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LIQUID CHROMATOGRAPHY ON SILICA USING MOBILE PHASES CONTAINING ALIPHATIC CARBOXYLIC ACIDS

I. EFFECTS OF CARBOXYLIC ACID CHAIN LENGTH ON SEPARATION EFFICIENCY AND SELECTIVITY

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SUMMARY

The effect of adding aliphatic linear chain carboxylic acids to mobile phases used in separation of solutes of widely varying functionality on microparticulate silica was investigated. Changes in peak shape and retention time of varied solutes as a function of mobile phase acid chain length are described. Peak shape generally improves with increasing acid chain length, especially for acidic solutes. Retention of acidic solutes decreases with decreasing acid chain length; basic solutes exhibit the opposite behavior. These and other effects are discussed in conjunction with a proposed retention mechanism. Potential for application of this type of chromatographic system for rapid assessment of chemical types in complex samples is discussed.

INTRODUCTION

Unlike gas chromatography (GC), few liquid chromatographic (LC) procedures are currently available which rapidly yield direct information on complex samples such as fossil fuel or environmental samples. For example, at the very least, GC will indicate the boiling range of the sample components whereas the most popular LC approach, reversed-phase separation using UV–visible detection, yields very little or no direct information. Molecular-size based LC separations nominally give the molecular-weight range of the compounds present, but are often suspect due to adsorption effects and lack of appropriate standards for complex samples of unknown or mixed chemical type.

Thus, as part of a program for evaluating different procedures for extracting polar compounds from fossil fuel liquids, work on a high-performance liquid chromatographic (HPLC) method for rapid evaluation of the chemical types present in the various extracts was begun. Although LC of polar materials on silica gel often yields tailing and/or broad peaks, work by Lawrence and Leduc[1] indicated that the peak shape and chromatographic efficiency of triazine heterocyclic compounds and

phenols was improved considerably by addition of small amounts of aliphatic carboxylic acids to the mobile phase. This technique appeared sufficiently promising for the separation of polar extracts of fuels for an in-depth study, reported here, to be undertaken to determine the general effects of carboxylic acids on separations performed on silica, and on the variation of separation efficiency and selectivity with carbon chain length of straight chain acids. Application of the technique to fossil fuels will be reported separately.

EXPERIMENTAL

Apparatus

A single HPLC system —consisting of two Waters Assoc. M6000 pumps, a Waters Assoc. M660 gradient programmer, a Waters Assoc. WISP autoinjector and a Schoeffel MSF770 variable-wavelength detector in series with a Perkin-Elmer M200 spectrophotometer equipped with 8-μl flow cell— was used throughout the study. Helium passed continuously through solvents in the pump reservoirs, thus removing dissolved gases. All columns were packed in this laboratory using essentially the method and apparatus described by Coq *et al.*[2].

Materials

Dichloromethane and 1,2-dichloroethane were Burdick & Jackson Labs. (Muskegon, MI, U.S.A.) "distilled in glass" grade. Anhydrous ethanol was obtained from U.S. Industrial Chemical Co. (New York, NY, U.S.A.) and the heptane used was Phillips Petroleum "pure" grade (Bartlesville, OK, U.S.A.). None of the solvents were used as received.

Each gallon (3.79 l) of heptane was passed through a 80 × 1.6 cm column filled with 400°C activated 13X molecular sieves and then through a 200 × 2.5 cm column filled with equal amounts of SiO_2 (activated at 150°C) and Al_2O_3 (activated at 400°C). Ethanol was treated similarly except that an activated carbon column (coconut charcoal; No. 5-690A; Fisher Scientific, Pittsburgh, PA, U.S.A.) was substituted for the 13X sieve column. Finally, the chlorinated solvents were treated only with the SiO_2–Al_2O_3 column.

Acetic acid (ACS grade; Fisher), propanoic acid (AR grade; Mallinckrodt, St. Louis, MO, U.S.A.) and decanoic acid (96%; Aldrich, Milwaukee, WI, U.S.A.) were vacuum distilled over a 30-cm glass-helices packed column. Further purification was achieved by precipitating the acids as their ammonium salts with gaseous NH_3 from a dichloromethane–pentane (1:4) solvent, filtering, suspending the salt in fresh solvent, and regenerating the acid with gaseous HCl. This step was absolutely necessary only with the decanoic acid. All purified acids were kept refrigerated under argon.

Column packing materials (LiChrosorb Si-60; E. Merck, Darmstadt, G.F.R.) were used as received. Columns were constructed from 1/4 in. (0.64 cm) "zero dead volume" fittings and 4.6 mm I.D. polished stainless-steel tubing commonly available from many suppliers. Column dimensions appear in Table I.

Except for a few known to be extremely impure, all chemicals used as solutes were used as received.

TABLE I
CHROMATOGRAPHIC CONDITIONS

Gradient number	1	2
Column length × diameter (cm)	25.0 × 0.46	16.3 × 0.46
Precolumn length × diameter (cm)	7.0 × 0.46	7.0 × 0.46
Column packing (SiO_2, Merck)	Si-60, 5 μm	Si-60, 10 μm
N (average plates/m)	10–15,000	8–10,000
Precolumn packing (SiO_2, Merck)	Si-60, 10 μm	Si-60, 10 μm
Flow-rate (ml/min)	2.0	2.0
Average back pressure (p.s.i.)	1200	1200
Chart speed (cm/min)	0.5	0.5
Gradient conditions:		
Initial %B → final %B;	1 → 100	6 → 100
Gradient time (min), time at 100%B (min), reequilibration time (min);	30, 2, 20	40, 0, 20
Gradient curve	Linear	Linear
Solvent A	Heptane	1,2-Dichloroethane containing 0.0335 *M* acid*
Solvent B	Dichloromethane containing 0.0268 *M* acid* and 0.3% (v/v) ethanol	1,2-Dichloroethane containing 0.0335 *M* acid* and 8.0% (v/v) ethanol

* Either acetic, propanoic, decanoic, or no acid.

Procedure

Accurately known solutions in the range 20–40 mg/50 ml dichloromethane were prepared of each solute and stored at −10°C. Several blends containing aliquots of 10–20 solutes were also prepared. The retention order of each of the solutes in a given blend was established by running each individually under gradient operation and comparing retention times and ratios of peak heights at two wavelengths with those of peaks in the blend. After elution order was determined for all blends, they were rerun as a set to obtain the most consistent group of retention data possible. Dual wavelength peak height ratios were used to check for any peak order reversals during the final rerunning of the blends. Rechecking was usually unnecessary except for solvent systems containing no acid. One of the blends was rerun at the end of the series to check for significant changes in column retention characteristics.

