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# ION-EXCHANGE PAPER CHROMATOGRAPHIC RESOLUTION OF SOME RACEMIC DL-AMINO ACIDS ON ALGINATE

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## SUMMARY

Sodium alginate in silica gel was tested as an adsorbent in the ion-exchange paper chromatography of some racemic DL-amino acids, using as eluent pyridine–water–amyl alcohol–$Na_2HPO_4 \cdot 7H_2O$ (0.5% aqueous solution) (7:7:2:6). The behaviour of amino acids on alginic acid seemed to depend on an ion-exchange mechanism in addition to other mechanisms. The complete resolution was obtained of the racemic acids into the L-forms, which showed faster mobilities, and the D-forms, which did not move from the starting line in most instances. This technique also appeared to be useful in differentiating sugars containing a galactose moiety.

## INTRODUCTION

The chemical resolution of the DL-amino acids on a quantitative basis for the purpose of their identification is of current interest. In 1970, Krause[1] attempted to distinguish pairs of optically isomeric amino acids by utilizing their different catalytic activities towards a mixture of indigo carmine and water. They found that phenylalanine, leucine and methionine were the most rapidly acting acids; the D-isomers were more active for valine, glutamic acid, threonine and methionine, while the L-isomers were more active for phenylalanine, leucine and serine. In 1969, plates coated with 4:1 (w/w) alginic acid–cellulose were used[2] to separate amino acids. Optical isomers of the amino acids were not separated by this mixture, but basic amino acids were well separated by hydrochloric acid. In addition, previous work[3] indicated the separation of primary aromatic amines on alginic acid and carboxymethylcellulose, both among isomers and among groups of amines that have different acid–base characteristics, such as diamines and monoamines. A quantitative relationship was found to exist between results obtained on a thin layer and those obtained on a column, although this only applied within a certain range of $R_F$ values.

The thin-layer chromatographic and electrophoretic behaviour of primary aromatic amines on weak ion exchangers has also been reported[4]; alginic acid exhibited a stronger retention power than carboxymethylcellulose, with a marked selectivity for amines. $R_F$ values and electrophoretic mobilities were obtained in organic and inorganic acid solutions, water, buffered media and aqueous organic

solvents. The $R_F$ values were correlated with $pK_b$ values[4]. The above investigation depended on a study of the hydrogen ion concentration gradient on thin layers of alginic acid[5].

The chromatographic resolution of racemates on natural optically active ion exchangers has been reported[6], and the racemic bases (±)-*threo*-$PhCH(NH_2)CH$ $PhCO_2Me$, (±)-*erythro*-$PhCH(NH_2)CH$ $PhCO_2Me$ and ritaline were resolved on alginic acid. The resolution depended on the degree of swelling of alginic acid, as previously described[7]. Through their reactions with L- and D-amino acids, specific oxidases and thin-layer chromatography, it was possible to identify[8] some L- and D-amino acids present in a mixture.

However, in all of the previous reports, there was no indication of the resolution of DL-amino acids into their L- and D-forms by simple chemical procedures. The present work was primarily concerned with this problem, taking advantage of the chromatographic characteristics of alginic acid, its exchange mechanism and its applications in organic and inorganic thin-layer and paper chromatography.

## EXPERIMENTAL

Unlike pure alginic acid, papers impregnated with a 2:1 (w/w) mixture of sodium alginate and silica gel retain their ion-exchange capacity virtually unchanged; they also have a better retention power and reproducibility than pure alginic acid plates. In this study, Whatman No. 1 paper was used and, in order to coat four $35 \times 20$ cm paper sheets, a dilute silica gel suspension was prepared (1 g per 100 ml of distilled water). The paper sheets were dipped into this suspension and allowed to dry completely at room temperature. The dried paper sheets were then dipped into a solution of sodium alginate in distilled water (2 g per 100 ml) and allowed to dry overnight at 18–22° prior to use.

Each amino acid was dissolved separately to form a 1% solution in a 21% aqueous solution of isopropanol–ethanol–methanol (1:1:1). In this way, no hydrochloric acid came into contact with tyrosine or cystine. The amount of each sample was 2 $\mu$g, chosen on the basis of the size of the spot and the sensitivity of the detection reagent[9] found in preliminary experiments. The samples were applied to the paper on the starting line, 5 cm from the lower edge. The papers were then developed for 24 h by the ascending technique using pyridine–water–amyl alcohol–$Na_2HPO_4 \cdot 7H_2O$ (0.5% aqueous solution) (7:7:2:6) as the eluent. The papers were then allowed to dry at 18–22° and finally sprayed with a 0.2% solution of ninhydrin in isopropanol or acetone.

