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EFFECTS OF VARIOUS STRENGTHS OF BASES ON THE ELECTROPHORETIC BEHAVIOR OF WEAK ACIDS IN A NON-AQUEOUS SOLVENT

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SUMMARY

Differential migrations of anions of weak acids in the form of phenols, naphthols and phenanthrol were obtained by high-voltage electrophoresis on paper, using the addition of an appropriate strength and concentration of base in acetonitrile–sulfolane (9:1) containing a small amount of tetraethylammonium perchlorate as background electrolyte. Positional isomers of nitrophenols and naphthols were successfully resolved using either low concentrations of strong bases like tetrabutylammonium hydroxide and tetramethylammonium hydroxide or higher concentrations of intermediate strength base like benzyltrimethylammonium hydroxide. It was also possible to separate stronger acids like carboxylic and sulfonic acid derivatives of naphthols and of naphthylamine using a weaker base like tributylamine. The use of potentiometric and spectrophotometric titration data to predict the electrophoretic behavior of very weak acids was limited, probably due to ion-pair formation and adsorption of the anionic forms of the acids on paper.

INTRODUCTION

The long-term goal of our work is to examine the feasibility of applying non-aqueous electrophoresis to separations of mixtures of weak acids and weak bases found in coal tars, petroleum pitches and crudes[1]. The present paper, however, deals only with the separations of weak acids such as phenols, naphthols and phenanthrol. Prior work with non-aqueous solvents has been done by Krawezyk[2], who separated mixtures of organic dyes and natural amino acids; adenosine mono-, di- and triphosphate and cytosin 2′-, 3′- and 5′-monophosphate by ionophoresis and electrophoresis on Whatman No. 1 paper and on silica gel or cellulose coated plates, each saturated with formamide, N-methylformamide or dimethylformamide. The good separation properties of systems containing formamide and its derivatives were probably due to their high dielectric constants and possibly specific interactions with the substances to be separated owing to the presence of the C=O group. Krawezyk[3]

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also compared the separations of quinoline bases and phenols in aqueous and non-aqueous media. They were separated by ionophoresis on Whatman No. 3 MM paper saturated with a dimethylformamide solution of benzoic acid and sodium carbonate. Quinoline bases and phenols were less mobile in dimethylformamide solution than in a corresponding aqueous solution. The conductance of a dimethylformamide solution of benzoic acid increased with the addition of quinoline bases owing to the formation of a hydrogen-bridge followed by proton transfer from benzoic acid to the base. Recent work[4] shows that electrophoretic separations of positional isomeric aromatic sulfonates, which are not possible in aqueous buffers, were achieved by using dimethylformamide as the solvent. Examples are given for the separations of naphthol sulfonates of food colors and azo dyes derived from the naphthol sulfonates. Non-aqueous electrophoretic studies of complex formation between catechol and technetium(V)[5] and of cationic metal chelates of 1,10-phenanthroline in non-aqueous systems[6] indicate the promise of non-aqueous solvents in the electrophoresis of organometallic complexes.

Most of the electrophoretic work reported to date for purely non-aqueous solvent systems appears to have been restricted to the solutes that ionized to a large extent in the non-aqueous medium or to the solutes that formed ions due to specific interactions with the non-aqueous media. However, the feasibility of separating weak acids and bases by high-voltage electrophoresis on paper after conversion into ionic species by adding either a strong base or acid to the non-aqueous solvent has been examined in our laboratory[7]. Migrations of model weak bases took place in 9:1 acetonitrile–sulfolane upon adding strong acids and, also, to migrations of weak acids upon adding strong bases. Recently, David *et al.*[8] observed that strong acids, such as trichloroacetic acid or strong organic bases, such as sodium methoxide in combination with a bulky, inert supporting electrolyte (*e.g.*, tetraalkylammonium perchlorates) enhanced the electric double layer and gave rise to reproducible migration times and higher separation efficiencies of weak organic bases or acids, respectively. As reported earlier[9], weak organic nitrogen bases showed differential migration in high-voltage paper electrophoresis upon the addition of an appropriate strength and concentration of acid in 9:1 acetonitrile–sulfolane containing a small amount of tetraethylammonium perchlorate as background electrolyte. Dichloro- and trichloroacetic acids were effective for separating 5,6-benzoquinoline, 7,8-benzoquinoline and 1,10-phenanthroline. The study also reported that the use of potentiometric and spectrophotometric titration data to predict the electrophoretic behavior was very limited due to adsorption of the free base by paper and by ion-pair formation of the protonated base.

The present investigation extends earlier studies on weak bases and acids[7,9] to the differential migration of weak acids in a non-aqueous medium. Various tetraalkylammonium hydroxides, tributylamine and piperidine were selected as reagents because they had little UV-visible absorbance, thereby minimizing interference with fluorometric measurements of the aromatic acids (as anions or ion-pairs). The mixed acids of carboxylic and sulfonic derivatives of naphthols and of naphthylamine were also selected as test solutes, for electrophoretic separations using a base of a weaker strength like tributylamine. The test solutes were selected to model those that occur in coal-tar pitches[1].

The concentration as well as the strength of the base proved to be important

in causing differential ionization of weak acids. Potentiometric and spectrophotometric titrations were performed so as to find the combination of concentration and strength of a base required to promote different partial ionizations of the weak acids. Attempts were then made to correlate that information with the electrophoretic data.

EXPERIMENTAL

Chemicals

Anthracene, 3-nitrophenol, 4-nitrophenol, 2-naphthol, 9-phenanthrol, 2-hydroxy-6-naphthoic acid (all from Aldrich, Milwaukee, WI, U.S.A.), 1-naphthol, 2-naphthol-7-sulfonic acid sodium salt and 2-naphthylamine-6-sulfonic acid (all from Eastman Kodak, Rochester, NY, U.S.A.) were used as received. Tetrabutylammonium hydroxide (0.1 *M* solution in 9:1 benzene–methanol), tetramethylammonium hydroxide (25% solution in methanol), benzyltrimethylammonium hydroxide (all from Eastman Kodak), tributylamine, piperidine (both from Aldrich) and pyridine (J. T. Baker, Philipsburg, NJ, U.S.A.) were used to promote ionization of the acids. Tetraethylammonium perchlorate (Eastman Kodak) was used as the background electrolyte in a 9:1 mixture of acetonitrile (J. T. Baker) and sulfolane (Eastman Kodak). This 9:1 mixture was used throughout this study and will be referred to as the standard solvent. Methanesulfonic acid (Eastman Kodak) was used to prepare buffer for standardizing the pH electrode.

The $5.0 \cdot 10^{-3}$ *M* solutions of acids and solutions of bases ranging from $2.0 \cdot 10^{-3}$ *M* to $40.0 \cdot 10^{-3}$ *M* were freshly prepared in standard solvent containing 0.25% tetraethylammonium perchlorate before each potentiometric, electrophoretic or paper chromatographic experiment. Stock solutions of bases were standardized in water against primary standard acid potassium hydrogen phthalate using phenolphthalein as an indicator. Stock solutions of acids were standardized against previously standardized base. Similarly, solutions of acids and bases ranging from $5.0 \cdot 10^{-5}$ *M* to $1.0 \cdot 10^{-3}$ *M* were freshly prepared in standard solvent containing 0.25% tetraethylammonium perchlorate before each spectrophotometric experiment. The solvent system containing 9:1 acetonitrile–sulfolane (standard solvent) and 0.25% tetraethylammonium perchlorate was used as a blank.

A buffer for standardizing the pH electrode was prepared in the standard solvent as described earlier[9].

Pure cellulose, Whatman 3 MM paper (Whatman, Clifton, NJ, U.S.A.), was used as the electrophoretic support.

Apparatus

A high-voltage electrophoresis apparatus, Model L-24 (Shandon Southern, Runcorn, U.K.), and its 10 kV d.c. power supply (SAE 3205) were employed with custom-made glass troughs[7].

Sample zones were detected by their fluorescence under UV or absorbance in the visible region using a Model CS-910 densitometer (Shimadzu, Columbia, MD, U.S.A.).

