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HIGH-VOLTAGE PAPER ELECTROPHORESIS OF ORGANIC COMPOUNDS IN ACETONITRILE

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SUMMARY

By the addition of a strong acid to a suitable background electrolyte in acetonitrile, organic bases were separated from acids and neutrals in high-voltage paper electrophoresis. By substituting a strong base for the acid one can likewise separate organic acids from bases and neutrals. On cation-exchange paper, and at moderate concentrations of acidified background electrolyte, it was possible not only to separate the bases from the acids and neutrals, but also to separate the bases from one another. Finally, by judicious choice of the location of the position of origin, one could optimize the separation of some acids and bases from one another.

INTRODUCTION

A large body of knowledge has been accumulated about the electrophoretic behavior of organic acids and bases in mixed water–alcohol solvents[1] as well as the role of evaporation and adsorption in the paper electrophoresis of amino-acids, dipeptides and sugars in water–formamide and water–urea solvent systems[2]. The factors which influence the movement of inorganic ions when subjected to an electric field on ion-exchange paper have also been examined[3]. However, since the pioneering work of Hayek[4] on the electrophoresis of carbon blacks in the presence of dispersants in kerosene, electrophoresis in purely non-aqueous solvent systems, as opposed to mixed organic–water solvents, has received relatively little attention as seen in a recent review[5].

Our long-term goal is to examine the feasibility of applying non-aqueous electrophoresis to the mixtures of weak acids and bases found in coal tars, petroleum pitches and crudes. Johansen *et al.*[6] subjected petroleum crudes to paper curtain electrophoresis using a nitrobenzene solvent system. Although they did not achieve

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sharp separation of the metals or metallo-porphyrins from the asphaltenes, they demonstrated the electrophoretic migration of the metal-rich fractions. Leighton *et al.*[7] studied the electrophoresis of lubricating oil additives in glacial acetic acid, and observed that the additive anions of the dialkyldithiophosphate group generally migrated ahead of sulfonates, salicylates and phenates. Their study also showed that it was sometimes difficult to ensure reproducibility between runs.

It is important to note that most of the electrophoretic work reported to date for purely non-aqueous solvent systems appears to have been restricted to solutes that ionized to a large extent in the non-aqueous medium. However, the feasibility of converting very weak acids and bases into ionic species by adding a strong base or acid respectively to the non-aqueous solvent does not appear to have been examined. Hence, we were also interested in seeing if, by judicious selection of the added species and its concentration, further electrophoretic selectivity might be gained.

In the present study, the electrophoretic behavior of model organic compounds, especially nitrogen bases and phenolic acids, has been explored in acetonitrile as the non-aqueous solvent because much information is available about potentiometric differentiation of bases in that medium. The results of such a study might then be applicable to the electrophoresis of difficult mixtures such as coal-tar pitches without prior separation of the neutral species, especially the hydrocarbons.

This study, therefore, is an extension of the work of Johansen *et al.*[6] to other purely non-aqueous solvent systems, with the important difference that the electrophoresis was carried out in the presence of a strong acid or base in addition to a neutral background electrolyte. The strong acid or base was added in just a sufficient amount to promote the ionization of one or more of the solutes by protonation or deprotonation.

An important part of this work, therefore, was a search for and testing of various organic solvents as well as suitable background electrolytes for the electrophoresis of model compounds. Acetonitrile was selected not only because of the information available about titration behavior of solutes in that solvent but also on the basis of its relatively high dielectric constant, aprotic character, low viscosity, convenient liquid range, purity and availability. The test compounds were selected to to model those which occur in coal-tar pitches[8]. Other criteria for selecting the model compounds included detectability by fluorescence, availability in anhydrous form, and solubility in acetonitrile. The background electrolytes were selected on the basis of their solubility in acetonitrile.

