Journal of Chromatography, 87 (1973) 543–553
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 6999

SEPARATION OF ANILINE, N-HETEROCYCLIC, AND SULFONAMIDE DERIVATIVES ON PAPER IMPREGNATED WITH STRONG ACID EXCHANGE RESIN

DONALD J. PIETRZYK and ELSA CHAN-SANTOS
University of Iowa Department of Chemistry, Iowa City, Iowa 52242 (U.S.A.)

(First received June 25th, 1973; revised manuscript received August 14th, 1973)

SUMMARY

The retention of aniline derivatives, sulfonamides, and several N-heterocyclics on paper impregnated with a strong acidic H^+-form resin was investigated. Solvent systems used were ethanol, acetonitrile, dimethylformamide, dimethylsulfoxide, pyridine, and butylamine mixed with water. Either aprotic, amphiprotic, basic, or acidic developing mixtures can be used for separations. The retention data can be correlated, in general, to the pK_a values for the weak bases and to column and batch retention data obtained with strongly acidic cation resin.

INTRODUCTION

The advantages and applications of sheet methods, such as paper and thin-layer chromatography, for qualitative and quantitative separations is well documented. These methods can also be used in preliminary studies to establish the eluting conditions for a liquid column chromatographic separation. Since each sheet can hold a large number of compounds, all of these can be studied at one time for a particular eluting condition. Hence, retention characteristics are identified faster than by the usual column techniques of determining retention volumes or distribution coefficients.

Paper impregnated with synthetic cation- and anion-exchange resins that are routinely used in column techniques have been used for the separation of inorganic ions[1–7] as well as for organic molecules[7–11], particularly amino acids and related compounds[12]. In many cases, the sheet retention data have been shown to correlate with retention data measured by batch or column methods for the corresponding cation or anion resin, particularly for the separation of inorganic ions[1–4].

Occasional differences between migration on synthetic cation- and anion-exchange resin impregnated paper and columns of the respective resins have been noted. This has been attributed to the basic differences in sheet and column methods and to the fact that the cellulose phase may influence the retention[2–6].

Column and thin-layer data for a series of aromatic amines and amino acids have been compared[13–15] through an equation relating R_F and the batch distribution coefficient, K_D (ref. 16). It was possible to predict retention volumes for the column from thin-layer R_F values providing the R_F values were in the range of 0.2 to 0.8. However, in these studies the weak cation exchangers alginic acid and carboxymethylcellulose, were used in the absence of a cellulose phase[13–15].

Recent work in this laboratory[17] demonstrated the potential of using nonaqueous solvents for the column separation of closely related weak organic bases on H^+-form ion-exchange resins. In this report paper impregnated with ion-exchange resin in the H^+-form was investigated for separation of weak bases under the same separation conditions with two goals in mind: (1) Development of the ion-exchange resin-paper as a suitable means for rapid qualitative and quantitative separation of the weak bases, and (2) to establish that the retention data on the ion-exchange resin-paper can be used to predict column behavior.

EXPERIMENTAL

Reagents

The aniline derivatives, including *p*-dimethylaminobenzaldehyde, were obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.). Heterocyclic amines and sulfonamides were purchased from Aldrich (Milwaukee, Wisc., U.S.A.) and Matheson, Coleman & Bell (East Rutherford, N.J., U.S.A.), respectively. The compounds were recrystallized or distilled by usual procedures. All solvents were the best commercial grade available and were used as received.

Paper impregnated with strong (SA-2) and weak (WA-2) cation resin in the Na^+- and H^+-forms, respectively, were obtained from H. Reeve Angle (Clifton, N.J., U.S.A.) in large sheets and cut according to the required size. The SA-2 was converted to the H^+- form by soaking the resin-paper in 4% aqueous HCl overnight, washing with water eight times for 30-min periods, and then air-drying overnight. Subsequently, the resin-paper was dried in an oven at 100° for about 30 min and cooled in a desiccator before use. Other resin papers were used as received or modified as described.

Apparatus

Typical ascending and descending chromatographic tanks of various sizes were used and were lined with filter paper. Spray equipment was obtained from Brinkmann (Westbury, N.Y., U.S.A.). Micro-syringes were used for spot application.

