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# LIQUID CHROMATOGRAPHY ON SILICA USING MOBILE PHASES CONTAINING TETRAALKYLAMMONIUM HYDROXIDES

## I. GENERAL SEPARATION SELECTIVITY AND BEHAVIOR OF TYPICAL POLAR COMPOUNDS IN FUELS

J. B. GREEN
*National Institute for Petroleum and Energy Research, P. O. Box 2128, Bartlesville, OK 74005 (U.S.A.)*
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SUMMARY

The known selectivity of base-modified silicas for acidic solutes led to development of the *in situ* tetraalkylammonium hydroxide (TAAH)-modified silica high-performance liquid chromatographic systems described here. These systems are generated by adding small concentrations of TAAH to normal phase eluents pumped through conventional silica columns. The *in situ* approach has the following advantages: the type and concentration of TAAH can be varied to control separation behavior and selectivity; greater column-to-column consistency and separation reproducibility is achieved; commercially available columns and packings are used; and the TAAH in the mobile phase also serves as an electrolyte for electrochemical detection of solutes, if desired. Retention of 180 solutes spanning several classes of compounds is measured in four different systems; the applicability of this approach to separation of acid concentrates from fuels as well as its compatibility with electrochemical detectors is demonstrated; and in addition, general chromatographic parameters such as the effect of the mobile phase composition on retention and selectivity are discussed.

## INTRODUCTION

During prior work with separations of fossil fuel liquids into acid, base and neutral concentrates[1], a need arose for a general method for separating acidic compounds like those found in fossil fuels according to compound class. Most of the available methods suffered from one or more of the following problems: use of out-of-date equipment and experimental methodology, applicability to only a narrow range of sample types, or too narrow a scope such as isolation of only one or two types of acidic compounds potentially in fuels. Based on this survey of existing methods[2], it was concluded that the most promising general liquid chromatographic (LC) method for subfractionating acidic classes was use of strongly basic stationary phases formed from the reaction of alkali-metal[3,4] or tetraalkylammonium[4] hydrox-

ides (TAAH) with silica in conjunction with a normal phase eluent.

The base-treated silica approach has a number of potential advantages including: (1) good correlation between solute acidity and solute retention[4]; (2) compatibility with fairly polar eluents[2–4]; (3) minimal sample losses from irreversible retention[3]; and (4) use of a packing material (silica) sufficiently low in cost to allow construction of relatively inexpensive preparative scale columns. Correlation of retention with acidity is of fundamental importance to successful fractionation of acidic classes in fuels because each major class present —*e.g.*, pyrrolic benzologs, amides, hydroxyaromatics and carboxylic acids— has a fairly narrow range of individual class-member acidities. Hence, a separation method with a good compound acidity–retention correlation has a high probability of having a good compound class–retention correlation. Secondly, the method should utilize a mobile phase with sufficient polarity to have good dissolution properties for acids in real fuel samples. One has only to attempt to apply one of several published high-performance liquid chromatographic (HPLC) methods utilizing predominantly *n*-alkane eluents[5–7] to separation of high boiling acid concentrates from either synfuels or petroleum to quickly realize the importance of eluent dissolution power; all but the lowest molecular weight members of each major acid class are largely insoluble in alkane solvents. The third point, low sample loss from irreversible adsorption, is the major fault of alumina, which has been used extensively to separate acids and other classes in fuels[8–12]. Stronger acids such as carboxylic acids must be preseparated or they are not recovered[8,12,13]. The final point, low cost, is especially important when separating nondistillable acids which are high in metals, particles and other contaminants which could potentially shorten column life.

Preliminary work[2] with alkali-metal and TAAH-treated silicas, other amine-silica systems, as well as commercial amino- and cyano-bonded-phase columns clearly, showed the superiority of TAAH-silica systems for compound class separations of acidic solutes typically found in fuels. In the interest of obtaining the most stable and flexible HPLC separation possible, TAAHs were added directly to mobile phases for dynamic coating of the silica instead of pretreatment as had been done previously[3,4]. Addition of TAAH to the mobile phase also gives the chromatographer greater ability to adjust and control separation selectivity and supplies an electrolyte enabling electrochemical detection (ED) of solutes, if desired.

Although they appear similar at first glance, the nonaqueous normal phase systems studied here are significantly different than previously described reversed-phase ion-pair systems utilizing tetraalkylammonium salts as pairing ions[14–17]. In those systems, anionic solutes interact with adsorbed tetraalkylammonium ions at the mobile phase–stationary phase interface[18,19]. Conversely, in the systems discussed here, un-ionized solutes in the nonaqueous mobile phase interact directly with the base-modified silica surface. Thus, a major difference in the two types of systems is the presence of an aqueous–nonpolar interface containing ionized species in the cited methods as opposed to the current system which is more analogous to normal liquid–solid chromatography.

The chromatographic behavior of four systems —one without TAAH in the mobile phase, two spiked with tetramethylammonium hydroxide (TMAH) and one containing tetrabutylammonium hydroxide (TBAH)— was investigated in detail. The individual systems discussed are optimized for application to fuel acids, but the gen-

eral method should be applicable to other sample types as well. The feasibility of amperometric detection of phenols in a TBAH-spiked eluent is demonstrated as well as potential extension of the method to separations of chlorophenols and other acids not found in fuels.

## EXPERIMENTAL

### *Apparatus*

All separations were carried out with a Waters Assoc. (Milford, MA, U.S.A.) HPLC system comprised of three M6000 pumps, an M680 gradient programmer, WISP autoinjector, M440 dual-wavelength UV detector and M730 peak integrator. Columns were 23 cm × 4.6 mm I.D. and were packed with Dupont (Wilmington, DE, U.S.A.) 7–8 μm silica after the procedure of Coq *et al.*[20]. In addition, an IBM (Danbury, CT, U.S.A.) M230 amperometric detector with a wall jet flow-cell was used during a brief evaluation of the feasibility of ED of phenols in TBAH-spiked mobile phases. An oxidizing potential of +0.4 V *vs* $Ag/AgNO_3$ (acetonitrile) was applied to the wall jet cell in all experiments.

### *Materials*

Stock methanolic solutions of TMAH and TBAH were purchased from either Alfa (Danvers, MA, U.S.A.) or Aldrich (Milwaukee, WI, U.S.A.). Results from gas chromatographic determination of water in these commercial stock solutions typically ranged from 5 to 15%. Hence, these solutions were not handled with special precautions often employed during use of strictly anhydrous substances.

Bulk mobile phase components were HPLC grade (Fisher Scientific, Pittsburgh, PA, U.S.A.) and used without further purification. Chromatographic solutes were obtained from a variety of sources and used without further purification.

### *Procedures*

Four chromatographic systems were extensively investigated by measuring

TABLE I

GRADIENT PROGRAM FOR THE HPLC SYSTEM CONTAINING NO TETRAALKYLAMMONIUM HYDROXIDES

A, MTBE (methyl *tert.*-butyl ether); B, 60% (v/v) MTBE–40% (v/v) methanol.

| *Time (min)* | *Volume percent** | |
|---|---|---|
| | *A* | *B* |
| 0 | 98 | 2 |
| 2 | 98 | 2 |
| 11 | 94 | 6 |
| 35 | 50 | 50 |
| 49 | 0 | 100 |
| 50 | 0 | 100 |
| 53 | 98 | 2 |
| 70 | 98 | 2 |

* All gradient segments were linear.

TABLE II

GRADIENT PROGRAM FOR MTBE-BASED HPLC SYSTEM CONTAINING TETRAMETHYLAMMONIUM HYDROXIDE

A, MTBE; B, 60% (v/v) MTBE–40% (v/v) methanol–$1.5 \cdot 10^{-3}$ *M* TMAH*.

| *Time (min)* | *Volume percent*** | |
|---|---|---|
| | *A* | *B* |
| 0 | 98 | 2 |
| 2 | 98 | 2 |
| 11 | 94 | 6 |
| 35 | 50 | 50 |
| 49 | 0 | 100 |
| 50 | 0 | 100 |
| 53 | 98 | 2 |
| 70 | 98 | 2 |

* This solution was prepared by adding 3.00 ml of stock TMAH–methanol (20:80) to 4 l of MTBE–methanol (60:40).

