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NOTE

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Abstract

Amino acids have been chromatographed in *n*-butanol, *n*-butanol + acetic acid, and methanol + hydrochloric acid systems on titanium arsenate and plain papers using the ascending technique. The effect of solvent composition on the migration of spots has been studied systematically. A number of important binary and ternary separations have been achieved.

INTRODUCTION

Chromatography on plain papers is very limited in scope. Therefore efforts have been made recently to use impregnated papers. Qureshi et al. have recently shown the importance and potentialities of such papers for separation of metal ions (*1–5*). However, no attempt has been made to utilize these papers for the separation of organic substances except the publications of Catelli (*6*) and Coussio et al. (*7*) who chromatographed the amino acids and alkaloids, respectively, on zirconium phosphate papers. However, their studies suffer from a number of limitations:

(1) Their approach is not systematic.
(2) Only a few compounds have been chromatographed in a limited number of solvent systems.

(3) Results have not been compared with those on plain papers.

It is, therefore, interesting and worthwhile to explore the separation potentialities of inorganic ion-exchange papers for the systematic separation of organic compounds. The following pages summarize our findings in this direction.

EXPERIMENTAL

Apparatus

Chromatography was performed on Whatman No. 1 paper strips of 15 × 3.5 cm using 20 × 5 cm glass jars.

Reagents

All chemicals were either E. Merck (Darmstadt) or B.D.H. (England) AnalaR reagents.

Preparation of Titanium Arsenate Papers

Whatman No. 1 paper strips 15 × 3.5 cm were first dipped in 0.5 *M* titanium(IV) chloride solution for 5 to 10 sec and then placed on a dry filter sheet to remove the excess reagent. After 15 to 20 min they were dipped in 0.5 *M* sodium arsenate solution for 20 to 25 sec. The excess solution was drained off and the strips were allowed to dry completely on a filter sheet at room temperature. These strips were then washed with demineralized water which removes the unreacted reagents. Finally, these papers were dried at room temperature (20 ± 3°C).

Test Solutions

Amino acids solutions (0.1 to 0.2%) were prepared in demineralized water.

Detector

A ninhydrin solution (0.2%) in *n*-butanol saturated with water was used for detection of the spot on the paper strips. The spots were generally visible on heating the strips at 60 to 80°C.

Procedure

The test solutions were applied to the strips two or three times, with subsequent drying, with the help of fine capillaries. The paper was first conditioned for 10 to 15 min in the jar. It was then dipped in the solvent, and the solvent ascent was kept to 11 cm in each case.

RESULTS AND DISCUSSION

The results are summarized in Table 1.

DISCUSSION

These studies have revealed some very interesting features. In order to make the discussion simpler, let us consider the behavior of amino acids in simple systems, i.e., *n*-butanol, 10% acetic acid, and *n*-butanol + acetic acid systems (Table 1).

(1) R_F values are higher in 10% acetic acid than in *n*-butanol on plain papers as well as on titanium arsenate papers.
(2) In mixed *n*-butanol + acetic acid systems, the R_F values in general increase with the increase in acetic acid content of the solvent mixtures.

Both the above facts may be interpreted as follows: Amino acids are less soluble in *n*-butanol than in acetic acid. The presence of an increased amount of acetic acid facilitates the partition of the solute in mobile phases and hence an increase in the R_F value is expected. However, the behavior of histidine, lysine, and glycine is different on titanium arsenate papers. In these cases the R_F values remain almost constant (≈ 0.05) and are therefore independent of the solvent composition. This peculiar behavior enhances the separation possibility of these amino acids from other amino acids.

For a comparison of the behavior of amino acids on two types of papers, a quantity, R_i (R_F on untreated papers $-$ R_F on treated papers), is plotted against the isoelectric point (pI) of the amino acids in different solvent systems (Figs. 1a, 1b, and 1c). The following points emerge from these curves.