Potentiometric titrations were performed in the H-type cell described earlier[9]. pH values were measured using a Corning pH meter, Model 130, equipped with Corning pH triple-purpose (silver/silver chloride internal standard) glass electrode

(Cat. No. 476022), and an automatic temperature compensator. A silver billet electrode (Fisher Scientific, Pittsburgh, PA, U.S.A.) was used as reference electrode. The pH meter does not display digital pH values beyond 20. Hence those pH values were read from the output of the pH meter onto a Linear® chart recorder.

The UV absorption spectra of titrated bases were recorded on a computer-controlled instrument that has been described elsewhere[10]. It consists of a GCA-McPherson Spectrophotometer (EU-700 Series) interfaced to a Digital Equipment Corp. PDP-11/34 computer.

Procedures

High-voltage electrophoresis. Each electrode trough was filled with 200 ml of background electrolyte solution containing the appropriate base. The preparation of the paper support and wicks has been described earlier[9]. A sample (*ca.* 2.5 μl) of $5.0 \cdot 10^{-3}$ *M* of each weak acid was applied to the paper along a midway line using a micropipette (Wiretol® II, Drummond Scientific, Broomall, PA, U.S.A.).

Electrophoresis was carried out for 5 min at a constant current of 4 mA (*ca.* 5 kV). Higher currents and longer times gave lower reproducibilities and caused charring of the paper[7]. Following each electrophoresis experiment, the paper was air-dried and the sample zones were scanned for their fluorescence in UV at 300/400 nm excitation/emission wavelengths using a 400-nm filter or for their absorbance in the visible at 400 nm. The mixture of acids was scanned for their fluorescence in the UV at 300–350/400 nm excitation/emission wavelengths using 400-nm filter and for their absorbance in the visible at 400 nm. The distance travelled by each compound was measured from the center of its zone of application at the origin to the center of the sample zone. The observed migration distance was corrected for transport due to electroendosmotic flow[11,12] as determined by anthracene[7]. In cases where the mean distance of travel was within ±0.3 cm of that for anthracene, the migration distance was interpreted as zero. Migration towards the anode was denoted as positive and that towards the cathode as negative. Each measurement represents the results of at least three replicate experiments.

Potentiometric measurements. The glass electrode was calibrated using a tetraethylammonium methanesulfonate–methanesulfonic acid buffer of apparent pH 4.402 before each titration[13]. The H-type cell[14] used for the potentiometric titrations and the titration procedure have been described earlier[9].

Under helium and with constant stirring, a 0.01 *M* solution of tetrabutylammonium hydroxide, tetramethylammonium hydroxide, piperdine, tributylamine or pyridine was added to the glass electrode compartment containing 50 ml of background electrolyte solution. The base was pumped at a rate of 3.0 ml/min for 10 min.

The titrations of acids using those bases were also carried out in the same set-up. A 50 ml of 0.005 *M* solution of each acid (3-nitrophenol, 2-naphthol, 9-phenanthrol, 2-naphthol-7-sulfonic acid sodium salt, 2-naphthylamine-6-sulfonic acid and 2-hydroxy-6-naphthoic acid) was also titrated against 0.01 *M* solution of base.

Spectrophotometric measurements. Titration of weak acids for the spectrophotometric measurement were performed by adding successive portions of piperidine, tetramethylammonium hydroxide or tetrabutylammonium hydroxide, which represented 50% to 1000% of the acid present, and diluting solutions to the same volume every time. Spectra were then recorded in the UV region 380–235 nm for these solutions containing different concentrations of bases.

Spectra for pure acids and pure bases were also recorded to correct for any excess acid or base present in the titration mixture. The data were taken on a PDP-11/34 computer and the manipulation was done through multiplication and subtraction techniques. The difference in the spectra for a weak acid before and after each addition of base was monitored at the wavelength of maximum absorbance for a particular acid.

Paper chromatography. These studies of the acids were also carried out using acetonitrile–sulfolane (9:1) containing a small amount of tetraethylammonium perchlorate and an appropriate amount of base as a mobile phase. The detailed procedure has been described earlier[9]. Each measurement represents the results of at least two replicate experiments.

RESULTS

Preliminary experiments

Electrophoresis. Three types of preliminary experiment were carried out using tetramethylammonium hydroxide, piperidine and tributylamine representing strong, intermediate and weak bases, respectively. Model compounds were restricted to 3-nitrophenol, 2-naphthol and 9-phenanthrol, containing one, two and three benzene rings, respectively, in order to check their electrophoretic behavior in bases of different strengths. pK values in water of these and other strong acids are given in Table I.

In the presence of 2.0 mM concentrations of tetramethylammonium hydroxide, both 3-nitrophenol and 2-naphthol did not ionize at all whereas 9-phenanthrol showed some ionization (see Table II). At the highest concentration, 40.0 mM, migrations of all the species were nearly the same. At intermediate concentration, the migration distances lay at intermediate values. The migrations of 3-nitrophenol and 2-naphthol correlated well with their pK_a values in water. No correlation could be drawn for 9-phenanthrol, as its pK_a value was not available. Note that there were

TABLE I

pK VALUES OF WEAK AND STRONG ACIDS IN WATER

Acid	*pK_a (25°C)*	*Acid*	*pK_a (25°C)*
3-Nitrophenol	8.36*	9-Phenanthrol	–
	25.60**		
4-Nitrophenol	7.16*	2-Naphthyl-7-sulfonic acid	3.74 (pK_1)§§
	20.70**		9.91 (pK_2)§§
	7.68***		9.10§§,§§§
1-Naphthol	9.30§	2-Naphthylamine-6-sulfonic acid	3.92***
2-Naphthol	9.57§	2-Hydroxy-6-naphthoic acid	3.69 (pK_1)§§
			9.78 (pK_2)§§

* Ref. 16.
** In acetonitrile; ref. 17.
*** In ethanol–water (1:1); ref. 18.
§ Ref. 19.
§§ Ref. 20.
§§§ Sodium salt of the acid.

TABLE II

MIGRATION DISTANCES IN DIFFERENT CONCENTRATIONS OF TETRAMETHYLAMMONIUM HYDROXIDE

Concentration (M)	*Corrected migration distances (cm)*					
	*Anthracene (cm)**	*3-Nitrophenol*	*4-Nitrophenol*	*1-Naphthol*	*2-Naphthol*	*9-Phenanthrol*
$2.0 \cdot 10^{-3}$	0 (−1.1)	+0.3 ± 0.1	+2.4 ± 0.3	+1.0 ± 0.1* +2.2 ± 0.2	0.0 ± 0.1	−0.4 ± 0.1** +1.8 ± 0.2
$20.0 \cdot 10^{-3}$	0 (−1.2)	+2.6 ± 0.3	+3.4 ± 0.3	+1.0 ± 0.1* +3.0 ± 0.2	+0.5 ± 0.1	−0.9 ± 0.1** +2.7 ± 0.2
$40.0 \cdot 10^{-3}$	0 (−1.2)	+2.9 ± 0.2	+4.4 ± 0.2	+1.0 ± 0.1* +3.6 ± 0.1	+2.2 ± 0.2	0.0 ± 0.1** +3.0 ± 0.1

* Observed migration distance.
** Intense peak.

two distinct peaks observed for 9-phenanthrol at all concentrations of tetramethylammonium hydroxide used. This may have been due to an impurity, which was also ionized to a different extent. The migration of that impurity was also concentration dependent.

A similar trend of migration was observed in the presence of piperidine (see Table III), except that the migrations of all the species at the highest concentration, 40.0 m*M*, were either the same or somewhat smaller, probably due to ion-pair formation of the acids at higher concentrations of base[15].

No significant ionization of any acids were observed using the weaker base tributylamine even at 40.0 m*M*.

Paper chromatography. Model organic acids showed no adsorption on paper when the mobile phase contained 2.0 m*M*, 20.0 m*M* and 40.0 m*M* tetramethylammonium hydroxide, piperidine or tributylamine. All of the acids travelled very close to the solvent front.

