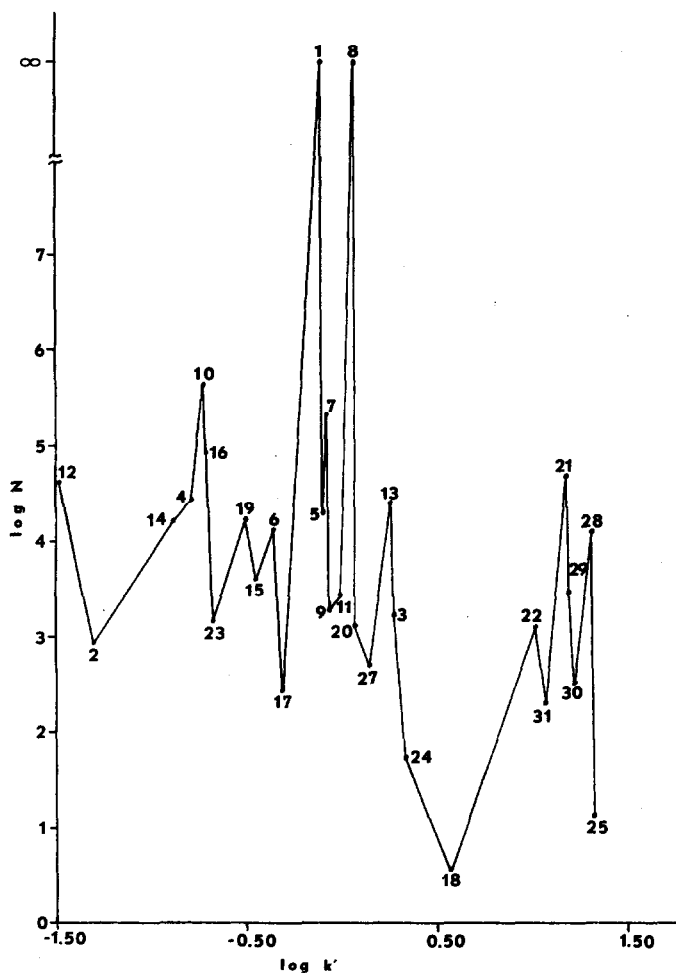


Fig. 3. Graph of  $\log N$  vs.  $\log k'$  for silica/*n*-heptane-2-propanol (95:5) system.

the molecule (Table II). Compound 13 (5-aminoindan) is also found at the beginning of the  $k'$  region for the non-basic type compounds and has a  $k'$  value of 0.52. 5-Aminoindan is probably retained less than expected due to its size and the presence of a saturated ring (Table II). Compound 20 (1-aminoanthracene) has a  $k'$  of 1.86 and is found near the middle of the  $k'$  region for the non-basic type nitrogen compounds. The shorter retention of 1-aminoanthracene is probably related to the size of the molecule and steric considerations which would prevent strong interaction of the amino group of the compound with the stationary phase.

The compound-class separation obtained with the amino-silica system was not observed with either the cyano-silica or silica systems. Figs. 2 and 3 present the  $\log N$  vs.  $\log k'$  plots for the cyano-silica and silica systems, respectively. The elution order of the model compounds was different for each system. A considerable amount of mixing was observed in both the cyano-silica and silica systems of all class types throughout the entire range of  $\log k'$  values.

### *Comparison of amino-silica with cyano-silica and silica chromatography systems*

As emphasized, the amino-silica system effected a compound-class separation while the cyano-silica and silica systems did not (Figs. 1-3). In comparing the  $\log k'$  range for the basic type compounds in the amino-silica system with the other two systems in this range the following observations were made. With the amino-silica system, ten compounds were present in the basic compound region. With the exception of compound 9, all of the compounds in this range were basic type compounds. Compound 9 contains both a basic type nitrogen and a non-basic type nitrogen, and eluted near the upper limit of the basic compound region. With the cyano-silica system, the first compound eluted was compound 9. All of the other compounds present in the basic compound range were basic type compounds, except for compound 13, an amino compound. With the silica system, a mixture of basic and non-basic type compounds was found.