Procedures

R_F values. Ethanol solutions containing 10 to 50 μg of the anilines and heterocyclics and 2 to 3 μg of the sulfonamides were applied by a micro-syringe onto a resin-paper (16 cm × 20 cm) at $1^1/_2$ cm apart. The chromatogram was developed by descending chromatography in a chromatographic tank using the appropriate solvent mixture. All solvent mixtures are per cent by volume. When the solvent front reached the appropriate point the chromatography was terminated by removing the resin-paper from the tank and air drying it under ambient conditions or by forced hot air. The developing period took from 30 min to 1 h. After drying the resin-paper the compounds spotted were detected by spraying or UV-scanning techniques. The anilines and sulfonamides were identified by spraying with *p*-dimethylaminobenzalde-

hyde solution, heterocyclic amines by UV, and caffeine, theophylline, and theobromine by an alcoholic I_2–KI spray. R_F values were calculated in the usual way.

RESULTS AND DISCUSSION

It has been suggested that when a weak organic base in solution and a strongly acidic H^+-form exchange resin are in contact, an acid–base interaction

$$\text{Resin } SO_3^-H^+ + B \rightleftharpoons \text{Resin } SO_3^-BH^+ \qquad (1)$$

is possible and the direction of the equilibrium is determined by the solvent or solvent mixture used[17]. The more basic the solvent becomes, the greater it competes with B for the resin site and, therefore, B will be retained less. Similarly, acidic sol-

TABLE I

R_F VALUES FOR ANILINE DERIVATIVES ON SA-2 AND WA-2 (H^+) PAPER IN ETHYL ALCOHOL–WATER MIXTURES

Aniline derivative	pK_a[21]	*% Ethyl alcohol*										
		100	*97*	*95*	*90*	*80*	*70*	*60*	*50*	*40*	*30*	*20*
		On SA-2 (H^+) paper										
4-Nitro-	1.00	0.04	0.06	0.07	0.11	0.14	0.13	0.10	0.06	0.03	0.01	0.00
2-Nitro-	−0.26	0.52	0.55	0.58	0.67	0.70	0.70	0.69	0.48	0.35	0.15	0.13
2,4-Dinitro-	−4.53	0.65	0.65	0.69	0.75	0.73	0.73	0.65	0.37	0.28	0.07	0.06
2,6-Dichloro-4-nitro-	−2.6	0.77	0.79	0.82	0.85	0.82	0.85	0.73	0.32	0.12	0.00	0.00
4-Chloro-2-nitro-	−1.03	0.71	0.72	0.82	0.83	0.81	0.83	0.85	0.49	0.34	0.11	0.08
N,N-Dimethyl-4-nitro-	0.61	0.24	0.27	0.27	0.34	0.38	0.34	0.27	0.16	0.12	0.02	0.01
4-Cyano-	1.74	0.01	0.02	0.02	0.02	0.03	0.03	0.03	0.02	0.01	0.01	0.00
N-Methyl-4-nitro-		0.23	0.28	0.29	0.37	0.43	0.39	0.33	0.18	0.10	0.03	0.02
4-Methyl-2-nitro-	0.43	0.37	0.41	0.43	0.54	0.59	0.57	0.49	0.25	0.16	0.05	0.03
2-Chloro-4-nitro-		0.70	0.69	0.75	0.80	0.81	0.81	0.81	0.52	0.32	0.14	0.09
Caffeine	1.22	0.04		0.06	0.08	0.14	0.21	0.21	0.23	0.17	0.11	0.11
		On WA-2 (H^+) paper										
4-Nitro-		0.82	0.81	0.77	0.78	0.75	0.72	0.62	0.43	0.40		0.15
3-Nitro-	2.47	0.79	0.79	0.76	0.76	0.70	0.67	0.57	0.36	0.33		0.13
2,6-Dimethyl-	3.95	0.79	0.78	0.72	0.67	0.63	0.59	0.15	0.10	0.05		0.00
2-Methyl-5-nitro-	2.32	0.81	0.80	0.76	0.75	0.70	0.66	0.53	0.31	0.28		0.10
4-Chloro-	4.00	0.78	0.76	0.72	0.70	0.67	0.60	0.45	0.25	0.21		0.05
4-Methoxy-	5.33	0.36	0.42	0.50	0.41	0.24	0.17	0.17	0.12	0.08		0.04
2-Methoxy-	4.52	0.68	0.67	0.66	0.60	0.55	0.47	0.35	0.27	0.11		0.08
2-Methyl-	4.44	0.73	0.69	0.66	0.62	0.55	0.52	0.33	0.24	0.11		0.04
3-Methyl-	4.69	0.63	0.65	0.63	0.57	0.48	0.38	0.26	0.21	0.07		0.04
N-Methyl-	4.85	0.65	0.67	0.64	0.55	0.46	0.41	0.25	0.16	0.06		0.00
3,5-Dimethyl-	4.91	0.63	0.68	0.64	0.56	0.44	0.35	0.22	0.15	0.04		0.02
4-H	4.60	0.67	0.65	0.64	0.56	0.52	0.46	0.33	0.27	0.12		0.04

vents will compete with BH^+ for the resin site and will influence the retention of the base. Aprotic and amphiprotic solvents will influence the equilibrium in reaction 1 according to the ability of the solvents to level the acidic and basic property of the resin and the solute B, respectively.