EXPERIMENTAL

Chemicals

Tetraethylammonium bromide (TEAB) (a background electrolyte), benzyltrimethylammonium hydroxide in methanol (BTAH) (a strong base), and seven test compounds, anthracene, 1-naphthol, trinitrophenol, 4-nitrophenol, N-ethyl carbazole, 5,6-benzoquinoline, and 1,10-phenanthroline were purchased from Aldrich, Milwaukee, WI, U.S.A. Tetraethylammonium perchlorate (TEAP) (another background electrolyte), tetramethylammonium hydroxide (another strong base) and pyrenebutyric acid (another test compound), were products of Eastman-Kodak, Rochester, NY, U.S.A. Acetonitrile and trichloroacetic acid (TCA), an intermediate-strength acid, were obtained from J. T. Baker, Phillipsburg, NJ, U.S.A. Trifluoromethanesulfonic acid (TFMSA), a strong acid, was obtained from PCR Research Chemicals, Gainesville, FL, U.S.A. Another background electrolyte, anhydrous lithium perchlorate was obtained from City Chemical Corporation, New York, NY, U.S.A. All solutions used as background electrolytes were made up on weight per volume basis.

The two grades of paper, Whatman 3MM and P81, were purchased in 46 × 57 cm sheets from Whatman, Clifton, NJ, U.S.A. 3MM is a pure cellulose while P81 is a cellulose-based cation-exchanger in the monoammonium form of monoesterified orthophosphoric acid.

Apparatus

A Model L24 high-voltage electrophoresis apparatus (Shandon Southern, Runcorn, Great Britain) was used together with its 10 kV d.c. power supply (SAE 3205). The troughs were custom-made of glass, and the upper and lower plates of the apparatus were relined with 0.025-in. thick Teflon® sheet. Micropipets were products of Corning Science Products Division, Corning, NY, U.S.A.

Procedures

Preparation of the paper support. The paper was cut into 10 × 57 cm strips and together with the wicks soaked in the appropriate electrophoresis solution in a covered beaker. Then, the electrode troughs were each filled with 200 ml of the same solution, and the wicks were placed in position. Then, the paper support was placed in position between the wicks. The wicks together with the paper support were again saturated with the solution by means of a pipet. Excess solution was squeezed out by momentarily clamping the apparatus lid down, followed by blotting lightly with a towel.

Sample preparation and application. Samples of *ca.* 0.5 mg/ml were prepared in carbon tetrachloride and applied by means of micropipets. For each run, a spot for each component was applied along a line midway between the electrodes. The volume of each sample was *ca.* 2 μl.

Electrophoresis. Electrophoresis was carried out for 5 min at a constant current of 4 mA. Higher currents and voltages sometimes caused charring of the paper, while longer times, *e.g.* 10 min, gave somewhat lower reproducibilities that probably resulted from differences in evaporative flow. Electrophoresis for 15 min or longer usually resulted in the loss of one or more species by diffusion beyond the end of the paper. All experiments were done in triplicate at least.

Detection of sample zone. Sample zones were usually detected by their fluorescence under UV. Trinitrophenol and 4-nitrophenol did not fluoresce but were readily detected by their yellowish color. 1-Naphthol, after 20 min under UV, developed a brownish color which was also easily detected. The positive-moving component of N-ethyl carbazole (see Results) gave a dark-blue color upon being sprayed with an acetonitrile solution of trichloroacetic acid.

Measurement of migration distance. The distance traveled by each compound was measured from its point of application at the origin to the leading edge of the sample zone following electrophoresis. Migration towards the anode was denoted as positive, and that towards the cathode as negative. The observed migration was corrected for transport due to electroendosmotic flow[9,10] as determined by means of anthracene. Since electroendosmosis is not the only driving force responsible for liquid flow[10], it is possible to overcorrect for transport due to liquid flow. Hence, in those cases where the mean distance of travel for a solute was found to be nearly the same as that for anthracene, its migration distance was interpreted as zero.