Preliminary work indicated that most compound types potentially in a fossil fuel could be resolved in one of the two basic gradient programs listed in Table I. Thus, relatively weakly retained compounds were run with gradient 1 and the rest with gradient 2. A new column and precolumn were used for each series of experiments with a given solvent system and gradient. All columns used in gradient 1 systems were packed on the same day using the same batch of silica. The same was true for columns used for gradient 2 experiments. Columns used for gradient 2 runs were shorter and contained larger particle size packing to avoid back pressures higher than 10.3 MPa (1500 p.s.i.) which can provoke column instability. As the theoretical plate data (Table I) show, the columns used were of only moderate efficiency.

RESULTS

Tables II and III contain retention data for gradients 1 and 2, respectively. As these tables indicate, each solute was run in mobile phase systems containing acetic,

TABLE II
RETENTION TIMES (t_R) OF COMPOUNDS RUN WITH GRADIENT 1
Retention times have a relative standard deviation of ±1%. NE = Not eluted.

Compound number	*Compound name*	t_R *(min)*			
		Acid added to mobile phase			
		Acetic	*Propanoic*	*Decanoic*	*None*
1	Unretained compound	2.60	2.60	2.60	2.60
2	Chrysene	4.76	5.20	5.22	5.44
3	Benzo[*a*]pyrene	4.88	5.32	5.54	5.64
4	Benzo[*ghi*]perylene	5.16	5.52	5.64	5.56
5	Coronene	5.22	5.74	5.88	5.84
6	1,2,3,4-Tetraphenylnaphthalene	6.88	7.64	6.98	7.66
7	3-Methylbenzo[*b*]thiophene	3.04	3.32	3.34	3.46
8	*o*-Thiocresol	3.50	3.44	3.94	4.40
9	Thianthrene	3.90	4.56	4.42	4.86
10	Dinaphthenothiophene	5.74	6.24	6.24	6.32
11	Dibenzofuran	3.54	3.82	3.90	4.08
12	1-Phenylpyrrole	5.46	6.16	6.04	6.26
13	9-Ethylcarbazole	6.03	6.48	6.24	6.58
14	9-Methylcarbazole	6.36	6.84	6.66	6.86
15	1-Methylindole	7.00	7.50	7.34	7.68
16	2,3,4,5-Tetraphenylpyrrole	10.1	10.4	10.1	11.0
17	1,2,3,4-Tetrahydrocarbazole	10.4	11.2	10.8	11.3
18	2,3-Dimethylindole	10.8	11.7	11.4	11.8
19	3-Methylindole	11.0	11.5	11.4	12.0
20	Carbazole	11.3	11.8	11.8	12.3
21	2-Methylcarbazole	11.8	12.4	12.4	12.8
22	Indole	12.2	12.6	12.7	13.2
23	2,5-Dimethylpyrrole	12.4	12.9	13.1	13.7
24	13H-Dibenzo[*a*,*i*]carbazole	12.6	12.7	13.0	13.5
25	Triphenylamine	4.86	5.68	5.64	6.06
26	N-Phenyl-1-naphthylamine	8.56	8.96	8.76	9.30
27	Diphenylamine	9.17	9.60	9.38	9.96
28	12H-benz[α]phenothiazine	9.62	9.82	9.96	10.0
29	N-Phenylbenzylamine	15.2	14.1	12.8	12.9
30	Phenyl salicylate	9.74	10.4	9.98	14.5
31	Diphenyl carbonate	10.6	11.1	10.7	11.4
32	Phenyl benzoate	10.8	11.3	10.9	11.7
33	*o*-Tolyl benzoate	10.8	11.4	10.8	11.8
34	Methyl benzoate	12.4	12.7	12.8	13.8
35	Coumarin	21.4	22.0	22.8	25.0
36	Dimethyl phthalate	21.6	23.0	24.0	26.2
37	Benzonitrile	11.9	12.4	12.2	13.0
38	4-Phenoxybutyronitrile	18.0	18.0	17.6	20.0
39	Benzophenone	14.4	14.7	14.4	15.7
40	Benzanthrone	17.3	17.3	17.0	19.1
41	2,4,6-Tri-*tert.*-butylphenol	4.00	4.38	4.04	4.66
42	2,6-Dimethylphenol	13.0	13.3	13.4	14.2
43	*o*-Ethoxyphenol	13.2	13.3	13.9	16.8
44	2,4-Di-*sec.*-butylphenol	14.8	15.2	14.9	16.1

TABLE II *(continued)*

Compound number	*Compound name*	t_R *(min)*			
		Acid added to mobile phase			
		Acetic	*Propanoic*	*Decanoic*	*None*
45	2,3,5-Trimethylphenol	17.7	18.0	17.5	20.0
46	2,3-Dimethylphenol	18.1	18.6	18.1	20.0
47	*o*-Cresol	18.2	18.6	18.3	20.0
48	2,4,5-Trimethylphenol	18.4	18.6	18.3	20.2
49	Hydroquinone	19.5	19.9	20.2	21.9
50	*p*-Nonylphenol	19.9	20.0	19.4	22.2
51	*m*-Ethylphenol	21.3	20.6	21.6	23.7
52	3,5-Dimethylphenol	21.6	21.2	22.2	24.6
53	*p*-Ethylphenol	21.6	21.5	22.5	24.7
54	3,4,5-Trimethylphenol	21.7	21.9	23.0	25.2
55	Phenol	21.7	21.7	22.8	25.0
56	3,4-Dimethylphenol	21.7	21.7	22.9	25.0
57	2-Naphthol	21.7	22.0	22.9	24.9
58	2,3-Dihydroxynaphthalene	NE	NE	NE	NE
59	1-Fluorenecarboxylic acid	21.7	20.0	23.7	NE
60	2,2-Diphenylpropanoic acid	21.7	23.1	26.0	NE
61	*p*-Toluic acid	21.7	21.5	27.3	NE
62	1-Naphthoic acid	21.7	21.5	28.7	NE
63	Thiobenzanilide	16.1	16.9	16.3	17.5
64	Benzanilide	21.8	24.1	24.0	25.5
65	Salicylamide	NE	NE	NE	NE
66	2-Methylmercaptobenzothiazole	15.3	16.0	15.9	16.9
67	Benzoxazole	21.7	21.7	22.6	24.8
68	Benzothiazole	22.3	24.0	25.1	27.2
69	2-Methyl[α]naphthothiazole	23.5	25.7	26.5	28.3
70	2,6-Diethylaniline	17.7	17.6	16.7	17.1
71	2,6-Dimethylaniline	20.8	19.8	19.3	20.6
72	6-Aminochrysene	21.1	20.8	21.5	22.5
73	1-Aminonaphthalene	21.9	21.4	21.9	23.2
74	*p*-Chloroaniline	21.9	21.5	22.1	22.7
75	2-Aminoanthracene	23.6	22.6	22.7	23.3
76	*o*-Ethylaniline	24.3	22.8	21.6	22.1
77	2,4,6-Trimethylaniline	27.5	25.0	23.0	23.1
78	2,5-Dimethylaniline	29.4	26.0	24.2	23.8
79	Aniline	30.3	26.8	26.3	26.0
80	2,3-Dimethylaniline	30.8	28.6	26.5	25.4
81	N-Methylaniline	31.0	27.8	22.7	17.7
82	2,4-Dimethylaniline	34.4	31.0	29.0	26.5
83	N-Ethylaniline	NE	30.9	25.0	16.2
84	*p*-*n*-Butylaniline	NE	31.2	30.0	27.2
85	*p*-Ethylaniline	NE	31.5	30.4	28.0
86	N,N-Dimethylaniline	NE	31.1	25.0	15.1
87	N,N-Diethylaniline	NE	NE	NE	14.7
88	Dibenzo[*a*,*c*]phenazine	10.0	10.5	10.2	10.9
89	2-Chloropyridine	20.7	20.4	20.7	22.2
90	2-Methoxypyridine	22.1	22.0	22.4	24.4
91	2-Quinolinethiol	25.8	27.8	28.0	28.9
92	7,8-Benzoquinoline	30.8	26.8	22.8	22.0