TABLE III

MIGRATION DISTANCES IN DIFFERENT CONCENTRATIONS OF PIPERIDINE

Concentration (M)	*Corrected migration distances (cm)*			
	*Anthracene (cm)**	*3-Nitrophenol*	*2-Naphthol*	*9-Phenanthrol*
$2.0 \cdot 10^{-3}$	0 (−1.1)	+0.8 ± 0.2	+0.8 ± 0.1	+0.5 ± 0.1** +1.3 ± 0.1
$20.0 \cdot 10^{-3}$	0 (−1.2)	+1.0 ± 0.2	+1.2 ± 0.1	+0.6 ± 0.1** +1.8 ± 0.2
$40.0 \cdot 10^{-3}$	0 (−0.9)	+0.9 ± 0.1	00.0 ± 0.2	0.0 ± 0.1** +1.8 ± 0.3

* Observed migration distance.
** Intense peak.

Potentiometric data

The objectives behind the potentiometric measurements were (a) to obtain the apparent pH values of the solvent alone and in the presence of excess base and (b) to determine the apparent pK values of model acids in the same solvent, acetonitrile–sulfolane (9:1). Then, an optimum concentration of base was to be selected for the electrophoresis using the titration curves of the model organic acids. At the optimum concentration, one should obtain the maximum differential ionization and, hence, the maximum differential migration of model compounds in electrophoresis, assuming that adsorption and ion-pair formation were negligible.

The capabilities of the bases to ionize weak acids were estimated from their pH profiles. The pH profile of each base obtained by titrations of blank solutions with 0.01 M base is shown in Fig. 1. Note that the apparent pH of the blank was close to 7.0; however, it increased very rapidly and then levelled off quickly. The pH profile of the blank for benzyltrimethylammonium hydroxide is not included as it was nearly identical with that for piperidine.

In electrophoresis, conditions have to be selected to produce very nearly the same pH value regardless of the (unknown) amount of acid present on the paper. Thus, it is necessary to use excess base under conditions where the amount of excess is not very critical. Since the change in the apparent pH was too rapid for strong bases, one should be better off running an electrophoresis in weaker bases whose plateau fell in the described region of pH. Thus, potentiometric titrations were then

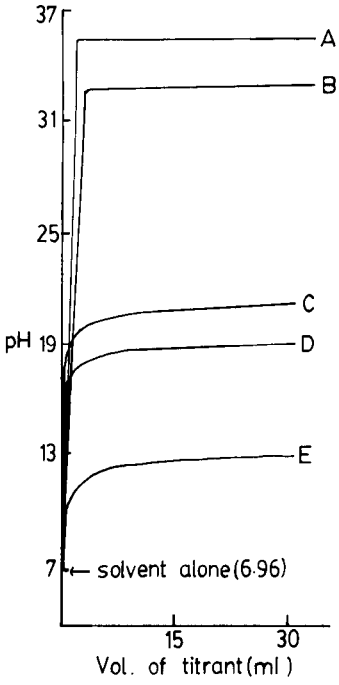
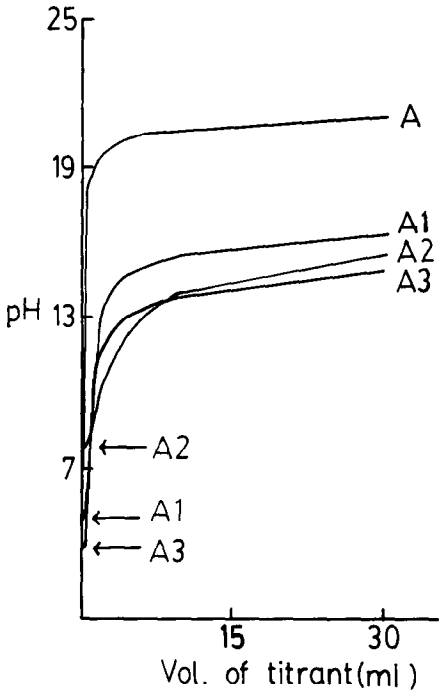


Fig. 1. Titration of 50 ml of blank solutions with 0.01 M solutions of different bases. Traces: A = tetrabutylammonium hydroxide, B = tetramethylammonium hydroxide; C = piperidine; D = tributylamine; E = pyridine.



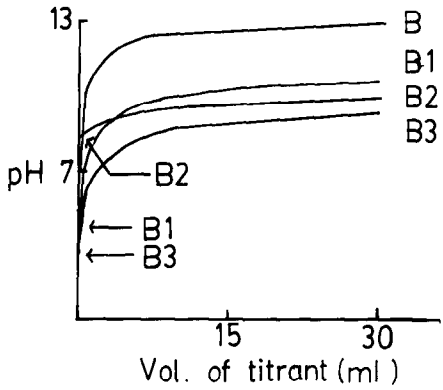


Fig. 2. The pH curves for the base alone relative to the titration curves of 50 ml of different weak acids (0.005 *M*) with 0.01 *M* piperidine. Traces: A = blank; A1 = 2-naphthol; A2 = 9-Phenanthrol; A3 = 3-nitrophenol.

Fig. 3. The pH curves for the base alone relative to the titration curves of 50 ml of different weak acids (0.005 *M*) with 0.01 *M* pyridine. Traces: B = blank; B1 = 2-naphthol; B2 = 9-phenanthrol; B3 = 3-nitrophenol.

performed using weaker bases. The locations of the pH curves for the blanks alone relative to the titration curves of 3-nitrophenol, 2-naphthol, and 9-phenanthrol with piperidine and pyridine are shown in Fig. 2 and Fig. 3, respectively. In the presence of piperidine at 30 ml of titrant (5 ml beyond the equivalence point), no equivalence point was obtained for any acids, and the pH was still far from that of the base obtained in the blank solvent. For 2-naphthol, for example, a three-fold excess of base was required to reach the pH of the solvent (see A1 in Fig. 2). 9-Phenanthrol (see A2 in Fig. 2) and 3-nitrophenol (see A3 in Fig. 2) showed similar behavior. Initial apparent pH values of the weak acids were in line with their pK_a values in water. 3-Nitrophenol started, as expected, at a more acidic pH whereas 9-phenanthrol started at the least acidic pH. However, these results did not correlate well with preliminary electrophoretic studies in tetramethylammonium hydroxide and piperidine, where 9-phenanthrol migrated more than 3-nitrophenol and 2-naphthol. Parallel behavior in all respects was found for pyridine (see Fig. 3). In addition, 2-naphthol now required nearly a 10-fold excess to reach 1 unit below the pH of solvent alone, and likewise for 3-nitrophenol and 9-phenanthrol.

Model compounds were increased by introducing the strong acids 2-naphthol-7-sulfonic acid sodium salt, 2-naphthylamine-6-sulfonic acid and 2-hy-

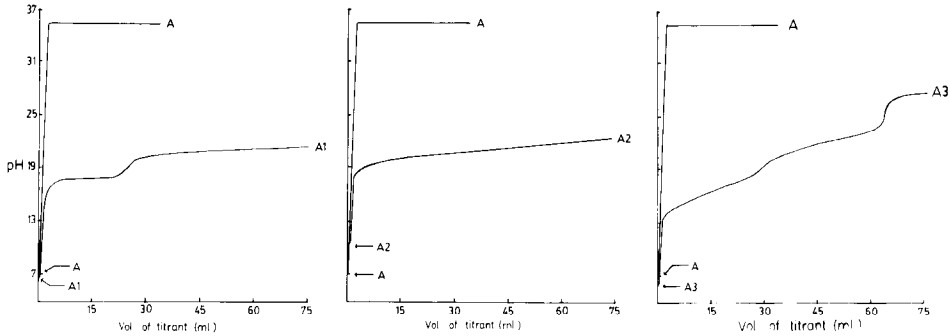


Fig. 4. The pH curves for the base alone relative to the titration curves of 50 ml of different acids (0.005 *M*) with 0.01 *M* tetrabutylammonium hydroxide. Traces: A = blank; A1 = 2-naphthyl-7-sulfonic acid; A2 = 2-naphthylamine-6-sulfonic acid; A3 = 2-hydroxy-6-naphthoic acid.

droxy-6-naphthoic acid to see if successful prediction about their ionization and electrophoretic behavior was possible using potentiometric titrations with a strong base. The results of such studies might then be used to carry out electrophoresis of these acids in the weaker base such as tributylamine. Stoichiometric 1:1 reactions were observed when 2-naphthol-7-sulfonic acid and 2-hydroxy-6-naphthoic acid were titrated against 0.01 *M* tetrabutylammonium hydroxide; however, a large excess of base did not attain the same pH as that for the same amount of excess base in solvent alone (see Fig. 4). 2-Naphthylamine-6-sulfonic acid did not show any equivalent point for titration. 2-Naphthol-7-sulfonic acid showed only one break (see A1 in Fig. 4) since it was available as a sodium salt of sulfonic acid having a p*K* value for the hydroxy group of 17.8 ± 0.1. As expected, 2-hydroxy-6-naphthoic acid showed two p*K* values (see A3 in Fig. 4), at 16.2 ± 0.2 and 21.5 ± 0.1.