If trace amounts of water are introduced it will influence the equilibrium in reaction 1 by competing with B for the resin sites. In addition, the swelling properties of the resin will change. The net result is a sharp decrease in retention of the base.

As the water concentration is increased further, an additional effect which influences retention is observed. Thus, at high water levels in mixed water–organic solvent mixtures the retention is influenced not only by the acid–base interaction but also by the solubility of the base in the internal solvent mixture in the resin. These solvent properties, which are discussed in more detail elsewhere[17–20], can be exploited for the column separation of weak organic bases, such as anilines, N-heterocyclics, and sulfonamides, on strongly acidic H^+-form resin[17].

Table I lists the R_F values for a series of aniline derivatives in water–ethanol mixtures on SA-2 paper. At high alcohol concentration the anilines are highly retained (low R_F) and as the alcohol concentration decreases the retention decreases and passes through a minimum at about 80% alcohol (high R_F). A continued decrease in alcohol concentration results in an increase in retention (low R_F). Although the solvent is listed at 100%, special efforts were not made to remove the last traces of water from the solvent nor is the ion-exchange paper completely anhydrous.

Data for acetonitrile–water and dimethylformamide–water mixtures are listed in Tables II and III. Acetonitrile does not level reaction 1 to the same extent as ethanol and, hence, retention (low R_F) in the more concentrated acetonitrile mixtures is higher than for comparable ethanol–water mixtures. Dimethylformamide provides a weakly basic condition and accordingly retention is less. If a more basic solvent is used, less retention is observed; several R_F values for pyridine–water and butylamine–water mixtures are also listed in Table III.

The general behavior for these anilines on ion-exchange paper is similar to

TABLE II

R_F VALUES FOR ANILINE DERIVATIVES ON SA-2 (H^+) PAPER IN ACETONITRILE–WATER MIXTURES

Aniline derivative	*% Acetonitrile*								
	100	*97*	*95*	*90*	*80*	*70*	*60*	*40*	*20*
4-Nitro-	0.02	0.02	0.06	0.16	0.34	0.30	0.22	0.07	0.02
2-Nitro-	0.30	0.39	0.51	0.59	0.76	0.83	0.94	0.47	0.19
2,4-Dinitro-	1.00	0.97	0.94	0.95	0.95	0.98	0.70	0.59	0.19
2,6-Dichloro-4-nitro-	0.96	0.97	0.96	0.96	0.96	0.98	0.99	0.45	0.12
4-Chloro-2-nitro-	0.71	0.75	0.83	0.86	0.92	0.96	0.98	0.53	0.16
N,N-Dimethyl-4-nitro-	0.03	0.05	0.13	0.25	0.59	0.65	0.69	0.14	0.02
4-Cyano-	0.00	0.01	0.02	0.05	0.07	0.07	0.06	0.02	0.01
N-Methyl-4-nitro-	0.04	0.06	0.14	0.23	0.58	0.66	0.51	0.16	0.03
4-Methyl-2-nitro-	0.09	0.12	0.24	0.35	0.63	0.77	0.69	0.25	0.05
2-Chloro-4-nitro-	0.71	0.75	0.79	0.84	0.92	0.95	0.98	0.53	0.18
3-Nitro-	0.00	0.00	0.00	0.01	0.02	0.01	0.01	0.00	0.00
4-Chloro-	0.00		0.00		0.00		0.00		0.00