** All gradient segments were linear.

retentions of a wide variety of compounds as well as separating over 100 fossil fuel samples in each system. Mobile phases and gradients for each system are specified in Tables I–IV; other conditions were: flow-rate, 2.0 ml/min; recorder chart speed, 0.5 cm/min; temperature, ambient; UV detection wavelengths, 254 and 280 nm (except for the chloroform-based system in Table III where 280 and 313 nm were used). A new silica column was used with each system; precolumns and/or guard columns were not used except with the chloroform-based system in Table III. With that system, 7-cm guard columns packed with pellicular silica were replaced daily. Except

TABLE III

GRADIENT PROGRAM FOR CHLOROFORM-BASED HPLC SYSTEM CONTAINING TETRAMETHYLAMMONIUM HYDROXIDE

A, *n*-Hexane; B, 95% (v/v) chloroform–5% (v/v) ethanol; C, $1.0 \cdot 10^{-3}$ *M* TMAH in methanol*.

| *Time (min)* | *Volume percent*** | | |
|---|---|---|---|
| | *A* | *B* | *C* |
| 0 | 70 | 30 | 0 |
| 2 | 70 | 30 | 0 |
| 12 | 0 | 99 | 1 |
| 30 | 0 | 85 | 15 |
| 50 | 0 | 50 | 50 |
| 51 | 0 | 50 | 50 |
| 52 | 0 | 100 | 0 |
| 54 | 70 | 30 | 0 |
| 67 | 70 | 30 | 0 |

* This solution was prepared by adding 2.00 ml of stock TMAH–methanol (20:80) to 4 l of methanol.

** All gradient segments were linear.

TABLE IV

GRADIENT PROGRAM FOR MTBE-BASED HPLC SYSTEM CONTAINING TETRABUTYLAMMONIUM HYDROXIDE

A, MTBE; B, 60% (v/v) MTBE–40% (v/v) methanol–$1.5 \cdot 10^{-3}$ *M* TBAH*.

| *Time (min)* | *Volume percent*** | |
|---|---|---|
| | *A* | *B* |
| 0 | 97 | 3 |
| 2 | 97 | 3 |
| 11 | 90 | 10 |
| 35 | 50 | 50 |
| 49 | 0 | 100 |
| 50 | 0 | 100 |
| 53 | 97 | 3 |
| 70 | 97 | 3 |

* This solution was prepared by adding 6.00 ml of stock TBAH–methanol (25:75) to 4 l of MTBE–methanol (60:40).

** All gradient segments were linear.

for the chloroform-based system, column performance after several hundred injections of both pure compounds and actual fuel samples was essentially unchanged. With the chloroform-containing system, the column's performance had significantly deteriorated after evaluation of that system. Guard columns from the latter system usually showed the presence of a yellow material which may have been a reaction product between chloroform and TMAH.

The concentration of TAAH in the mobile phase at a given point in a gradient program was determined by (1) the level added to the second or third solvent used in the gradient system, (2) the relative ratios of the two or three solvents used at that point in the program, and (3) by the distribution coefficient of the TAAH between silica and the particular mobile phase composition corresponding to that point in the gradient. Thus, TAAH concentration was dynamic and controlled ultimately by actual gradient conditions.

Sets of retention data of standard compounds in each chromatographic system were obtained as follows. 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. One of the blends was rerun at the end of the series to check for significant changes in column retention characteristics.

Fossil fuel samples used to characterize each of the four HPLC systems were largely acid, base, or neutral concentrates of coal liquid, shale oil, or petroleum liquids isolated with published methods[1]. Because fuels contain a much larger num-

ber of compounds than could be practically tested on an individual compound basis, they represent excellent "test mixtures" for a given chromatographic method. The utility of this approach was extended further by separating fuels of widely varying type and boiling range. Results from fuel separations were then correlated with retention data of pure compounds for an overall evaluation of each of the chromatographic systems investigated.

## RESULTS AND DISCUSSION

Table V shows retentions of standard compounds in the four chromatographic systems studied. Given the rather large volume of data in the table, a brief summary along with salient conclusions is given below.

### *Neutral and basic compounds*

Neutral and basic compound classes without an active hydrogen eluted essentially unretained in all four systems. However, some of the more condensed-ring aromatic hydrocarbons (PAHs) and azaaromatics were retained slightly in the TAAH-spiked systems. Basic compounds with active hydrogens, such as primary and secondary arylamines, exhibited significant retention in the TAAH-spiked systems whenever the amino group was attached to a condensed aromatic nucleus. This behavior is consistent with the increased hydrogen-donor capability of condensed-ring amino-PAHs thereby producing a stronger interaction with the base-treated silicas. As expected, the presence of an electron withdrawing functionality in active-hydrogen compounds served to further increase their retention.

### *Acidic compounds*

In the TAAH-containing systems the acidic classes investigated generally eluted in the order of their inherent acidity: pyrrolic benzologs $\simeq$ alcohols $<$ amides $<$ hydroxyaromatics $<$ carboxylic acids $\leqslant$ difunctional acids $<$ trifunctional or chelating acids. This retention order is again consistent with the ability of the respective classes to donate a hydrogen(s) to the basic TAAH-silica surface. Carboxylic acids were the only compound class which showed even approximately equivalent retention in the TAAH-free LC system. Virtually all other acids were essentially unretained, thus showing the profound effect of the TAAH in the mobile phase of the other systems. Within a given class, structural features which increased acidity also increased retention and *vice versa*. For example, alkylation, especially in *ortho*- and *para*-positions, decreased retention whereas greater aromaticity, *e.g.* naphthol *vs.* phenol, increased retention in TAAH-spiked systems.

Amides showed the most complex pattern of retention of any class, primarily because they have the greatest number of possible structural variations. For example, there are N,N-disubstituted, N-substituted and unsubstituted amides, as well as aliphatic, aromatic, linear, cyclic, sulfur-containing analogs, etc. This structural complexity causes amides as a group to cover a wide range of acidities and hence potentially to overlap with virtually all other compound classes. A wide range of amide retentions was observed in all four LC systems.

The only acid types poorly eluted or not eluted at all in any system were multifunctional compounds capable of a chelation type of interaction with the silica

TABLE V

COMPARATIVE RETENTION TIMES ($t_R$) OF STANDARD ACIDIC, BASIC, AND NEUTRAL COMPOUNDS IN HPLC SYSTEMS I–IV*

| *Compound number* | *Compound name* | $t_R$ *(min)* | | | |
|---|---|---|---|---|---|
| | | *I No added base* | *II TMAH–MTBE* | *III TMAH–chloroform* | *IV TBAH–MTBE* |
| *Hydrocarbons, ethers, thiophenes* | | | | | |
| 1 | Benzene | 1.65 | 1.73 | ND** | 1.96 |
| 2 | 1,2,3,4-Tetraphenylnaphthalene | 1.70 | 1.63 | 2.16 | 1.73 |
| 3 | Fluoranthene | 1.73 | 1.86 | 2.40 | 2.10 |
| 4 | Chrysene | 1.73 | 1.83 | 2.26 | 2.10 |
| 5 | Benzo[*a*]pyrene | 1.76 | 1.96 | 2.36 | 2.26 |
| 6 | Benzo[*ghi*]perylene | 1.73 | 1.83 | 2.26 | 2.63 |
| 7 | Coronene | 1.76 | 2.16 | 2.26 | 2.83 |
| 8 | Dibenzofuran | 1.73 | 1.70 | 2.20 | 1.83 |
| 9 | 3-Methylbenzo[*b*]thiophene | 1.83 | 1.70 | 2.46 | 1.80 |
| 10 | Dinaphtho[2,3-*b*:2′,3′-*d*]thiophene | 1.83 | 1.70 | 2.46 | 2.63 |
| *Miscellaneous* | | | | | |
| 11 | Thianthrene | 1.66 | 1.60 | 2.10 | 1.93 |
| 12 | Benzonitrile | 1.93 | 2.60 | 2.56 | 2.66 |
| 13 | 4-Phenoxybutyronitrile | 1.80 | 2.56 | 2.76 | 2.56 |
| 14 | 9-Nitroanthracene | 1.70 | 2.13 | 2.73 | 2.56 |
| *Azaaromatics* | | | | | |
| 15 | 2-Methylpyridine | 3.20 | 1.63 | 2.10 | 2.10 |
| 16 | 3-Methylpyridine | 3.56 | 1.63 | 2.10 | 2.06 |
| 17 | 2-*n*-Amylpyridine | 2.15 | 1.80 | 2.53 | 2.03 |
| 18 | Quinoline | 2.83 | 1.80 | 2.63 | 2.53 |
| 19 | Isoquinoline | 3.23 | 2.03 | 2.70 | 2.53 |
| 20 | 3-Methylisoquinoline | 2.93 | 1.80 | 2.63 | 2.33 |
| 21 | 2,6-Dimethylquinoline | 2.50 | 1.76 | 2.10 | 2.10 |
| 22 | 4-Azafluorene | 2.50 | 2.03 | 2.70 | 2.50 |
| 23 | Acridine | 2.30 | 2.06 | 2.70 | 2.53 |
| 24 | 7,8-Benzoquinoline | 1.83 | 1.93 | 2.46 | 2.23 |
| 25 | 2-Methylacridine | 2.30 | 2.03 | 2.70 | 2.53 |
| 26 | 2-Azafluoranthene | 2.46 | 2.03 | 2.70 | 2.53 |
| 27 | 1-Azapyrene | 2.66 | 2.06 | 2.70 | 2.50 |
| 28 | 2-Methoxypyridine | 1.83 | 1.70 | 2.46 | 1.80 |
| 29 | 4-Chloroquinoline | 2.30 | 1.73 | 2.36 | 1.96 |
| 30 | 2-Methylpyrazine | 2.73 | 1.80 | 2.53 | 2.33 |
| 31 | 2,3,5,6-Tetramethylpyrazine | 2.73 | 1.63 | 2.10 | 1.80 |
| 32 | 2,2′-Bipyridyl | 2.23 | ND | ND | 2.13 |
| 33 | 1,10-Phenanthroline | ND | 8.43 | ND | 11.6 |
| 34 | 2,2′-Biquinoline | 1.76 | ND | 2.46 | 2.13 |
| 35 | Dibenzo[*a*,*c*]phenazine | 1.73 | 1.83 | 2.26 | 2.10 |
| *Ketones, esters* | | | | | |
| 36 | Benzophenone | 1.73 | 1.70 | 2.20 | 1.83 |
| 37 | Benzanthrone | 1.80 | 2.56 | 2.76 | 3.00 |
| 38 | Methyl benzoate | 1.66 | 1.60 | 2.10 | 1.93 |

