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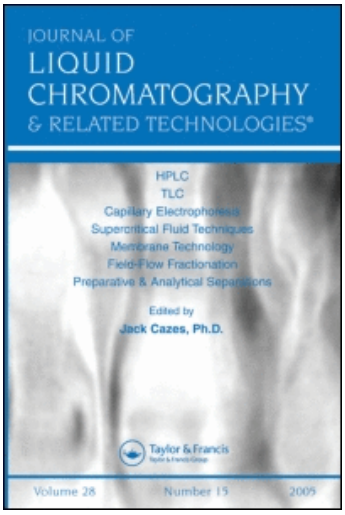

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THIN-LAYER CHROMATOGRAPHIC SEPARATIONS OF AMINO ACIDS ON STANNIC TUNGSTATE

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ABSTRACT

Thin-layer chromatography of 24 important amino acids in aqueous and mixed solvent systems has been performed on stannic tungstate ion-exchange material. Results of these studies reveal that the stannic tungstate thin-layers offer promising potentialities for the separation of amino acids. The various solvent system which have been studied, acetone-formic acid-water and ethylacetate-formic acid are found to be most useful. It is interesting to note that DL-3,4 dihydroxyphenylalanine (DHPA*) has been selectively separated from a mixture of a number of amino acids in ethylacetate formic acid systems. Moreover, specific separations of DL-methionine has been achieved from a synthetic mixture of other amino acids chromatographed. Aspartic acid and glutamic acid which belong to monoaminodicarboxylic acid type have been sharply separated from each other in n-butanol- acetic acid and acetone-formic acid-water systems. A large number of other important and difficult ternary and binary separations have also been practically achieved.

INTRODUCTION

Papers impregnated with inorganic ion-exchange material have been widely used for the separation of

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metal ions (1-5). Very limited studies have been made for the separation of organic compounds on such type of ion-exchange papers. However, titanium arsenate and zirconium phosphate papers have been used for the separation of few amino acids (6-9) and alkaloids (10). Thin-layers of pure inorganic ion-exchange material such as stannic antimonate (11) and stannic arsenate (12-13) have been found useful for the separation of metal ions. The use of such layers without any binder makes it easier to have a clear interpretation of the mechanism of the separation. A survey of literature revealed that almost no work has been reported for the separation of organic compounds on thin-layers prepared from inorganic ion-exchange materials. It is therefore, worthwhile to explore the importance of the layers of inorganic ion exchangers for the systematic separation of organic compounds. Stannic tungstate thin-layers have been tried because this material has been found to be quite stable in acids, bases and other organic solvents and possess excellent separation potentialities (14). Amino acids especially, have been chosen for the chromatographic studies because of their biomedical, physiological and pharmaceutical importance.

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EXPERIMENTAL

Reagents and Chemicals

Stannic chloride pentahydrate (Poland), sodium tungstate (Reidel, Germany), n-butanol, dioxane, acetic acid, formic acid (B.D.H., England), pyridine, acetone (E. Merck Darmstadt) were used. All other chemicals and solvents used were of analytical grade from B.D.H., England.

Apparatus

A thin-layer chromatography (TLC) Desaga (Germany) applicator was used to prepare thin-layers on 20 x 20 cm glass plates. Large mouth (Toshniwal) chamber were used for the development.

Micro-Capillary Pipettes were used for the spotting purposes.

Detector

Ninhydrin solution (0.2%) in n-butanol saturated with water was used for the detection of amino acids on TLC plates.

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Preparation of Ion-Exchange Material and Thin-Layer Plates

Stannic tungstate was prepared by mixing 0.05M solutions of stannic chloride and sodium tungstate in the volume ratio of 1:1 at pH = 1 and digesting the resulting precipitate at room temperature for 24 hours. The precipitate was filtered under suction and completely dried in an oven at 40 $\pm$ 4°C. The material so obtained was cracked in DMW (demineralized water) and then placed in $IMHNO_3$ for 24 hours to convert it to the H^+ form. The material was washed with DMW to remove excess acid and finally dried at 40°C. Ten grammes of stannic tungstate granules thus obtained were mixed in about 5 ml of distilled water and slurry was made by grinding the mixture vigorously in a glass mortar for a long time. This step proves to be very much important for the complete adhesion. The grinding of the granules must be complete and slurry should be in the form of a fine paste without any solid particles being left. The slurry was then spread over the clean glass plates with

the help of an applicator to give 0.10 mm thick layers. The plates were ready for use after drying at room temperature.