TABLE III

R_F VALUES FOR SOME ANILINE DERIVATIVES ON SA-2 (H$^+$) PAPER IN DIMETHYLFORMAMIDE–WATER MIXTURES

Aniline derivative	*Solvent composition*									
	% Dimethylformamide									
	100	*97*	*95*	*90*	*80*	*70*	*60*	*50*	*40*	*20*
4-Nitro-	0.95	0.95	0.89	0.91	0.85	0.85	0.62	0.41	0.24	0.05
2-Nitro-	1.00	1.00	0.92	0.98	0.95	0.95	0.85	0.72	0.61	0.32
2,4-Dinitro-	1.00	1.00	1.00	0.96	0.95	0.95	0.82	0.69	0.56	0.23
2,6-Dichloro-4-nitro-	0.98	1.00	0.95	0.98	0.97	0.98	0.84	0.65	0.57	0.12
4-Chloro-2-nitro-	1.00	1.00	0.95	0.97	0.96	0.96	0.84	0.68	0.56	0.22
N,N-Dimethyl-4-nitro-	1.00	1.00	1.00	0.96	0.93	0.92	0.78	0.60	0.43	0.10
4-Cyano-	0.74	0.75	0.63	0.68	0.66	0.53	0.34	0.16	0.07	0.02
N-Methyl-4-nitro-	1.00	1.00	1.00	0.95	0.93	0.91	0.78	0.65	0.44	0.12
4-Methyl-2-nitro-	1.00	1.00	0.96	0.95	0.93	0.91	0.79	0.68	0.51	0.17
2-Chloro-4-nitro-	1.00	1.00	0.96	0.97	0.96	0.95	0.84	0.69	0.59	0.27
3-Nitro-	0.26	0.25	0.22	0.23	0.15	0.09	0.04	0.02	0.01	0.00
2-Methyl-5-nitro-	0.29	0.29	0.27	0.25	0.19	0.10	0.05	0.03	0.02	0.01
4-Chloro-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	% Pyridine									
	100	*90*	*80*	*70*	*60*	*50*	*40*	*30*	*20*	
4-Nitro-	0.91	0.90	0.93	0.91	0.84	0.75	0.58	0.37	0.21	
3-Nitro-	0.93	0.91	0.84	0.90	0.90	0.80	0.64	0.45	0.28	
2,6-Dimethyl-	0.97	0.95	0.96	0.95	0.95	0.90	0.74	0.56	0.39	
4-Chloro-	0.95	0.87	0.89	0.87	0.84	0.79	0.60	0.41	0.24	
2-Methyl-	0.91	0.90	0.86	0.85	0.82	0.76	0.62	0.45	0.27	
3,5-Dimethyl-	0.82	0.82	0.77	0.76	0.70	0.61	0.50	0.32	0.15	
4-H	0.82	0.79	0.76	0.74	0.70	0.63	0.53	0.40	0.24	
4-Methoxy-	0.37	0.37	0.41	0.37	0.34	0.31	0.24	0.17	0.10	
4-Hydroxy- (5.6)*	0.10	0.04	0.17	0.16	0.14	0.11	0.08	0.06	0.03	
	% Butylamine									
	100	*95*	*90*	*80*	*70*	*60*	*40*	*20*	*10*	
4-Anisidine (5.31)	0.79	0.78	0.79	0.79	0.80	0.78	0.79	0.47	0.35	
Hydroxylamine (5.65)	0.88	0.85	0.83	0.83	0.85	0.85	0.84	0.72	0.69	
N,N-Dimethyl-4-phenylene diamine (6.59)	0.85	0.81	0.82	0.81	0.83	0.82	0.80	0.65	0.51	

* pK_a.

that observed when using strongly acidic cation resin in the H$^+$-form in the same solvent medium[17]. Fig. 1 compares the change in the batch distribution coefficient[17] for *p*-nitroaniline in several solvent mixtures to $1/R_F-1$ for the same medium. The value $1/R_F-1$ is used so that the relative direction of change is the same as for K_D and because it has been shown for several systems that K_D and $1/R_F-1$ are related[13–16].

The magnitude of the retention in 100% organic solvent, the minimum at about 80% organic solvent, the increase in retention as the water concentration increases, and the effect of a solvent with basic properties is consistent with the batch

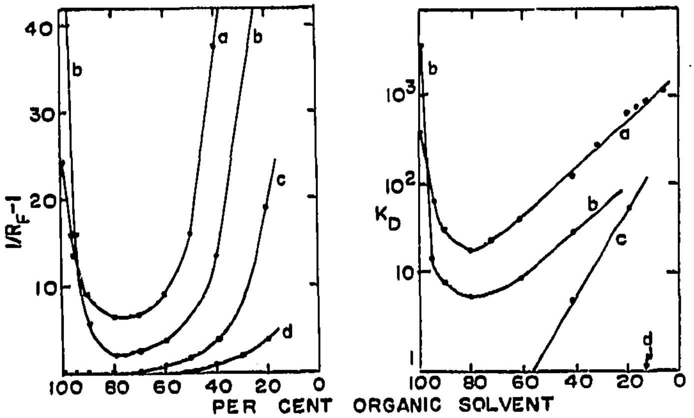


Fig. 1. Comparison of retention of *p*-nitroaniline on SA-2 and strong acid H^+-form cation resin as a function of the solvent–water composition. a = Ethanol; b = acetonitrile; c = dimethylformamide; d = pyridine.

experiments with cation resin and can be explained by the influence of the solvent on the equilibrium in reaction 1 (ref. 17).