*(Continued on p. 60)*

TABLE V *(continued)*

| *Compound number* | *Compound name* | $t_R$ *(min)* | | | |
|---|---|---|---|---|---|
| | | *I No added base* | *II TMAH–MTBE* | *III TMAH–chloroform* | *IV TBAH–MTBE* |
| 39 | Phenyl benzoate | 1.66 | 1.60 | 2.10 | 1.93 |
| 40 | *o*-Tolyl benzoate | 1.66 | 1.60 | 2.10 | 1.93 |
| 41 | Coumarin | 2.10 | 2.73 | 3.73 | 3.23 |
| 42 | Dimethyl phthalate | 1.66 | 2.46 | 2.80 | 2.60 |
| 43 | Diphenyl carbonate | 1.80 | 23.2 | ND | 17.5 |
| *Azoles* | | | | | |
| 44 | Benzothiazole | 1.76 | 1.76 | 2.26 | 1.90 |
| 45 | 2-Methylmercaptobenzothiazole | 1.76 | 1.76 | 2.26 | 1.90 |
| 46 | 2-Methyl[*a*]naphthothiazole | 1.76 | 1.76 | 2.26 | 1.96 |
| 47 | Benzoxazole | 1.76 | 1.76 | 2.26 | 1.90 |
| 48 | Benzimidazole | 9.86 | 30.1 | 24.7 | 28.0 |
| 49 | 2-Phenylbenzimidazole | 2.66 | 22.4 | 19.3 | 19.4 |
| *Diarylamines, triarylamines* | | | | | |
| 50 | Diphenylamine | 1.73 | 2.62 | 3.10 | 2.63 |
| 51 | N-Phenylbenzylamine | 1.73 | 2.13 | 2.26 | 2.20 |
| 52 | N-Phenyl-1-naphthylamine | 1.73 | 2.73 | 3.10 | 2.63 |
| 53 | Triphenylamine | 1.60 | 1.60 | 2.10 | 1.70 |
| 54 | 12*H*-benz[*a*]phenothiazine | 1.76 | 4.50 | 4.80 | 5.13 |
| *Monoarylamines* | | | | | |
| 55 | Aniline | 1.93 | 3.23 | 4.63 | 3.06 |
| 56 | N-Methylaniline | 1.73 | 2.13 | 2.73 | 2.63 |
| 57 | N,N-Dimethylaniline | 1.73 | 1.83 | 2.26 | 1.90 |
| 58 | 2,3-Dimethylaniline | 1.96 | 2.63 | 3.33 | 2.76 |
| 59 | 2,4-Dimethylaniline | 2.00 | 2.63 | 3.33 | 2.76 |
| 60 | 2,5-Dimethylaniline | 1.93 | 2.63 | 3.33 | 2.76 |
| 61 | 2,6-Dimethylaniline | 1.86 | 2.53 | 2.73 | 2.63 |
| 62 | N-Ethylaniline | 1.66 | 1.60 | 2.10 | 1.93 |
| 63 | *o*-Ethylaniline | 1.93 | 2.60 | 3.30 | 2.66 |
| 64 | *p*-Ethylaniline | 2.03 | 2.80 | 3.96 | 2.76 |
| 65 | 2,4,6-Trimethylaniline | 1.86 | 2.14 | 2.40 | 2.33 |
| 66 | N,N-Diethylaniline | 1.70 | 1.63 | 2.16 | 1.73 |
| 67 | 2,6-Diethylaniline | 1.70 | 1.96 | 2.16 | 2.06 |
| 68 | *p*-*n*-Butylaniline | 1.93 | 2.70 | 3.80 | 2.66 |
| 69 | 1,2,3,4-Tetrahydroquinoline | 1.76 | 2.06 | 2.70 | 2.50 |
| 70 | 1-Amino-5,6,7,8-tetrahydronaphthalene | 1.90 | 2.53 | 3.03 | 2.53 |
| 71 | 1-Aminonaphthalene | 1.83 | 4.13 | 6.16 | 4.23 |
| 72 | 2-Aminonaphthalene | ND | ND | 6.66 | 4.46 |
| 73 | 2-Aminoanthracene | 2.03 | 5.40 | 7.96 | 5.93 |
| 74 | 1-Aminopyrene | ND | ND | 9.53 | 8.73 |
| 75 | 3-Aminofluoranthene | ND | ND | 10.40 | 9.95 |
| 76 | 6-Aminochrysene | 2.06 | 7.10 | 8.86 | 8.53 |
| 77 | *p*-Chloroaniline | 1.96 | 3.76 | 6.20 | 3.90 |
| *Alcohols* | | | | | |
| 78 | 1-Indanol | ND | ND | 6.31 | 4.18 |
| 79 | 2-Indanol | ND | ND | 8.45 | 5.36 |

