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

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SUMMARY

By the addition of an appropriate strength and concentration of acid which is in acetonitrile–sulfolane (9:1) containing a small amount of tetraethylammonium perchlorate as background electrolyte, weak organic nitrogen bases showed differential migration in high-voltage paper electrophoresis. Dichloro- and trichloro-acetic acids were effective for separating 5,6-benzoquinoline, 7,8-benzoquinoline and 1,10-phenanthroline. The use of potentiometric and spectrophotometric titration data to predict the electrophoretic behavior was very limited due to adsorption of the free bases by paper.

INTRODUCTION

The long-term goal of this study is to examine the feasibility of applying non-aqueous electrophoresis to the separation of mixtures of weak acids and weak bases found in coal tars, petroleum pitches and crudes. The feasibility of converting very weak acids and bases into ionic species by adding either a strong base or acid to the non-aqueous solvent has been examined in our laboratory[1]. This provided information on conditions that led to migration of model weak bases in acetonitrile–sulfolane (9:1) upon adding strong acids and, also, to migrations of weak acids upon adding strong bases.

The present study deals with the attempts to obtain, by electrophoresis, a differential migration of those weak nitrogen bases on paper by using acids of different strengths. In strong acids, the change in apparent pH with a concentration of acid was too rapid; the optimal apparent pH was difficult to adjust and properly maintain. Acetic acid and its halogenated derivatives were selected for testing because they had little UV–visible absorbance, thereby minimizing interference with fluorometric measurements of the aromatic bases. The sulfolane in the acetonitrile served to reduce the volatility of acetonitrile and to improve the reproducibility of the electrophoretic

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data. Anthracene was used to measure and correct for electroendosmotic flow[2,3].

Potentiometric and spectrophotometric titrations were performed so as to find the combination of concentration and strength of an acid needed to promote partial ionization of the weak base. Attempts were then made to correlate this information with the electrophoretic data.

EXPERIMENTAL

Chemicals

Anthracene (Anth), 5,6-benzoquinoline (5,6-BQ), 7,8-benzoquinoline (7,8-BQ) and 1,10-phenanthroline (1,10-Phen) were used as received (Aldrich, Milwaukee, WI, U.S.A.). Trifluoromethanesulfonic acid (TFMSA), methanesulfonic acid (MSA), dichloroacetic acid (DCA), monochloroacetic acid (MCA) (all from Eastman Kodak, Rochester, NY, U.S.A.) and trichloroacetic acid (TCA) plus acetic acid (HAC) (both from J. T. Baker, Phillipsburg, NJ, U.S.A.) were used to promote ionization of the bases. Tetraethylammonium perchlorate (TEAP) (Eastman Kodak, Rochester, NY, U.S.A.) 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.

The $5.0 \cdot 10^{-3}$ *M* solutions of bases and solutions of acids ranging from $1.0 \cdot 10^{-3}$ *M* to $20.0 \cdot 10^{-3}$ *M* were freshly prepared in standard solvent containing 0.25% TEAP before each potentiometric, electrophoretic or paper chromatographic experiments. Similarly, solutions of bases and acids ranging from $5.0 \cdot 10^{-5}$ *M* to $30.0 \cdot 10^{-5}$ *M* were freshly prepared in standard solvent containing 0.25% TEAP before each spectrophotometric experiment. The solvent system containing acetonitrile–sulfolane (9:1) (standard solvent) and 0.25% TEAP, hereafter referred to as a background electrolyte, was used as a blank.

A buffer for standardizing the pH electrode was prepared in the standard solvent according to the procedure of Kolthoff and Chantooni[4] using tetraethylammonium methanesulfonate–MSA. Tetraethylammonium methanesulfonate was prepared by neutralizing 1 *M* aqueous solution of tetraethylammonium hydroxide (Aldrich, Milwaukee, WI, U.S.A.) using aqueous phenolphthalein as an external indicator. The solution was evaporated to an oil which was then continuously extracted with ethyl acetate for 24 h. The extract was evaporated to dryness and the salt washed twice with anhydrous diethyl ether (J. T. Baker), dried at 65°C *in vacuo* for 6 h and stored in a closed bottle in a desiccator until used. NMR and IR spectra confirmed the purity of the salt.

Pure cellulose, Whatman 3MM 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, Great Britain), and its 10-k V d.c. power supply (SAE 3205) were employed with custom-made glass troughs[1].

Sample zones were detected by their fluorescence under UV light using a Model CS-910 densitometer (Shimadzu Scientific Instrument, Columbia, MD, U.S.A..

Potentiometric titrations were performed using a Corning pH meter, Model

130, equipped with Corning pH triple-purpose (Ag–AgCl internal standard) glass electrode (cat. No. 476022), and an automatic temperature compensator. A silver–0.01 *M* silver nitrate (in acetonitrile) electrode, designed by Kolthoff and Reddy[5], and a silver billet electrode (Fisher Scientific, Pittsburgh, PA, U.S.A.) were used as reference electrodes. The silver–0.01 *M* silver nitrate electrode was made by fusing a platinum wire into the bottom of the cell and plating it with silver from a 0.05 *M* silver cyanide solution in the conventional way[3]. The reference electrode and its side arm were filled with 0.010 *M* silver nitrate solution in acetonitrile.

The UV absorption spectra of titrated bases were recorded on a computer-controlled instrument that has been described[6]. It consists of a GCA-McPherson spectrophotometer (EU-700 series) interfaced to a Digital Equipment PDP11/34 computer.

Procedures

High-voltage electrophoresis. Paper was cut into 10 × 57 cm strips. Each electrode trough was filled with 200 ml of background electrolyte solution containing the appropriate acid. The paper wicks and supports were placed in position and saturated with solution. Excess solution was removed by blotting lightly with a cotton towel and clamping shut the apparatus lid for a few seconds. Then, approximately a 2.5-μl sample of $5.0 \cdot 10^{-3}$ *M* of each compound 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[1]. Following each electrophoresis experiment, the distance traveled 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[2,3] as determined by anthracene[1]. 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.

The sample zones were scanned at 250–350/400 nm excitation/emission wavelengths using a 400-nm filter. The mixture of bases was scanned at 250–350/400 nm excitation/emission wavelengths using "UV-D_2" filters, and a 400-nm filter.