In separate experiments using conventional paper chromatography, it was shown that all solutes, with the exception of trinitrophenol and 1,10-phenanthroline, had R_F values of 1.0, *i.e.*, they were not adsorbed by paper under the experimental conditions employed in the electrophoretic studies. The R_F values for the trinitrophenol and 1,10-phenanthroline were *ca.* 0.96 and 0.85 on the 3MM paper when the electrolyte was 0.2% in TFMSA, and 0.88 and 0.78 when the electrolyte was 0.4% in TMAH. On P81 paper, only 5,6-benzoquinoline showed any appreciable adsorption, with an R_F value of 0.89, when the electrolyte was 0.2% in TFMSA. Because we were not interested in the electrophoretic mobility *per se*, no correction was made for adsorption of the trinitrophenol, 1,10-phenathroline and 5,6-benzoquinoline along the lines suggested by Alberti *et al.*[3].

Uncertainties in the corrected migration distances were calculated by subtracting the distance traveled by the anthracene from that of the compound of interest in a given run, and reporting the mean as the variation in the corrected migration distance for that particular compound. That value, therefore, reflected the reproducibility of migration relative to an internal standard, anthracene. Hence, the uncertainties reported in the tables are often smaller for the corrected distances than for the uncorrected distances.

RESULTS

Preliminary studies

As discussed later, it was possible to make weak acids migrate by adding a strong base and to make weak bases migrate by adding a strong acid. However, the introductory experiments emphasized the fact that other variables needed to be controlled in order to improve the reproducibility from run to run. Both the position of the origin and the concentration of electrolyte proved to be important, even when an internal standard was present.

Effect of the position of origin. The migration of various compounds placed in parallel rows transverse to the electrical field is summarized in Table I. In these experiments, sample spots were applied along the midway line, and along the 10- and 15-cm lines on either side of this line. Other pertinent experimental conditions are indicated in the table.

It was observed that the closer the position of origin was to the cathode, the greater the migration distances of the anodic-moving compounds. On the contrary, the extent of migration of the cathodic-moving compounds decreased and even changed direction, the closer the position of the origin was to the cathode. Analogously, the closer the position of the origin was to the anode, the greater the migration distances of the cathodic-moving compounds, and conversely, the extent of migration of the anodic-moving compounds decreased and even changed direction.

Salts as background electrolytes. The migration behavior of some model compounds in various background electrolytes is summarized in Tables II and III. In these experiments, sample spots were applied only along the midway line.

TABLE I

EFFECT OF LOCATION OF ORIGIN ON UNCORRECTED MIGRATION DISTANCES ON 3MM, OF MODEL COMPOUNDS IN ACETONITRILE THAT WAS 0.25% IN TEAP AND 3.2% IN TCA

Compound	*Distance (cm)*				
	Origin and midline	*Origin on cathodic side*		*Origin on anodic side*	
		10	*15*	*10*	*15*
Anthracene	+0.4	+1.1	+1.7	0.0	−1.5
Pyrenebutyric acid	+0.5	+1.4	+1.9	0.0	−1.4
1-Naphthol	+0.5	+1.5	+2.3	0.0	−1.4
Trinitrophenol	+1.6	+2.1	+3.0	+1.2	−1.0
4-Nitrophenol	+0.7	+1.8	+2.5	+0.5	−1.7
5,6-Benzoquinoline	−2.7	−1.1	+1.6	−2.3	−4.0
1,10-Phenanthroline	−2.8	−1.3	+0.4	−2.2	−4.1
N-Ethyl carbazole	−2.4	−0.8	+0.7	−2.2	−3.4
	+0.8*	+1.9*	+2.8*	+0.6*	−1.8*

* Impurity.