propanoic, decanoic, and no acid. As shown in Table III, some of the components run with the gradient 1 systems were rerun in gradient 2 systems in order to indicate the approximate difference in polarity between the two gradients. For example, compound No. 59 (1-fluorenecarboxylic acid) was retained 21.7 min in the gradient 1 system containing acetic acid and was eluted after only 3.3 min in the acetic acid mobile phase system run in gradient 2.

Several conclusions were drawn from detailed analysis of data in Tables II and III. The most obvious one was that adding relatively small amounts of carboxylic acids to mobile phases affects the retention of compounds of widely varying chemical type in a manner which can be correlated with the structures of both solute and acid. The general effect of adding a carboxylic acid to the mobile phase on the retention of solutes of varying chemical type is summarized in Table IV. In general, solutes with acidic or neutral functional groups are retained less in mobile phase systems containing carboxylic acids compared to the same system without acids. Conversely, basic substances have enhanced retention in acid containing systems.

For basic substances, an approximately linear correlation exists between basicity and retention time. As an approximate measure of basicity, the only parameter available for a large number of compounds is the aqueous acid dissociation constant: $BH^+ \overset{K_a}{\leftrightarrows} B + H^+$. Table V shows results of correlations for the aniline and pyridine series of compounds. pK_a data were obtained from several tabulations[3–6]. In general, the results in Tables II and III show greater retention of basic compounds in systems containing acids of short chain length due to decreased steric hindrance and greater inherent acidity. Unfortunately, this trend is not obvious from inspection of the correlations in Table V. Thus, the physical significance of the slope and intercept data is not clear. However, it should be noted that the relative standard deviations for the correlations decrease with decreasing chain length of the acid additive. Of course, this implies that retention of basic solutes is governed more by their inherent basicity in mobile phase systems containing shorter chained carboxylic acids. Of the basic compounds tested, N-alkylated anilines exhibited the greatest shift in retention, with t_R sometimes doubling when the strongest acid (acetic) was added to the mobile phase. As shown in Table V, their poor retention in the non-acid-containing system resulted in a negative pK_a *vs.* retention time correlation coefficient for the aniline series. In fact, a satisfactory pK_a correlation with retention of anilines in the non-acid-containing system could only be obtained by excluding data for N-alkylated species.

The effect of chain length of the mobile phase acid on retention of sterically hindered solutes was also investigated. In the alkylated pyridine series of compounds, basicity [as measured by pK_a (H_2O)] changes very little with the size of the pyridine alkyl substituent[4]. Thus, Figs. 1 and 2 show the effect of steric hindrance alone on retention of 2- and 4-substituted pyridines. Fig. 1 clearly shows a slower decrease in t_R with increasing 2-substituent chain length as the chain length of the acid in the mobile phase decreases. On the other hand, Fig. 2 does not show such a clear trend, thereby indicating greater enhancement of retention by mobile phase acids for highly hindered solutes such as 2-hexylpyridine. It should be noted, however, that the 4-alkylpyridine compounds used to obtain data in Fig. 2 were not all normal chain types (as were those in Fig. 1); the extent to which this may influence the comparison between the two figures is not known.

TABLE III
RETENTION TIMES (t_R) OF COMPOUNDS RUN WITH GRADIENT 2
Except for the compound 2,2′-biquinoline, retention times have a relative standard deviation of ±1 %. The standard deviation of retention times for 2,2′-biquinoline is approximately ±10 %. NE = Not eluted; ND = not determined.

*Compound number**	*Compound name*	t_R *(min)*			
		Acid added to mobile phase			
		Acetic	*Propanoic*	*Decanoic*	*None*
1	Unretained compound	1.26	1.34	1.24	1.26
3	Benzo[*a*]pyrene	1.28	1.36	1.26	1.16
4	Benzo[*g,h,i*]perylene	1.26	1.38	1.24	1.24
5	Coronene	1.26	1.42	1.40	1.32
6	1,2,3,4-Tetraphenylnaphthalene	1.20	1.34	1.18	1.16
11	Dibenzofuran	1.22	1.38	1.36	1.16
13	9-Ethylcarbazole	1.30	1.36	1.24	1.28
20	Carbazole	1.34	1.42	1.42	1.36
24	13H-Dibenzo[*a,i*]carbazole	1.30	1.42	1.28	1.38
25	Triphenylamine	1.20	1.34	1.36	1.30
37	Benzonitrile	1.58	1.74	1.66	1.64
39	Benzophenone	1.46	1.46	1.68	1.78
46	2,3-Dimethylphenol	2.16	2.14	2.44	2.52
47	*o*-Cresol	2.10	2.18	2.44	2.64
48	2,4,5-Trimethylphenol	2.10	2.06	2.44	2.54
51	*m*-Ethylphenol	2.60	2.58	3.02	3.24
55	Phenol	2.62	2.68	3.14	3.64
56	3,4-Dimethylphenol	2.62	2.70	3.14	3.46
57	2-Naphthol	2.50	2.62	2.89	3.38
58	2,3-Dihydroxynaphthalene	NE	NE	NE	NE
59	1-Fluorenecarboxylic acid	3.30	3.34	4.72	~43
60	2,2-Diphenylpropanoic acid	3.38	3.46	5.06	~44
61	*p*-Toluic acid	4.30	4.32	6.56	NE
62	1-Naphthoic acid	3.82	3.86	6.66	NE
67	Benzoxazole	ND	3.58	ND	ND
68	Benzothiazole	ND	3.90	ND	ND
69	2-Methyl[α]naphthothiazole	3.98	4.44	4.90	5.36
81	N-Methylaniline	3.56	3.70	2.96	2.06
83	N-Ethylaniline	5.04	ND	ND	ND
84	*p-n*-Butylaniline	6.82	ND	ND	ND
85	*p*-Ethylaniline	7.16	ND	ND	ND
86	N,N-Dimethylaniline	5.74	4.96	3.34	2.06
87	N,N-Diethylaniline	11.02	ND	ND	ND
89	2-Chloropyridine	2.84	3.12	3.40	3.46
90	2-Methoxypyridine	3.54	3.98	4.28	4.36
91	2-Quinolinethiol	2.68	2.98	3.14	3.04
93	4-Chloroquinoline	7.32	ND	8.62	8.70
94	2-Mercaptopyridine	7.72	8.24	9.22	8.98
95	2-Hydroxyquinoline	12.3	ND	14.6	20.5
96	6-Methoxyquinoline	12.4	12.3	11.7	11.5
97	4-Mercaptopyridine	24.2	25.7	26.1	27.3
98	2-Ethanolpyridine	27.5	30.1	28.0	28.2
99	2,3-Dihydroxypyridine	NE	NE	NE	NE