TABLE 1

Comparison of R_F Values of Amino Acids on Titanium

Compound	a		b		c	
	TiAs	PP	TiAs	PP	TiAs	PP
Glycine	0.05	0.05	0.05	—	0.05	0.17
DL-α-Alanine	0.15	0.05	0.20	0.23	0.10	0.27
L-Serine	0.05	0.05	0.05	0.20	0.07	0.30
L-Cystine	0.05	0.05	0.05	0.05	0.05	0.10
DL-Threonine	—	0.07	0.10	—	0.05	0.10
DL-Methionine	0.55	0.34	0.60	0.50	0.50	0.50
L-Valine	0.12	0.12	0.50	0.60	0.60	0.52
L-Leucine	0.82	0.43	0.85	0.85	0.92	0.65
L-Aspartic acid	0.05	0.05	0.05	0.12	0.10	0.30
L-Glutamic acid	0.15	0.10	0.12	0.22	—	0.22
L-Asparagine	0.05	0.05	0.05	0.10	0.05	0.15
L-Lysine	0.05	0.05	0.05	—	0.05	0.15
L-Histidine	0.05	0.05	0.05	0.05	0.05	0.15
L-Phenylalanine	—	0.35	0.50	0.50	0.62	0.80
L-Tyrosine	0.05	0.05	0.10	0.10	0.05	0.12
L-Tryptophan	0.70	0.30	—	—	0.72	0.52
L-Proline	0.25	0.11	0.35	0.40	0.20	0.32
β-Alanine	0.07	0.05	0.05	0.40	0.37	0.62
L-Citrulline	0.05	0.05	0.15	0.15	0.05	0.20
N-Phenylglycine	0.85	0.85	—	—	—	0.90
Cyclopentylglycine	0.72	0.42	0.65	0.72	0.88	0.80
Cyclohexylglycine	—	—	0.62	—	0.90	0.82
β-1-Naphthylalanine	0.75	0.48	0.70	—	0.90	0.75
β-Piperonylalanine	0.20	0.35	0.68	0.55	0.80	0.62
3, 4-Dihydroxyphenylalanine	0.10	0.12	—	0.22	0.12	0.38

[a]Solvents: a = *n*-butanol saturated with water; b = *n*-butanol saturated with water + acetic acid (3:1); c = *n*-butanol–acetic acid + water (5:1:4); d = *n*-butanol + acetic acid + water (5:2:3); e = *n*-butanol + acetic acid + water (5:4:1); f = glacial acetic acid; g = 10% acetic acid; and h = methanol + 2 *M* HCl + water (2:1:2).

Arsenate (TiAs) and Whatman No. 1 Plain Papers (pp)

Solvent[a]									
d		e		f		g		h	
TiAs	PP	TiAs	PP	TiAs	PP	TiAs	PP	TiAs	PP
0.10	0.40	0.06	0.21	0.05	—	—	—	—	—
0.25	0.45	0.35	0.35	0.70	—	0.72	0.95	0.77	0.90
0.30	0.52	0.10	0.52	0.05	—	0.72	0.95	—	—
0.55	0.40	0.05	0.27	0.05	0.05	0.70	—	—	—
0.35	0.45	0.24	0.46	—	—	—	0.45	0.70	0.92
0.65	0.65	0.60	0.60	0.07	—	0.85	0.95	0.72	0.85
0.67	0.72	0.62	0.65	—	—	0.95	0.90	—	—
0.64	0.73	0.83	0.77	—	0.82	0.77	0.95	0.80	0.85
—	0.62	0.12	0.40	0.10	—	0.55	0.95	—	—
0.25	0.45	0.15	0.26	0.10	—	0.80	0.90	0.65	0.92
0.11	0.36	0.07	0.27	0.10	—	0.75	0.95	0.37	—
0.05	0.31	0.05	0.10	0.05	—	0.67	0.95	0.10	0.58
0.05	0.34	0.05	—	0.07	—	0.05	0.90	0.12	0.87
0.85	0.80	0.90	0.82	—	—	—	—	—	—
0.12	0.50	0.32	0.45	0.05	0.10	0.90	0.95	—	—
0.60	0.70	—	0.40	—	—	0.52	0.70	0.65	0.70
0.25	0.50	0.42	0.44	0.10	—	0.70	0.90	0.85	—
0.17	0.65	0.30	—	0.07	0.80	0.72	0.95	—	—
0.27	0.40	0.37	0.29	0.05	—	0.65	0.95	0.70	0.85
—	0.95	—	0.92	—	—	0.80	0.95	—	—
0.85	0.90	0.95	0.92	—	—	0.85	0.95	—	—
0.85	0.95	0.95	0.90	—	—	—	—	—	—
0.92	0.90	—	0.87	—	—	0.60	0.70	—	—
0.75	0.78	0.82	0.75	—	—	0.70	0.95	—	—
0.15	0.72	0.50	0.42	0.10	—	0.60	0.85	—	—