Procedure

Approximately 0.04 ml of test solutions of amino acids were applied with the help of glass capillary on the plates. After drying the spots the plates were developed in various solvent systems and solvents were allowed to ascend upto 12 cm in all the cases from the point of application.

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RESULTS AND DISCUSSION

Results of these studies reveal that stannic tungstate thin-layers offer promising potentialities for the systematic separation of amino acids. The major advantage of using stannic tungstate layer is that 'ion-exchange' and 'adsorption' take place simultaneously. As a result compact and well defined spots are obtained. It is clear from tables (1-7) that a large number of binary and ternary separation of amino acids are possible on thin-layers of stannic tungstate. The various solvent systems which have been studied, acetone-formic acid-water; n-butanol - acetic acid - water and ethyl acetate - formic acid systems are found to be most useful for the separation of amino acids. It is very interesting and worthwhile to note that DL-3,4 dihydroxyphenylalanine (DHPA*) has been selectively separated from the mixture of a number of amino acids in ethylacetate-formic acid. An striking feature emerges when pure dioxane; dioxane-nitric acid are used as developers. In this most of the amino acids remain at the point of application except DL-methionine which behaves

TABLE - I

R_F Values of Amino Acids on Stannic Tungstate Layers.

COMPOUND	SOLVENT SYSTEM A (4.30 hr)	B (5.15 hr)
DL-Alanine	0.53	0.87
DL-2 Amino n-butyric acid	0.74	0.70
L-Arginine Monohydrochloride	0.78	0.38
DL-Aspartic acid	0.86	0.85
L-Cystine HCl	0.54	0.73
L-Cystine	0.48	-
DL-3,4 Dihydroxyphenylalanine	-	0.20
L-Glutamic acid	0.51	0.56
Glycine	0.55	0.69
L-Histidine HCl	0.95	0.67
L-Hydroxyproline	0.87	0.85
L-Leucine	0.52	0.40
DL-Isoleucine	0.63	0.84
DL-Nor Leucine	0.85	0.62
L-Lysine Mono HCl	0.48	0.85
DL-Methionine	0.71	0.52
L-Ornithine HCl	0.39	0.23
DL-Phenylalanine	-	-
L-Proline	0.32	0.45
DL-Serine	0.82	0.52
DL-Threonine	0.88	0.84
DL-Tryptophan	0.50	-
L-Tyrosine	0.53	0.25
DL-Valine	0.63	0.62

A = n-butanol saturated with water: acetic acid (3:1) system;

B = acetone: formic acid: water (2:2:1).

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TABLE - II

R_F values of Amino Acids on Stannic Tungstate Layers.

COMPOUND	SOLVENT SYSTEM C (5.50 hr)	D (5.00 hr)
DL- Alanine	0.86	-
DL-2 Amino n-butyric acid	0.72	0.66
L-Arginine Monohydrochloride	0.48	0.50
DL-Aspartic acid	0.55	0.85
L-Cystine HCl	-	0.72
L-Cystine	0.75	0.76
DL-3,4 Dihydroxyphenylalanine	0.39	0.33
L-Glutamic acid	0.85	0.98
Glycine	0.52	0.52
L-Histidine HCl	-	0.60
L-Hydroxyproline	0.68	0.44
L-Leucine	0.86	0.77
DL-Isoleucine	0.53	0.45
DL-Nor Leucine	-	0.56
L-Lysine Mono HCl	0.92	0.85
DL-Methionine	0.57	0.24
L-Ornithine HCl	-	0.53
DL-Phenylalanine	0.62	0.77
L-Proline	0.95	0.59
DL-Serine	0.48	0.85
DL-Threonine	-	0.60
DL-Tryptophan	0.80	0.45
L-Tyrosine	0.45	-
DL-Valine	0.79	0.72

C = n butanol: acetic acid: water (5:4:1);

D = ethylacetate: formic acid (6:4).