(Continued on p. 218)

TABLE III *(continued)*

*Compound number**	*Compound name*	t_R *(min)*			
		Acid added to mobile phase			
		Acetic	*Propanoic*	*Decanoic*	*None*
100	8-Hydroxyquinoline	NE	NE	NE	NE
64	Benzanilide	ND	2.58	ND	ND
65	Salicylamide	6.80	ND	8.84	12.0
101	Oxindole	9.48	10.2	10.9	11.9
102	Acetanilide	9.70	10.5	11.6	11.4
103	1-Benzyl-2-pyrrolidineone	11.7	ND	13.0	14.0
104	3-Methoxy-2-[1H]-pyridone	26.0	ND	30.5	37.8
88	Dibenzo[*a*,*c*]phenazine	1.30	1.40	1.30	1.36
105	Quinoxaline	8.94	9.90	10.3	10.3
106	1-Azacarbazole	9.00	ND	9.38	12.7
107	4-Phenylpyrimidine	9.44	9.98	10.7	11.0
108	2,3-Dimethylquinoxaline	9.74	10.6	11.0	11.2
109	2,2′-Biquinoline	11.2	8.8	8.8	~5
110	*m*-Phenanthroline	13.3	12.5	13.2	13.8
111	2-Methylpyrazine	12.2	13.8	15.2	15.8
112	Quinazoline	13.3	13.5	15.1	15.5
113	2,5-Dimethylpyrazine	14.3	15.0	16.1	16.8
114	2,3,5-Trimethylpyrazine	15.3	15.8	17.5	17.7
115	2,3,5,6-Tetramethylpyrazine	15.5	15.0	17.5	17.9
116	5-Aminoindole	17.5	16.4	14.8	10.1
117	3-Methylpyridazine	20.6	22.1	23.1	23.6
118	4,5-Diphenylimidazole	31.0	31.4	28.6	27.8
119	Benzimidazole	32.0	32.7	30.2	29.9
120	Harmane	NE	47.4	42.0	37.4
121	3,5-Diphenylpyrazole	NE	NE	NE	NE
122	2,2′-Bipyrridyl	NE	NE	NE	NE
123	1,10-Phenanthroline	NE	NE	NE	NE
124	4-Aminoquinaldine	NE	NE	NE	NE
92	7,8-Benzoquinoline	3.38	3.46	3.12	2.86
125	2-Phenylpyridine	7.90	7.96	6.52	5.30
126	2-Azafluoranthene	8.08	8.26	8.80	8.74
127	13-Azafluorene	8.84	8.90	9.26	9.24
128	1-Azapyrene	8.96	9.04	9.20	9.32
129	8-Methylquinoline	9.30	9.38	8.64	6.44
130	4-Azafluorene	10.1	9.82	9.68	9.54
131	5,6-Benzoquinoline	10.8	10.7	10.3	10.1
132	Quinoline	11.2	11.1	10.8	10.8
133	2-Benzylpyridine	11.4	11.2	10.6	9.78
134	Acridine	11.6	11.3	10.5	8.96
135	6-Methylquinoline	12.4	12.1	11.5	11.4
136	2-Methylacridine	12.4	12.1	11.1	9.32
137	7-Methylquinoline	13.3	12.7	11.9	11.4
138	4-Phenylpyridine	13.6	13.5	12.8	13.1
139	Isoquinoline	13.9	13.4	12.8	12.6
140	3,5-Dibutylpyridine	14.7	14.3	12.6	12.5
141	4-(5-nonyl)-Pyridine	14.9	14.1	13.2	13.6
142	4-Methylquinoline	15.2	14.2	13.2	12.4
143	Pyridine	15.4	14.9	14.7	14.6

TABLE III *(continued)*

*Compound number**	*Compound name*	t_R *(min)*			
		Acid added to mobile phase			
		Acetic	*Propanoic*	*Decanoic*	*None*
144	2-*n*-Hexylpyridine	15.5	14.0	13.0	10.8
145	3-Methylisoquinoline	15.8	14.9	14.0	13.0
146	2-*n*-Amylpyridine	15.9	14.7	13.3	11.2
147	3-Ethylpyridine	16.1	15.5	14.7	14.6
148	2-Methylquinoline	16.9	14.8	13.4	11.4
149	3-Methylpyridine	17.1	16.6	15.7	15.3
150	2,6-Dimethylquinoline	17.2	16.5	15.3	12.0
151	4-*tert.*-Butylpyridine	17.4	16.8	15.8	15.7
152	2-Ethylpyridine	17.5	17.0	15.7	13.6
153	4-Isopropylpyridine	17.6	17.8	16.0	16.0
154	2,7-Dimethylquinoline	18.2	17.8	16.0	12.1
155	3,5-Dimethylpyridine	18.7	18.7	16.9	15.9
156	4-Ethylpyridine	18.8	18.7	17.1	16.4
157	2-Methylpyridine	19.7	19.7	17.9	16.3
158	4-Methylpyridine	20.1	20.6	18.4	17.2
159	2-Methyl-5-ethylpyridine	20.5	20.3	18.1	16.2
160	2,4-Dimethylquinoline	20.5	20.7	17.8	14.0
161	3-Ethyl-4-methylpyridine	21.7	21.7	18.9	17.4
162	3,4-Dimethylpyridine	22.8	23.2	20.3	18.3
163	2,4-Dimethylpyridine	26.6	28.8	23.7	20.2
164	2,6-Dimethylpyridine	27.1	28.7	23.6	18.3
165	4-Phenylpiperidine	NE	NE	NE	NE

* If the compound also appears in Table II, it carries the same number here as in Table II.

Generally, retention of acidic or neutral solutes decreased with decreasing carbon chain length of the acid added to mobile phase. This effect was greatest for carboxylic acid solutes (compounds 59–62 in Table II), which did not elute at all in the gradient I mobile phase system not containing a carboxylic acid. In fact, Table II shows that the retention region spanned by carboxylic acid solutes decreases with decreasing mobile phase acid chain length to the point where they all coelute in the acetic acid-containing system.