Potentiometric measurements. The glass electrode was calibrated using a tetraethylammonium methanesulfonate–MSA buffer of apparent pH 4.402 before each titration[4]. When not in use, the electrode was kept in water. Just prior to use, the glass bulb was rinsed several times with acetonitrile and then inserted into the glass electrode compartment of the H-type cell containing the solution to be titrated.

The H-type cell as shown in Fig. 1 was similar to one used by Coetzee and Padmanabhan[7]. It consisted of three compartments. The glass electrode compartment held the glass electrode, a temperature compensator, an inlet for Teflon® tubing (0.16-cm I.D.) that was connected to a helium tank and another inlet for Teflon tubing (0.16-cm I.D.) that was connected to a Model 396-31, pump (Milton Roy, Riviera Beach, FL, U.S.A.), so as to add the titrant. The reference electrode compartment (10-mm O.D. tubing) dipped into a 0.1 *M* TEAP solution contained in the salt bridge compartment (12-mm O.D. tubing). This compartment was connected to the glass electrode compartment (12-mm O.D. tubing) by a fine porosity sintered glass disk.

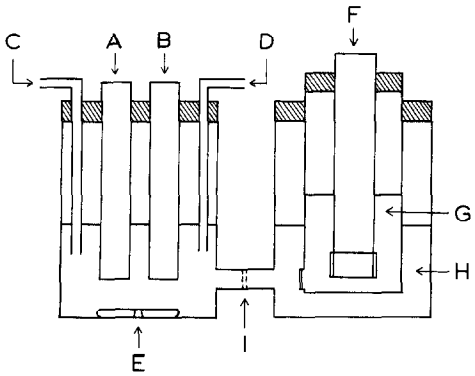


Fig. 1. H-type cell for potentiometric titration. A = Corning pH triple-purpose (Ag–AgCl internal standard) glass electrode; B = automatic temperature compensator; C = inlet helium; D = inlet for titrant; E = bar magnet; F = reference electrode; G = 0.01 *M* silver nitrate solution in acetonitrile; H = 0.1 *M* TEAP solution used as a salt bridge; I = fine porosity sintered glass disk.

Under helium and with constant stirring (bar magnet), a. 0.01 *M* or a 0.10 *M* solution of TFMSA, MSA, TCA, DCA, MCA or HAC was added independently to the glass electrode compartment containing 50 ml of background electrolyte solution. The acid was pumped at a rate of 3.0 ml/min for 10 min. The pH values were fed to a Linear® recorder.

The titrations of bases using those acids were also carried out in the set-up described above. A 50-ml 0.0050 *M* solution of each base (5,6-BQ, 1,10-Phen and 7,8-BQ) was also titrated against a 0.010 *M* or a 0.10 *M* solution of acid.

To study the possible effect of paper on the acid–base reactions, pure cellulose paper (5 × 10 cm), similar to that used as the electrophoretic support, was added in the glass electrode compartment of the H-type cell. The titrations of both the blank solvent and the bases using TFMSA and MSA were carried out.

Paper chromatography. These studies of bases were also carried out using acetonitrile–sulfolane (9:1) containing a small amount of TEAP and an appropriate amount of a weak acid as mobile phase. Paper strips (Whatman 3 MM Paper) of 12 × 10 cm were cut and a small amount of each 0.005 *M* base was spotted by a micropipette. The paper strips were placed in a covered jar containing the mobile phase. After the mobile phase had been allowed to run to a preestablished distance, the paper strips were taken from the jar, dried in air, and the migrations of the bases observed under the UV lamp.

Spectrophotometric measurements. Titrations of weak bases for the spectrophotometric measurements were performed by adding successively 50–300% of different portions of acids TFMSA, TCA and MCA, and diluting solutions to the same volume every time. Spectra were thenr ecorded in the UV region for these solutions (380–235 nm) containing different concentration of acids. Spectra for pure acids and pure bases were also recorded to correct for any excess amount of acid or base present in the titration mixture. The data were taken on a PDP-11/34 computer and the manipulation was done through digital multiplication and subtraction techniques. The difference in spectra of weak base alone and the base after titration was monitored at the wavelength of maximum absorbance for a particular base.

RESULTS

Preliminary experiments

Electrophoresis. Two types of preliminary experiments were carried out using the strong acids, TFMSA and MSA. In the first experiment, the number of model compounds was restricted to 5,6-BMQ and 1,10-Phen and checking their electrophoretic behaviors in TFMSA where complete ionization had previously been obtained[1]. At 2.0 m*M*, 5.0 m*M* and 10.0 m*M* concentrations of TFMSA, both 5,6-BQ and 1,10-Phen ionized to the same extent, within experimental error (Table I). At the highest concentration, 20.0 m*M* TFMSA, migrations of all species were somewhat less, probably due to a smaller fraction of the current being carried by those ions. There were two distinct peaks observed for 5,6-BQ alone and in a mixture at 1.0 m*M* and 2.0 m*M* concentrations of TFMSA. The peak intensity at the origin increased as the concentration of the acid decreased. At the same time, onset of differential migration was observed at 1.0 m*M* TFMSA. Thus, there is a trade-off between the complete ionization and adsorption.

TABLE I

MIGRATION DISTANCES IN DIFFERENT CONCENTRATIONS OF TFMSA

TFMSA ($\times 10^{-3}$ M)	*Observed migration distance, Anth (cm)*	*Corrected migration distance (cm)*		
		5,6-BQ	*1,10-Phen*	*Mixture**
1.0	0 (+0.7)	−0.6 ± 0.1**	−4.2 ± 0.1	−0.1 ± 0.1**
		−2.5 ± 0.3		−2.1 ± 0.3
				−3.8 ± 0.1
2.0	0 (+0.8)	−0.5 ± 0.3**	−4.2 ± 0.3	−0.3 ± 0.1**
		−4.2 ± 0.3		−4.1 ± 0.2
5.0	0 (+0.9)	−4.8 ± 0.4	−4.9 ± 0.3	−3.5 ± 0.1
10.0	0 (+1.2)	−4.8 ± 0.2	−5.2 ± 0.2	−4.2 ± 0.1
20.0	0 (+0.3)	−2.6 ± 0.1	−3.0 ± 0.1	−2.3 ± 0.1

* Equimolar mixture of 5,6-BQ and 1,10-Phen.
** Intense peak.

The second experiment was done using a weaker acid, MSA. The trend of migration with MSA appeared to be similar to the one obtained by TFMSA except that the maximum migration occurred at 5.0 m*M* MSA (Table II) compared to 10.0 m*M* in the case of TFMSA.