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TABLE - III

R_F Values of Amino Acids on Stannic Tungstate Layers.

COMPOUND	SOLVENT E (6.45 hr)	SYSTEM F (6.30 hr)
DL-Alanine	0.0	0.0
DL- 2 Amino n-butyric acid	0.0	0.0
L-Arginine Monohydrochloride	0.0	0.0
DL-Aspartic acid	0.0	0.0
L-Cystine HCl	0.0	0.0
L-Cystine	0.10	0.0
DL-3,4 Dihydroxyphenylalanine	0.0	-
L-Glutamic acid	0.0	0.22
Glycine	0.0	0.0
L-Histidine HCl	0.0	0.13
L-Hydroxyproline	0.23	0.0
L-Leucine	0.0	0.0
DL-Isoleucine	0.0	0.0
DL-Nor Leucine	0.0	0.35
L-Lysine Mono HCl	0.0	0.0
DL-Methionine	0.40	0.15
L-Ornithine HCl	0.0	0.0
DL-Phenylalanine	0.0	0.0
L-Proline	0.0	0.0
DL-Serine	0.0	0.0
DL-Threonine	0.0	0.46
DL-Tryptophan	0.0	-
L-Tyrisine	0.18	0.0
DL-Valine	-	0.0

E = Dioxane; F = Dioxane + 0.1M HNO_3

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TABLE - IV

R_F Values of Amino Acids on Stannic Tungstate Layers

COMPOUND	SOLVENT SYSTEM G (5.15 hr)	H (5.45 hr)
DL-Alanine	0.61	0.62
DL-2 Amino n-butyric acid	0.87	0.85
L-Arginine Monohydrochloride	0.80	0.53
DL-Aspartic acid	0.50	0.67
L-Cystine HCl	0.72	0.87
L-Cystine	-	0.67
DL-3,4 Dihydroxyphenylalanine	0.96	0.95
L-Glutamic acid	0.63	0.67
Glycine	0.86	0.84
L-Histidine HCl	0.74	0.20
L-Hydroxyproline	0.91	-
L-Leucine	0.45	0.59
DL-Isoleucine	0.68	0.86
DL-Nor Leucine	0.77	0.42
L-Lysine Mono HCl	0.33	0.76
DL-Methionine	0.82	0.78
L-Ornithine HCl	0.47	0.35
DL-Phenylalanine	0.86	0.61
L-Proline	0.74	0.78
DL-Serine	0.38	0.86
DL-Threonine	0.78	0.56
DL-Tryptophan	0.55	0.71
L-Tyrosine	-	-
DL-Valine	0.89	0.91

G = Acetic acid + formic acid + water (4:3:2)

H = Ethylalcohol + ethylacetate + n-butanol (3:4:2)

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TABLE - V

R_F Values of Amino Acids on Stannic Tungstate Layers

COMPOUND	SOLVENT SYSTEM	
	I (5.45 hr)	J (5.00 hr)
DL-Alanine	0.70	0.62
DL-2 Amino n-butyric acid	0.84	0.85
L-Arginine Mono HCl	0.41	0.69
DL-Aspartic acid	0.78	0.41
L-Cystine HCl	0.61	0.80
L-Cystine	0.84	0.85
DL-3,4 Dihydroxyphenylalanine	0.95	0.92
L-Glutamic acid	0.77	0.29
Glycine	0.86	0.67
L-Histidine HCl	0.61	0.65
L-Hydroxyproline	-	0.86
L-Leucine	0.86	0.49
DL-Isoleucine	0.24	0.76
DL-Nor Leucine	0.56	0.58
L-Lysine Mono HCl	0.85	0.84
DL-Methionine	0.92	-
L-Ornithine HCl	0.85	0.94
DL-Phenylalanine	0.47	0.69
L-Proline	0.60	0.20
DL-Serine	0.59	0.53
DL-Threonine	-	0.72
DL-Tryptophan	0.85	0.45
L-Tyrosine	0.45	0.76
DL-Valine	0.77	0.86

I = Ethylacetate + Pyridine + Water (2:1:2);
J = acetone + ethanol + water (6:1:3).