Although the effect of the concentration of acid added to the mobile phase is not included in Tables II or III, it stands to reason that as the acid becomes more dilute the solute retention times should approach those of the system containing no acid. However, one item of interest was whether the retention times of the decanoic acid-containing systems, for example, could be matched using a solvent system containing dilute acetic acid. If that were the case, then the chromatograms obtained using longer-chain acids could be duplicated by using more dilute, shorter chain acids. However, as Table VI shows, small but significant differences exist in retention times of the more retained compounds for the closest match obtained between a dilute acetic acid- and a decanoic acid-containing system. Thus, some specific effects induced by chain length are implied.

Also not indicated in Tables II and III are the effects of chain length on peak shape and separation efficiency. As indicated by Lawrence and Leduc[1], the chroma-

TABLE IV
SEMI-QUANTITATIVE EFFECTS OF CARBOXYLIC ACIDS ON RETENTION TIME
t_{R_0} = retention time using mobile phase system without acid; t_{R_A} = Retention time obtained with the analogous acetic acid-containing mobile phase. NC = No change.

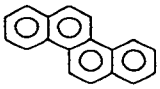
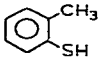
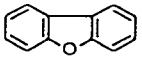
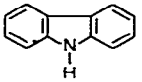
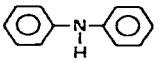
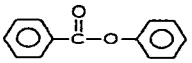
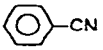
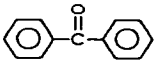
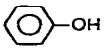
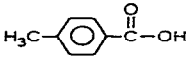
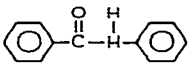
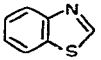
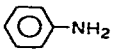
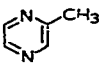
Chemical class	*Typical structure*	*Gradient number*	$\frac{t_{R_0} - t_{R_A}}{t_{R_0}} \times 100\%$
Hydrocarbon		1	NC
S-containing		1	+20%
Ether		1	+10%
Pyrrole		1	+10%
N-Phenylaniline		1	+10%
Ester		1	+15%
Nitrile		1	+10%
Ketone		1	+10%
Phenol		1	+10%
Carboxylic acid		1, 2	> +100%
Amide		1, 2	+20%
Thiazole		1	+20%
Aniline		1	−10% (non-alkylated) −20% (ring-alkylated) > −100% (N-alkylated)
Pyridine		2	+10% (X = electron withdrawing group) −10% (X = H) −20 to −50% (X = alkyl group(s))
Dinitrogen types		2 2 2	+20% (pyrazines) −10% (imidazoles) −70% (aminopyrroles)

TABLE V
RESULTS OF LINEAR pK_a *VS.* t_R REGRESSIONS FOR PYRIDINE AND ANILINE CHEMICAL GROUPS
S.D. = Standard deviation.

Group	*Acid added to mobile phase*			
	Acetic	*Propanoic*	*Decanoic*	*None*
(A) Pyridine group				
Slope (min^{-1})	0.128	0.117	0.149	0.170
Slope S.D. (min^{-1})	0.008	0.009	0.010	0.014
y-Intercept	3.51	3.70	3.40	3.29
y-Intercept S.D.	0.14	0.15	0.15	0.19
Correlation coefficient	0.947	0.937	0.946	0.925
(B) Aniline group				
Slope (min^{-1})	0.0757	0.102	0.102	−0.068*
Slope S.D. (min^{-1})	0.005	0.006	0.031	0.042
y-Intercept	2.36	1.87	2.08	6.26
y-Intercept S.D.	0.15	0.17	0.77	0.94
Correlation coefficient	0.986	0.983	0.741	−0.46

* Recalculation of data without N-alkylated anilines yielded the following: slope = 0.171 ± 0.026 min^{-1}, y-intercept = 0.27 ± 0.42, correlation coefficient = 0.935.

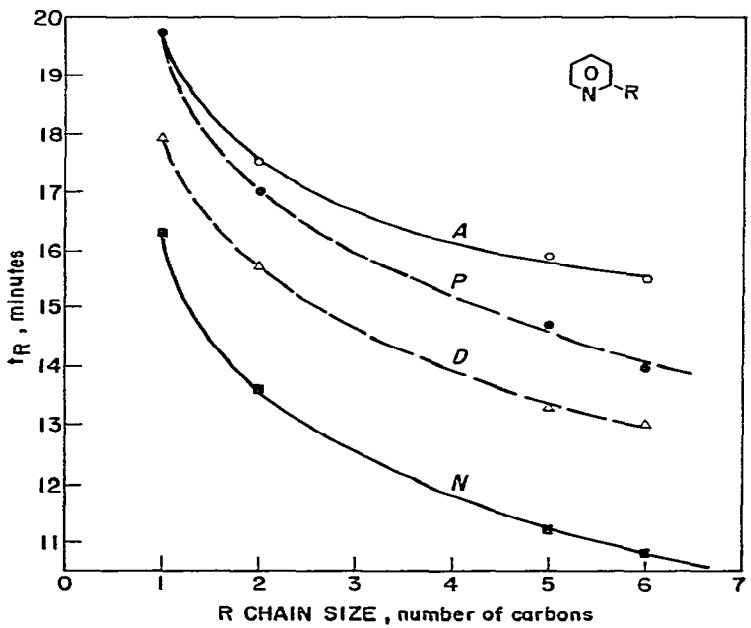


Fig. 1. Effect of alkyl chain size on retention of 2-alkylated pyridines. Data for compound numbers 144, 146, 152 and 157 (Table III) were used for R = 1, 2, 5 and 6, respectively.

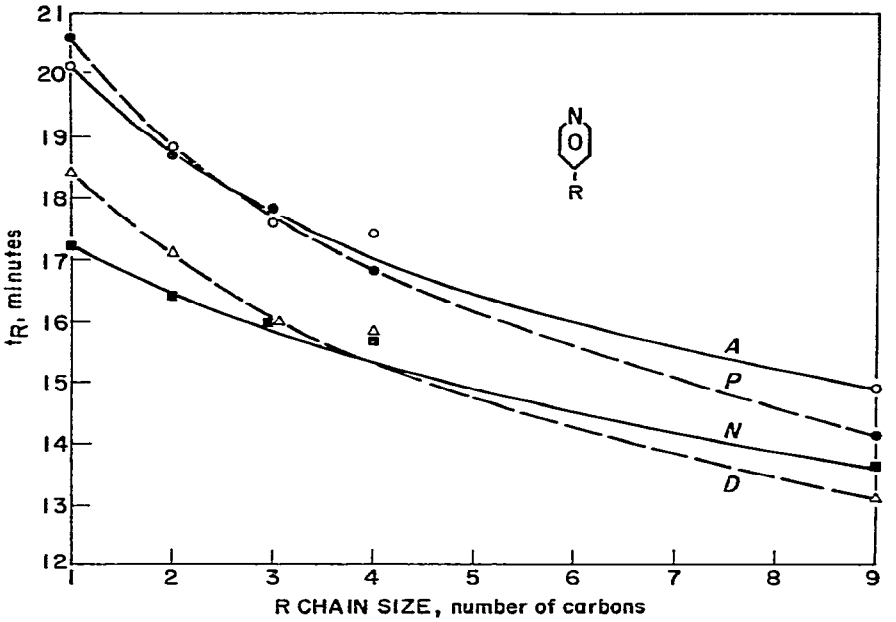


Fig. 2. Effect of alkyl chain size on retention of 4-alkylated pyridines. Data for compound numbers 141, 151, 153, 156 and 158 (Table III) were used for R = 1, 2, 3, 4 and 9, respectively.