Paper chromatography. Model organic bases showed no adsorption on paper when the mobile phase contained 1.0 m*M* and 2.0 m*M* TFMSA. All the bases were very close to the solvent front.

Potentiometric data on bases and acids

The objectives behind the potentiometric measurements were (a) to determine the apparent p*K* values of model bases in acetonitrile–sulfolane (9:1) and (b) to obtain the apparent pH in blanks as a function of the concentration of each acid alone.

TABLE II

MIGRATION DISTANCES IN DIFFERENT CONCENTRATIONS OF MSA

MSA ($\times 10^{-3}$ M)	*Observed migration distance, Anth (cm)*	*Corrected migration distance (cm)*		
		5,6-BQ	*1,10-Phen*	*Mixture**
1.0	0 (−0.7)	−0.3 ± 0.1**	−0.3 ± 0.1**	0.0 ± 0.1**
		−3.4 ± 0.2	−4.1 ± 0.1	−3.3 ± 0.2
2.0	0 (−0.2)	−0.4 ± 0.1**	−0.4 ± 0.1	−0.5 ± 0.1**
		−3.5 ± 0.1		−3.2 ± 0.3
5.0	0 (−0.7)	−3.7 ± 0.2	−4.3 ± 0.3	−3.6 ± 0.2
10.0	0 (−0.6)	−3.2 ± 0.1	−4.1 ± 0.2	−3.2 ± 0.1
20.0	0 (−0.5)	−2.9 ± 0.1	−3.6 ± 0.1	−3.0 ± 0.3

* Equimolar mixture of 5,6-BQ and 1,10-Phen.
** Intense peak.

Then, an optimum concentration of acid was to be selected for the electrophoresis by comparing the pH curves of pure acids with the titration curves of the model organic bases. At the optimum concentration, one should obtain the maximum differential ionization and, hence, the maximum differential migration of the model compounds in electrophoresis, assuming adsorption is negligible.

The capabilities of the acids to ionize weak bases were estimated from their pH profiles. The pH profile of each acid obtained by titration of blank solutions with 0.01 *M* solutions of different acids is shown in Fig. 2. Note that the apparent pH of the blank was close to 7.0 and that the only unexpected behavior was found with MCA. It repeatedly titrated as a somewhat weaker acid than HAC, probably due to the small amount of water present in glacial acetic acid.

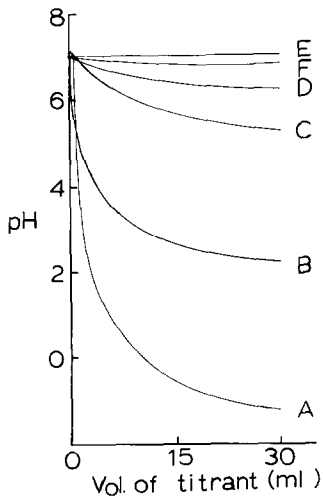


Fig. 2. Titration of 50 ml of blank solutions with 0.01 *M* solutions of different acids. A = TFMSA; B = MSA; C = TCA; D = DCA; E = MCA; F = HAC.

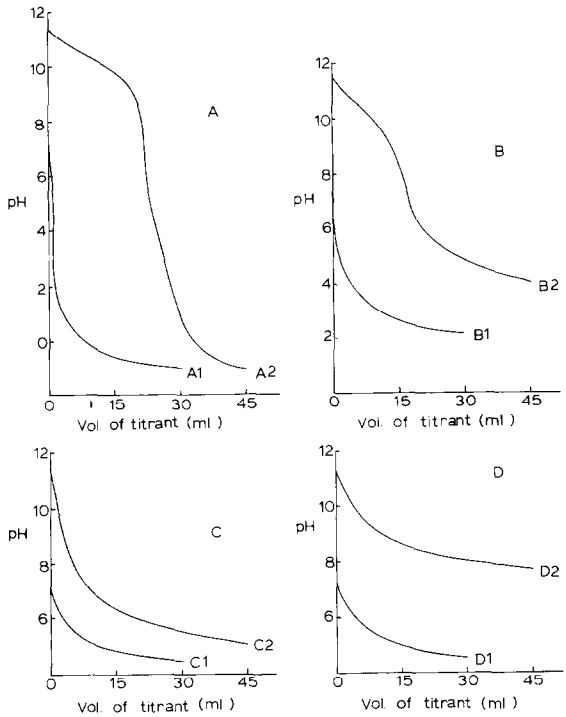


Fig. 3. pH curves for the acids alone relative to the titration curves of 50 ml of 5,6-BQ with different acids: (A) A1 = blank with 0.01 *M* TFMSA, A2 = 0.005 *M* 5,6-BQ with 0.01 *M* TFMSA; (B) B1 = blank with 0.01 *M* MSA, B2 = 0.005 *M* 5,6-BQ with 0.01 *M* MSA; (C) C1 = blank with 0.1 *M* TCA, C2 = 0.005 *M* 5,6-BQ with 0.1 *M* TCA; (D) D1 = blank with 0.1 *M* DCA, D2 = 0.005 *M* 5,6-BQ with 0.1 *M* DCA.

The location of pH curves for the acids alone relative to the titration curves of 5,6-BQ and 1,10-Phen with different acids are shown in Figs. 3 and 4, respectively. Titration of 5,6-BQ with TFMSA showed signs of second p*K* (Fig. 3A), whereas MSA of the same concentration showed only one p*K* value followed by a region of moderately rapid change in pH (Fig. 3B). Even after using ten-fold more concentrated solutions of TCA (Fig. 3C) and DCA (Fig. 3D), a three-fold excess of TCA was required to reach the pH of the solvent, whereas even at a nine-fold excess of DCA, the pH was still approximately one unit more alkaline than that of the solvent alone. The curve of 1,10-Phen showed similar behavior (Fig. 4A–D) to that of 5,6-BQ. The 1,10-Phen started, as expected, at a more alkaline pH. Again, the corresponding ratios for excess acid produced somewhat less acidic pH values. In other words, a higher ratio of the excess TCA was needed to attain pH of the solvent alone (four- to five-fold) than for TFMSA.