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TABLE - VI

Separations Actually Achieved On Stannic Tungstate Thin Layers in Important Solvent Systems

Solvent Systems: Acetone : Formic acid : Water

"Ternary Separations" In Mixture of Amino Acids

(I) DL-Alanine (10.4-11.3) — DL-3,4 DHPA*(3.0-3.6) — L-Leucine (5.0-5.2)
(II) DL-Alanine (10.7-11.2) — DL-3,4 DHPA* (2.8-3.1)— L-Proline (5.8-6.1)
(III) DL-Alanine (10.5-10.8) — DL-3,4 DHPA*(1.0-1.8) — DL-Serine (5.4-6.3)
(IV) DL-Alanine (10.7-11.4) — DL-3,4 DHPA*(2.4-2.9) — DL-Methionine (8.8-10.2)
(V) DL-Alanine (7.0-7.4) — DL-3,4 DHPA*(3.0-3.2) — L-Cystine HCl (7.8-8.1)
(VI) DL-Alanine (10.5-10.7) — DL-3,4 DHPA*(0.0-0.0) — L-Glutamic acid (7.0-7.2)
(VII) L-Tyrosine (0.0-0.0) — DL-Alanine (8.6-9.3) — DL-Methionine (6.6-7.1)
(VIII) DL-Aspartic acid (10.5-10.7) — DL-3,4 DHPA*(0.0-0.00)— L-Glutamic acid (7.0-7.5)

"Binary Separations"

(I) L-Tyrosine (0.0-0.0) — 2 Amino n-Butyric acid (7.8-8.2)
(II) L-Tyrosine (1.2-1.4) — DL-Valine (7.9-8.3)
(III) L-Tyrosine (1.7-2.0) — DL-Aspartic acid (9.5-9.8)
(IV) L-Tyrosine (1.8-2.3) — L-Hydroxy Proline (9.3-10.0)
(V) DL-3,4 DHPA* (0.0-0.0) — L-Lysine Mono HCl (10.9-11.2)
(VI) DL-3,4 DHPA* (0.0-0.0) — DL-2 Amino n-Butyric acid (8.0-8.3)
(VII) L-Ornithine HCl (1.5-1.9) — DL-2 Amino n-Butyric acid (9.4-9.8)
(VIII) L-Ornithine HCl (1.0-1.2) — DL-Valine (8.8-9.0)
(IX) L-Ornithine HCl (0.00-0.00) — DL-Threonine (10.2-10.4)
(X) DL-3,4 DHPA* (0.0-0.0) — DL-Threonine (9.4-9.8)
(XI) DL-3,4 DHPA* (0.0-0.0) — L-Lysine Mono HCl (8.3-8.7)
(XII) L-Arginine Mono HCl (5.0-5.3) — DL-Alanine (10.3-10.7)
(XIII) L-Arginine Mono HCl (4.8-5.1) — DL-2 Amino n-Butyric acid (9.3-10.7)
(XIV) L-Arginine Mono HCl (4.3-5.2) — L-Lysine Mono HCl (9.3-10.1)
(XV) L-Arginine Mono HCl (3.8-4.0) — DL-Threonine (9.5-9.9)

DHPA* = dihydroxyphenylalanine

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TABLE - VII

List of Important and Difficult Ternary and Binary Separations Achieved on Stannic Tungstate Layers

Solvent System: Butanol : Acetic acid : Water

"Ternary Separations"