TABLE VI
COMPARISON OF RETENTION WITH A MOBILE PHASE CONTAINING DECANOIC ACID WITH THAT OF A DILUTE ACETIC ACID SYSTEM —GRADIENT 1
Standard deviation of retention times is ±1%.

Compound number	*Compound name*	t_R *(min)*	
		Acetic acid (0.0080 M)	*Decanoic acid (0.0268 M)*
1	Unretained compound	2.50	2.60
19	3-Methylindole	11.6	11.4
20	Carbazole	11.7	11.8
22	Indole	12.4	12.7
24	13H-Dibenzo[*a*,*i*]carbazole	13.0	13.0
26	N-Phenyl-1-naphthylamine	8.84	8.76
27	Diphenylamine	9.54	9.38
29	N-Phenylbenzylamine	13.1	12.8
30	Benzophenone	14.4	14.4
47	*o*-Cresol	17.9	18.3
48	2,4,5-Trimethylphenol	17.9	18.3
51	*m*-Ethylphenol	20.8	21.6
55	Phenol	21.6	22.8
57	2-Naphthol	22.0	22.9
59	1-Fluorenecarboxylic acid	25.3	23.7
61	*p*-Toluic acid	28.1	27.3
62	1-Naphthoic acid	26.5	28.7
70	2,6-Dimethylaniline	18.8	19.3
72	6-Aminochrysene	20.3	21.5
75	2-Aminoanthracene	22.2	22.7
79	Aniline	23.5	26.3
81	N-Methylaniline	21.6	22.7
86	N,N-Dimethylaniline	23.1	25.0

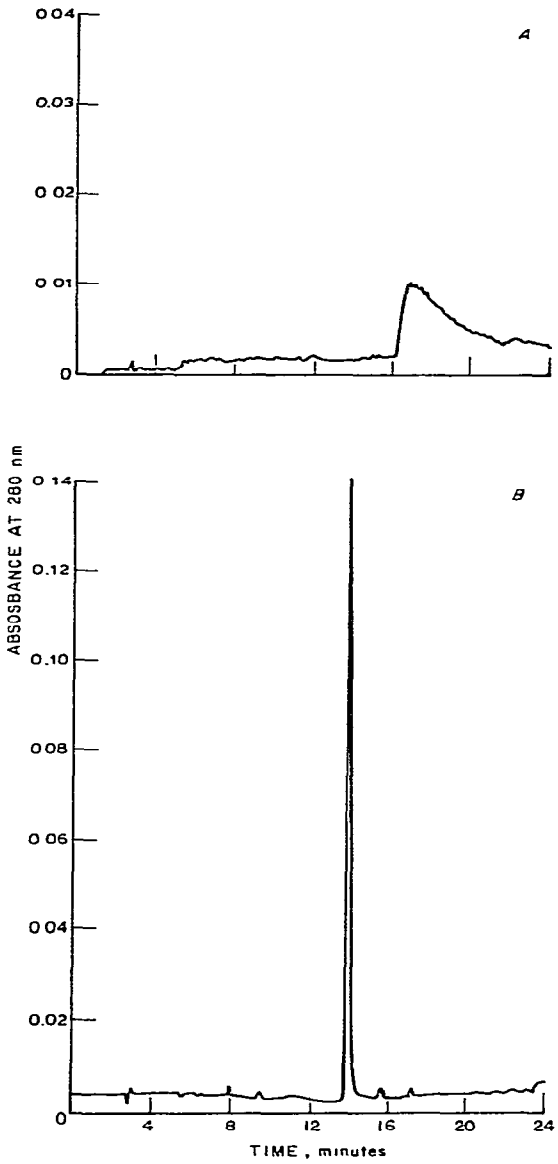


Fig. 3. Chromatograms of *o*-ethoxyphenol, gradient 1. A, no acid; B, decanoic acid added to mobile phase.

tographic efficiency of separations of most solutes is considerably improved via addition of carboxylic acids to mobile phases. This effect is illustrated in Fig. 3 for an acidic compound, *o*-ethoxyphenol. Fig. 4 shows chromatograms of a series of basic compounds. A rapid visual comparison confirms the superiority of either Fig. 4A or B over Fig. 4C, the system without acid. Also, careful examination of Fig. 4A and B reveals slightly improved peak shape (but, of course, less retention) in the chromatogram (B) where decanoic acid was used. This was generally observed to be the case,

especially for acidic solutes. The most obvious explanation for the superiority of the longer chain acid would be that it better protects or "blocks" SiO_2 sites which cause peak tailing. However, it could also be due to a kinetic effect, with the stronger acid (acetic) interacting at slower rates with solutes and thus resulting in broader peaks.

Fig. 5 summarizes the retention data by indicating the approximate retention times for functional group types investigated here. Retention data for the propanoic acid-containing systems are shown because they represent an approximate average be-

