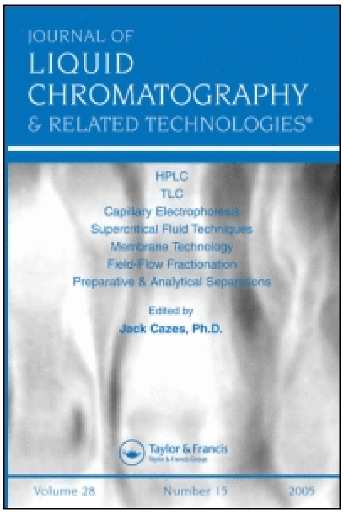

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Chromatographic Studies on Hydrous Oxides of Polyvalent Metals. II. Electrochromatographic Studies of Amino Acids on Hydrous Zirconium (IV) Oxide Papers: Separation of Acidic Amino Acids

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CHROMATOGRAPHIC STUDIES ON HYDROUS OXIDES OF POLYVALENT METALS. II. ELECTROCHROMATOGRAPHIC STUDIES OF AMINO ACIDS ON HYDROUS ZIRCONIUM (IV) OXIDE PAPERS: SEPARATION OF ACIDIC AMINO ACIDS+

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ABSTRACT

Electrochromatographic behaviour of some amino acids on hydrous zirconium (IV) oxide impregnated papers have been carried out. Five background electrolytes of different pH were used for these studies at fixed potential difference and time

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intervals. On the basis of differential mobility of amino acids which depends on the ion exchange properties of hydrous zirconium oxide and the nature of complexes formed with electrolytes, some useful binary, ternary and quarternary separations have been achieved.

INTRODUCTION

The proteins are among the most important componants of all living systems, their function range from catalysts (enzymes) to regulators to structural componants. The building blocks and language of proteins are most of the amino acids. Separation, identification and determination of amino acids has received significant attention in view of its importance in bio-chemistry, medicine and chemistry. Some attempts have been earlier made in this direction using resin beads[1-2].

The papers impregnated with olive oil, paraffin, greeas, vaseline etc. have been used for the separation of fatty acids[3]. The papers impregnated with calcium sulphate have been used for the separation of 34 organic acids[4].

The earlier work on electrochromatography[5-10] reveals that the papers impregnated with synthetic inorganic ion-exchangers have good analytical potential for the separation of inorganic ions. Although some paper chromatographic studies of amino acids have also been performed on hydrous zirconium (IV) oxide[11], titanium (IV) arsenate[12] and Tin (IV) and Thorium (IV) phosphosilicates[13]. Tin (IV) arsinosilicate[14] has also been utilised in the thin layer chromatographic studies of amino acids.

The hydrous zirconium (IV) oxide acts as a cation[15-16] as well as anion[17-19] exchanger. It has been proved useful in, column[18-22], paper[23,24], Thin layers[16] and electrochromatography[10,24] for the separation of inorganic ions. All these studies has given us impetus to extend our work to study the electrochromatographic behaviour of some amino acids on hydrous zirconium oxide impregnated papers.

In the present study the electrochromatographic behaviour of some amino acids on hydrous zirconium oxide impregnated papers is discussed. The same operation was repeated with plain papers to find the role of the ion exchanger under an electrical potential on migration of amino acids. On the basis of differential mobility of amino acids, a large number of analytically important separations of binary, ternary and multicomponant mixtures of amino acids have been achieved.

EXPERIMENTAL

Apparatus

Electrophoretic studies were performed on whatman No. 1 filter paper strips (46.4 x 2.75 cm) using a horizontal type electrophoresis apparatus run on an electronically regulated power supply unit (Systronics Ltd. India).

Reagents and Chemicals

Chemicals and solvents used for the studies were obtained from BDH (England). E. Merck and Romali (India).