The significance of the resin being in the H^+-from is illustrated by using SA-2 (Na^+) paper. In general, the anilines were hardly retained in high ethanol concentration and migrated almost with the solvent front. As the alcohol concentration decreased a slight decrease in migration was observed. The influence of the strong acid character of the resin-paper is illustrated by comparison of the R_F data on SA-2 to WA-2 (H^+) (–COOH exchange group) paper. Some retention is observed but it is considerably less than for the SA-2 paper. Some of these data for WA-2 paper are included in Table I. When the same experiments are repeated, except that paper minus cation resin is used (ordinary grade chromatographic paper), almost complete migration of the anilines is observed. If the R_F values for the aniline derivatives in Tables I, II, and III are plotted *versus* % solvent composition, the tendency, in general, is that the stronger the base, the smaller will be its R_F value at all solvent compositions. This observation was made for ethanol–, acetonitrile–, DMF–, and pyridine–water mixtures. All of these experiments are analogous to batch observations with cation-exchange resins and support the view that the major factor contributing to the retention is represented by reaction 1 (ref. 17).

Examination of all R_F data reveals that migration is strongly influenced by the basicity of the developing mixture. Table III lists R_F data for the more basic anilines in basic solvent–water mixtures. If R_F is plotted *versus* pK_a (see Fig. 2), a qualitative relationship between migration and solvent mixture as a function of the strength of the base is obtained. Hence, as the strength of the bases that are to be separated increases, a stronger developing mixture than that provided by ethanol or acetonitrile is required.

An alternate developing mixture is to combine a basic solvent with a hydrophilic solvent or to use a ternary system in which the basic solvent is held constant. These types of eluting conditions or their variations permit[17] a shift in the R_F according to the strength of the bases being separated. Table IV lists R_F data for a select group of anilines in acetonitrile–DMF mixtures and in ethanol–water–pyridine

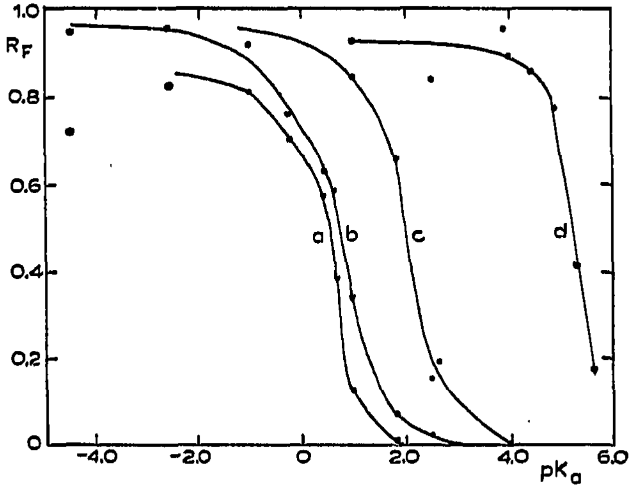


Fig. 2. Relationship between R_F and pK_a for aniline derivatives on SA-2 paper in organic solvent–water (8:2). a = Ethanol; b = acetonitrile; c = dimethylformamide; d = pyridine.