TABLE II

MIGRATION DISTANCES OF MODEL COMPOUNDS IN 0.25% $LiClO_4$

Compound	*Distance (cm)*			
	3MM paper		*P81 paper*	
	Observed	*Corrected*	*Observed*	*Corrected*
Anthracene	+1.9 ±0.3	0.0	+1.0 ±0.1	0.0
1,10-Phenanthroline	−2.0 ±0.2	−3.9 ±0.1	−4.3 ±0.5	−5.3 ±0.5
5,6-Benzoquinoline	−1.9 ±0.2	−3.7 ±0.3	−2.8 ±0.3	−3.8 ±0.3
N-Ethyl carbazole	NV*	—	−3.8 ±0.4	−4.8 ±0.4
	+2.0 ±0.3**	+0.1 ±0.1**	+1.4 ±0.2**	+0.4 ±0.2**
Trinitrophenol	+4.2 ±0.6	+2.3 ±0.5	+4.5 ±0.3	+3.5 ±0.4
Pyrenebutyric acid	+1.5 ±0.2	−0.4 ±0.1	+0.9 ±0.1	−0.1 ±0.1
1-Naphthol	+1.8 ±0.3	−0.1 ±0.1	+1.2 ±0.2	+0.2 ±0.1

* Not visible.
** Impurity.

First, a series of three neutral salts were examined. Table II shows that when $LiClO_4$ alone was used as the background electrolyte the migration behavior depended upon the support. Although the acids and the neutral indicator compound, anthracene, always migrated towards the cathode, the grade of paper made a significant difference only in the case of 1,10-phenanthroline, where the migration distance increased almost two-fold on P81 compared to 3MM.

When TEAP was used as the background electrolyte (Table III), all the test compounds except trinitrophenol migrated towards the anode. Although there was no significant difference in the migration directions for the bases on the two supports, P81 clearly differentiated 1,10-phenanthroline from other bases. Regardless of the support, trinitrophenol was still significantly ionized compared with the other acids.

When TEAB was used as the background electrolyte and 3MM as the support,

TABLE III

MIGRATION DISTANCES OF MODEL COMPOUNDS IN 0.25% TEAP

Compound	*Distance (cm)*			
	3MM paper		*P81 paper*	
	Observed	*Corrected*	*Observed*	*Corrected*
Anthracene	−1.5 ±0.1	0.0	+0.4 ±0.2	0.0
1,10-Phenanthroline	−1.3 ±0.2	+0.3 ±0.1	−4.4 ±0.8	−4.7 ±1.0
5,6-Benzoquinoline	−1.3 ±0.2	+0.2 ±0.1	−2.4 ±0.4	−3.1 ±1.0
N-Ethyl carbazole	−1.5 ±0.3	+0.1 ±0.3	−3.2 ±0.6	−3.6 ±0.8
	—	—	+0.6* ±0.2	+0.2* ±0.3
Trinitrophenol	+5.6 ±0.2	+7.1 ±0.2	+7.0 ±0.8	+6.6 ±0.4
Pyrenebutyric acid	−1.2 ±0.1	+0.4 ±0.1	+0.3 ±0.2	0.2
1-Naphthol	−1.3 ±0.1	+0.3 ±0.1	+0.6 ±0.2	+0.2 ±0.4

* Impurity.

there was again a significant difference in the migration of the trinitrophenol from all the rest.

Effect of acid

Addition of acid to the background electrolyte showed different effects on the migration behavior of the compounds depending upon the acid added as well as the background electrolyte. In studying the effect of the strong acid, TFMSA, no additional neutral background electrolyte was added because that acid solution showed adequate conductivity. By contrast, the effect of the intermediate-strength acid, TCA, was studied in the presence of a neutral background electrolyte because without the latter, that acid solution showed inadequate conductivity.