(I) L-Proline (10.6-10.8) — DL-3,4 DHPA* (3.7-4.0) — L-Hydroxy Proline (8.2-8.5)
(II) L-Proline (10.2-10.6) — DL-3,4 DHPA* (2.8-3.2) — DL-Aspartic acid (5.8-6.2)
(III) Glycine (7.5-7.9) — DL-3,4 DHPA* (3.0-3.3) — L-Proline (10.8-11.0)
(IV) L-Proline (9.7-10.1) — DL-3,4 DHPA* (0.0-0.0) — DL-Isoleucine (6.5-6.7)
(V) L-Proline (10.4-10.6) — DL-3,4 DHPA* (0.0-0.0) — DL-Methionine (5.6-6.0)
(VI) L-Proline (10.6-11.1) — DL-3,4 DHPA* (0.0-0.0) — DL-Phenyl alanine (5.7-6.3)

"Binary Separations"

(I) L-Proline (11.0-11.2) — L-Tyrosine (7.0-7.6)
(II) L-Proline (10.8-11.0) — DL-Serine (6.0-6.2)
(III) L-Proline (11.2-11.5) — DL-Isoleucine (7.0-7.3)
(IV) L-Proline (10.0-10.3) — DL-3,4 DHPA* (0.00-0.00)
(V) L-Proline (9.8-10.2) — L-Arginine Mono HCl (5.8-6.0)
(VI) L-Proline (11.5-11.7) — DL-Aspartic acid (6.8-7.3)
(VII) L-Lysine Mono HCl (10.8-11.1) — L-Tyrosine (6.0-6.6)
(VIII) L-Lysine Mono HCl (10.8-11.3) — DL-Serine (6.3-6.9)
(IX) L-Lysine Mono HCl (10.8-11.2) — L-Arginine Mono HCl (6.0-6.4)
(X) L-Lysine Mono HCl (9.5-9.8) — DL-Aspartic acid (7.8-8.0)
(XI) L-Leucine (9.8-10.0) —DL-Serine (6.0-6.2)
(XII) L-Lucine (10.0-10.3) — L-Tyrosine (5.4-5.6)
(XIII) DL-2 Amino n-Butyric acid (7.8-8.1) — DL-Serine (5.8-6.0)
(XIV) DL-2 Amino n-Butyric acid (8.0-8.3) — DL-Tyrosine (5.6-5.8)
(XV) DL-2 Amino n-Butyric acid (8.6-9.0) — DL-DHPA* (0.0-0.0)
(XVI) DL-2 Amino n-Butyric acid (9.5-9.7) — L-Arginine Mono HCl (5.8-6.3)

DHPA* = dihydroxyphenylalanine

in a peculiar way. As a result of this specific separation of DL-methionine has been selectively achieved from a synthetic mixture of a number of amino acids. Aspartic acid, glutamic acid both belonging to monoamino-dicarboxylic acid type have been sharply separated from each other in n-butanol: acetic acid (3:1) and acetone: formic acid: water (2:2:1) systems. Furthermore, certain separations of important and difficult pairs of mono-aminomonocarboxylic acid types such as glycine-leucine; leucine-DL-serine; alanine-serine; alanine-leucine; DL-serine- DL-isoleucine; leucine-threonine; DL-serine-DL-valine; leucine-isoleucine; DL-Norleucine and also valine from DL-threonine have been conveniently achieved utilizing stannic tungstate layers. Distinct separations of heterocyclic amino acids from one another have been obtained. Thus separation of tryptophan from hestidine and hydroxyproline have been realized in many systems i.e. n-butanol- acetic acid-water; acetic acid-formic acid-water; n-butanol saturated with water-acetic acid and also n-butanol-ethylalcohol-ethylacetate solvent systems. It is interesting to observe in the case of heterocyclic amino acids. The R_F value increases with the increase in molecular weight of the amino acids. In ethylacetate-ethylalcohol-n-butanol and ethylacetate-pyridine-water systems. R_F value decreases in the following sequence -

Tryptophan Histidine Hydroxyproline

while the order of R_F values are reversed in acetone-ethylalcohol-water system i.e. with the increase in molecular weight of amino acids R_F value decreases. Thus chromatography of amino acids on thin-layers of stannic tungstate offers a large number of important and difficult separations of amino acids.

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