TABLE IV

R_F VALUES FOR SOME ANILINE DERIVATIVES ON SA-2 (H^+) PAPER IN HYDROPHILIC–BASIC SOLVENT MIXTURES

Aniline derivative	*Solvent composition*									
	Acetonitrile–dimethylformamide									
	*100**	*97*	*95*	*90*	*80*	*70*	*60*	*50*	*40*	*20*
4-Nitro-	0.02	0.05	0.17	0.36	0.55	0.71	0.75	0.88	0.89	0.93
2-Nitro-	0.30	0.33	0.34	0.45	0.61	0.79	0.86	1.00	1.00	1.00
2,4-Dinitro-	0.97	0.97	0.97	0.95	0.97	0.98	1.00	1.00	1.00	1.00
2,6-Dichloro-4-nitro-	1.00	0.98	1.00	0.97	0.98	0.99	1.00	1.00	1.00	1.00
4-Chloro-2-nitro-	0.75	0.69	0.67	0.72	0.70	0.89	0.90	1.00	1.00	1.00
N,N-Dimethyl-4-nitro-	0.03	0.07		0.44	0.56	0.78	0.84	1.00	1.00	1.00
4-Cyano-	0.00	0.02	0.04	0.06	0.12	0.22	0.28	0.45	0.47	0.64
3-Nitro-	0.00	0.00	0.00	0.01	0.02	0.04	0.05	0.07	0.07	0.14
4-Carboxy-	0.00	0.00	0.00	0.00	0.01	0.02	0.03	0.04	0.05	0.11
4-Acetyl- (2.2)**	0.00	0.00	0.00	0.00	0.01	0.03	0.05	0.06	0.07	0.13
	Ethanol–water–pyridine									
	80:0:20	*70:10:20*	*60:20:20*	*40:40:20*	*30:50:20*	*20:60:20*				
4-Nitro-	0.73	0.60	0.62	0.70	0.51	0.37				
3-Nitro-	0.80	0.66	0.68	0.63	0.58	0.45				
2,6-Dimethyl-	0.88	0.72	0.71	0.67	0.62	0.51				
4-Chloro-	0.81	0.64	0.62	0.60	0.64	0.43				
2-Methyl-	0.70	0.47	0.41	0.46	0.44	0.36				
4-H	0.68	0.34	0.36	0.35	0.33	0.27				
3,5-Dimethyl-	0.59	0.33	0.37	0.32	0.31	0.22				
4-Methoxy-	0.17	0.07	0.07	0.09	0.09	0.08				
4-Hydroxy-	0.04	0.01	0.02	0.02	0.01	0.00				

* % Acetonitrile.
** pK_a.

(20%) mixtures. In the first case, a broad change in R_F is obtained by increasing the concentration of the basic solvent; a more rapid change is possible if a solvent more basic than DMF is used. For the latter mixture the presence of the basic solvent fixes the magnitude of the retention and as the alcohol concentration decreases (water increases) retention increases. Holding the pyridine constant at a higher or lower level will cause the R_F values to shift in the same way except from an initial lower or higher value, respectively.

Introduction of strong acid (HCl) into the developing solvent mixture leads to an increase in the R_F value and offers an additional developing mixture. For example, typical R_F changes that were observed are 0.07 to 0.17 (4-nitroaniline) and 0.18 to 0.57 (4-chloro-2-nitroaniline) for 0.4 F HCl and 1.0 F HCl in ethanol–water (8:2), respectively.

Several other organic weak bases were investigated. As observed previously[17] any general relationship between pK_a and retention appears to hold only for derivatives of a specific type of base (for example, the anilines). Therefore, only the parent compounds of a series of N-heterocyclic compounds were examined. Table V lists R_F data based on a multiple pass of the eluting mixture. The relative order of retention is the same as for batch cation resin experiments[17], however, the magnitude of retention on the SA-2 paper appears to be greater in comparison to the batch experiments. Based on the anilines it would be predicted that R_F values for substituted derivatives of the N-heterocyclic compounds would be greater or less than the parent compound according to how the substituent influences its basicity.

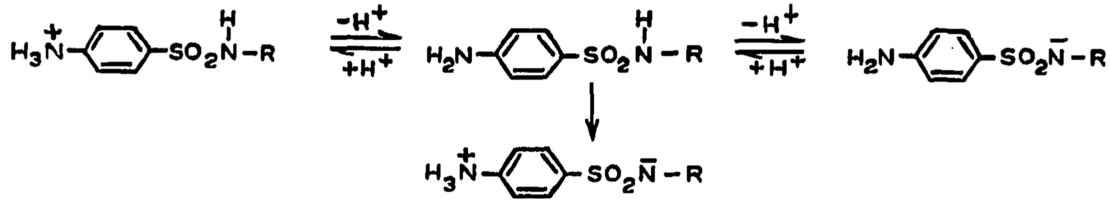
TABLE V

R_F VALUES FOR SOME HETEROCYCLIC AMINES ON SA-2 (H^+) PAPER IN VARIOUS SOLVENTS

Compound	*pK$_a$*	*% Acetonitrile*				*% Ethyl alcohol*		
		90	*80*	*70*	*50*	*80*	*70*	*50*
Pyrazine	0.60	0.02	0.05	0.05	0.02	0.09	0.08	0.05
Quinoxaline	0.72	0.05	0.10	0.11	0.09	0.19	0.18	0.14
Phenazine	1.20	0.03	0.07	0.07	0.04	0.15	0.13	0.07
Pyrimidine	1.30	0.07	0.16	0.18	0.17	0.27	0.24	0.21
Benzotriazole	1.60	0.01	0.01	0.01	0.00	0.02	0.02	0.01
Number of developments		5	4	3	2	2	2	2

R_F data were measured for caffeine, theophylline, and theobromine in ethanol–water mixtures. In general, the R_F values were approximately the same for the three compounds. (Data for caffeine are listed in Table I.) In contrast, a difference, although not large, is observed in the batch cation resin experiments[17].