When enough TFMSA was dissolved in acetonitrile to yield an adequately conducting solution in the 3MM paper, the test compounds migrated in a fashion very similar to that in $LiClO_4$ solution. There were, however, two very significant differences as shown in Table IV. First, the test bases migrated a considerably greater

TABLE IV

MIGRATION DISTANCES OF MODEL COMPOUNDS IN 0.2% TEMSA

Compound	*Distance (cm)*			
	3MM paper		*P81 paper*	
	Observed	*Corrected*	*Observed*	*Corrected*
Anthracene	+1.6 ±0.0	0.0	+1.4 ±0.2	0.0
1,10-Phenanthroline	−3.9 ±0.3	−5.5 ±0.3	−3.2 ±0.4	−4.6 ±0.5
5,6-Benzoquinoline	−3.8 ±0.2	−5.4 ±0.2	−2.5 ±0.7	−3.9 ±0.8
N-Ethyl carbazole	−3.7 ±0.2	−5.3 ±0.2	−3.1 ±0.4	−4.5 ±0.6
	+1.6 ±0.1*	0.0 ±0.1*	+1.7 ±0.2**	+0.3 ±0.2*
Trinitrophenol	+1.9 ±0.1	+0.3 ±0.1	+4.5 ±0.6	+3.1 ±0.6
Pyrenebutyric acid	+1.3 ±0.1	−0.2 ±0.1	+1.2 ±0.1	−0.2 ±0.1
1-Naphthol	+1.5 ±0.0	0.0	+1.3 ±0.2	−0.1 ±0.0

* Impurity.

TABLE V

MIGRATION DISTANCES OF MODEL COMPOUNDS IN 0.25% $LiClO_4$ THAT WAS 3.2% IN TCA

Compound	*Distance (cm)*			
	3MM paper		*P81 paper*	
	Observed	*Corrected*	*Observed*	*Corrected*
Anthracene	+1.2 ±0.0	0.0	+0.6 ±0.2	0.0
1,10-Phenanthroline	−2.5 ±0.1	−3.6 ±0.1	−1.8 ±0.2	−2.3 ±0.0
5,6-Benzoquinoline	−2.5 ±0.3	−3.7 ±0.2	−1.3 ±0.2	−1.9 ±0.1
N-Ethyl carbazole	−2.2 ±0.2	−3.4 ±0.2	−1.3 ±0.2	−1.9 ±0.1
	+1.2 ±0.1[*]	0.0[*]	+1.1 ±0.3[*]	+0.5 ±0.1[*]
Trinitrophenol	+1.5 ±0.1	+0.3 ±0.1	+1.4 ±0.1	+0.9 ±0.1
Pyrenebutyric acid	+1.2 ±0.1	0.0	+0.5 ±0.2	0.0
1-Naphthol	+1.3 ±0.0	+0.1 ±0.0	+0.8 ±0.1	+0.2 ±0.1

[*] Impurity.

distance towards the cathode, and, second, the test acids migrated towards the anode to much the same extent as the anthracene. On P81, however, the trinitrophenol behaved as a species that was nearly entirely ionized.

In $LiClO_4$ solution acidified with TCA, the effect of the acid was not noticeable until its concentration had been increased to 3.2%. As summarized in Table V, addition of TCA produced different effects on the migration behavior of the test bases depending upon the paper carrier. On 3MM, the bases migrated a slightly greater distance towards the cathode compared to $LiClO_4$ solution alone. On the contrary, on P81, the migration distances of the bases showed a dramatic decrease relative to those in $LiClO_4$ solution alone, and, instead of being well separated from one another, 5,6-benzoquinoline and N-ethyl carbazole migrated to the same extent. The acids again migrated towards the anode more or less to the same extent as neutral anthracene on 3MM, but on P81 the trinitrophenol migrated as though it were partly ionized.