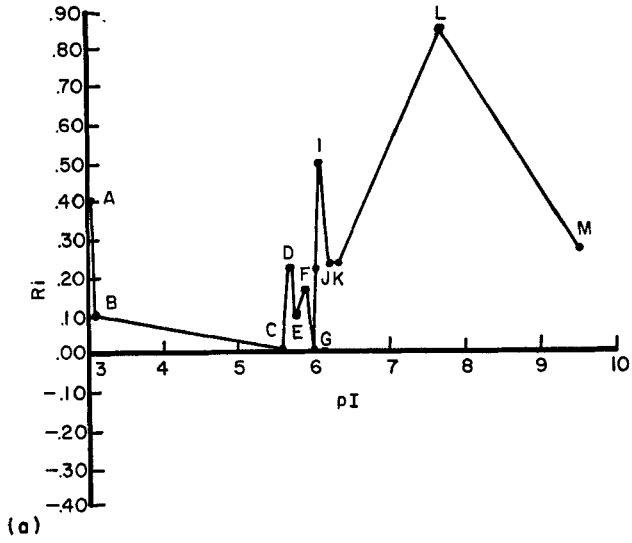

FIG. 1a. Plot of pI vs R_i (10% acetic acid): (A) aspartic acid, (B) glutamic acid, (C) tyrosine, (D) serine, (E) methionine, (F) tryptophane, (G) valine, (H) leucine, (I) glycine, (J) α-alanine, (K) proline, (L) histidine.

1. Amino acids are adsorbed more strongly on the ion-exchange paper than on the plain paper in most solvents except for butanol saturated with water where the reverse is true. R_i is positive in all solvents except *n*-butanol saturated with water, in which case it is negative (Fig. 1c). This important feature is due to the fact that two different mechanisms are operative in the two cases. In butanol it is the adsorption mechanism, while in other cases it is the ion-exchange mechanism because in butanol the ion exchanger is not ionized and hence acts merely as a sorbent.
2. In butanol saturated with water, all the R_i values are negative because only the adsorption mechanism is working. However, on increasing the percentage of acetic acid, i.e., in *n*-butanol + acetic acid (3:1), R_i is positive in most cases but it is sometimes negative (Fig. 1b). However, in acetic acid medium the R_i values are all positive because now the ion-exchange mechanism is working.

R_F values of amino acids with aliphatic side chains are in the order leucine > valine > alanine > glycine in most cases. That is to say, R_F increase with an increase in the side chain. This is probably due to an