TABLE V *(continued)*

| *Compound number* | *Compound name* | $t_R$ *(min)* | | | |
|---|---|---|---|---|---|
| | | *I No added base* | *II TMAH–MTBE* | *III TMAH–chloroform* | *IV TBAH–MTBE* |
| 80 | 4-Chromanol | 2.03 | 6.71 | 8.72 | 6.72 |
| 81 | 9-Hydroxyfluorene | 1.76 | 6.20 | 8.83 | 5.76 |
| 82 | 1-Acenaphthol | 1.76 | 6.66 | 9.10 | 6.73 |
| *Pyrrolic benzologs* | | | | | |
| 83 | 1-Phenylpyrrole | 1.93 | 1.76 | 2.26 | 1.80 |
| 84 | 2,3,4,5-Tetraphenylpyrrole | 1.80 | 2.56 | 2.76 | 2.20 |
| 85 | Indole | 1.76 | 8.10 | 13.6 | 8.43 |
| 86 | 1-Methylindole | 1.73 | 1.83 | 2.26 | 2.20 |
| 87 | 2-Methylindole | 1.70 | 6.26 | 9.60 | 6.76 |
| 88 | 3-Methylindole | 1.73 | 6.06 | 10.6 | 5.93 |
| 89 | 7-Methylindole | 1.73 | 6.30 | 8.83 | 6.73 |
| 90 | 2,3-Dimethylindole | 1.76 | 4.80 | 8.00 | 5.13 |
| 91 | 1,2,3,4-Tetrahydrocarbazole | 1.93 | 4.13 | 6.96 | 4.46 |
| 92 | Carbazole | 1.76 | 7.70 | 12.2 | 8.73 |
| 93 | 2-Methylcarbazole | 1.76 | 6.36 | 10.3 | 7.00 |
| 94 | 9-Methylcarbazole | 1.76 | 1.96 | 2.36 | 2.26 |
| 95 | 9-Ethylcarbazole | 1.73 | 1.83 | 2.26 | 2.10 |
| 96 | 13*H*-Dibenzo[*a*,*i*]carbazole | 1.76 | 9.43 | 12.9 | 10.9 |
| *Amides, lactams* | | | | | |
| 97 | Benzamide | 3.73 | 14.7 | 15.0 | 13.2 |
| 98 | 1-Methyl-2-pyridone | 12.70 | ND | 5.50 | 9.39 |
| 99 | N,N-Diethyl-*m*-toluamide | ND | 2.26 | 2.76 | 2.63 |
| 100 | Acetanilide | 2.86 | 10.5 | 13.6 | 12.8 |
| 101 | Oxindole | 2.46 | 11.3 | 9.63 | 7.93 |
| 102 | 2-Hydroxyquinoline | 5.80 | 18.5 | 13.6 | 13.9 |
| 103 | 3,4-Dihydrocarbostyril | ND | ND | ND | 4.70 |
| 104 | 1-Naphthaleneacetamide | 5.46 | ND | 13.2 | 17.3 |
| 105 | Benzanilide | 1.93 | 5.73 | 7.33 | 6.43 |
| 106 | Thiobenzanilide | 1.83 | 27.5 | 13.6 | 14.4 |
| 107 | 2-Mercaptopyridine | 2.23 | 2.12 | 3.20 | 2.93 |
| 108 | 4-Mercaptopyridine | 7.91 | 39.1 | 4.40 | ND |
| 109 | 3-Methoxy-2(1*H*)-pyridone | 23.6 | 29.0 | 22.9 | 25.1 |
| *Hydroxyaromatics* | | | | | |
| 110 | Phenol | 1.76 | 23.2 | 24.3 | 17.6 |
| 111 | *o*-Cresol | 1.76 | 18.8 | 20.8 | 13.3 |
| 112 | *m*-Cresol | 1.73 | 21.8 | 22.6 | 16.3 |
| 113 | *p*-Cresol | 1.76 | 21.0 | 22.5 | 15.4 |
| 114 | *m*-Ethylphenol | 1.76 | 20.7 | 22.0 | 15.4 |
| 115 | *p*-Ethylphenol | 1.80 | 20.7 | 22.2 | 15.1 |
| 116 | 2,3-Dimethylphenol | 1.76 | 16.8 | 19.2 | 11.6 |
| 117 | 2,4-Dimethylphenol | 1.73 | 16.6 | 18.8 | 11.6 |
| 118 | 2,6-Dimethylphenol | 1.80 | 9.63 | 7.13 | 8.10 |
| 119 | 3,4-Dimethylphenol | 1.76 | 19.7 | 21.1 | 14.4 |
| 120 | 3,5-Dimethylphenol | 1.80 | 20.3 | 21.3 | 14.9 |
| 121 | 2,3,5-Trimethylphenol | 1.80 | 14.5 | 16.7 | 10.7 |

*(Continued on p. 62)*

TABLE V *(continued)*

| *Compound number* | *Compound name* | $t_R$ *(min)* | | | |
|---|---|---|---|---|---|
| | | *I No added base* | *II TMAH–MTBE* | *III TMAH–chloroform* | *IV TBAH–MTBE* |
| 122 | 2,4,5-Trimethylphenol | 1.76 | 14.3 | 16.3 | 10.8 |
| 123 | 3,4,5-Trimethylphenol | 1.80 | 18.7 | 19.8 | 13.4 |
| 124 | 4-*sec.*-Butylphenol | 1.73 | 19.9 | 21.7 | 14.3 |
| 125 | 2-*tert.*-Butyl-4-methylphenol | 1.70 | 8.93 | 15.1 | 7.50 |
| 126 | 2,4-Di-*sec.*-butylphenol | 1.93 | 9.20 | 14.3 | 7.75 |
| 127 | *p*-Nonylphenol | ND | 18.7 | 20.2 | 12.7 |
| 128 | 2,4,6-Tri-*tert.*-butylphenol | 1.80 | 1.66 | 2.76 | 1.86 |
| 129 | 5-Indanol | ND | ND | 20.8 | 14.2 |
| 130 | 1-Naphthol | 1.70 | 27.6 | 27.6 | 22.0 |
| 131 | 2-Naphthol | 1.76 | 28.3 | 26.8 | 23.0 |
| 132 | 2-Methyl-1-naphthol | ND | ND | 21.6 | 17.8 |
| 133 | 2-Phenylphenol | ND | ND | 21.6 | 18.9 |
| 134 | 3-Phenylphenol | ND | ND | 23.8 | 20.2 |
| 135 | 4-Phenylphenol | ND | ND | 24.8 | 21.3 |
| 136 | 2-Benzylphenol | ND | ND | 21.9 | 16.5 |
| 137 | 9-Phenanthrol | ND | ND | ND | 25.1 |
| 138 | *p*-Tritylphenol | 1.76 | 23.0 | 21.5 | 17.7 |
| 139 | *o*-Thiocresol | 1.70 | 35.4 | ND | 30.5 |
| 140 | 4-(Methylthio)-phenol | 1.73 | 27.5 | 25.4 | 21.7 |
| 141 | 4-(Methylthio)-*m*-cresol | 1.73 | 25.3 | 23.7 | 19.9 |
| 142 | *o*-Ethoxyphenol | 1.83 | 17.8 | 7.73 | 11.7 |
| 143 | *o*-Chlorophenol | ND | ND | ND | 28.5 |
| 144 | *m*-Chlorophenol | ND | ND | ND | 24.8 |
| 145 | *p*-Chlorophenol | ND | ND | ND | 23.6 |
| 146 | 2,3-Dichlorophenol | ND | ND | ND | 28.5 |
| 147 | 2,4-Dichloro-1-naphthol | ND | ND | ND | 26.5 |
| 148 | 2,4,5-Trichlorophenol | ND | ND | ND | 26.2 |
| 149 | 2,3,4,5-Tetrachlorophenol | ND | ND | ND | 25.2 |
| 150 | Pentachlorophenol | ND | ND | ND | 24.5 |
| 151 | *p*-Nitrophenol | 9.26 | 37.5 | 33.1 | 29.3 |
| 152 | Resorcinol | ND | ND | 44.1 | 35.4 |
| 153 | 5-*n*-Pentadecylresorcinol | 1.70 | ND | ND | 29.2 |
| 154 | Hydroquinone | 2.23 | 41.1 | ND | 31.2 |
| 155 | 1,3-Dihydroxynaphthalene | 2.83 | 38.7 | 38.9 | 35.6 |
| 156 | 1,7-Dihydroxynaphthalene | 2.23 | 48.6 | ND | 38.2 |
| 157 | 2,3-Dihydroxynaphthalene | ND | NE*** | NE | NE |
| 158 | 2,2'-Dihydroxybiphenyl | 21.0 | 38.7 | 40.8 | 30.3 |
| 159 | Pyrogallol | NE | NE | NE | NE |
| *Carboxylic acids* | | | | | |
| 160 | 2,2-Diphenylpropanoic acid | 29.4 | 35.7 | 36.0 | 32.0 |
| 161 | *p*-Toluic acid | 26.5 | 36.2 | 36.0 | 30.8 |
| 162 | 1-Naphthoic acid | 31.7 | 37.8 | 36.0 | 31.7 |
| 163 | 1-Naphthylacetic acid | 35.6 | 36.9 | 36.1 | 32.5 |
| 164 | 1-Fluorenecarboxylic acid | 26.5 | 35.6 | 36.0 | 29.9 |
| 165 | Anthracene-9-carboxylic acid | 37.0 | 38.9 | 34.2 | 32.3 |
| *Difunctionals* | | | | | |
| 166 | 5-Aminoindole | 3.23 | 19.9 | 18.3 | 16.4 |

TABLE V *(continued)*

| *Compound number* | *Compound name* | $t_R$ *(min)* | | | |
|---|---|---|---|---|---|
| | | *I No added base* | *II TMAH–MTBE* | *III TMAH–chloroform* | *IV TBAH–MTBE* |
| 167 | 4-Aminoquinaldine | 31.9 | 10.5 | 14.2 | 13.6 |
| 168 | 2-Amino-9-hydroxyfluorene | 2.66 | 21.5 | 19.0 | 17.8 |
| 169 | Harmane | 6.66 | 12.9 | 15.3 | 15.5 |
| 170 | 1-Azacarbazole | 2.40 | 5.20 | 7.06 | 8.4 |
| 171 | 5-Hydroxyindole | 3.80 | 45.0 | 27.9 | 38.2 |
| 172 | 5-Hydroxyquinoline | 3.65 | 35.0 | 31.2 | 27.7 |
| 173 | 2-(*o*-Hydroxyphenyl)-benzothiazole | 1.70 | 30.2 | ND | 21.9 |
| 174 | 2-Hydroxycarbazole | 2.66 | 38.3 | 39.3 | 31.7 |
| 175 | 2-Ethanolpyridine | 5.80 | 6.06 | 6.20 | 7.60 |
| 176 | 2,3-Dihydroxypyridine | ND | NE | ND | NE |
| 177 | Phenyl salicylate | 1.96 | 23.2 | 24.2 | 17.5 |
| 178 | Salicylamide | 7.3 | 39.8 | 38.8 | 33.5 |
| 179 | Salicylic acid | ND | 37.5 | 42.8 | 29.0 |
| 180 | Fluorescein | 38.5 | 42.2 | 40.8 | 38.2 |
| 181 | Indole-5-carboxylic acid | 23.8 | 43.2 | 43.8 | 38.6 |

* System numbers refer to Tables specifying respective experimental conditions.
** ND = Not determined.
*** NE = Not eluted.

surface. Examples of this type of compound are 1,2-dihydroxyaromatics and 8-hydroxyquinoline. These compounds are not expected to be present at significant levels in most fuels.