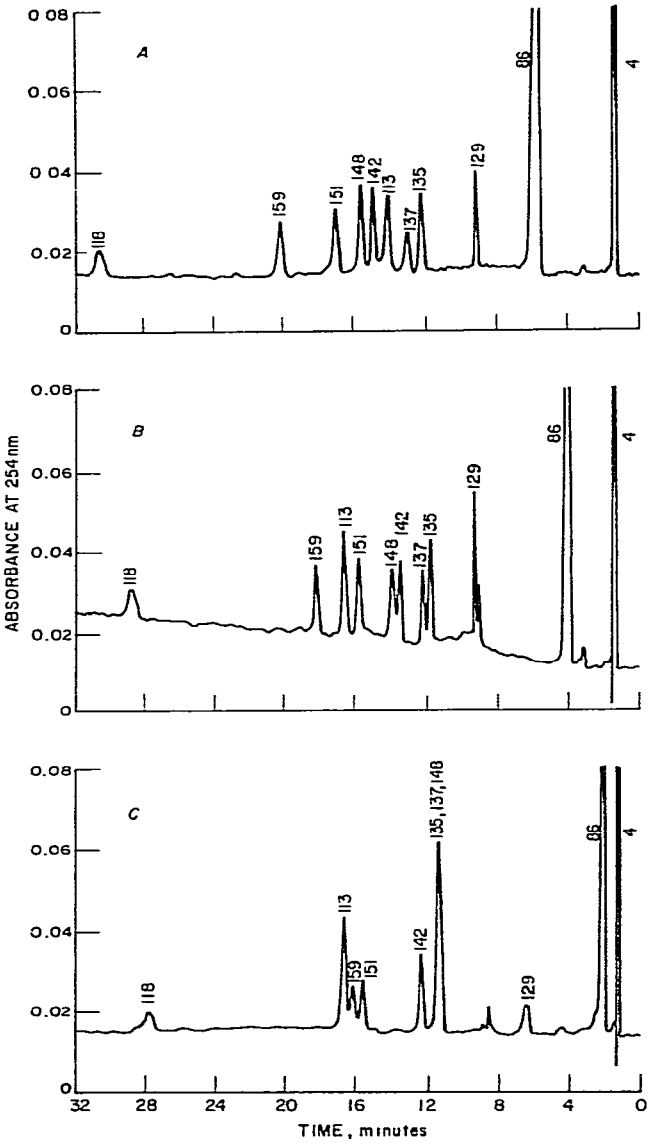


Fig. 4. Chromatograms of N-containing compounds, gradient 2. A, acetic acid; B, decanoic acid; C, no acid added to mobile phase. Compound numbers refer to those in Table III. Chromatogram obtained with propanoic acid was very similar to A.

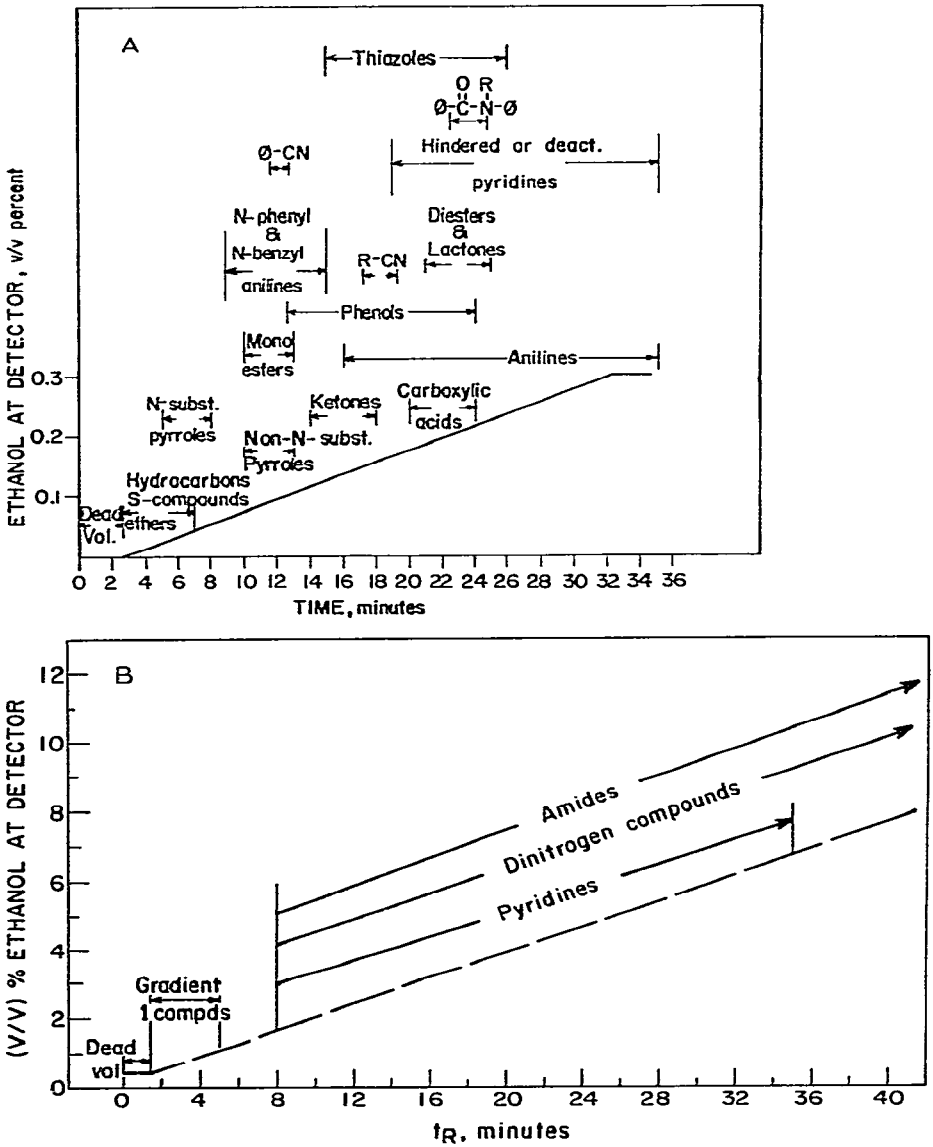


Fig. 5. Approximate retention regions for compound classes eluting with propanoic acid-containing mobile phase systems. A, gradient 1; B, gradient 2.

tween the acetic and decanoic acid data sets. As indicated in Table IV, an analogous diagram for the no-acid series of data would differ most from Fig. 5 in the retentions of carboxylic acid, aniline, and pyridine functionalities.

DISCUSSION

Retention mechanism

Classically, the retention mechanism appropriate to separations on silica gel is

thought to involve direct competition of the solute and solvent for active adsorption sites on the SiO_2 surface[7,8]. However, recent work by Scott and Kucera[9,10] has indicated that the classical mechanism does not hold in cases where the mobile phase contains solvents of high polarity. They proposed two similar mechanisms in which solutes interacted with one or more layers of polar solvent molecules adsorbed onto the silica surface.

The results obtained here are totally incompatible with a retention mechanism involving direct competition of solutes and polar solvents for active sites on silica. For example, according to traditional thinking, a polar mobile phase component such as acetic acid should displace all solutes toward decreased retention, when in fact it was observed to substantially increase retention of basic solutes.

The mechanism most consistent with the results presented here is one analogous to those of Scott and Kucera. Thus, the silica surface is probably coated with the two most polar components of the mobile phase system: ethanol and the carboxylic acid. Solutes in turn interact with these adsorbed mobile phase molecules. Of course, the concentration of the alcohol and acid in the liquid phase as well as their inherent adsorption energies determine the proportion of active sites on the silica occupied by each. A secondary solvent layer made up of less strongly bound mobile phase molecules is also possible[10]. In this case, solutes displace components in the secondary layer in order to interact with the innermost, silica-bound layer.

Snyder's work[8] shows that the adsorption energy for carboxylic acids is substantially higher than that for alcohols on silica. Hence, except possibly for active sites which are sterically unsuitable, the silica surface should be largely coated with carboxylic acids in the two systems used in this work. Only in the latter stages of gradient 2 would one expect significant primary adsorption of ethanol. Therefore, the probable effect of increasing the concentration of ethanol during gradient operation was to more effectively displace solutes bound to the adsorbed carboxylic acid layer and cause them to elute in more timely fashion.