The  $\log k'$  range for non-basic type compounds in the amino-silica system was compared to the other two chromatographic systems in this range. With the amino-silica system, fifteen compounds were present in this region. Eight of the compounds were non-basic type compounds. Compound 11 contains a nitrogen-nitrogen double bond and was found at the beginning of this region. Compound 13, an amino type compound, was found towards the beginning of this region also. Compound 20, also an amino type compound, was present towards the upper end of this region. Compounds 21, 22, and 24 were mixed-functional types containing both basic and non-basic types of nitrogen, and were present in this range near the upper end of the range. Compound 25 was an amino mixed-functional type and contained both an amino group and a basic nitrogen. Compound 25 was found at the upper limit of this  $\log k'$  range. With the cyano-silica and silica systems, basic, non-basic, amino and mixed functional type compounds were found in this range.

In the  $\log k'$  range for the amino type compounds, the following observations were made. With the amino-silica system, the rest of the model compounds were found. Amino type compounds and amino mixed-functional type compounds which contained amino groups, non-basic and basic type nitrogens were present. With the cyano-silica system, the same type compounds were noticed in this range as with the amino-silica system. With the silica system, mixed-functional type compounds, which contained both basic and non-basic nitrogens, were found along with amino and amino mixed-functional type compounds.

### *Hydrogen bonding interactions*

Snyder<sup>27</sup> has discussed the general molecular interactions that can occur with silica, amino-silica, and cyano-silica. He has classified these stationary phases in terms of his solvent selectivity triangle. This classification shows that silica appears to function as a Group VIII type which is acidic, but weakly basic and weakly dipolar. Aminopropyl functions as a Group I phase which is basic, but weakly acidic and weakly dipolar, and the cyanopropyl stationary phase behaves as a Group VI dipolar adsorbent. In addition, the strengths of the adsorbents are in the following order: silica > amino-silica > cyano-silica<sup>27</sup>.

It is believed that the order of retention and subsequent separation into compound-class types with the amino-silica/*n*-heptane-2-propanol system can be explained, partially, on the basis of hydrogen bonding of the solutes to the stationary

phase. Based on the chromatographic data from Table I, it appears that the hydrogen bonding interaction increases in the order basic type solutes, non-basic type solutes, amino type solutes. As the retention volume increases, it appears the degree of hydrogen bonding interaction between the solute and the amino-silica stationary phase also increases.

The pyridine type solutes do not have a hydrogen which can hydrogen bond to the nitrogen of the amino groups in the stationary phase, whereas the hydrogen bonded to the nitrogen in a pyrrolic type compound can form a hydrogen bond with the stationary phase amine nitrogens. The solutes which contain an amino group can form a hydrogen bond with the amine nitrogen in the stationary phase through one of the hydrogens on the amino group in the solute. In addition, hydrogen bonding between the hydrogens of the amines on the stationary phase and the nitrogen of the amino solute is expected.

The previous discussion is certainly an oversimplification, and competition between the solute and the strong polar solvent in the mobile phase with the stationary phase amino groups is expected to occur especially with an increasing amount of the strong polar solvent present in the mobile phase<sup>28</sup>. It is assumed that Snyder's displacement model is in operation; however, additional work is needed to elucidate the significance of the solute-strong solvent hydrogen bonding interactions in the mobile phase<sup>29</sup>. In related work, Hennion *et al.*<sup>30</sup> have shown that the amino group in an amino-propyl-bonded silica can adsorb a different number of alcohol molecules depending on which alcohol is used in a *n*-hexane-alcohol binary mobile phase. The results were verified by plots of the inverse of capacity factor values *versus* the concentration of the polar solvent in the mobile phase. In addition, Chang and Huang<sup>31</sup> have suggested several interaction modes of anilines with a silica-amino column and *n*-heptane-2-propanol mobile phases.

## CONCLUSIONS

By comparing three normal-phase chromatographic systems with the same model compounds, only one system provided a good compound-class type separation. The amino-silica/*n*-heptane-2-propanol (85:15) system effected a separation of aromatic nitrogen containing model compounds into three main compound-classes with the three compound-class fractions containing various multifunctional compounds. However, the ability to separate aromatic nitrogen containing compounds into three main classes with one stationary phase and one mobile phase is relatively rapid and straightforward. With the ability to separate one sample into three main fractions, each fraction of a given class type could then be further characterized by other chromatographic or spectral techniques.

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