In TEAP acidified with TCA (Table VI), the effect of the acid was greatly

TABLE VI

MIGRATION DISTANCES OF MODEL COMPOUNDS IN 0.25% TEAP AND 3.2% TCA

Compound	*Distance (cm)*			
	3MM paper		*P81 paper*	
	Observed	*Corrected*	*Observed*	*Corrected*
Anthracene	+0.7 ±0.1	0.0	0.0 ±0.2	0.0
1,10-Phenanthroline	−4.5 ±0.3	−5.2 ±0.3	−2.4 ±0.2	−2.2 ±0.3
5,6-Benzoquinoline	−4.4 ±0.3	−5.1 ±0.3	−1.9 ±0.0	−1.9 ±0.1
N-Ethyl carbazole	−3.8 ±0.2	−4.5 ±0.2	−1.7 ±0.2	−1.8 ±0.1
	+0.8 ±0.2[*]	+0.1 ±0.1[*]	+0.4 ±0.2[*]	+0.3 ±0.2[*]
Pyrenebutyric acid	+0.6 ±0.1	−0.1 ±0.0	0.0 ±0.2	0.0
1-Naphthol	+0.8 ±0.1	+0.1 ±0.0	0.0 ±0.2	0.0
Trinitrophenol	+1.4 ±0.1	+0.7 ±0.2	+1.5 ±0.1	+1.4 ±0.2
4-Nitrophenol	+0.7 ±0.1	0.0 ±0.1	+0.4 ±0.2	+0.3 ±0.2

[*] Impurity.

amplified. On 3MM the bases migrated a relatively greater distance towards the cathode compared to TEAP solution alone. Furthermore, the anthracene changed its direction, and migrated a short distance towards the anode. All the acids except trinitrophenol traveled more or less to the same extent as anthracene towards the anode. On P81, the bases migrated a shorter distance towards the cathode compared with TEAP solution alone, and again they were not as well separated from one another as was the case in TEAP solution alone. While the weaker test acids, pyrenebutyric acid, 1-naphthol and 4-nitrophenol in the presence of TCA, traveled more or less the same short distance towards the anode as anthracene, the stronger test acid trinitrophenol suffered a dramatic decrease in migration distance compared with TEAP alone.

Effect of base

In the absence of an additional neutral background electrolyte, each base alone showed inadequate conductivity. Of the two neutral background electrolytes tested, TEAP was found to be the more suitable because $LiClO_4$ tended to form a gel-like precipitate.

As summarized in Table VII, the addition of TMAH caused a slight decrease in the migration distance of the strong test acid trinitrophenol (probably because of precipitation) and a dramatic change in the migration direction of 1-naphthol and pyrenebutyric acid. The migration distance of pyrenebutyric acid was not as great as expected and even showed a slight decrease upon doubling the TMAH concentration. An interesting change in the migration direction of the anthracene was also observed. It traveled a relatively greater distance towards the cathode compared with when it was in TEAP alone. The test bases on the other hand traveled a relatively shorter distance towards the cathode compared with their migrations in TEAP alone. However, doubling the TMAH concentration, resulted in a slight increase in their migration distances. It was also observed that at the higher TMAH concentration, the bases traveled closer to the anthracene than at the lower concentration.

TABLE VII

MIGRATION DISTANCES ON 3MM OF MODEL COMPOUNDS IN 0.25% TEAP PLUS 0.2% AND 0.4% TMAH

Compound	*Distance (cm)*			
	0.2% TMAH		*0.4% TMAH*	
	Observed	*Corrected*	*Observed*	*Corrected*
Anthracene	−2.5 ±0.2	0.0	−2.2 ±0.0	0.0
Pyrenebutyric acid	+0.7 ±0.2	+3.1 ±0.1	+0.4 ±0.3	+2.6 ±0.4
1-Naphthol	+0.7 ±0.2	+3.0 ±0.2	+1.4 ±0.1	+3.6 ±0.3
	−1.4 ±0.1*	+1.0 ±0.2*	−1.2 ±0.0	+1.0 ±0.2*
4-Nitrophenol	NI**	—	+2.2 ±0.1	+4.4 ±0.2
Trinitrophenol	+3.6 ±0.5	+6.1 ±0.3	+3.2 ±0.1	+5.4 ±0.2
5,6-Benzoquinoline	−1.5 ±0.3	+1.0 ±0.5	−1.9 ±0.1	+0.3 ±0.2
1,10-Phenanthroline	−1.1 ±0.4	+1.3 ±0.4	−1.7 ±0.1	+0.5 ±0.2
N-Ethyl carbazole	−1.3 ±0.3	+1.2 ±0.4	−1.9 ±0.1	+0.3 ±0.3

* Impurity.