*Comparison of the four LC systems*

A brief glance at Table V shows great differences between the TAAH-containing and the TAAH-free system, but an intercomparison of the three TAAH systems is difficult with only the numerical data in the table. On the other hand, Figs. 1 and 2 provide a convenient means for contrasting the four systems.

Fig. 1 (A1–D1) show chromatograms in each LC system of one of the compound blends used to obtain the data in Table V. This mixture contains representatives of the four major acid classes in petroleum[9,21]: pyrrolic benzologs, amides, hydroxyaromatics and carboxylic acids. Fig. 1 (A2–D2) show traces from separation of an actual petroleum acid concentrate in each of the four LC systems. The following general conclusions may be drawn from Fig. 1.

(1) Patterns in peak shape and retention characteristics of the four systems suggested by the relatively few compounds in the synthetic mixture are borne out very well in the analogous separation of the petroleum acids. For example, retention and hence class separation of hydroxyaromatics and carboxylic acids is best in the methyl *tert.*-butyl ether (MTBE)–TMAH system (Fig. 1B1 and 1B2). Secondly, peak shape and resolution within the carboxylic acid class is best in the MTBE–TBAH system (Fig. 1D1 and 1D2). Finally, as mentioned previously, only carboxylic acids were retained appreciably in the TAAH-free system (Fig. 1A1 and 1A2).

(2) The compositional complexity of the individual acid classes in petroleum

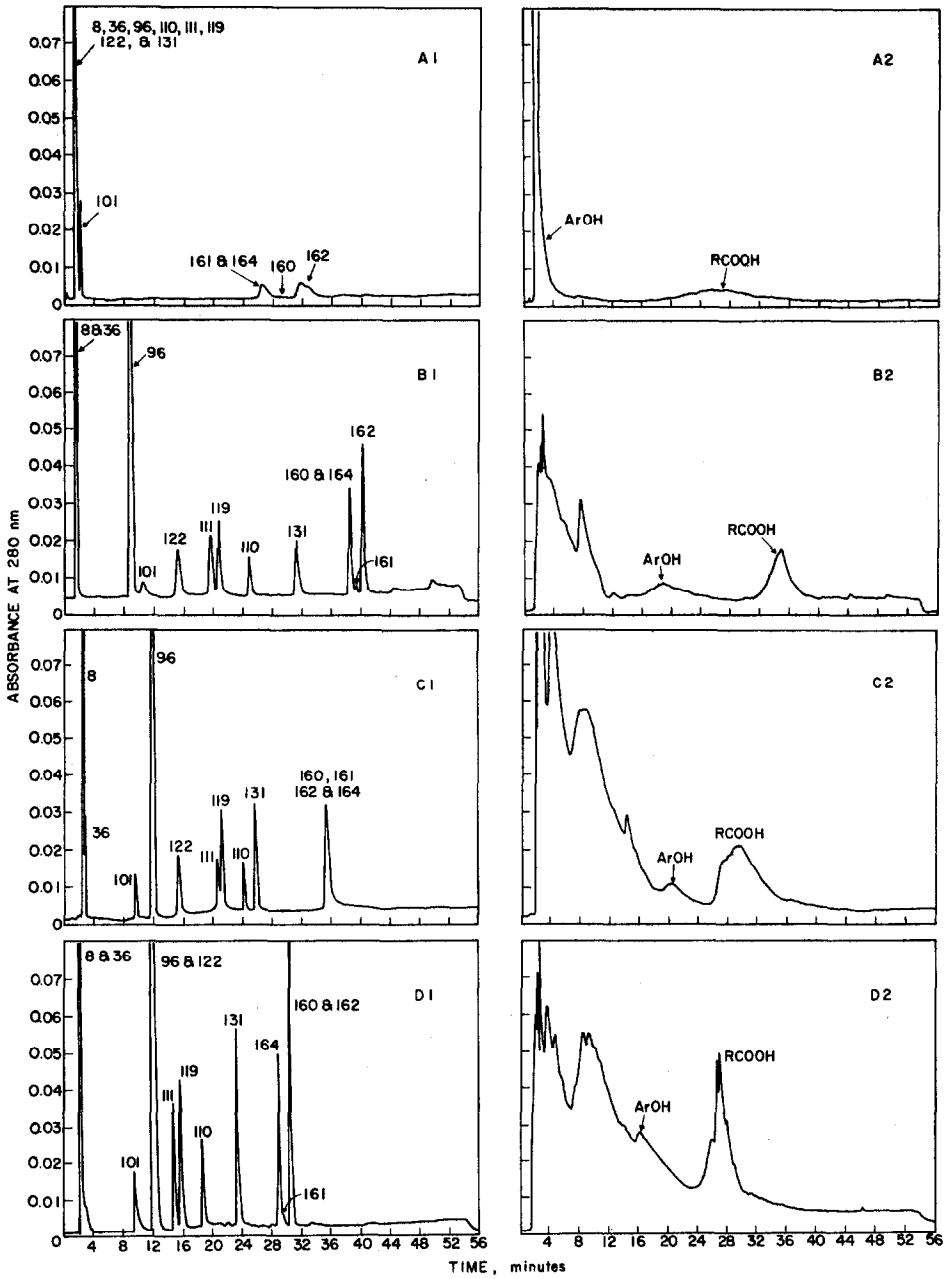


Fig. 1. Separation of a synthetic blend of acidic solutes (A1–D1) and an acid concentrate from a Wilmington 535–675°C heavy distillate (A2–D2) using each of the four HPLC systems in Tables I–IV (A–D, respectively). Compound numbers in A1–D1 refer to Table V. See text for discussion. About 1 μg of each component (A1–D1) and 30 μg (A2, B2) or 60 μg (C2, D2) of each concentrate were injected.