The possible effects of paper on the acid–base reactions were studied using TFMSA and MSA as shown in Fig. 5. In the absence of paper, TFMSA showed evidence for two p*K* values for both 5,6-BQ and 1,10-Phen (Fig. 5A and B): 10.3 ± 0.1 and 3.1 ± 0.2 for 5,6-BQ; 12.1 ± 0.1 and 3.8 ± 0.2 for 1,10-Phen. Also, the ratios for the endpoint volumes were roughly 3:1 for 5,6-BQ and 1.5:1 for 1,10-Phen.

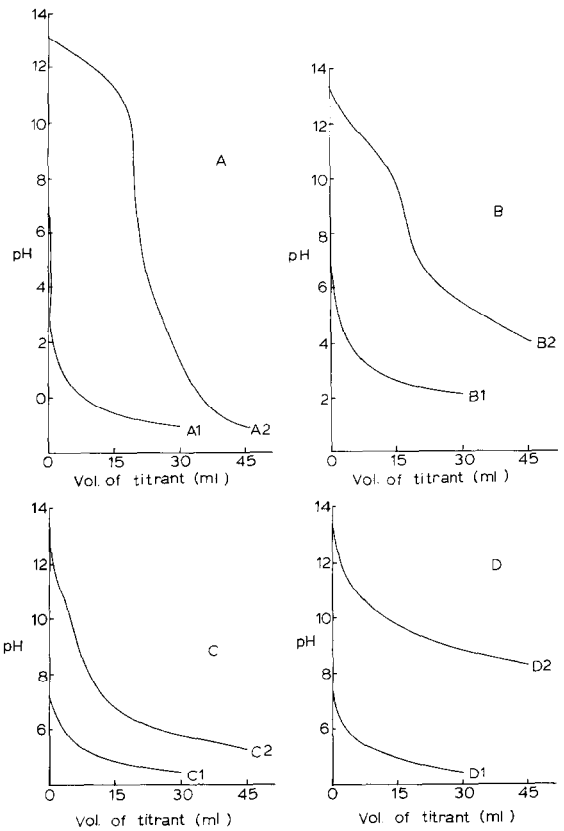


Fig. 4. pH curves for the acids alone relative to the titration curves of 50 ml of 1,10-Phen with different acids: (A) A1 = blank with 0.01 *M* TFMSA, A2 = 0.005 *M* 1,10-Phen with 0.01 *M* TFMSA; (B) B1 = blank with 0.01 *M* MSA, B2 = 0.005 *M* 1,10-Phen with 0.01 *M* MSA; (C) C1 = blank with 0.1 *M* TCA, C2 = 0.005 *M* 1,10-Phen with 0.1 *M* TCA; (D) D1 = blank with 0.1 *M* DCA, D2 = 0.005 *M* 1,10-Phen with 0.1 *M* DCA.

Only one p*K* value could be determined using MSA (Fig. 5C and D). In the presence of paper, solvent blanks showed that each of the acids was reacting to a significant extent with the paper, presumably, for the most part, by adsorption. Titrations of weak bases with the strong acids in the presence of paper showed extremely complex results. The p*K* value of the base, especially 5,6-BQ, showed that it became weaker. Also, the second p*K* fused into the first. In the case of 5,6-BQ, the break was large and occurred at a volume for the endpoint of the second p*K*. In the case of 1,10-Phen, the break occurred at a volume slightly greater than that for the endpoint of the first p*K* but less than that for the endpoint of the second p*K*. Titrations with MSA in the presence of paper showed no significant effect on either the endpoint volumes or the p*K* values.

Electrophoresis

The conditions were selected as described below on the basis of potentiometric studies, where 1.0 m*M*, 2.0 m*M* and 5.0 m*M* of acid corresponded to 5.6 ml, 12.5 ml

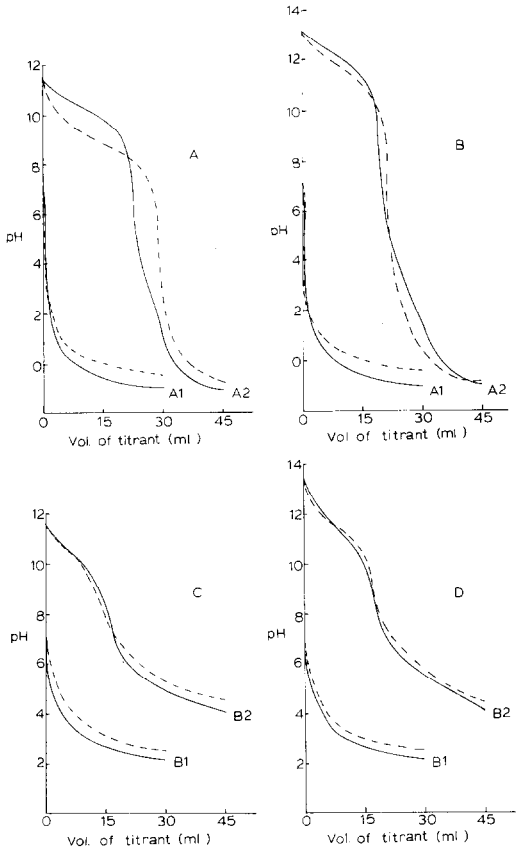


Fig. 5. Effects of paper on the acid-base reactions. (——) Represents titration curves in the absence of paper; (----) represents titration curves in the presence of paper (5 × 10 cm); (A) A1 = blank with 0.01 *M* TFMSA, A2 = 0.005 *M* 5,6-BQ with 0.01 *M* TFMSA; (B) A1 = blank with 0.01 *M* TFMSA, A2 = 0.005 *M* 1,10-Phen with 0.01 *M* TFMSA; (C) B1 = blank with 0.01 *M* MSA, B2 = 0.005 *M* 5,6-BQ with 0.01 *M* MSA; (D) B1 = blank with 0.01 *M* MSA, B2 = 0.005 *M* 1,10-Phen with 0.01 *M* MSA.

and 50.0 ml respectively of titrant in the potentiometric studies. At this volumes maximum change in the apparent pH values were observed. For 5,6-BQ they were 10.6, 10.0 and −1.0 respectively, and for 1,10-Phen, 12.3, 11.8 and −1.0, respectively. Thus, acids ranging from 1.0 m*M* to 20.0 m*M* in concentration were used in the background electrolyte solution, since, in the blank solution all the acids in this range of concentration reached the pH values where ionization was expected. The electrophoretic results with MCA and HAC showed no migration of model bases (Tables III and IV) indicating that MCA and HAC did not ionize the model bases enough to cause migration from the origin. Thus, the predictions based upon potentiometric measurements were misleading.