Comparing the pH curves of blanks in the presence of different bases (see Fig. 1) and the titrations of stronger acids in the presence of base tetrabutylammonium

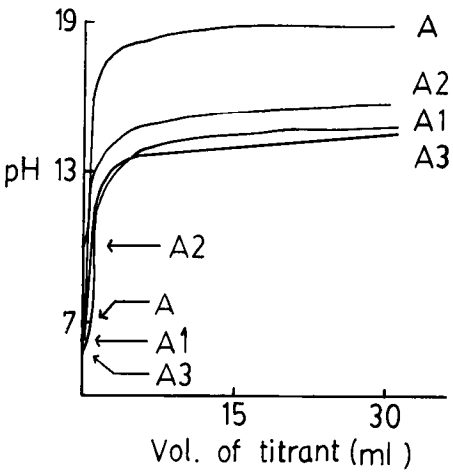


Fig. 5. The pH curves for the base alone relative to the titration curves of 50 ml of different acids (0.005 *M*) with 0.01 *M* tributylamine. Traces as in Fig. 4.

hydroxide (see Fig. 4) suggests that a weaker base, tributylamine, should be in the proper range. Titration of 0.005 *M* of these acids with 0.01 *M* tributylamine (see Fig. 5) showed that 2-hydroxy-6-naphthoic acid, 2-naphthol-7-sulfonic acid and 2-naphthylamine-6-sulfonic acid were in their decreasing order of strength, as seen from their initial pH values in the solvent alone. These results and those obtained from stronger base correlated well with their pK_a values in water. However, all the three acids failed to show any end-point of titration. Furthermore, even after the addition of 800% excess base, tributylamine failed to reach the pK_a of the base alone in acetonitrile–sulfolane (9:1).

Spectrophotometric data

The spectrophotometric measurements were designed to examine (a) the changes in the spectra for weak acids upon the addition of various amounts of different bases, (b) the change in absorbance at the wavelength of peak absorbance and determine the stoichiometries for the titrations of weak acids by a weaker base, piperidine, were the same as predicted from potentiometric measurements and (c) the stoichiometries observed for those same acids in the stronger bases, tetramethylammonium hydroxide and tetrabutylammonium hydroxide, which were not possible with potentiometric measurements. Spectrophotometric data represent the changes in absorbance between the solution of pure acid and that of the acid after the addition of base (with the correction of excess acid or base)[9]. The measurement indicated that, when the difference in absorbance became zero or slightly positive or negative, no acid was left in the solution.

When piperidine was used to titrate three weak acids 3-nitrophenol, 2-naphthol and 9-phenanthrol, the presence of a large excess (see Fig. 6) did not lead to complete

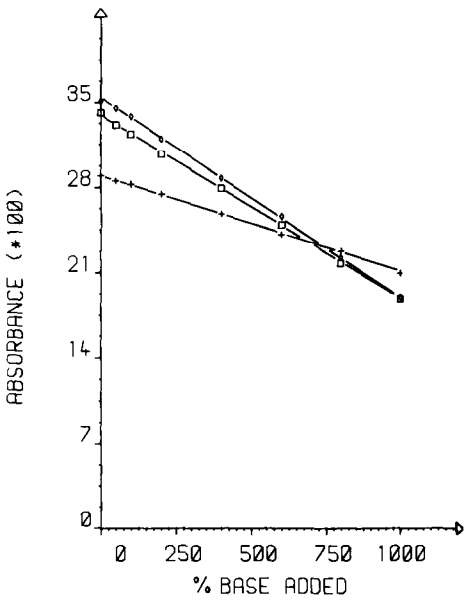


Fig. 6. Spectrophotometric measurements of different acids with different percentages of base (piperidine) added. Traces: □ = 3-nitrophenol; + = 2-nitrophenol; ◇ = 9-phenanthrol.

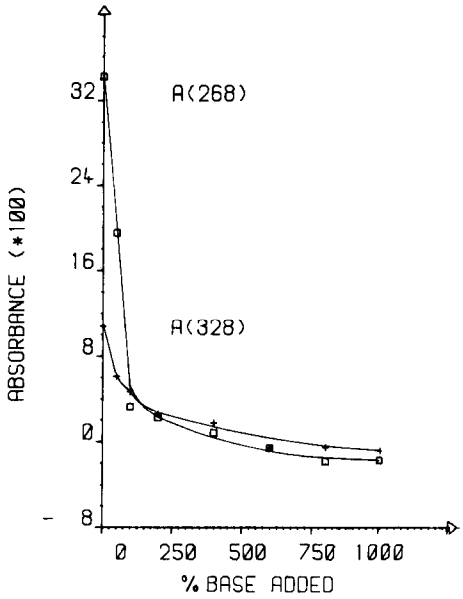
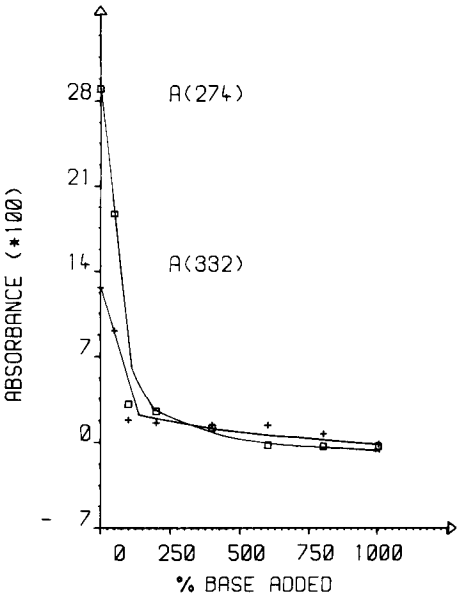


Fig. 7. Spectrometric measurements of 3-nitrophenol at different wavelengths with different percentages of base (tetramethylammonium hydroxide) added. Traces: □ = 268 nm; + = 328 nm.



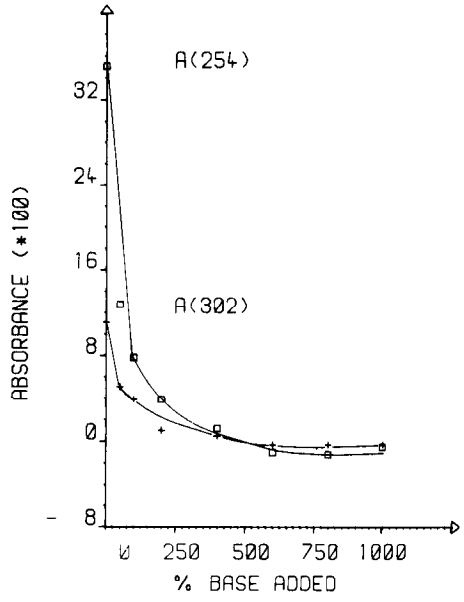


Fig. 8. Spectrophotometric measurements of 2-nitrophenol at different wavelengths with different percentages of base (tetramethylammonium hydroxide) added. Traces: □ = 274 nm; + = 332 nm.