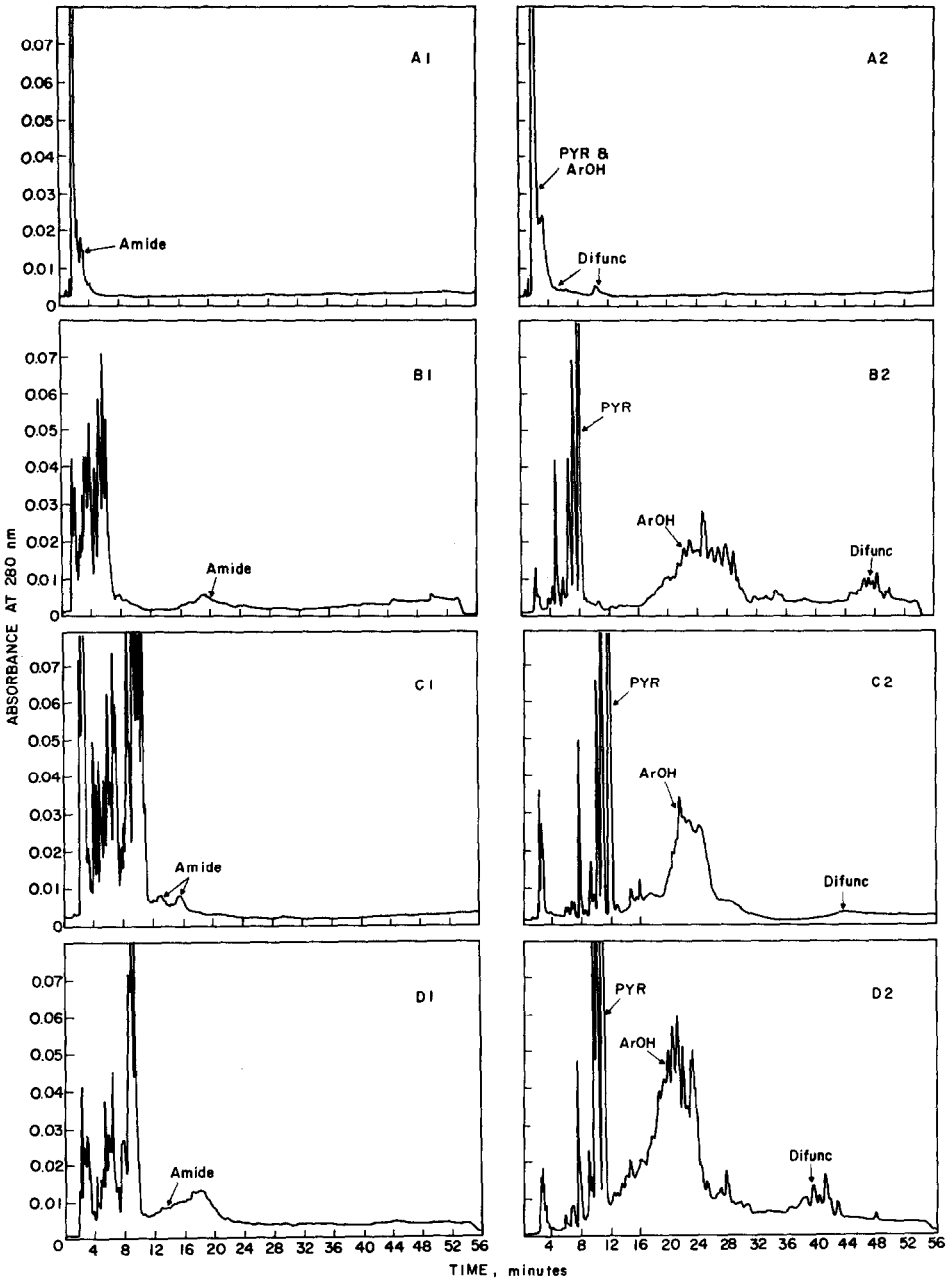


Fig. 2. Separation of SRC-II 325–425°C coal liquid weak (A1–D1) and strong (A2–D2) acid concentrates using each of the four HPLC systems in Tables I–IV (A–D, respectively); PYR = pyrrolic benzologs. See text for discussion. About 25 μg injected in each.

causes each class to appear as a rather featureless "hump". As will be seen later, less complex fuels like coal liquids exhibit considerably more fine structure within each class.

(3) Overlap between the pyrrolic and amide classes is suggested by the rather ambiguous pattern in the early (less than 12 min) portion of the petroleum acid separations. The best resolution of N–H types occurs in Fig. 1C2, the chromatogram obtained with the chloroform–TMAH system. As will be seen later, this system typically performed best in separations of compounds with N–H functionalities.

Chromatograms in Fig. 2 confirm and add to the conclusions drawn from Fig. 1. The eight traces shown are of weak (Fig. 2(A1–D1)) and strong (Fig. 2(A2–D2)) acid concentrates isolated from a 325–425°C distillate from an SRC-II coal liquid. The major acid types present in the weak acids are pyrrolic benzologs, with small amounts of amides and hydroxyaromatics also present[16–18]. The strong acid concentrate contains largely pyrrolic benzologs, hydroxyaromatics and difunctional compounds[22–24]. The following conclusions may be drawn from Fig. 2.

(1) Retention and resolution of pyrrolic benzologs are best in Fig. 2C1 and 2C2 (chloroform–TMAH system) but good in the other TAAH-containing systems.

(2) Retention and resolution of hydroxyaromatics is best in Fig. 2B2 (MTBE–TMAH system), but acceptable in the other TAAH-spiked systems.

(3) Separation of difunctional acids is evident in all TAAH-spiked systems, but peak shape is poor with the chloroform–TMAH system (Fig. 2C2).

(4) No appreciable separation of coal liquid acids is obtained in the system without any TAAH.

(5) Overall, the MTBE–TMAH system provides the best compound class separation of weak and strong SRC-II acids.

The indicated compound type assignments for peaks in separation of Wilmington and SRC-II acid concentrates in Figs. 1 and 2 were initially set from correlations based on Table V. These assignments were later confirmed via preparative scale (1–3 g) separations of these and other acid concentrates followed by analysis of the resulting fractions by infrared spectroscopy, low and high resolution mass spectrometry, gas chromatography and gas chromatography–mass spectrometry. Results from analysis of selected hydroxyaromatic concentrates were recently published[25,26]; reports describing compositions of other fractions are in preparation.

As stated in the Experimental section, over 100 fuel samples were separated in each of the four LC systems as well as the standard blends used to derive the retention data in Table V. Since it is clearly impossible to show all the results in a manner similar to Figs. 1 and 2, an attempt has been made to semiquantitatively rate each system's performance in separating compounds from all the major compound classes investigated. This information is summarized in Table VI. As can be seen by the overall total rating or by the rating for acid classes only, the MTBE–TMAH system (Table II) gives the best overall class separations while the MTBE–TBAH system (Table IV) provides the best overall peak shape.

*Need for the chloroform–TMAH system*

Although the chloroform–TMAH system gives superior performance in separating compounds with N–H functionalities, that is not the reason it was developed. Instead, the driving force for development of the chloroform-based system was the

TABLE VI

PERFORMANCE RATINGS OF THE LC SYSTEMS AS A FUNCTION OF COMPOUND CLASS

| *Compound class* | *Rating category** | *System*** | | | |
|---|---|---|---|---|---|
| | | *I No added base* | *II TMAH–MTBE* | *III TMAH–chloroform* | *IV TBAH–MTBE* |
| *Neutral species* | A | 5 | 4 | 4 | 4 |
| | B | 5 | 5 | 5 | 5 |
| *Bases* | | | | | |
| Azaarenes | A | 5 | 4 | 4 | 4 |
| | B | 5 | 5 | 5 | 5 |
| Aminoarenes | A | 5 | 3 | 3 | 3 |
| | B | 5 | 2 | 1 | 2 |
| Strong bases | A | 5 | 3 | 3 | 3 |
| | B | 5 | 3 | 2 | 1 |
| *Acids* | | | | | |
| Alcohols | A | 5 | 3 | 3 | 3 |
| | B | 5 | 3 | 3 | 3 |
| Pyrrolic benzologs | A | 5 | 2 | 2 | 3 |
| | B | 5 | 2 | 1 | 2 |
| Amides | A | 4 | 3 | 3 | 3 |
| | B | 5 | 2 | 3 | 1 |
| Hydroxyarenes | A | 5 | 1 | 2 | 3 |
| | B | 5 | 1 | 3 | 2 |
| Carboxylic acids | A | 1 | 1 | 2 | 2 |
| | B | 4 | 3 | 4 | 2 |
| Multifunctional acids | A | 4 | 1 | 2 | 1 |
| | B | 5 | 2 | 5 | 1 |
| *Totals* | | | | | |
| Overall | A | 44 | 25 | 28 | 29 |
| | B | 49 | 28 | 32 | 24 |
| Acids only | A | 24 | 11 | 14 | 15 |
| | B | 29 | 13 | 19 | 11 |

* Rating categories are (A) the ability of the system to separate a given class from others and (B) overall peak shape and resolution of individual members within the class; 1 denotes best performance, 5 worst.

** System numbers refer to Tables specifying respective experimental conditions.

failure of either MTBE-based TAAH-spiked system to provide acceptable separations of very high boiling concentrates, which in turn was due to their inherently poor solubility in MTBE. Fig. 3 contrasts the separation of a strong acid concentrate from a Wilmington, CA, 675+°C petroleum residue in chloroform–TMAH *vs.* MTBE–TMAH systems. The elution pattern in the MTBE-based system is largely based on solubility and hence of no value.