Paper chromatography showed that the model organic bases remained at the origin in a mobile phase that contained 20.0 m*M* MCA or 20.0 m*M* HAC. This indicated adsorption by the paper of neutral species in acid solutions. Thus, it confirmed the results of electrophoresis which showed that organic bases did not move in the presence of MCA or HAC.

TABLE III

MIGRATION DISTANCES IN DIFFERENT CONCENTRATIONS OF MCA

MCA (× 10^{-3} M)	*Observed migration distance, Anth (cm)*	*Corrected migration distance (cm)*			
		7,8-BQ	*5,6-BQ*	*1,10-Phen*	*Mixture**
1.0	0 (0.0)	−0.1 ± 0.1	−0.2 ± 0.1	−0.8 ± 0.1	−0.2 ± 0.1
2.0	0 (0.0)	−0.3 ± 0.1	−0.3 ± 0.1	−1.1 ± 0.1	−0.5 ± 0.1
5.0	0 (+0.3)	−0.2 ± 0.1	−0.1 ± 0.1	−1.3 ± 0.1	−0.5 ± 0.1
10.0	0 (−0.1)	0.0 ± 0.1	0.0 ± 0.1	−0.3 ± 0.1	−0.1 ± 0.1 −0.5 ± 0.1
20.0	0 (−0.4)	−0.1 ± 0.1	−0.1 ± 0.1	−0.2 ± 0.1	−0.1 ± 0.1 −0.4 ± 0.1

* Equimolar mixture of 7,8-BQ, 5,6-BQ and 1,10-Phen.

TABLE IV

MIGRATION DISTANCES IN DIFFERENT CONCENTRATIONS OF HAC

HAC (× 10^{-3} M)	*Observed migration distance, Anth (cm)*	*Corrected migration distance (cm)*			
		7,8-BQ	*5,6-BQ*	*1,10-Phen*	*Mixture**
1.0	0 (+0.1)	0.0 ± 0.1	0.0 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1
2.0	0 (+0.1)	0.0 ± 0.1	0.0 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1
5.0	0 (+0.2)	0.0 ± 0.1	0.0 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1
10.0	0 (+0.1)	−0.1 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1
20.0	0 (+0.3)	−0.3 ± 0.1	0.0 ± 0.1	−0.3 ± 0.1	−0.4 ± 0.1

* Equimolar mixture of 7,8-BQ, 5,6-BQ and 1,10-Phen.

Potentiometric studies suggested that TCA and DCA should be able to ionize model bases in the concentration range of 2.5 m*M* to 4.0 m*M*. Electrophoresis of model bases in TCA and DCA showed significant migrations in this range of concentration; however, there was no differential migration. Hence, it was assumed that model bases when spotted on the paper were not behaving as expected for the concentration present in the background electrolyte. Hence, higher concentrations of TCA and DCA were used to test the possibility of obtaining differential migration. Electrophoresis of model bases in TCA and DCA now showed significant differential migration (Figs. 6 and 7, Tables V and VI). The number of model compounds was expanded by adding 7,8-BQ and checking its electrophoretic behavior in TCA and DCA since complete ionization had previously been obtained in TFMSA[1]. The results (Figs. 6 and 7, Tables V and VI) suggested that, in the presence of TCA and DCA, 7,8-BQ migrated less than 5,6-BQ which, in turn, migrated less than 1,10-Phen. This was confirmed by running an equimolar mixture of 7,8-BQ, 5,6-BQ and 1,10-Phen. It, too showed differential migration at concentrations 5.0 m*M* or higher when scanned at 250–350 nm excitation and 400 nm emission wavelengths using "UV-D_2" filters and a 400-nm filter.

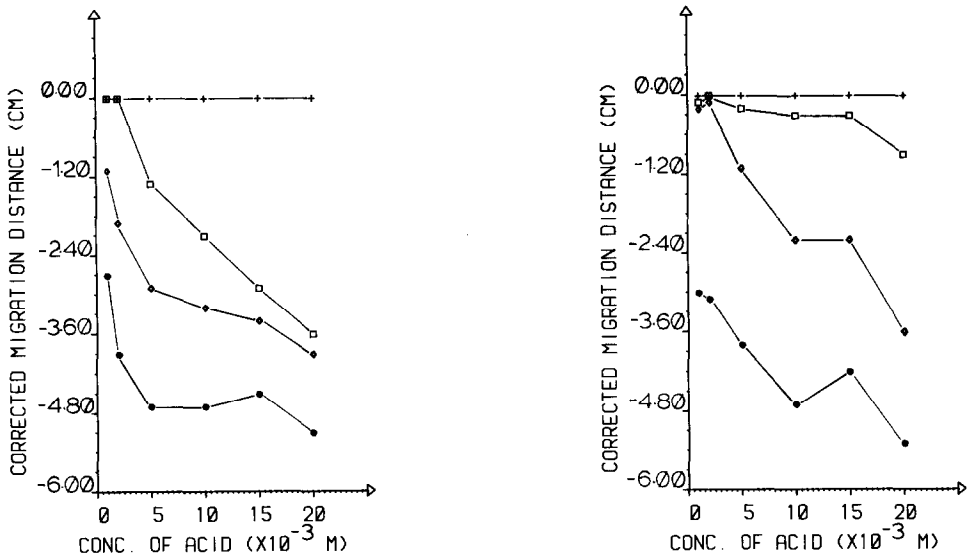


Fig. 6. Electrophoresis of model bases in different concentrations of TCA. +, Anthracene; □, 7,8-BQ; ◇, 5,6-BQ; ●, 1,10-Phen. See Table V for detailed results.

Fig. 7. Electrophoresis of model bases in different concentrations of DCA. +, Anthracene; □, 7,8-BQ; ◇, 5,6-BQ; ●, 1,10-Phen. See Table VI for detailed results.

In the presence of increasing concentrations of TCA, the migration distances of model bases showed a definite trend (Fig. 6, Table V). There was a sharp increase in migration distances for 5,6-BQ and 1,10-Phen where as it increased gradually for 7,8-BQ. However, 1,10-Phen migrated a much larger distance compared to 7,8-BQ and 5,6-BQ. The best separation of all three species in an equimolar mixture was observed at 5.0 m*M* TCA (Fig. 8a), a two-fold excess over that predicted from potentiometric measurements. Again, there were two distinct peaks observed for 5,6-BQ alone and in a mixture at 1.0 m*M* and 2.0 m*M* TCA, as observed earlier in the cases of TFMSA and MSA. Thus, adsorption of an uncharged base occurred on the paper at low concentrations of acids, making it difficult to predict from pH measurements, the concentrations in order to effect mobility.