** Not included.

In the presence of BTMAH (Table VIII) the acids showed a behavior very similar to that in TMAH except for trinitrophenol, which suffered a relatively greater decrease in migration distance at the higher concentration. 1-Naphthol traveled very close to the neutral anthracene at both the lower and higher concentrations, indicating that the solution was less basic than that of TMAH. Again, the bases traveled very close to the anthracene at both the lower and higher concentrations of BTMAH.

TABLE VIII

MIGRATION DISTANCES ON 3MM OF MODEL COMPOUNDS IN 0.25% TEAP PLUS 0.2% AND 0.4% BTMAH

Compound	*Distance (cm)*			
	0.2% BTMAH		*0.4% BTMAH*	
	Observed	*Corrected*	*Observed*	*Corrected*
Anthracene	−1.9 ±0.4	0.0	−2.9 ±0.3	0.0
Pyrenebutyric acid	+0.7 ±0.1	+2.4 ±0.2	0.0 ±0.0	+2.9 ±0.3
1-Naphthol	−1.7 ±0.3	+0.2 ±0.1	−2.2 ±0.2	+0.7 ±0.4
Trinitrophenol	+3.8 ±0.3	+5.6 ±0.1	+2.2 ±0.1	+5.1 ±0.3
5,6-Benzoquinoline	−1.6 ±0.4	+0.3 ±0.1	−2.5 ±0.2	+0.4 ±0.1
1,10-Phenanthroline	−1.6 ±0.4	+0.4 ±0.1	−2.4 ±0.2	+0.6 ±0.1
N-Ethyl carbazole	−1.7 ±0.3	+0.3 ±0.2	−2.6 ±0.3	+0.4 ±0.0

DISCUSSION

This study has demonstrated that it is possible by the addition of a strong acid to a suitable background electrolyte in acetonitrile to separate weak organic bases from acids and neutrals in high-voltage paper electrophoresis. By substituting a strong base for the acid one can likewise separate weak organic acids from bases and neutrals.

Addition of a strong acid, TFMSA, to the solvent produced the desired effect of protonating the test bases 1,10-phenanthroline, 5,6-benzoquinoline and N-ethyl carbazole, as illustrated by the enhanced migration of those compounds toward the cathode. And by the same token, that acid suppressed the dissociation of the strong test acid, trinitrophenol, resulting in the latter migrating more or less to the same extent towards the anode as the uncharged standard, anthracene. It was also interesting to note that under those conditions the electroendosmotic liquid flow was directed towards the anode, presumably because the trifluoromethane-sulfonate anion was a relatively harder base, capable of dragging the solvent molecules towards the anode.

The effect of addition of the intermediate-strength acid, TCA, was very similar to that of TFMSA except that the electroendosmotic flow was not pronounced. Also, the TCA was not strong enough an acid to suppress the dissociation of the strong test acid, trinitrophenol. Hence, that acid still showed an appreciable migration towards the anode.

The presence of the base, TMAH, in addition to producing a cathodic electroendosmotic flow, resulted in ionizing even the weaker test acids, 4-nitrophenol, 1-naphthol and pyrenebutyric acid. That was illustrated by the fact that those acids migrated a very short distance towards the anode, upstream against the cathodic electroendosmotic flow. The strongest test acid, trinitrophenol, traveled an even greater distance toward the anode, as expected. On the other hand the test bases, although they traveled in the direction of the cathode with the anthracene, trailed that compound, indicating that they suffered some retardation by the paper. Although increasing the concentration of the base did not greatly affect the migration distances of the test compounds, it greatly improved the shape of the sample zones so that they remained well defined and symmetrical instead of tailed.