## RESULTS

The results in Table I show that in a control experiment on paper sheets not impregnated with alginate or alginate–silica gel, all the amino acids listed moved from the origin with different mobilities in a run period of 48 h, although the L-forms and the DL-forms moved as a single spot. However, on the paper impregnated with alginate, the mobilities of these amino acids were different. The mobilities of the basic amino acids L-arginine and L-lysine were slower, and racemic DL-aspartic acid and L-cystine did not move from the origin in 48 h. Racemic DL-methionine and

TABLE I

$R_F$ VALUES OF AMINO ACIDS (2 μg) ON ALGINATE–SILICA GEL PAPER CHROMATOGRAPHY AT 18–22°.

| *Amino acid* | *Specific rotation*, $[\alpha]_w^{22°}$* | | *Control experiment, n-butanol–acetic acid–water, 48 h* | *Alginated paper, n-butanol–acetic acid–water, 48 h* | *Alginate–silica gel paper, pyridine–water–amyl alcohol–$Na_2HPO_4 \cdot 7H_2O$, 24 h* |
|---|---|---|---|---|---|
| | *L-* | *D-* | | | |
| L-Arg | +12.3 | | 0.25 | 0.10 | 0.00 |
| L-Lys | +26.1 | | 0.25 | 0.05 | 0.00 |
| DL-Asp | + 4.2 | −25.1 (in HCl) | 0.30 | 0.00 | 0.52 (D-)<br>0.78 (L-) |
| L-Cys | −70.5 (0.2 *N* NaOH) | | 0.37 | 0.00 | 0.87 |
| DL-Met | − 8.5 | + 8.4 | 0.95 | 0.00 (L-)<br>0.95 (D-) | 0.30 (D-)<br>0.75 (L-) |
| DL-Ser | − 6.0 | + 6.5 | 0.28 | 0.00 (D-)<br>0.15 (L-) | 0.00 (L-)<br>0.55 (D-) |
| L-Ser | − 6.7 | | | 0.15 | |
| L-Tyr | −12.8 (3.0 *N* NaOH) | | 0.65 | 0.35 | 0.00 |
| DL-Ala | + 3.1 | −13.8 | 0.60 | 0.60 | 0.07 (D-)<br>0.58 (L-) |
| DL-Leu | −10.3 | +10.7 | 0.92 | 0.80 | 0.00 (D-)<br>0.95 (L-) |
| DL-Ile | +11.09 | −10.9 | 0.90 | 0.76 | 0.00 (D-)<br>0.70 (L-) |
| DL-Phe | −34.0 | +33.9 | 0.97 | 0.95 | 0.00 (D-)<br>0.85 (L-) |
| L-Gly | | | 0.40 | 0.20 | 0.00 |

* Using sodium light (5893 Å).

DL-serine, however, showed fast mobilities and were able to be resolved into the two optically active isomers. D-Methionine separated from the starting line moved with a high velocity with $R_F$=0.95 while its L-form did not show any movement from the origin in 48 h. DL-Serine was resolved into two well separated spots, one moving with $R_F$=0.15, corresponding to L-serine (eluted and identified by the oxidases technique[8]), and the other not moving from the origin, identified[8] as D-serine. In addition, the mobilities of the racemic amino acids DL-alanine, DL-leucine, DL-isoleucine and DL-phenylalanine were slightly decreased or were unchanged, and there was no resolution of their DL-forms into the two isomers, each moving on the alginated paper as a single spot. Also, the $R_F$ values of L-tyrosine and L-glycine were reduced by half in the control experiment (Table I). These results show that even on alginated paper there was some resolution of some, but not all, amino acid racemates, when the developing medium was *n*-butanol–acetic acid–water (4:1:5).

However, after studies with various constituents and concentrations in the stationary phase and in the developing solvent, we were able to separate the D- and L-forms by resolving the DL-amino acids by a simple paper chromatographic technique, using a mixture of sodium alginate extracted[10] from natural sources (Egyptian fisheries) with silica gel. The addition of silica gel contributed much to the resolving

power of this system, in spite of the fact that it was unsuccessful when used alone. Also, the eluent used contributed partially to the resolution of the racemates; in this eluent, phosphate ions were added in the form of a dibasic sodium salt; Table I indicates the $R_F$ values of the resolved D- and L-components shown in Fig. 1. Under these conditions, the basic amino acids L-arginine and L-lysine did not move, while the acidic amino acid DL-aspartic acid was resolved into two well separated spots, the D-form with $R_F=0.52$ and the L-form with $R_F=0.78$. Also, D-methionine was resolved and separated from the L-form but with different mobilities from those on the alginated paper. DL-Alanine was resolved into the L-form with $R_F=0.58$ and the D-form with $R_F=0.07$.