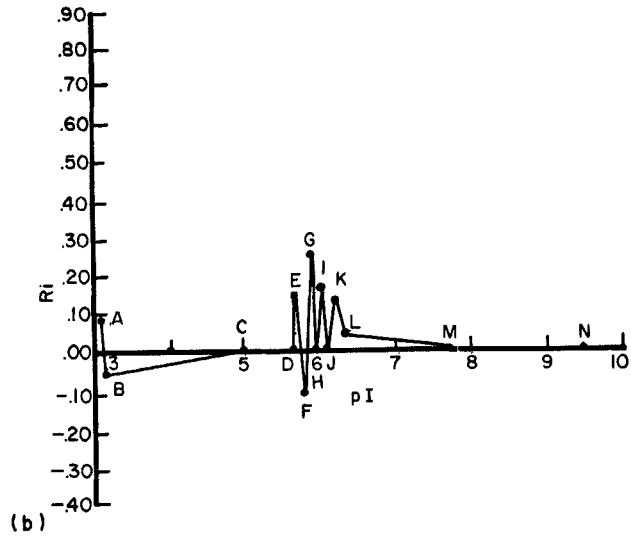

FIG. 1b. Plot of pI vs R_f (*n*-butanol–acetic acid, 3:1): (A) aspartic acid, (B) glutamic acid, (C) cystine, (D) tyrosine, (E) serine, (F) methionine, (G) tryptophane, (H) phenylalanine, (I) valine, (J) leucine, (K) α-alanine, (L) proline, (M) histidine, (N) lysine.

increase in the size of the molecule. The same fact was also observed in the cases of cellulose phosphate papers (*8*).

Let us consider the methanol + 2 *M* HCl + water system in Table 1. The pH of this solvent will be approximately 1. At this pH the amino acids will fully protonated. However, in the fully protonated state, lysine and histidine will have two proton charges and are more strongly adsorbed. Similarly leucine, threonine, α-alanine, and proline have only one positive charge, and hence they are not so strongly adsorbed ($R_F \approx 0.9$).

The superiority of titanium arsenate papers over plain papers can be easily judged from the resolving capabilities and the compactness of the spots. These special features make these papers promising for the separations of amino acids. A large number of important ternary and binary separations have been practically realized on titanium arsenate papers in various solvent systems. Thus methionine and leucine have been separated from glutamic acid, lysine, proline, glycyl glycine, threonine, α-alanine, histidine, leucine, citrulline, serine, tyrosine, valine, and aspartic acid. Other separations which are evident from Table 1 have also been achieved.

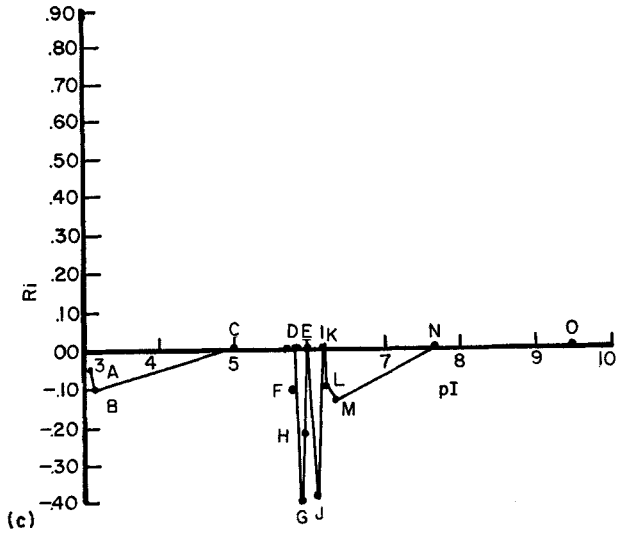

FIG. 1c. Plot of pI vs R_i (*n*-butanol): (A) aspartic acid, (B) glutamic acid, (C) cystine, (D) tyrosine, (E) serine, (F) methionine, (G) tryptophane, (H) phenylalanine, (I) valine, (J) leucine, (K) glycine, (L) α-alanine, (M) proline, (N) histidine, (O) lysine.

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REFERENCES

1. M. Qureshi, J. P. Rawat, and V. Sharma, *Talanta, 20,* 267 (1973).
2. M. Qureshi and S. D. Sharma, *Anal. Chem., 45*(7), 1283 (1973).
3. M. Qureshi, K. G. Varshney, and F. Khan, *Separ. Sci. 8*(2), 279 (1973).
4. M. Qureshi, K. N. Mathur, and A. H. Israili, *Talanta, 16,* 503 (1969).
5. M. Qureshi and W. Husain, *Separ. Sci., 4,* 197 (1969).
6. P. Catalli, *J. Chromatogr., 9,* 534 (1962).
7. I. D. Coussio, C. B. Marini Bettolo, and V. Mascatteli, *Ibid., 11,* 238 (1963).
8. M. Lederer, *Chromatogr. Rev., 4,* 83 (1962).

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