The other base, BTMAH, was not strong enough to ionize the weak test acid, 1-naphthol, but otherwise it affected the electrophoretic behavior of the test compounds in much the same fashion as TMAH.

The effect of the grade of paper proved difficult to interpret, probably because the ammonium ion in the exchanger was undoubtedly displaced to different extents by the different electrolytes added to the acetonitrile. The migration distances of the test bases in all of the background electrolytes were generally greater on P81 than on 3MM. That trend was reversed when acids were added to the background electrolytes. By contrast, the migration distances of the test acids were generally greater on 3MM than on P81. The exception was trinitrophenol, which sometimes showed a decrease or no change at all in its migration distance on P81 compared with 3MM.

The migration behavior of anthracene suggested that on P81, the electroendosmotic flow was always directed towards the anode independent of the nature of the background electrolyte. In the presence of TCA, however, the electroendosmotic flow must have been very small because the anthracene remained at the origin.

The electrophoretic behavior of charged species in zone electrophoresis is influenced by a number of parameters[10] including: electroendosmosis, a liquid flow which in aqueous systems is usually directed towards the cathode; evaporation, brought about by the heating effect of the electrical current, giving rise to a flow of liquid which carries the electromigrating species along with it; structural effects due to the stabilizing medium alone; and adsorption and molecular sieving phenomena, which depend on both medium and migrant, and produce a retardation of its migration.

In this study, the interplay of these parameters in affecting the electrophoretic behavior of organic compounds in a non-aqueous solvent system was elucidated. As illustrated in the study on the effect of the location of the origin on the paper, the principal causes of liquid flow in acetonitrile-based solvent systems appear to be electroendosmosis and evaporation. The latter gave rise to flow from the electrodes towards the center of the paper, while the direction of flow due to electroendosmosis depended on the nature of the background electrolyte. Hence, depending on whether the location of the origin was at the center or off-center and closer to either one of the electrodes, liquid flow would either be negligible or directed to either one of the electrodes. Thus, in the presence of TCA, where the electroendosmotic flow was directed towards the anode, the liquid flow was greatly enhanced when the location of the origin was closer to the cathode than when it was at the center. That was the result of the additive effects of both evaporation and electroendosmosis. On the contrary, when the position of origin was located close to the anode, the electroendosmotic flow was opposed to the flow owing to evaporation, and even closer to the anode the latter effect was so great that it even resulted in a complete reversal of the migration direction of the indicator compound, anthracene. A similar effect was noticed even with the test bases. Under those conditions the migration of the test bases not only suffered a decrease but was also reversed when the location of the origin was closer to the cathode. On the contrary, when the location of the origin was closer to the anode, the test bases showed an enhancement of their respective migration distances.

The study of the effect of the nature of the background electrolyte demonstrated that the direction of liquid flow due to electroendosmosis was determined to a great extent by the nature of the cation. It was observed that the lithium cation caused liquid flow directed towards the cathode, while the tetraethylammonium cation (TEA cation) caused liquid flow directed towards the anode. This behavior may be related to the difference between those cations' affinity for the solvent molecules. The lithium cation, being the harder of the two acid cations, showed a greater propensity for attracting the solvent molecules than the TEA cation. Hence, in the case of the TEA cation, most of the solvent molecules were dragged by the anion rather than the cation.

The electrophoretic behavior of the test compounds in the presence of bromide anion as opposed to perchlorate anion supported the theory that electroendosmotic flow is determined by the affinity of the ions of the background electrolyte for the solvent molecules. Bromide ion, being a softer base than perchlorate ion, showed less affinity for the solvent molecules, and hence the electroendosmotic flow was strongly biased towards the cathode, presumably because the TEA cation, though a soft acid, had a greater tendency to be solvated by the molecules of acetonitrile.

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