Fig. 9. Spectrometric measurements of 9-phenanthrol at different wavelengths with different percentages of base (tetramethylammonium hydroxide) added. Traces: □ = 254 nm; + = 302 nm.

TABLE IV

MIGRATION DISTANCES IN DIFFERENT CONCENTRATIONS OF TETRABUTYLAMMONIUM HYDROXIDE

Mixture A was an equimolar mixture of 3-nitrophenol, 2-naphthol and 9-phenanthrol; mixture B was an equimolar mixture of 3- and 4-nitrophenol and 1- and 2-naphthol.

Concentration (M)	*Corrected migration distances (cm)*							
	*Anthracene (cm)**	*3-Nitrophenol*	*4-Nitrophenol*	*1-Naphthol*	*2-Naphthol*	*9-Phenantrol*	*Mixture A*	*Mixture B*
$2.0 \cdot 10^{-3}$	0 (−2.8)	+4.4 ± 0.2	+6.4 ± 0.3	+1.0 ± 0.1** +5.2 ± 0.2	+3.4 ± 0.1	−0.6 ± 0.1** +3.4 ± 0.1	−0.5 ± 0.1** +3.4 ± 0.2 +4.5 ± 0.2	+1.0 ± 0.1** +3.5 ± 0.2 +4.5 ± 0.1 +5.5 ± 0.1 +6.5 ± 0.1
$20.0 \cdot 10^{-3}$	0 (−3.0)	+5.1 ± 0.2	+7.1 ± 0.3	+1.1 ± 0.1** +6.3 ± 0.2	4.5 ± 0.1	−0.5 ± 0.1** +3.1 ± 0.1	−0.5 ± 0.1** +2.9 ± 0.1 +4.4 ± 0.1 +5.1 ± 0.2	+1.0 ± 0.1** +4.2 ± 0.2 +5.1 ± 0.2 +6.3 ± 0.1 +7.1 ± 0.2

* Observed migration distance.
** Intense peak.

conversion into the ionic forms of the weak acids. In contrast, nearly a stoichiometric 1:1 reaction of the stronger base tetramethylammonium hydroxide was observed (see Figs. 7–9). Nevertheless, 3-nitrophenol was only 90% in ionic form at the equivalent point; 300% excess (4 equivalent amounts) was required to reach the absorbance corresponding to 100% of the anionic form (see Fig. 7). For 2-naphthol, nearly 95% was in ionic form at the equivalence point, but 400% excess (5 equivalent amounts) was required to reach 100% conversion (see Fig. 8). 9-Phenanthrol was only 80% ionic at the equivalence point and it required 525% excess (6.25 equivalent amounts) to get to 100% anionic form (see Fig. 9). Thus, excess base was required to reach 100% anionic form even with the stronger base tetramethylammonium hydroxide, though, the results were still in line with their pK_a values in water. Even though there was evidence of a 1:1 reaction for the strongest base, tetrabutylammonium hydroxide, nearly 100% excess base was required to reach 100% of the anionic form of 3-nitrophenol (see Fig. 10). However, the results with tetrabutylammonium hydroxide may be misleading as more than 100% anionic form was observed at higher percentage of excess base, probably owing to our inability to correct adequately for the benzene–alcohol in the tetrabutylammonium hydroxide.

Electrophoresis

The conditions were selected as described below on the basis of spectrophotometric studies (see Figs. 7–9) where 2.0 m*M*, 20.0 m*M* and 40.0 m*M* of base corresponded to 50%, 500% and 1000% of base, respectively, added in spectrophotometric studies. For example, this also corresponded to, 55%, 100% and 100%, respectively, of the anionic form for 3-nitrophenol (see Fig. 7). However, for isomeric compounds, small differences in the percentages of their anionic forms were expected from their pK_a values in water. For that reason, the electrophoretic behaviors in tetramethylammonium hydroxide of 4-nitrophenol and 1-naphthol were compared with those of 3-nitrophenol, 2-naphthol and 9-phenanthrol (see Table II). The results suggested that 4-nitrophenol migrated more than 3-nitrophenol, and that 1-naphthol migrated more than 2-naphthol. At a lower concentration of tetramethylammonium hydroxide, differential ionization was obtained for the isomeric compounds, whereas at higher concentration, complete ionization of all the species minimized the differences. Note that 1-naphthol showed an impurity peak, the migration of which did not depend on the concentration of the base, indicating non-ionization of the impurity present. The later study with tetrabutylammonium and benzyltrimethylammonium hydroxide also confirmed this assumption.

Next, tetrabutylammonium hydroxide was used to show that a stronger base showed greater mobilities than those in tetramethylammonium hydroxide, and to see if ion-pairing led to discrepancies similar to those observed in the preliminary electrophoretic study with tetramethylammonium hydroxide. Spectrophotometric measurements suggested that the strongest base tetrabutylammonium hydroxide should be able to ionize 3-nitrophenol (see Fig. 10) in the concentration range 2.0 m*M* to 20.0 m*M*. Electrophoresis showed significant migration of all the model acids even in 2.0 m*M* base (see Table IV). These results confirmed the spectrophotometric study, *e.g.*, 3-nitrophenol was nearly 70% in the anionic form at that concentration of tetrabutylammonium hydroxide. Though an equimolar mixture of 3-nitrophenol, 2-naphthol and 9-phenanthrol was well resolved only at 20.0 m*M* tetrabutylammo-

TABLE V

MIGRATION DISTANCES IN DIFFERENT CONCENTRATIONS OF TETRABUTYLAMMONIUM HYDROXIDE

Concentration (M)	*Corrected migration distances (cm)*				
	*Anthracene (cm)**	*2-Naphthyl-7-sulfonic acid*	*2-Naphthylamine-6-sulfonic acid*	*2-Hydroxy-6-naphthoic acid*	*Mixture***
$2.0 \cdot 10^{-3}$	0 (−2.8)	+3.0 ± 0.1*** +4.0 ± 0.2	+3.4 ± 0.2	2.9 ± 0.1*** +3.7 ± 0.3	+2.5 ± 0.1*** +3.4 ± 0.2 +4.2 ± 0.1
$20.0 \cdot 10^{-3}$	0 (−3.0)	+6.3 ± 0.3 +8.9 ± 0.3	+7.1 ± 0.2	+6.4 ± 0.3 +8.1 ± 0.2	6.2 ± 0.2*** 7.0 ± 0.1 7.7 ± 0.2 9.0 ± 0.3

* Observed migration distance.
** Equimolar mixture of all three acids.
*** Intense peak.

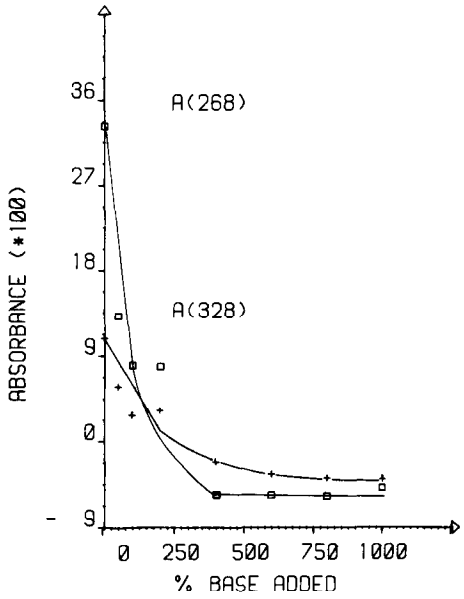


Fig. 10. Spectrophotometric measurements of 3-nitrophenol at different wavelengths with different percentages of base (tetrabutylammonium hydroxide) added. Traces: □ = 268 nm; + = 328 nm.

nium hydroxide, isomeric forms of naphthols and nitrophenols were well resolved at both 2.0 m*M* and 20.0 m*M* tetrabutylammonium hydroxide (see Table IV). These results were in qualitative agreement with the pK_a values of weak acids in water (and in acetonitrile) and also with our spectrophotometric data. Potentiometric results also confirmed the electrophoretic mobilities of 3-nitrophenol, 2-naphthol and 9-phenanthrol in 2.0 m*M* and 20.0 m*M* where the pH values reached 19.0 beyond the equivalence point (*i.e.* complete ionization was expected). Separated zones were much sharper in tetrabutylammonium hydroxide than in tetramethylammonium hydroxide. This was confirmed by running an equimolar mixture of 3-nitrophenol, 2-naphthol and 9-phenanthrol (Mixture A in Table IV, Fig. 11) and equimolar mixture of nitrophenols and naphthols (Mixture B in Table IV, Fig. 12).