Preparation of Ion Exchanger Papers

Hydrous Zirconium (IV) oxide papers were prepared as described earlier[9,24] by successively dipping the paper strips in freshly prepared 0.1M aqueous zirconium oxychloride solution in 0.1M HCl and 1M aqueous ammonia solution. The strips were allowed to drain, washed twice with deionized water to remove excess reagent and finally dried at 100±5° for 1 h in an air oven. Ordinary paper strips were also dried in an air oven at 100±5° for 1 h for better comparison. Dried papers were washed several times with deionised water and finally dried at room temperature before use.

Test solution and detection reagent

2% solutions of amino acids were prepared in demineralized water. The detection was made with a 2% alcohlic ninhydrin on warming for a few minute or by keeping for 12 hrs at room temperature.

Back ground electrolytes

The following background electrolyte solutions were used in these studies:

(i) 0.05M HCl+0.09M KCl (pH2),
(ii) 0.2M CH_3COOH+0.2M CH_3COONa (pH.4)
(iii) 0.01M CH_3COOH+0.1M CH_3COONa (pH-6),
(iv) 0.1M NH_4OH+0.1M NH_4Cl(pH8) and
(V) 0.1M NH_4OH+0.1M NH_4Cl (pH.10).

Procedure

The electrode chambers after washing several times with distilled water were filled with equal volumes of aqueous background electrolytes. The electrophoresis was then carried out by usual procedure[6]. A potential difference of 200 V was applied for 4h. The migration of amino acids was determined by measuring the spot from the point of application to the middle of the zone. On the basis of the result observed in the case of individual amino acids, the experiment was repeated with synthetic binary, ternary or multicomponent mixtures of amino acids of interest for separation.

RESULTS

In the present study, electrochromatographic migration of amino acid, in combination with the ion exchange behaviour of the hydrous zirconium oxide yield a number of important separations. The migration of an amino acid is the distance of the centre of the spot from the middle of the paper where it is applied originally. A plus sign is used for the distance travelled by the amino acids towards the anode and a minus sign for distance migrated towards the cathode. The mark 0.0 indicates no migration of amino acid under the specific condition. The exchanger material is sparingly soluble in background electrolytes used in the experiment. The rate of the migration on the impregnated paper under a given potential depends on, the size and charge of the componants, relative molecular mass, electrolyte concentration and adsorption as well as ion exchange behaviour of the exchanger.

The electrophoretic migration distances (in cm) of 24 amino acids in all the above background electrolyte solutions were measured. On the basis of migration distances and direction of movement of amino acids several important and analytically difficult binary, ternay and quarternary separations have been possible some of which are listed in Table 2. while Table 1. summarises the migration distances of 24 amino acids on plain as well as on ion exchange papers.

DISCUSSION

Hydrous zirconium oxide behaves as an anion exchanger in acidic medium and its anion exchange properties decrease with the increase of pH. This is evident with the increasing migration in case of Aspartic acid and glutamic acid which show higher movements at pH 6,8 and 10. A close examination of the data reveals, at low pH both the acidic amino

Table 1: Movement (cm)** of Amino Acids in various electrolytes on Hydrous Zirconium Oxide Papers and on ordinary Whatman No. 1 papers.
Time -4 hrs Voltage applied - 200 V.