Retention of sulfonamides can not be represented by reaction 1 because of the presence of both an acidic and basic group in the molecule. However, the nature of the R group influences the acidity of the molecule and retention data by batch cation resin experiments can be qualitatively correlated to the pK_a; the stronger the acid the lower the K_D. Table VI lists R_F data for the sulfonamides on SA-2 paper. In general, the order of retention is the same as for the batch experiments; but since re-



(2)

tention is high stronger eluting conditions than provided by ethanol are required. Basic solvents would also provide migration on the SA-2 paper.

On the basis of these series of experiments it can be concluded that eluting conditions for a column separation of weak organic bases on a H$^+$-form strongly acidic cation resin can be rapidly established, semiquantitatively, by first using paper impregnated with H$^+$-form strongly acidic resin. Because of the influence of the cellulose phase and the basic differences in a sheet and column method a simple quantitative relationship between the two methods is not apparent particularly as the structures of the weak organic bases become more complicated. This point is illustrated by comparing the K_D and R_F data for the series of N-heterocyclic compounds.

TABLE VI

R_F VALUES FOR SOME SULFONAMIDES ON SA-2 (H$^+$) PAPER

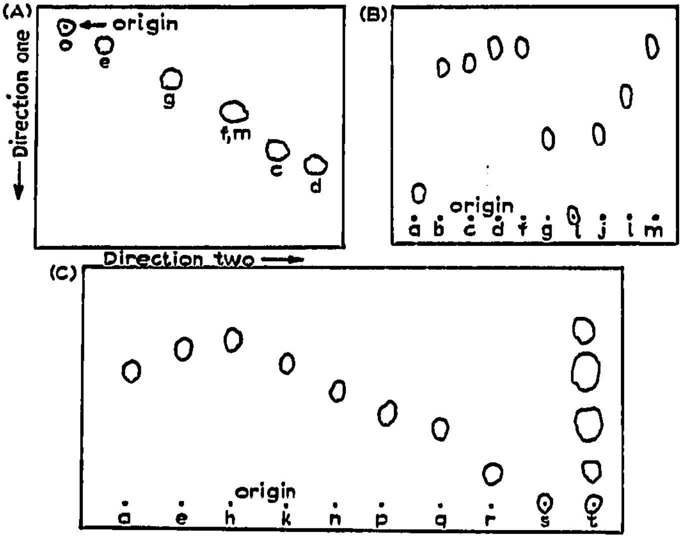
Compound	*pK$_a$ (ref. 22)*	*Developing solvent**	
		1 F HCl (H$_2$O)	*DMSO (100%)*
Sulfabenzamide	4.57	0.01	
Sulfacetamide	5.38	0.11	
Sulfadiazine	6.48	0.05	0.77
Sulfamerazine	7.06	0.02	0.68
Sulfathiazole	7.12	0.02	0.38
Sulfamethazine	7.37	0.01	0.53
Sulfapyridine	8.43	0.01	0.25
Sulfanilamide	10.43	0.15	0.29

* $R_F = 0$ for all the sulfonamides in 100% ethyl alcohol and 1 *F* acetic acid.

The SA-2 paper can also be used for the separation of mixtures of weak organic bases. Examination of the R_F values in Tables I–VI suggests a variety of eluting conditions for the separation. In general, a useful guideline for selecting the eluting conditions is to consider the basic strength of the organic bases. Also, it should be noted that eluting mixtures for the weaker bases do not include any kind of electrolytes and, therefore, recovery of the weak base from the impregnated paper in preparative applications is simplified.

Figs. 3 and 4 list several chromatograms illustrating typical separations. For separation of weak bases aprotic or amphiprotic solvents or their mixtures should be used. Either of two techniques can be used for separation of stronger bases. In one, a weak acid resin paper is used while in the other, a more basic or strongly acidic developing mixture is used with the SA-2 paper.