When electrophoresis of strong acids was carried out in tetrabutylammonium hydroxide, conditions in 2.0 m*M* and 20.0 m*M* excess corresponded to 30.0 ml and 75.0 ml of titrant, respectively, which produced pH values of 19.5–20.5 and 21.5–27.5, respectively (see Fig. 4). Thus, complete ionization was expected. Significant migrations of all the species were observed at 2.0 m*M* and 20.0 m*M* tetrabutylammonium hydroxide (see Table V). Migration decreased from 2-naphthol-7-sulfonic acid to 2-hydroxy-6-naphthoic acid to 2-naphthylamine-6-sulfonic acid. An equimolar mixture of the three acids was well resolved at 20.0 m*M* tetrabutylammonium hydroxide (see Fig. 13). The relatively small rate of migration of 2-naphthylamine-6-sulphonic acid was expected from its pK_a value in water and due to its presence as a zwitterion. However, 2-hydroxy-6-naphthoic acid, which was more ionized than 2-naphthol-7-sulfonic acid in aqueous and non-aqueous solutions migrated somewhat less in spite of the fact that a sulfonate group was present. Both these acids had

TABLE VI

MIGRATION DISTANCES IN DIFFERENT CONCENTRATIONS OF BENZYLTRIMETHYLAMMONIUM HYDROXIDE

Mixture A was an equimolar mixture of 3-nitrophenol, 2-naphthol and 9-phenanthrol; mixture B was an equimolar mixture of 3- and 4-nitrophenol and 1- and 2-naphthol.

Concentration (M)	*Corrected migration distances (cm)*							
	*Anthracene (cm)**	*3-Nitrophenol*	*4-Nitrophenol*	*1-Naphthol*	*2-Naphthol*	*9-Phenanthrol*	*Mixture A*	*Mixture B*
$2.0 \cdot 10^{-3}$	0 (−1.3)	+1.8 ± 0.1	+2.3 ± 0.2	+1.4 ± 0.1**	+1.5 ± 0.4	+0.3 ± 0.1*	+0.1 ± 0.1**	+1.4 ± 0.1**
				+1.8 ± 0.2		+1.3 ± 0.1	+1.4 ± 0.1	+1.8 ± 0.2
							+1.8 ± 0.1	+2.3 ± 0.1
$20.0 \cdot 10^{-3}$	0 (−1.4)	+3.1 ± 0.1	+3.7 ± 0.3	+1.4 ± 0.1**	+2.5 ± 0.4	+0.4 ± 0.1**	0.0 ± 0.1**	+1.4 ± 0.1**
				+3.0 ± 0.2		+2.0 ± 0.1	+2.0 ± 0.2	+2.2 ± 0.3
							+2.4 ± 0.1	+3.0 ± 0.1
							+3.3 ± 0.1	+3.8 ± 0.1
$40.0 \cdot 10^{-3}$	0 (−1.9)	+4.1 ± 0.1	+4.9 ± 0.2	+1.4 ± 0.1**	+3.8 ± 0.3	0.0 ± 0.1**	−0.1 ± 0.1**	+1.3 ± 0.1**
				+4.0 ± 0.2		+2.7 ± 0.1	+2.7 ± 0.1	+3.8 ± 0.1
							+3.9 ± 0.3	+4.1 ± 0.1
							+4.6 ± 0.1	+4.8 ± 0.2

* Observed migration distance.
** Intense peak.

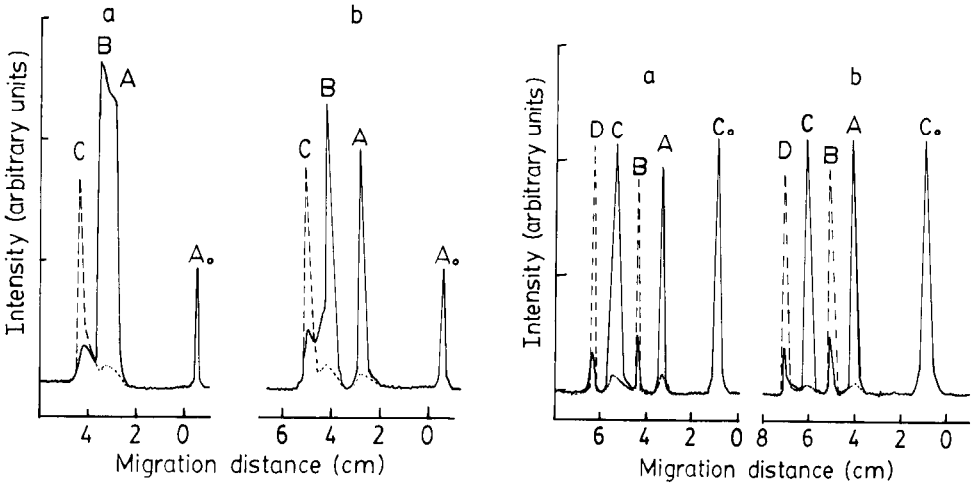


Fig. 11. The electrophoretic separation of an equimolar mixture of model acids in (a) 2.0 m*M* and (b) 20.0 m*M* tetrabutylammonium hydroxide. Model acids were separated in the order A = 9-phenanthrol, B = 2-naphthol and C = 3-nitrophenol. A_0 is an impurity peak. Traces: ——— = fluorescence scan at 300–350 nm excitation and 400 nm emission wavelengths using 400-nm filter; --- = absorbance scan at 400 nm wavelength.

Fig. 12. The electrophoretic separation of an equimolar mixture of model acids in (a) 2.0 m*M* and (b) 20.0 m*M* tetrabutylammonium hydroxide. Model acids were separated in the order A = 2-naphthol, B = 3-nitrophenol, C = 1-naphthol, and D = 4-nitrophenol. A_0 is an impurity peak. Traces as in Fig. 11.

considerable amounts of impurity, whose migrations were concentration-dependent, indicating that they were ionizable.

Benzyltrimethylammonium hydroxide appeared to be very similar to piperidine in strength in the potentiometric titrations. Spectrophotometric studies suggested that these intermediate strength bases should be unable to ionize the weak acids even at 40.0 m*M* (see Fig. 6). However, electrophoresis of model acids in benzyltrimethylammonium hydroxide showed significant migration even at 2.0 m*M* (see Table VI). Furthermore, this base appeared noticeably stronger than piperidine in electrophoresis: the migrated zones were very sharp compared with those obtained with piperidine and tetramethylammonium hydroxide. This was confirmed by running an equimolar mixture of 3-nitrophenol, 2-naphthol and 9-phenanthrol (Mixture A in Table VI, Fig. 14) which showed differential migration at 20.0 m*M* or higher concentration. Isomeric forms of the nitrophenols were well resolved at 40.0 m*M* benzyltrimethylammonium hydroxide (see Table V, Fig. 15), whereas isomeric forms of weaker naphthols showed good separation at 20.0 m*M* benzyltrimethylammonium hydroxide. Proper selection of detection mode permitted spectroscopic resolution of overlapping zones of naphthols and nitrophenols.