Amino acids	0.09M KCl + 0.05 MHCl pH 2	0.2M CH_3COOH + 0.2M CH_3COONa pH 4	0.1M CH_3COOH + 0.2M CH_3COONa pH 6	0.1M NH_4OH + 0.1M NH_4Cl pH 8	0.1M NH_4OH + 0.1M NH_4Cl pH 10
1. DL-Alanine	+1.2(+5.3)	+1.3(0.0)	+0.8(+1.0)	+3.5(+2.3)	+2.1(+2.3)
2. DL-2 amino-n-butryic acid	+1.3(+4.1)	+1.6(+1.8)	+0.5(+1.8)	+2.7(+2.6)	+2.6(+2.0)
3. L-Arginine monohydrochloride	+2.4(+8.9)	+4.1(+6.0)*	+5.4(+5.3)	+3.8(+5.7)*	+3.9(+3.1)
4. DL-Aspartic acid	0.0(+3.6)	0.0(-2.5)	-2.5(-3.4)	-1.5(-5.5)	-3.3(+2.2)
5. L-Cystein hydrochloride	-1.2(+1.5)	0.0(+1.8)	+1.1(+8.8)	+1.2(-4.2)*	-1.4(+2.4)
6. L-Cystine	-1.0(0.0)	0.0 (-)	+0.8(+1.0)	- (-)	0.0(-1.8)
7. DL-3,4,Dihydroxy phenyl alanine	0.0(+1.2)	0.0(-1.1)	0.0(+2.3)	0.0(0.0)	0.0(+1.0)
8. L-Glutamic acid	0.0(+5.0)	0.0(+1.4)	-2.0(-5.7)	-1.6(-4.7)*	-4.1(+0.9)
9. Glycine	+1.4(+4.5)	+3.5(+2.1)	+1.3(+1.1)	0.0(+1.2)	+0.5(+0.2)
10. L-Histidine monohydrochloride	+3.3(+7.1)	0.0(+6.2)*	+1.0(+2.6)	+1.2(-1.0)	+1.3(+2.2)

11. L-Hydroxyproline	-1.3(+5.2)	+2.2(+1.7)	-2.0(+1.8)	+1.2(+1.4)	+2.0(+1.6)
12. L-Leucine	+0.7(+5.2)	-0.2(+1.9)	+1.3(+0.5)	+2.8(0.0)	+3.1(+1.6)
13. DL-isoleucine	-0.5(+1.4)	-0.2(+1.6)	+1.6(+1.6)	+0.3(0.0)	0.0(+1.2)
14. DL-norleusine	+0.7 (+1.5)	0.0(0.0) *	+2.0(+0.9)	+0.7(+2.2)	+3.9(+2.5)
15. L-Lysine mono hydrochloride	+2.7 (+6.8)	+6.2(+9.5)	+5.4(+6.4)	+4.5(+5.5)	+2.6(+3.2)
16. DL-Methionine	+2.6 (-0.7)*	+1.5(+1.5)*	+0.8(+8.8)	+1.3(0.0) *	0.0(+2.0)
17. DL-Ornithine	+2.6(+5.9)	+4.5(+5.4)	+4.6(+8.7)	+3.1(+6.0)	+3.2(+3.0)
18. DL-B-Phenyl alanine	0.0(+2.6)	0.0(+1.0)	+0.7(+2.1)	+1.4(0.0)	+3.2(+1.6)
19. L-Proline	-0.5(+6.0)	-2.0(+1.3)*	0.0(+0.9)	+2.0(-2.4)	+2.6(+1.9)
20. DL-Serine	0.0(+5.8)	+0.7(+2.3)	+0.7(+2.0)	+2.4(0.0)	0.0(+2.5)
21. DL-Threonine	+1.0 (+5.0)	-1.1(+1.9)	+0.8(+2.2)	+1.6(+1.5)	0.0(+1.2)
22. DL-Tryptophane	0.0(+2.8)	0.0(+1.1)	-0.0(+1.5)	0.0(+1.3)	0.0(+1.6)
23. L-Tyrosine	0.0(+2.8)	0.0(-8.8)	0.0(+1.7)	0.00(0.0)	0.0(+2.5)
24. DL-Valine	+1.0(-5.4)	0.0 (+3.0)	0.0(+2.0)	+1.6(0.0)	+2.1(+2.5)

** Values on ordinary whatman paper in parenthesis, * Tailing, - Decomposed or not detected

Table 2. : **Separation Achieved on Hydrous Zirconium Oxide impregnated papers**

Voltage applied = 200V Time 4 hrs.