The obvious question arises: if the silica is coated with carboxylic acid molecules, then why is the effect on retention not greater than that observed for the wide variety of compounds studied? First, ethanol (the assumed silica-bound mobile phase component in the absence of added carboxylic acid), silica, and carboxylic acids all have hydroxyl groups which probably interact similarly with most solutes. Furthermore, silica coated with the more acidic carboxylic acid group would logically give rise to the observed increased retention of basic solutes and slightly ($\approx 10\%$) reduced retention of acidic and neutral solutes. Second, the retention order of different chemical classes of solutes is often not greatly different from that of the parent silica even on covalently derivatized silica. For example, Gilpin and Sisco[11] observed nearly the same elution order of hydrocarbons, phenols, and anilines on *n*-butyl-, 2-carbomethoxyethyl- and 3-cyanopropyl-modified silica as on the parent silica. Of course, they observed changes in capacity factors for the different solutes, but the changes were of similar magnitude to those reported here.

The observed increase in retention of basic solutes with shorter chain length carboxylic acids can be explained by the effect of chain length on the acidity of the COOH group (Table VII) and by steric considerations. Similarly, the slight decrease in retention of acidic and neutral solutes with decreasing acid chain length is likely due to the same factors.

TABLE VII
VARIATION OF NORMAL CHAIN CARBOXYLIC ACID ACIDITY WITH CHAIN LENGTH
After ref. 5.

Acid	pK_a
Formic	3.77
Acetic	4.76
Propanoic	4.88
Nonanoic	4.95

Other work has been reported which probably involved retention mechanisms similar to the one above. For example, Ghaemi and Wall[12] found that adding quaternary ammonium surfactants to water–methanol mobile phases produced separations on plain silica equivalent to those of conventionally bonded reversed-phase columns. Similarly, charge transfer agents have been used to effect hydrocarbon-type separations of fuel samples on silica and alumina[13]. Also, separations of sugars on silica coated *in situ* with polyamines are similar to those on covalently bonded amine columns[14]. Finally, Guillemin[15] has reported several separations on silica using mobile phases containing acids and bases and also a separation of phenol and *m*-cresol on silica using pure water as the mobile phase.

Exceptional molecules

The reader may have noticed upon scanning Tables II and III that some molecules eluted much differently than most of those of the same chemical type. A good example is 2,4,6-tri-*tert.*-butylphenol (compound No. 41) which elutes much earlier than other phenols. In general, unusually hindered molecules will elute much earlier than the majority in their chemical class.

Molecules capable of intramolecular hydrogen bonding will also elute earlier, for example 2-quinolinethiol (No. 91) and *o*-ethoxyphenol (No. 43). Tautomeric molecules usually elute as the major tautomer would be expected to unless the minor one has much different retention characteristics. For example, 2-hydroxyquinoline (No. 95) is retained similarly to members of the amide family.

Some classes of compounds have wide variability of retention due to inherent chemical properties. A good example is the dinitrogen "class". As a group they are not especially basic, and hence are not usually retained more than the pyridine class. However, imidazole has an exceptionally high pK_a of nearly 7 (ref. 4), which is reflected in the strong retention of benzimidazole (No. 119) and 4,5-diphenylimidazole (No. 118). On the other hand, phenazine has a very low basicity[4], and dibenzo[*a,c*]phenazine (No. 88) must be extremely low as evidenced by its surprisingly early elution. Finally, molecules capable of chelation [*e.g.* 1,10-phenanthroline (No. 123)] are not eluted at all.

Problems and advantages of solvent systems containing carboxylic acids

The most obvious disadvantages of adding carboxylic acids to the mobile phase are the problems in purification of commercially available acids and the nuisance of adding them to bulk solvents. The first problem of reagent purity is decreased somewhat by the fairly recent availability of a commercial HPLC-grade acetic acid (J.

T. Baker, Phillipsburgh, NJ, U.S.A.). Purification of other acids according to the procedures described here will yield good quality products but is time consuming. Even reagent grade acids contain considerable amounts of impurities which cause deactivation of silica columns and have high UV absorptivities.

For preparative work, presence of acid in the fractions can sometimes be a problem. However, often acids are conveniently removed from non-polar solvents as ammonium salts.

Finally, since carboxylic acids do cause some components to elute earlier and in much sharper bands, more scrupulous purification of the bulk solvents is often needed to reduce detector background.

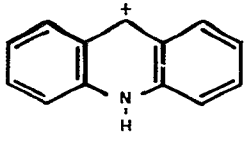
To offset the above disadvantages, use of carboxylic acids yields better resolution, peak symmetry, selectivity, chromatographic stability and column-to-column reproducibility in many cases. The first three advantages are discussed in the Results section. Chromatographic stability is enhanced by addition of acids because the effects of trace acidic or basic components in solvents which ordinarily cause gradual change in solute retention as they buildup on the column are largely swamped by the relatively large concentration of added acid.

Similarly, column-to-column reproducibility is improved by the "acid-coating" of the silica surface which is largely dependent on the mobile phase composition. Thus, different brands of packing give very similar separations, taking into account differences in surface area and pore size.

General comments

Although no data were presented in the Results section on the effect of acids on the UV response of various solutes, sometimes a slight effect was observed. The usual effect of the carboxylic acid was to increase the ratio of the response at lower wavelengths (*e.g.* 254 nm) to that at higher wavelengths (*e.g.* 280 nm). No systematic shift was observed with compound class or other variables, and it usually did not exceed +20% for 254/280 ratios. The effect of added acid on other detectors was not investigated.

As indicated in the Results section, the only commonly available measure of basicity is pK_a (H_2O). Since the solvent plays a strong role in properties such as basicity, use of aqueous constants in all probability contributed to the scatter of data in the pK_a *vs.* t_R correlations in Table V. An extreme example of this effect is the abnormally high pK_a of acridine compared to other benzoquinolines resulting from resonance stabilization from the paraquinoidal cation[4]:



which would be expected to make a more important contribution in an aqueous solvent than in non-aqueous systems. A total of four exceptionally high or low pK_a molecules including acridine were excluded from the correlations in Table V.

As shown in Fig. 5, considerable overlap of many functionalities would exist in a single separation of a complex sample performed on silica. Combination of this

technique with other types to obtain more discrete fractionation will be discussed in subsequent publications. However, a single separation analogous to the ones described here would rapidly give one an idea of the functional types present and of the complexity of the sample. Such information may be particularly useful in monitoring processes such as fuel refining. The chain length and concentration of carboxylic acid added to the mobile phase can be optimized for a given sample type to provide maximum resolution and best peak shape. Use of acids with chains longer than decanoic acid or use of branched chain acids may provide advantages for some applications and could be an area of future investigation.

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