Except for 4-nitrophenol, no significant migrations of weak acids were observed when tributylamine was used. Thus, it was still possible to separate a mixture of 4-nitrophenol and 3-nitrophenol in 20.0 m*M* and 40.0 m*M* base (see Table VII). Likewise, significant migrations of the strong acids were obtained in tributylamine. In 2.0 m*M* tributylamine, 2-naphthylamine-6-sulfonic acid, 2-naphthyl-7-sulfonic acid and 2-hydroxy-6-naphthoic acid migrated in their decreasing order (see Table

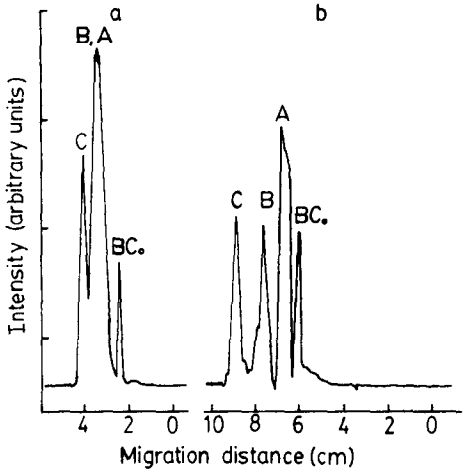


Fig. 13. The electrophoretic separation of an equimolar mixture of model acids in (a) 2.0 m*M* and (b) 20.0 m*M* tetrabutylammonium hydroxide. Model acids were separated in the order A = 2-naphthylamine-6-sulfonic acid, B = 2-hydroxy-6-naphthoic acid, and C = 2-naphthyl-7-sulfonic acid. C_0 is an impurity peak. The signal represents fluorescence scan at 250 nm excitation and 400 nm emission wavelengths using 400-nm filter.

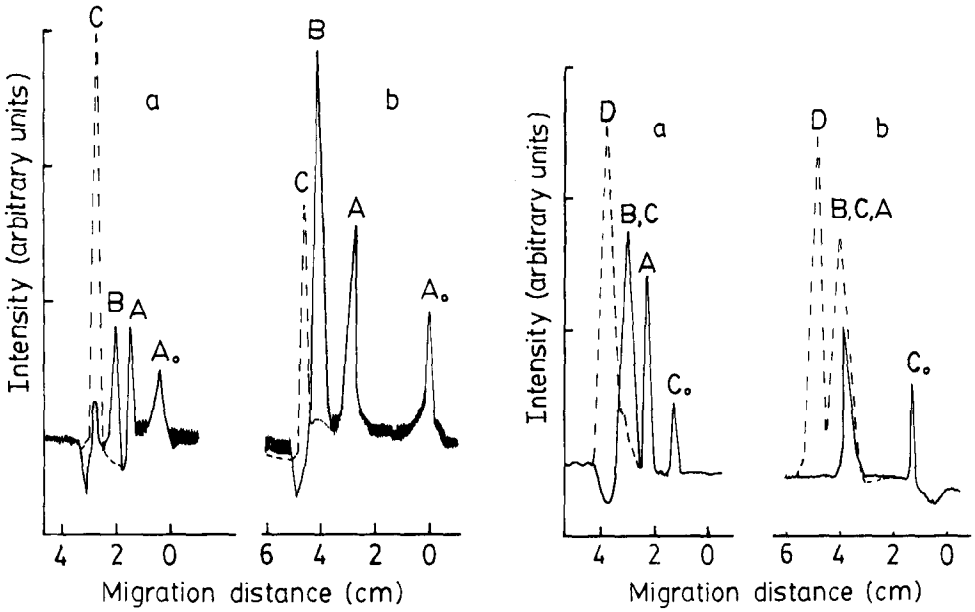


Fig. 14. The electrophoretic separation of an equimolar mixture of model acids in (a) 20.0 m*M* and (b) 40.0 m*M* benzyltrimethylammonium hydroxide. Details as in Fig. 11.

Fig. 15. The electrophoretic separation of an equimolar mixture of model acids in (a) 20.0 m*M* and (b) 40.0 m*M* benzyltrimethylammonium hydroxide. Details as in Fig. 12.

TABLE VII

MIGRATION DISTANCES IN DIFFERENT CONCENTRATIONS OF TRIBUTYLAMINE

Concentration (M)	*Corrected migration distances (cm)*			
	*Anthracene (cm)**	*3-Nitrophenol*	*4-Nitrophenol*	*Mixture***
$2.0 \cdot 10^{-3}$	0 (−1.3)	+0.2 ± 0.1	+0.7 ± 0.1	+0.3 ± 0.1
$20.0 \cdot 10^{-3}$	0 (−1.6)	+0.4 ± 0.1	+2.9 ± 0.2	0.0 ± 0.1 +3.0 ± 0.2
$40.0 \cdot 10^{-3}$	0 (−1.7)	+0.6 ± 0.1	+3.3 ± 0.1	+0.5 ± 0.1 +3.3 ± 0.2

* Observed migration distance.
** Equimolar mixture of 3- and 4-nitrophenol.

VIII, Fig. 16). These data did not correlate well with their pK_a values in water or with our potentiometric data in non-aqueous media. However, differential migration was observed best at 20.0 m*M* tributylamine. Decrease in migration of 2-naphthylamine-6-sulfonic acid at 20.0 m*M* in electrophoresis was unexpected.

Paper chromatography

Significant adsorption was observed for the weak acids 3-nitrophenol, 2-naphthol and 9-phenanthrol in the presence of 2.0 m*M* and 20.0 m*M* tetrabutylammonium and benzyltrimethylammonium hydroxide as seen in Table IX. Migrations of these weak acids, however, increased as the concentration of base was increased, except for 9-phenanthrol, which was more adsorbed at the higher concentration. This also confirmed their electrophoretic behavior in the presence of tetrabutylammonium hydroxide (see Table IV). The results indicated that differentiation in the latter was probably due to the formation and adsorption of ion-pairs, whereas that in benzyl-

TABLE VIII

MIGRATION DISTANCES IN DIFFERENT CONCENTRATIONS OF TRIBUTYLAMINE

Concentration (M)	*Corrected migration distances (cm)*				
	*Anthracene (cm)**	*2-Naphthyl-7-sulfonic acid*	*2-Naphthylamine-6-sulfonic acid*	*2-Hydroxy-6-naphthoic acid*	*Mixture***
$2.0 \cdot 10^{-3}$	0 (−1.3)	+1.3 ± 0.1*** +4.0 ± 0.1	+1.3 ± 0.1*** +4.3 ± 0.1	0.0 ± 0.1*** +0.6 ± 0.1	0.0 ± 0.1*** +0.6 ± 0.1 +1.3 ± 0.2*** +3.9 ± 0.1 +4.2 ± 0.1
$20.0 \cdot 10^{-3}$	0 (−1.6)	+1.5 ± 0.1*** +4.0 ± 0.2	+1.5 ± 0.1*** +3.5 ± 0.1	0.0 ± 0.1*** +0.7 ± 0.1	+0.7 ± 0.1 +1.3 ± 0.1*** +3.3 ± 0.2 +4.0 ± 0.2

* Observed migration distance.
** Equimolar mixture of all three acids.
*** Intense peak.

TABLE IX

R_F VALUES DETERMINED BY PAPER CHROMATOGRAPHY OF WEAK ACIDS IN THE BASES OF DIFFERENT STRENGTHS

Solvent: acetonitrile-sulfolane (9:1) containing a small amount of tetraethylammonium perchlorate and an appropriate amount of base. Length of development, 57 cm; temperature, 25°C.

Base	*Concentration (M)*	*Anthracene**	*3-Nitrophenol*	*4-Nitrophenol*	*1-Naphthol***	*2-Naphthol*	*9-Phenanthrol***
Blank***	–	1.0 ± 0.01	1.0 ± 0.01	1.0 ± 0.01	1.0 ± 0.01	1.0 ± 0.01	1.0 ± 0.01
Tetrabutylammonium hydroxide	$2.0 \cdot 10^{-3}$	1.0 ± 0.01	0.64 ± 0.02	0.89 ± 0.01	1.0 ± 0.01	0.48 ± 0.02	0.69 ± 0.03
	$20.0 \cdot 10^{-3}$	1.0 ± 0.01	0.89 ± 0.01	1.00 ± 0.01	1.0 ± 0.01	0.78 ± 0.03	0.63 ± 0.01
Benzyltrimethylammonium hydroxide	$2.0 \cdot 10^{-3}$	1.0 ± 0.01	0.90 ± 0.02	1.0 ± 0.01	1.0 ± 0.01	0.89 ± 0.01	0.80 ± 0.01
	$20.0 \cdot 10^{-3}$	1.0 ± 0.01	1.00 ± 0.02	1.0 ± 0.01	1.0 ± 0.01	1.00 ± 0.01	0.90 ± 0.03

* Neutral species.
** Additional spot at the origin not reported.
*** No base added.