Background electrolyte	Separation achieved (cm)
0.09M HCl+0.05M KCl (pH 2)	DL-Aspartic acid (0.0)/L-Glutamic acid (0.0) —— L-Arginine HCl (+2.4)/L-Histidine (+3.3)/L-Lysine HCl (+2.7)/DL-Ornithine (+2.6) L-cystin HCl(-1.2)/Glycine (-1.4)/L-Hydroxy prolin(-1.3)/ DL-valine (-1.0)——DL-Aspartic acid (0.0)/L-Glutamic acid (0.0)——L-Arginine HCl(+2.4)/L-Histidine (+3.3)/L-Lysine HCl (+2.7)/DL-Ornithine (+2.6)/DL-methionine (+2.6)
0.2M CH_3COOH+0.2M CH_3COONa (pH 4)	DL-Threonine(-1.1)/L-Proline (-2.0)—— L-Leucine (+0.2)/DL-isoleucine(+0.2)/DL-nor Leucine (0.0)/DL-B-phenyl alanine(0.0)/DL-Typotophane (0.0)/L-Tyrosine (0.0)/DL-valine(0.0)/L-cystein HCl (0.0)/ L-cystine (0.0) —— DL-Alanine (+1.3)/DL-2-aminobutyric acid (+1.6)/DL-methionine (+1.5) ——Glycine (+3.5)/LArginine HCl(+4.1)/DL-Ornithine (+4.5)—— L-Lysine HCl(+6.2)

0.1M CH_3COOH+0.1M CH_3COONa (pH 6)	DL-Aspartic acid(-2.5)/L-Glutamic acide(-2.0)/L-hydroxy proline (-2.0) —— L-proline (0.0)/DL-Serine (+0.7)/DL - Threonine(+0.8)/DL-B-phenyl alanine (0.0)/DL - Tyrosine (0.0)——L-Leucine (+1.3)/DL-isoleucin(+1.6)/DL-nor leucine(+2.0)—— L-Lysine HCl (+5.4)/L-Arginine HCl (+5.4)
0.1M NH_4OH+0.1M NH_4Cl (pH 8)	DL-isoleucine(+0.3)—— L- leucine (+2.8)/DL-2-amino-butyric acid (+2.7)/DL-Serine (2.4) —— DL-Alanine (+3.5)/L-Arginine HCl (3.8)——L-Lysine HCl (+4.5)
0.1M NH_4OH+0.1M NH_4Cl (pH 10)	L-Glutamic acid (-4.1)/DL-Aspartic acid(-3.3)—— methionine (0.0)/DL-Serine (0.0)/ L-tryptophane (0.0)——DL-Valine (+2.1)/L-proline (+2.6)/L-Hydroxyproline (+2.0)—— L-Arginine HCl (+3.9)/ DL-ornithine (+3.2)/DL-nor Leucine (+3.9)/DL-B phenyl alanine (+3.2).

acids (Aspartic acid and glutamic acid) are absorbed strongly and show no movement on hydrous zirconium oxide papers while basic amino acids such as Arginine, Histidine, Lysine and Ornithine move appreciably. It may be either due to the strong anion exchange behaviour of the exchanger which absorbs the two carboxylic groups present in them are strongly bounded to the exchanger matrix or due to the formation of anionic species which are strongly absorbed on the ion exchanger. All the four basic amino acids formed the cationic species which cause higher movement at acidic pH (2 to 6) but show decrease in the movement when the pH is again increased because at higher pH (above 6) the exchanger behaves as cation exchanger and the movement of acidic amino acids increases.

The phenyl group containing amino acids do not move on exchange paper at any pH except DL-phenyl alanine which show slight movement at higher pH. It may be either due to the strong absorption by the exchanger or due to the non ionisible nature of the phenyl group containing acid or due to the formation of neutral species with the electrolytes.

The sulphur containing amino acid show high movement due to the formation of different ionic species in various mediums.

On the basis of electrophoretic mobilities of amino acids on hydrous zirconium oxide paper a large number of separation have actually been achieved (Table 2.) The method is useful for separating the different types of amino acids from drugs, appetizers and plants and helpful in determining the composition of various amino acids in these materials.

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