In the presence of DCA, separation of model bases were observed at various concentrations (Fig. 7, Table VI). The best separation for all the species in a mixture was observed at 10.0 m*M* DCA (Fig. 8b), a four-fold excess over that predicted from potentiometric measurements. At 5.0 m*M* DCA, 5,6-BQ and 7,8-BQ whereas at 20.0 m*M* DCA, 5,6-BQ and 1,10-Phen were not base-line separated.

The 15.0 m*M* concentration of TCA and DCA was chosen to see if there was a decrease in the migrations at this concentration as observed in the earlier studies. A decrease in migration distance was noticed in the case of 1,10-Phen at 15.0 m*M* TCA and DCA whereas 5,6-BQ and 7,8-BQ moved at the same distance as in the 10.0 m*M* concentration of the acid (Figs. 6 and 7, Tables V and VI). The migration distance again increased sharply at 20.0 m*M* DCA whereas it increased only moderately for TCA.

TABLE V

MIGRATION DISTANCES IN DIFFERENT CONCENTRATIONS OF TCA

TCA (× 10^{-3} M)	*Observed migration distance, Anth (cm)*	*Corrected migration distance (cm)*			
		7,8-BQ	*5,6-BQ*	*1,10-Phen*	*Mixture**
1.0	0 (0.0)	0.0 ± 0.1*	0.0 ± 0.1**	−2.7 ± 0.1	−0.5 ± 0.1**
			−1.1 ± 0.1		−2.7 ± 0.1
2.0	0 (0.0)	0.0 ± 0.1	0.0 ± 0.1**	−3.9 ± 0.1	0.0 ± 0.1
			−1.9 ± 0.1		−1.9 ± 0.1
					−3.9 ± 0.1
5.0	0 (+0.3)	−1.3 ± 0.1	−2.9 ± 0.1	−4.7 ± 0.1	−1.4 ± 0.1
					−2.8 ± 0.1
					−4.7 ± 0.1
10.0	0 (−0.1)	−2.1 ± 0.1	−3.2 ± 0.1	−4.7 ± 0.01	−1.7 ± 0.2
					−3.0 ± 0.1
					−4.7 ± 0.1
15.0	0 (+0.5)	−2.9 ± 0.2	−3.4 ± 0.1	−4.5 ± 0.1	−2.8 ± 0.1
					−3.5 ± 0.2
					−4.6 ± 0.1
20.0	0 (+0.6)	−3.6 ± 0.3	−3.9 ± 0.2	−5.1 ± 0.2	−3.6 ± 0.3
					−5.1 ± 0.2

* Equimolar mixture of 7,8-BQ, 5,6-BQ and 1,10-Phen.
** Intense peak.

TABLE VI

MIGRATION DISTANCES IN DIFFERENT CONCENTRATIONS OF DCA

DCA (× 10^{-3} M)	*Observed migration distance, Anth (cm)*	*Corrected migration distance (cm)*			
		7,8-BQ	*5,6-BQ*	*1,10-Phen*	*Mixture**
1.0	0 (−0.-5)	−0.1 ± 0.1	−3.0 ± 0.4	−0.2 ± 0.1	−2.0 ± 0.1
				−2.9 ± 0.3	−2.9 ± 0.3
2.0	0 (−0.3)	0.0 ± 0.1	−0.1 ± 0.1	−3.1 ± 0.2	−0.2 ± 0.1
					−3.1 ± 0.2
5.0	0 (−0.3)	−0.2 ± 0.1	−1.1 ± 0.2	−3.8 ± 0.2	−0.5 ± 0.1
					−3.8 ± 0.2
10.0	0 (0.0)	−0.3 ± 0.1	−2.2 ± 0.1	−4.7 ± 0.4	−0.6 ± 0.2
					−2.3 ± 0.3
					−5.0 ± 0.4
15.0	0 (+0.1)	−0.3 ± 0.1	−2.2 ± 0.1	−4.2 ± 0.2	−0.6 ± 0.1
					−2.3 ± 0.1
					−4.2 ± 0.1
20.0	0 (+0.6)	−0.9 ± 0.3	−3.6 ± 0.3	−5.3 ± 0.4	−0.9 ± 0.2
					−3.7 ± 0.3
					−5.3 ± 0.4

* Equimolar mixture of 7,8-BQ, 5,6-BQ and 1,10-Phen.

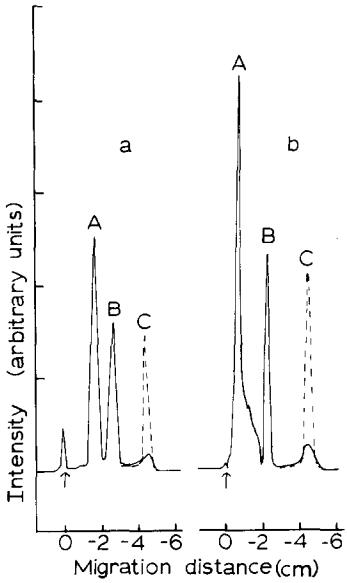


Fig. 8. The separation of an equimolar mixture of model bases in (a) 5.0 m*M* TCA and (b) 10.0 m*M* DCA. Model bases were separated in the order A = 7,8-BQ, B = 5,6-BQ and C = 1,10-Phen. (——) Represents fluorescence scan at 350 nm excitation and 400 nm emission wavelengths using 400-nm filters; (----) represents fluorescence scan at 250 nm excitation and 400-nm emission wavelengths using "UV-D_2" filters.

In order to confirm the electrophoretic behavior and justify possible separation of 7,8-BQ from 5,6-BQ, potentiometric titration of 7,8-BQ was performed with TFMSA. In the absence of paper, there were two p*K* values for 7,8-BQ, (Fig. 9A) 8.6 ± 0.1 and 2.5 ± 0.4. The ratio for the endpoint volumes were 5:1 compared to 1.5:1 for 1,10-Phen and 3:1 for 5,6-BQ. The 7,8-BQ showed behavior similar to that of 5,6-BQ except it started at a less alkaline pH and corresponding ratios for excess acid produced somewhat more acidic pH values (Fig. 9B). The p*K* value for 7,8-BQ is also smaller (less alkaline) compared to 5,6-BQ, confirming the electrophoresis results. In the presence of paper, however, the second p*K* value for 7,8-BQ became more apparent. This may be the reason why, in the presence of paper, 7,8-BQ did not appear weak (Fig. 9A). The ratio for the endpoint volume also reduced to 3:1.