The zones did not tail and were well-defined. In general, the time for one



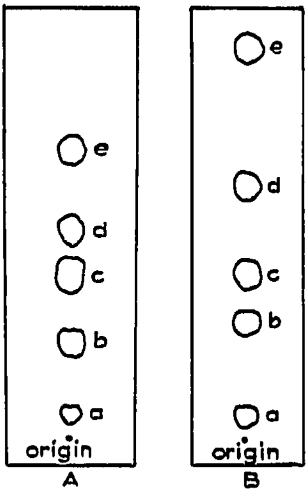


Fig. 3. Several typical separations on SA-2 paper (18 cm × 18 cm). (A) Acetonitrile–water (4:1); two-dimensional; (B) ethanol–water (4:1); (C) Ethanol–water–pyridine (5:3:2). a = 4-Nitroaniline; b = 2-nitroaniline; c = 2,4-dinitroaniline; d = 2,6-dichloro-4-nitroaniline; e = 3-nitroaniline; f = 4-chloro-2-nitroaniline; g = N,N-dimethyl-4-nitroaniline; h = 2,6-dimethylaniline; i = 4-cyanoaniline; j = N-methyl-4-nitroaniline; k = 4-chloroaniline; l = 4-methyl-2-nitroaniline; m = 2-chloro-4-nitroaniline; n = 2-methylaniline; o = 2,6-dichloro-1,4-diaminobenzene; p = aniline; q = 3,5-dimethylaniline; r = 4-methoxyaniline; s = 4-hydroxyaniline; t = mixture of a, e, h, k, n, p, q, r, and s.

Fig. 4. Separation of N-heterocyclics on SA-2 paper (6 cm × 16 cm). (A) Ethanol–water (4:1); two developments. (B) Acetonitrile–water (4:1); four developments. a = Pyrimidine; b = pyrazine; c = phenazine; d = quinoxaline; e = benzotriazole.

solvent pass for the paper sizes shown required from 30 to 60 min. No attempts were made to do an analysis based on peak area or by scanning techniques. However, preliminary studies with an anion resin paper, where the compound is isolated from the paper, suggest that separations are quantitative[23].

Improvement in the separation is possible by multiple development without adverse effects on the zones, see Figs. 3A and 4. Finally, in choosing spraying solutions for detection it must be remembered that the paper will provide a high level of acidity due to the presence of the H$^+$-form resin and/or will participate in exchange. These factors can influence the action of the detection agent.

REFERENCES

1 M. Lederer and C. Majani, *Chromatogr. Rev.*, 12 (1970) 239.
2 J. Sherma and K. M. Rich, *J. Chromatogr.*, 26 (1967) 327.
3 J. Sherma and F. VanLenten, *Separ. Sci.*, 6 (1971) 199.
4 M. Lederer and L. Ossicini, *J. Chromatogr.*, 13 (1964) 188.
5 L. Ossicini and M. Lederer, *J. Chromatogr.*, 17 (1965) 387.
6 J. Sherma and C. W. Cline, *Anal. Chim. Acta*, 30 (1964) 139.
7 H. F. Walton, *Anal. Chem.*, 44 (1972) 256R(5).
8 A. Lewandowski, *Anal. Chim. Acta*, 23 (1960) 317.

9 M. M. Tuckerman, *Anal. Chem.*, 30 (1958) 231.
10 M. M. Tuckerman, R. A. Osteryoung and F. C. Nachod, *Anal. Chim. Acta*, 19 (1958) 251.
11 L. M. Rinaldini, *Anal. Biochem.*, 36 (1970) 352.
12 C. S. Knight, *Chromatogr. Rev.*, 4 (1962) 49, 69.
13 L. Lepri, P. G. Desideri, V. Coas and D. Cozzi, *J. Chromatogr.*, 49 (1970) 239.
14 D. Cozzi, P. G. Desideri, L. Lepri and V. Coas, *J. Chromatogr.*, 40 (1969) 138.
15 D. Cozzi, P. G. Desideri, L. Lepri and V. Coas, *J. Chromatogr.*, 43 (1969) 463.
16 M. Lederer, *Bull. Soc. Chim. Fr.*, (1966) 16.
17 T. C. Gilmer and D. J. Pietrzyk, *Anal. Chem.*, 43 (1971). 1585.
18 D. J. Pietrzyk, *Talanta*, 16 (1969) 169.
19 A. Wilks and D. J. Pietrzyk, *Anal. Chem.*, 44 (1972) 676.
20 W. J. Casey and D. J. Pietrzyk, *Anal. Chem.*, 45 (1973) 1404.
21 D. D. Perrin, *Dissociation Constants of Organic Bases in Aqueous Solution*, Butterworths, London, 1965.
22 E. H. Northey, *The Sulfonamides and Allied Compounds*, Reinhold, New York, 1948.
23 D. J. Pietrzyk and E. Chan-Santos, unpublished results.