The overall objective during the development of the chloroform–TMAH system was to achieve nearly identical class selectivity to that of the MTBE–TMAH system with superior dissolution properties for heavy petroleum fractions. To a large extent this goal was met as may be seen from Figs. 1 and 2 and Table V. However,

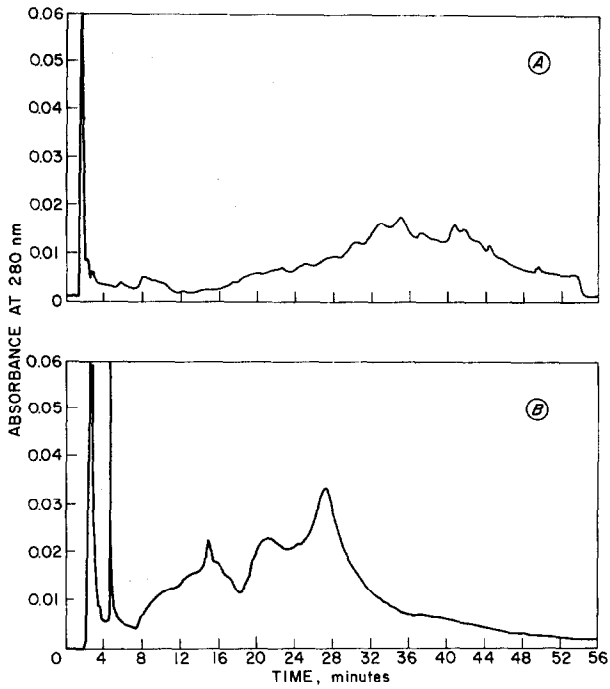


Fig. 3. Comparison of Wilmington 675+°C strong acid concentrate separation using (A) MTBE–TMAH (Table II) and (B) chloroform–TMAH (Table III) systems. Separation in (A) is solubility-limited. About 50 μg injected in (A) and (B).

this system has a number of significant disadvantages, including (1) the high toxicity of chloroform, (2) both poorer column life and overall system stability, and (3) poorer elution characteristics for certain difunctional compounds —especially dihydroxy-aromatics. Thus, it is routinely used only to separate very high boiling fractions.

*Separation of strong bases*

Both petroleum and synfuels contain small amounts of compounds which, based on their strong retention by silica, possess basic properties considerably in excess of those of alkylpyridines, alkylanilines and other typical basic classes in fuels. For example, in one separation scheme[2], these compounds are recovered by back-flushing silica with pyridine at 80°C. Prior attempts by the author to further fractionate these strongly basic compounds with normal phase LC have met with little success owing to their strong interaction with typical adsorbents and bonded phase columns. However, as shown in Fig. 4, the TAAH-spiked systems show promise for further separation of strong bases. Furthermore, an analogous separation of a strong base fraction from a Wilmington, CA, 675+°C residue showed a trace equivalent to Fig. 4. To the author's knowledge, an equivalent separation of strongly basic components from fuels has not been published. Finally, as indicated in Table VI, qualitatively superior separations of strong bases were obtained with the TBAH-spiked system.

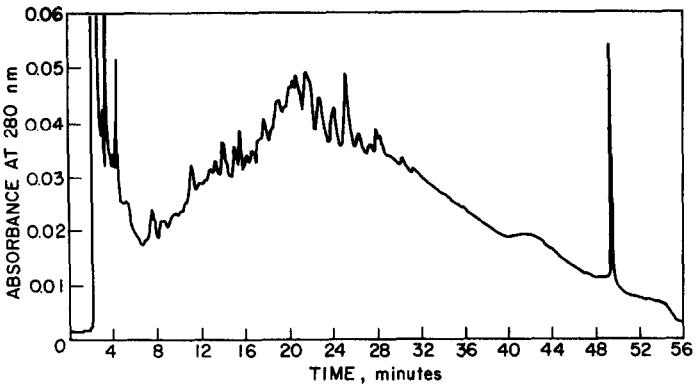


Fig. 4. MTBE–TBAH (Table IV) chromatogram of strong base subfraction isolated from SRC-II >325°C base concentrate. Satisfactory separation of this material has not been achieved with any other HPLC method. Amount injected, 79 μg.

*Electrochemical detection with TAAH-spiked eluents*

Use of electrochemical detectors in nonaqueous systems is rare[27,28], primarily because of minimum dielectric and/or conductivity requirements for mobile phases used in HPLC–ED systems. Since the TAAH-spiked systems contained both a high dielectric component, methanol, and an electrolyte, TAAH, they were potentially compatible with electrochemical detectors.

Fig. 5 shows a sample chromatogram where dual ED–UV was employed. As expected, the electrochemical detector detected only the phenolic compounds present whereas the UV detector responded to all aromatic solutes.

Obviously, the ability to selectively detect given classes of compounds such as phenols would be extremely useful when studying complex mixtures such as fuels. However, at least with the amperometric electrochemical detector investigated here, constraints on the mobile phase composition imposed by the detector would greatly limit its application to real samples. For example, the detector baseline showed excessive drift during gradient operation with either the MTBE–TMAH or MTBE–TBAH systems described in Tables II and IV, especially near the beginning of the gradients where the methanol concentration was only 2 or 3%. Even under isocratic conditions, the minimum methanol concentration for satisfactory detector operation was 5%. In addition, mobile phase impurities resulted in considerable background current which prevented operation of the detector at sensitivities below $10^{-6}$ A. On the other hand, addition of small amounts of other amines such as 1-butylamine to the mobile phase significantly improved the overall behavior of the electrochemical detector with little or no effect on the solutes' chromatographic behavior.

Thus, use of ED with TAAH-spiked normal phase systems is technically feasible, but considerably more work would be necessary to develop an optimum normal phase–TAAH–ED system. For example, an improved amperometric cell was recently described[28] which might greatly improve ED performance in TAAH-spiked normal phase systems. Similarly, use of longer chain TAAHs may promote improved ED performance, since TBAH was found to be definitely superior to TMAH in this preliminary ED study. Also, substitution of ethanol for methanol would allow a

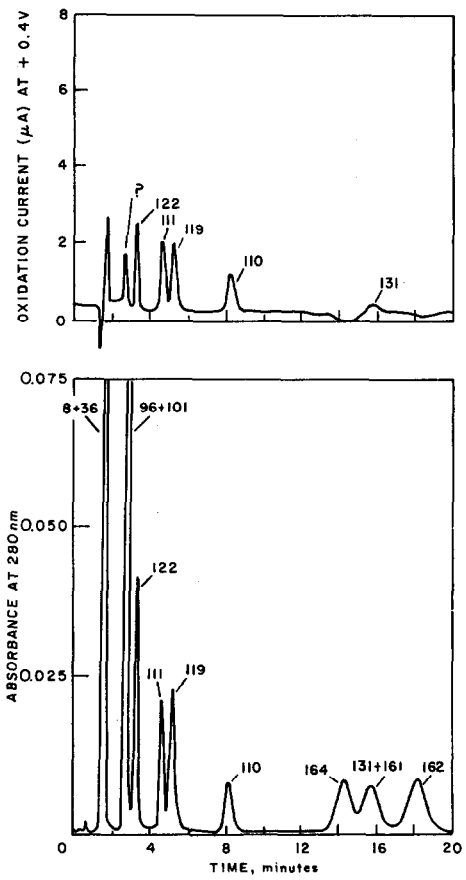


Fig. 5. Isocratic separation of the synthetic mixture of acidic solutes in Fig. 1 detected by ED and UV. Compound numbers refer to Table V. Separation conditions: 22 cm LiChrosorb Si60 10 μm $SiO_2$ column; flow-rate, 2.0 ml/min; mobile phase, MTBE–methanol–1-butylamine (94:5.9:0.1) containing 0.014% TBAH. About 1 μg of each component was injected.

higher alcohol concentration for a given eluent strength, which may in turn yield better ED performance. However, once a satisfactory HPLC–ED system were obtained, numerous other parameters such as the uniformity of detector response over representative members of each compound class would require investigation.