Spectrophotometric data on bases and acids

The objectives behind the spectrophotometric measurements were (a) to see if any change in the spectra for model bases occurred by the addition of various amounts of acids of different strengths and (b) to examine the change in absorbance at the wavelength of peak absorbance to see if the stoichiometries for the titrations of the various model bases were the same as predicted from potentiometric measurements. This could help in finding the concentrations of even weaker acids suitable for ionizing model bases for which the spectra for protonated and unprotonated bases would be expected to differ.

Three acids TFMSA, TCA and MCA were selected representing strong, me-

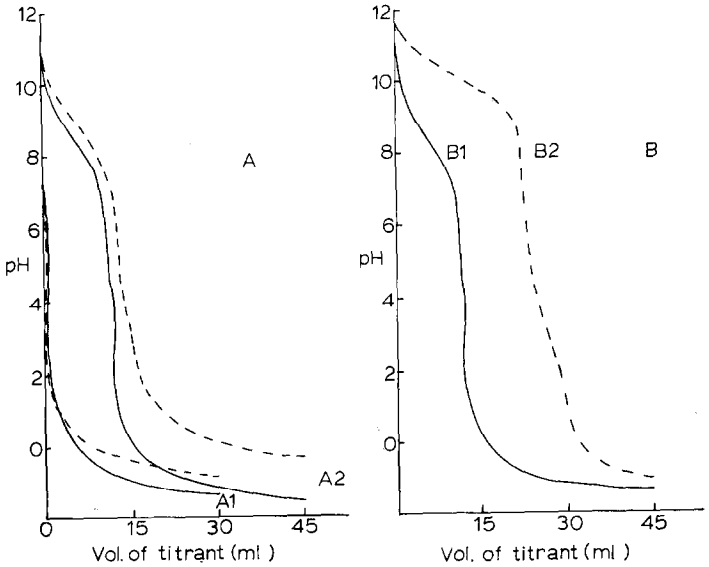


Fig. 9. Titration of 50 ml 7,8-BQ with TFMSA: (A) the pH curve for 0.01 *M* TFMSA alone, A1, relative to the titration curve of 0.005 *M* 7,8-BQ with 0.01 *M* TFMSA, A2. The dotted line represents titration curves in the presence of paper (5 × 10 cm). (B) titration of 0.005 *M* 7,8-BQ with 0.01 *M* TFMSA, B1, relative to the titration of 0.005 *M* 5,6-BQ with 0.01 *M* TFMSA, B2.

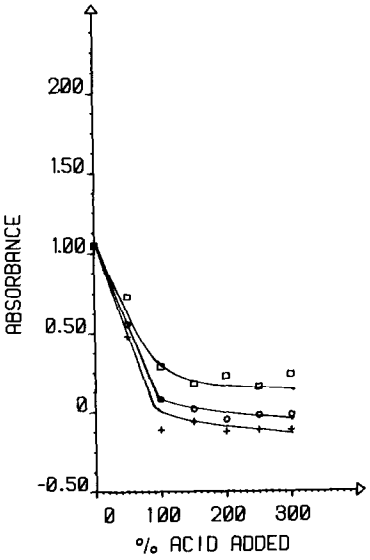
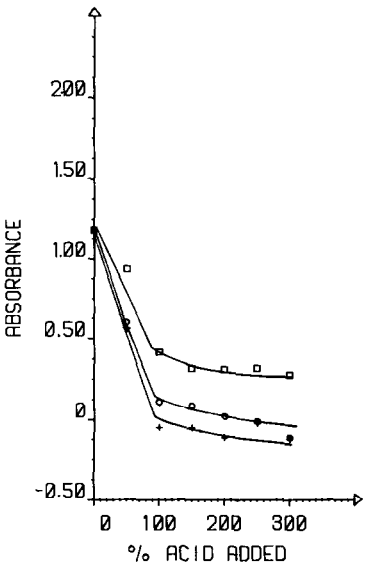


Fig. 10. Spectrophotometric measurements of 5,6-BQ at 279 nm with different percentage of acid added +, TFMSA; O, TCA; □, MCA.



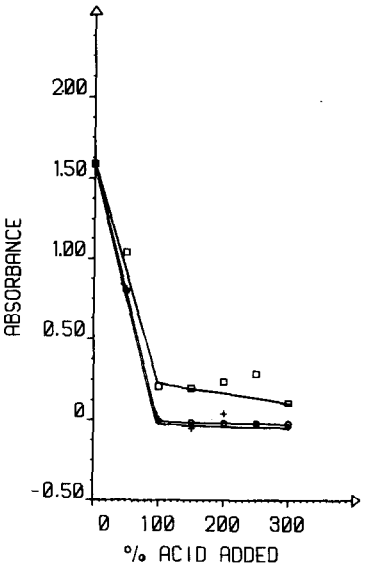


Fig. 11. Spectrophotometric measurements of 7,8-BQ at 279 nm with different percentage of acid added. +, TFMSA; (○), TCA; (□), MCA.

Fig. 12. Spectrophotometric measurements of 1,10-Phen at 271 nm with different percentage of acid added. +, TFMSA; ○, TCA; □, MCA.

dium and weak acids. The spectrophotometric data (Figs. 10–12) represent the difference in absorbance between the solution of pure base and that of the base after the addition of acid (with the correction for excess acid or base). The measurement indicated that when the difference in absorbance became slightly negative, no base was left in the solution. This suggested a stoichiometric 1:1 reaction with different acids. In the case of the strong acid, TFMSA, at a 1:1 ratio, the absorbance for each of the three base forms reached a zero value (*i.e.*, all of the base form had disappeared). For the intermediate strength acid, TCA, this was true only for 1,10-Phen. Excess acid was required to reach the zero absorbance, *e.g.*, for 5,6-BQ, it was 62% excess; for 7,8-BQ, it was 130% excess. The weak acid, MCA, was unable to produce zero absorbance for any of the three model bases even at 200% excess acid, even though it did give evidence of a 1:1 reaction in each case.