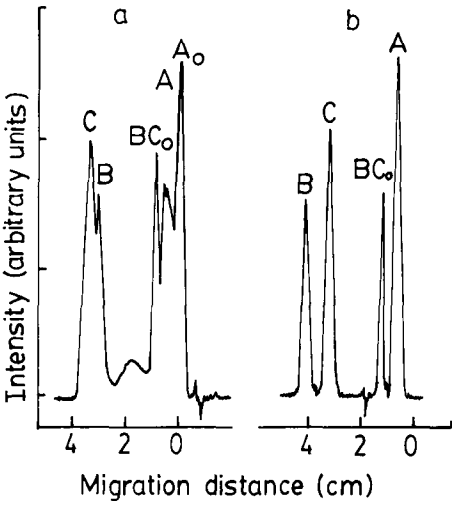


Fig. 16. The electrophoretic separation of an equimolar mixture of model acids in (a) 2.0 m*M* and (b) 20.0 m*M* tributylamine. Details as in Fig. 13.

trimethylammonium hydroxide it was probably due primarily to differences in the partial ionizations.

Considerable movement of the strong acids 2-naphthyl-7-sulfonic acid, 2-naphthylamine-6-sulfonic acid and 2-hydroxy-6-naphthoic acid was observed when the mobile phase contained 2.0 m*M* and 20.0 m*M* tetrabutylammonium hydroxide or tributylamine (see Table X). An additional spot at the origin for all of the acids is not reported in the table. Migrations of strong acids also increased as the concentration of base was increased, except for 2-hydroxy-6-naphthoic acid, which was more adsorbed at the higher concentration. This indicated why the latter migrated less in tributylamine than in tetrabutylammonium hydroxide. 2-Naphthylamine-6-

TABLE X

R_F VALUES DETERMINED BY PAPER CHROMATOGRAPHY OF STRONG ACIDS IN THE BASES OF DIFFERENT STRENGTHS

Conditions as in Table IX.

Base	*Concentration (M)*	*Anthracene**	*2-Naphthyl-7-sulfonic acid***	*2-Naphthylamine-6-sulfonic acid***	*2-Hydroxy-6-naphthoic acid***
Blank***	–	1.00 ± 0.01	1.00 ± 0.01	0.76 ± 0.02	1.00 ± 0.01
Tetrabutylammonium hydroxide	$2.0 \cdot 10^{-3}$	1.00 ± 0.01	0.25 ± 0.01	0.86 ± 0.01	0.86 ± 0.01
	$20.0 \cdot 10^{-3}$	1.00 ± 0.01	0.90 ± 0.02	1.00 ± 0.01	0.85 ± 0.01
Tributylamine	$2.0 \cdot 10^{-3}$	1.00 ± 0.1	0.74 ± 0.01	0.86 ± 0.02	0.65 ± 0.01
	$20.0 \cdot 10^{-3}$	1.00 ± 0.01	0.81 ± 0.01	0.90 ± 0.01	0.47 ± 0.02

* Neutral species.
** Additional spot at the origin not reported.
*** No base added.

sulfonic acid was the only species that adsorbed on paper even when the mobile phase did not contain any base. A decrease in its electromigration at higher concentrations of tributylamine also failed to reflect its chromatographic behavior.

DISCUSSION

The previous study[9] concluded that the use of potentiometric and spectrophotometric titration data for predicting the electrophoretic behavior was very limited, owing to adsorption of the free bases and to ion-pair formation of the protonated bases. However, chromatographic studies for weak acids indicated that adsorption phenomena could largely be neglected in the present study. Thus, potentiometric and spectrophotometric measurements should provide better predictions about the electrophoretic behavior of weak acids in the present non-aqueous solvent system and more nearly reflect the ideal behavior found in aqueous systems[21].

This study showed that it was possible to make weak acids migrate differentially by adding a strong base to acetonitrile–sulfolane (9:1) containing a background electrolyte, tetraethylammonium perchlorate. The concentration and strength of the base proved to be important. Although, non-aqueous potentiometry provided information on the relative ionizing powers of different bases, pK_a data in aqueous solution were more useful in predicting relative electrophoretic behaviors of the weak acids. Spectrophotometry gave qualitative agreement with aqueous pK_a values for 3- and 2-nitrophenol and on the extent the weak acids reacted with the strong bases tetramethyl- and tetrabutylammonium hydroxide. However, chromatographic data suggested some adsorption of the unreacted acids and/or ion-pair formation of the ionized acids.

Potentiometric studies in non-aqueous media provided information on the ionization behavior of stronger acids, such as sulfonic and carboxylic acid derivatives of naphthols and naphthylamine, but this did not lead to successful prediction of electrophoretic behaviors of these acids in the presence of the stronger base tetrabutylammonium hydroxide. The more readily ionizable 2-hydroxy-6-naphthoic acid (in aqueous and in our non-aqueous medium) migrated less than 2-naphthyl-7-sulfonic acid. Isomeric mixtures of these strong acids were still separable with tetrabutylammonium hydroxide and tributylamine. However, in tributylamine total reversal of migration behavior was observed from that predicted from pK_a values in aqueous and non-aqueous solution, probably due to ion-pair formation of one or more species in a mixture.

The bases that did effect differential ionization of weak acids were tetrabutyl- and benzyltrimethylammonium hydroxide. In the former, differential migration was observed even at 2.0 m*M*. The stronger 4-nitrophenol migrated ahead of its isomer, 3-nitrophenol, and the stronger 1-naphthol ahead of its isomer, 2-naphthol. The results were in qualitative and semiquantitative agreement with results from potentiometry and spectrophotometry. In this base, the acids separated in part according to charge but more so according to their chromatographic properties. This was confirmed when an equimolar mixture of 3-nitrophenol, 2-naphthol and 9-phenanthrol was separated well at 20.0 m*M* tetrabutylammonium hydroxide. In addition, the formation of an ion-pair of the weakly stabilized anion with tetraalkylammonium ion[15] was more likely since the concentration of base in acetonitrile was more than

0.01 M[14]. In benzyltrimethylammonium hydroxide, a relatively higher concentration of weaker base was needed to obtain approximately the same differential migration of all the weak acids.

This study also showed that an equimolar mixture of nitrophenols and naphthols could be well separated by electrophoresis using either a moderately strong base, tetrabutylammonium hydroxide, at a relatively lower concentration or a somewhat weaker base, benzyltrimethylammonium hydroxide, at a relatively higher concentration. Ion-pair formation and/or adsorption was used to advantage to separate these weak acids. The migrations could be followed easily by scanning with the proper excitation and emission wavelengths, together with proper selection of the detection mode so as to resolve overlapping zones spectroscopically.

Spectrophotometric and potentiometric results for tetramethylammonium hydroxide and tributylamine did not lead to successful prediction of conditions for electrophoresis of model weak acids. Their migrations contradicted pK_a values in water and in acetonitrile. However, separation of an isomeric mixture of nitrophenols and of naphthols was possible in both bases.

It was interesting to attempt to correlate the electrophoretic results of weak acids and bases with their pK values in aqueous and in non-aqueous solution. However, detailed calculations involving equilibria in acetonitrile using an autoprotolysis constant of $6 \cdot 10^{-33}$ may have been misleading because of the difficulty of obtaining acetonitrile and sulfolane in pure form. Traces of water, ammonia or ammonium acetate from solvent decomposition can affect the equilibria. As shown by Kolthoff *et al.*[17], alcohol in tetraalkylammonium hydroxide can increase the formation of conjugated pairs of weak acids. Also, the adsorption and/or ion-pair formation can change the equilibria, but they may be used as to advantage in the separation of a complex mixture.

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