*Retention mechanism*

Although a quantitative model cannot be derived from the data obtained from this study, a level of understanding sufficient for practical use of these systems was obtained. For example, it is obvious from the results and discussion presented thus far that the TAAH is the key component which governs retention and selectivity in these chromatographic systems. Thus, the roles of TAAH in controlling chromatographic properties of acidic solutes are summarized in the following equilibria:

$$R_4NOH + \begin{matrix} O \\ | \\ SiOH[CH_3OH]_x \\ | \\ O \end{matrix} \overset{\text{slow}}{\rightleftharpoons} \begin{matrix} O \\ | \\ SiONR_4[CH_3OH]_y \\ | \\ O \end{matrix} + H_2O + (x - y)CH_3OH \quad (1)$$

$$HA + \begin{matrix} O \\ | \\ SiONR_4[CH_3OH]_y \\ | \\ O \end{matrix} \rightleftarrows \begin{matrix} O \\ | \\ SiONR_4[CH_3OH]_z\,[HA] \\ | \\ O \end{matrix} + (y - z)CH_3OH \quad (2)$$

$$HA + R_4NOH \rightleftarrows R_4NOH[HA] \quad (3)$$

$$RCOOH + \begin{matrix} O \\ | \\ SiOH[CH_3OH]_x \\ | \\ O \end{matrix} \rightleftarrows \begin{matrix} O \\ | \\ SiOH[CH_3OH]_y\,[RCOOH] \\ | \\ O \end{matrix} + (x - y)CH_3OH \quad (4)$$

Eqn. 1 describes the reaction of the TAAH with the silica surface silanol groups to form tetraalkylammonium silicate. Stalling *et al.*[4] reported infrared evidence for the formation of silicate salts from reaction of tetramethylammonium and alkali-metal hydroxides with silica in methanol. Salt formation was also implied in this work by the chromatographic behavior of columns which had been exposed to TAAH. That is, a silica column which had been previously used with a TAAH-spiked mobile phase would initially provide essentially the same degree of retention of acidic solutes with mobile phases not containing TAAH. After several runs, the column would begin to lose its ability to retain acids and probably would eventually reach the point where its retention characteristics were the same as a column which had never been exposed to TAAH.

As with any equilibrium, increasing the concentration of TAAH in the mobile phase drives eqn. 1 to the right until all available silanol groups exist as silicates. The maximum surface coverage thereby obtained would result in maximum retention of acidic solutes through interaction with the silicates as shown in eqn. 2. However, a competing reaction, shown in eqn. 3, between free TAAH in the mobile phase and acidic solutes is also enhanced by increasing the concentration of TAAH in the mobile phase. Thus, the logical conclusion from examination of this simple model is that retention of acidic solutes will increasse as per eqns. 1 and 2 with increasing TAAH concentration up to the point where eqn. 3 begins to predominate. Once the TAAH concentration increases to the point where mobile phase TAAH–solute interactions become predominant, retention of acidic compounds should decrease with further increase in TAAH concentration.

The prediction of an optimum TAAH concentration was experimentally verified in preliminary work to obtain the optimum separation conditions listed earlier in Tables II–IV. One additional finding from the initial experiments was that each class of compounds has a different optimum TAAH concentration. The major factor

which determines a given class's optimum TAAH is its ability to interact with TAAH in the mobile phase (eqn. 3). For example, carboxylic acids readily form TAAH salts whereas very weak acids like pyrrolic compounds do not. In addition, carboxylic acids elute near the end of the gradient program when the TAAH concentration in the mobile phase is nearing its maximum.

Thus, the optimum TAAH concentration for class separations of a given sample is limited by the tendency of the most acidic class present to interact with TAAH in the mobile phase. For example, the TAAH concentrations reported here reflect a trade-off between separations of the pyrrolic and phenolic compound classes *vs.* the phenolic and carboxylic acid classes in fuels. A higher TAAH concentration would improve the pyrrole–phenol separation but would decrease carboxylic acid retention to the point where they would overlap with phenolic compounds. Lowering the TAAH concentration would induce the opposite problem of overlap between pyrrolic and phenolic classes. Therefore, it should be recognized that the TAAH concentrations given here would probably not be optimum for other types of samples containing different sets of acid classes.

Eqns. 1–4 also attempt to show the role of the polar modifier, methanol, used in this work. First, methanol competes with TAAH in eqn. 1 for free silanols to effectively decrease retention of acids. This effect was observed by comparing retention of acidic solutes under gradient conditions where the concentration of TAAH was held constant but the concentration of methanol at the end of the gradient program was varied. For example, if the second solvent (B) in a binary gradient system was methanol containing 0.002 *M* TMAH, retention of phenols increased if a gradient program of 0–40% B in 40 min was used instead of 0–100% B in 100 min. Even though the gradient ramp was identical, the fact that the column was exposed to 100% methanol in the second case caused a shift of eqn. 1 to the left which resulted in decreased retention of phenols. The presence of small amounts of water in methanol would also be expected to shift eqn. 1 to the left.

Methanol also competed directly with acidic solutes by hydrogen bonding with TAAH-silicates as shown in eqn. 2. Of course, methanol was used to control the elution of solutes in this manner during gradient elution. Methanol probably also played an important solvation role in eqn. 3.

Eqn. 4 specifies carboxylic acids because, with the exception of a few amides and multifunctional compounds, only carboxylic acids showed appreciable retention in the chromatographic system which did not contain TAAH. Therefore, only these compounds are potentially retained by both residual silanols and TAAH-silicates in a dual retention mode. The competitive role of methanol is similar in either type of interaction as shown in eqns. 2 and 4.

The role of the bulk mobile phase component, either MTBE or chloroform, is probably minor. Some differences in chromatographic performance, such as improved resolution of N–H-containing solutes and poorer peak shape for multifunctional compounds in the chloroform-containing system, may be attributed to the bulk component. However, the overall selectivities of the systems are controlled by the TAAH and methanol.

As discussed earlier, the major differences between TMAH- and TBAH-spiked systems were greater retention with TMAH and improved chromatographic efficiency with TBAH. These observations are consistent with the expected increase in steric

hindrance between acidic solutes and TBAH. The improved chromatographic efficiency observed with TBAH may imply faster kinetics for the acid solute–TBAH-silicate interaction (eqn. 2). If so, this property in turn relates to steric factors. Both TAAHs give chromatographic efficiencies superior to alkali-metal silicates investigated earlier[4]. However, the plate heights measured for TAAH-modified silicas were a factor of *ca.* 2 larger than equivalent columns tested under more conventional conditions.

Finally, an alternate retention mechanism based on retention of TAAH–solute pairs was also considered. This model was similar to some earlier models proposed for reversed-phase ion-pair chromatography[29,30]. However, numerous inconsistencies between this model and observed chromatographic behavior lead to its abandonment. For example, the simple fact that columns previously exposed to TAAH showed retention behavior characteristic of TAAH-spiked systems after switching back to non-TAAH-containing mobile phases contradicts this model. Also, the previously discussed decreased retention of carboxylic acids at higher levels of TAAH in the mobile phase indicates that formation of solute–TAAH pairs in the mobile phase probably decreases retention rather than promoting it.

*Applications*

Base-modified silicas have been used extensively for isolation of acids from fuels[3,31], environmental samples[4], and lipids[32]. *In situ* TAAH-modified silicas could potentially be used in place of the potassium or cesium silicates used in the above methods, but their main application probably lies in higher resolution HPLC separations rather than batch isolation of fatty acids from lipids, for example. On the other hand, a method for isolating carboxylic acids from crude oils using a scaled-up version of the chloroform–TMAH system discussed here has been developed[33].

Analytical scale MTBE–TMAH HPLC has been used to follow changes in composition of acidic compounds as a function of hydrogenation severity for an SRC-II coal liquid[23]. In that work and similar studies requiring process monitoring, rapid functional group-type separations such as those provided by normal phase TAAH-modified silica are valuable screening tools. On the other hand, preparative scale separations analogous to those described here have been used in the author's laboratory to provide fractions of fuels suitable for detailed analysis by mass spectrometry (MS) and other methods. In the case of MS, better quantitative results are obtained if compounds are separated into classes rather than analyzed as a gross mixture, and representative structures can be assigned to the various MS series with much greater confidence.

Future applications to nonfuel samples, evaluation of unusual detectors such as electrochemical and light scattering detectors, and basic research into TAAH-spiked chromatographic systems are all potentially fruitful areas. For example, the retention data on pure compounds indicated a clear separation of alkylphenols, chlorophenols and neutral compounds. That type of separation is potentially useful for environmental applications. Similarly, potential for electrochemical detection of phenols was demonstrated. Finally, an improved understanding of the retention mechanism as well as investigation of other TAAH systems may lead to expanded applications and further optimization of these systems.

## CONCLUSIONS

Any of the TAAH-modified silica HPLC systems meet most of the requirements listed in the Introduction for a suitable method for subfractionating acid concentrates from fuels. Generally, the MTBE–TMAH system gave the best overall performance for compound class-type separations; but it was not suitable for very high boiling acids because of their limited solubility in MTBE. The major application of these systems will probably be in class- or group-type separations, but they also separate geometrical isomers (*e.g.*, *o-*, *m-*, and *p*-cresol) and can be used for other typical normal phase separations.

Additional of small amounts of TAAHs to medium polarity mobile phases dramatically increases the retention of most acidic and some basic solutes on silica columns. Retention of bases is increased only if they have a N–H or similar functionality which can hydrogen bond to the TAAH-silicate surface.

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