Paper chromatography

The model organic bases showed considerable movement on paper when the mobile phase contained 20.0 m*M* DCA or TCA. In the case of DCA, 7,8-BQ gave an R_F value of 0.4; 5,6-BQ, 0.6 and 1,10-Phen, 0.8. In TCA, all bases were close to the solvent front. However, both at 1.0, and 2.0 m*M* of DCA and TCA, the R_F value for each of the bases was much smaller. In the case of DCA, 7,8-BQ gave an R_F value of 0.2, 5,6-BQ; 0.3 and 1,10-Phen, 0.5. In TCA, 7,8-BQ gave an R_F value of 0.3; 5,6-BQ, 0.4 and 1,10-Phen, 0.6. These results are in qualitative agreement with electrophoretic results.

DISCUSSION

This study showed that it was possible to make weak bases migrate differentially by adding a moderately weak acid to a mixture of acetonitrile–sulfolane (9:1) containing a background electrolyte, TEAP. The concentration as well as the strength of the acid proved to be important. Potentiometric studies provided information on the ionization behavior of the bases, but the results for HAC and MCA did not lead to successful predictions of conditions for electrophoresis of model bases in spite of the fact that in the blank they reached the pH values where ionization was expected. It was confirmed later by spectrophotometric studies that the bases even reacted extensively with MCA even though the data from paper chromatography suggested that these acids were too weak to cause equivalent migration of the bases.

An important clue about the behavior of model bases in the different strengths of acid was obtained by combining the results of spectrophotometric, paper chromatographic and potentiometric experiments. The percentage of the unreacted base form was obtained from both the spectrophotometric and potentiometric titrations at the level of 1.0 m*M* excess TCA used in the corresponding electrophoresis experiments. At this concentration of TCA or DCA, different amounts of adsorption were observed in paper chromatography as determined by R_F values. Assuming that it was uncharged base that was absorbed, it was possible to multiply the ratio of the ionized to the unionized form of a base from either spectrophotometric or potentiometric titration by its R_F value in order to obtain the net ratio of protonated base to the base in the electrophoretic system. A sample calculation is shown below:

$$\text{Net.}\ \frac{\text{protonated base}}{\text{base}} = \frac{\text{fraction in ionic form}}{\text{fraction in base form}}$$

$$\left(\text{or}\ \begin{matrix}\text{spectrophotometry}\\ \text{potentiometry}\end{matrix}\right) \cdot R_F\ \text{(paper chromatography)}$$

Combining the spectrophotometric and paper chromatographic results for 5,6-BQ at 1.0 m*M* excess TCA, 5.8% was uncharged base and the R_F was 0.4.

$$\text{Net.}\ \frac{\text{protonated base}}{\text{base}} = \frac{94.2}{5.8} \cdot \frac{4}{10} = 6.5$$

Similarly, combining the potentiometric and paper chromatographic results for 5,6-BQ, 16.3% was uncharged base, so that the net ratio was 2.1. Hence, the results from potentiometry and from spectrophotometry differed by a factor fo 3 for 5,6-BQ. This discrepancy is somewhat larger than expected.

One can also estimate the ratio of protonated to unprotonated base on the basis of electrophoresis alone by assuming that 1,10-Phen was completely ionized and that the fraction of its distance that 5,6-BQ traveled represents the effective ionization of the 5,6-BQ. That ratio was 0.69 at 1.0 m*M* excess TCA. Thus, the agreement between the electrophoretic data and the spectrophotometric or potentiometric data, corrected for adsorption, does not appear to be good. Similar cal-

culations for 7,8-BQ gave net protonated base to base ratios of 3.0 and 2.4 respectively from spectrophotometric and potentiometric results. The ratio of protonated to unprotonated base for 7,8-BQ from electrophoresis could not be estimated as it was completely adsorbed on the paper.

The discrepancies in these calculations suggested that, in addition to adsorption of the base, there is at least one other mechanism in operation. First, there is also some ion exchange of the protonated base (on the acidic sites of the paper) at the lower concentrations of the acid. At higher concentrations of acid, displacement of the protonated base takes place so that the maximum electrophoretic migration rate is observed. Another factor, as pointed out by one of the reviewers, is ion pairing, which has been shown to be important in paper electrophoresis[8–11]. However, the extent of ion pair formation may be small in water for solutions that are less than 0.1 *M* (ref. 10) or in acetonitrile below 0.01 *M* (ref. 7).

The acids which did effect differential ionization of the organic bases were DCA and TCA. In the case of DCA, the differential migration of bases in electrophoresis started at or above 5.0 m*M*. The stronger base 1,10-Phen usually migrated ahead of the two isomers, 5,6-BQ and 7,8-BQ. In DCA, the test bases appeared to be separated according to charge, with 1,10-Phen appearing to be doubly charged. Singly charged benzoquinolines would, therefore, trail it. The two benzoquinolines were probably separated because of their structure differences. The more readily ionizable 7,8-BQ in aqueous systems appeared to be weaker in the present nonaqueous system. It also very strongly adsorbed onto the paper. In addition, ion pairing and/or ion exchange of the protonated bases on the paper probably occurred. Hence, these results were only qualitatively in agreement with those from the potentiometric and spectrophotometric studies. In the case of TCA, differential migration of the bases was quite pronounced at 5.0 m*M* followed by a steep rise at higher concentrations. The reason for this last unexpected change is unknown.

This study also showed that equimolar mixtures of 7,8-BQ and 5,6-BQ in the presence of 1,10-Phen could be separated by electrophoresis using a moderately weak acid DCA or TCA at a relatively high concentration. The migrations could be followed easily by scanning with the proper excitation and emission wavelengths.

It is interesting to speculate on the general relationship between the results in this study and those reported by Haber[12]. Some of the combinations he reported appeared to be related to the present results in the sense that his experimental system often consisted fo a "neutral" solvent plus acidic and/or basic species. (Sometimes a given species might be viewed either as a solute or as a solvent, depending upon the relative amounts present.) If so, Haber's results can be understood in terms of the generalizations based upon pH curves that are useful in conventional (aqueous) electrophoresis[13].

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We are indebted to a reviewer for referring us to papers in which ion pairing was shown to be important in modifying the electrophoretic behavior of ions. Help from Pankaj Shah in running some electrophoretic experiments is greatly appreciated. We thank Dr. L. A. Carreira for the use of his GCA-McPherson spectrophotometer and PDP11/34 computer.

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