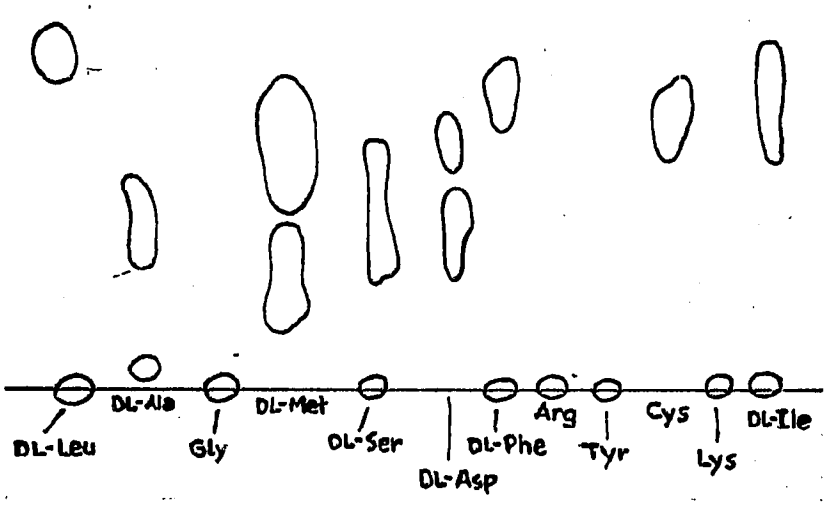


Fig. 1. Alginate–silica gel (2:1) paper chromatography of some DL-amino acids and L-amino acids using as eluent pyridine–water–amyl alcohol–$Na_2HPO_4 \cdot 7H_2O$ (0.5% solution) (7:7:2:6) for 24 h at 18–22°.

On the other hand, the L-forms of hydroxyamino acids, whether aromatic or aliphatic, such as tyrosine and serine, did not move from the origin, while D-serine moved with a relatively high $R_F$ value of 0.55. This was the greatest difference between the mobilities of pairs of L- and D-forms on the alginated paper (Table I), on which L-serine moved with $R_F=0.15$ (for the D-form $R_F=0.00$) and L-tyrosine moved with $R_F=0.35$ (for the D-form $R_F=0.00$).

Also, the D-forms of leucine, isoleucine and phenylalanine did not move from the origin, while their L-forms moved with $R_F$ values of 0.95, 0.70 and 0.85, respectively.

All the resolved L- and D-components were eluted and identified by the specific oxidases[8].

Staining of the separated spots with ninhydrin produced definite coloured spots with no tailing; the colour was stable for over 1 year, while the colour in control experiments faded within a few days.

The resolving power of alginic acid does not seem to depend only on its ion-exchange properties, as indicated by the $R_F$ values obtained for the separated isomers of amino acids, but must also depend on another mechanism; either a steric or a polarity mechanism, or even both, may contribute to the action of alginic acid. This is confirmed by the results obtained in the chromatography of sugars on alginated papers; Fig. 2 shows the separation of some monosaccharides and disaccharides on alginated paper (0.5% sodium alginate). Two effects occurred: (1) a reduction in mobilities compared with the control experiment, and (2) the galactose mobility was reversed and its spot moved downwards below the starting line and not above it.

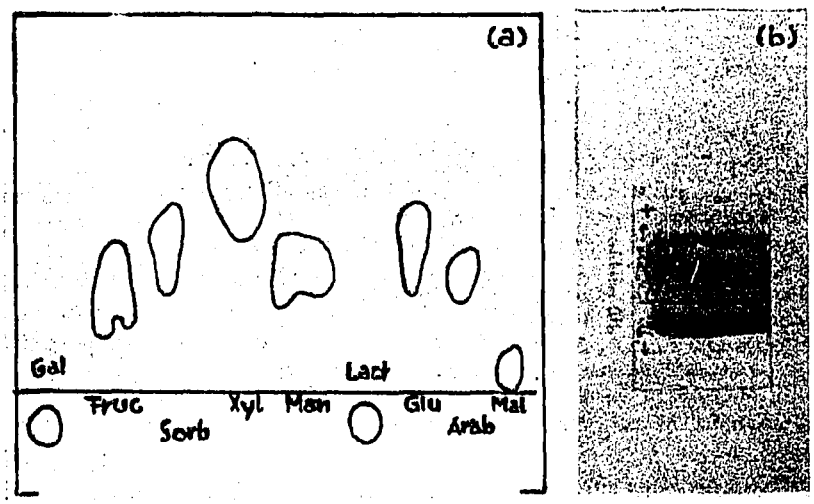


Fig. 2. (a) Alginate paper chromatography (0.5%) of some sugars using *n*-butanol–acetic acid–water (4:1:5) as eluent for 12 h at 18–22°. (b) Alginate paper electrophoresis of human serum at 220 V for 16 h.

The mobility of the disaccharide lactose, which also contains galactose in its molecule, was also reversed. Of the sugars used, galactose is the only one that differs in the position of the hydroxyl group on $C_4$, which may be helpful in explaining the mechanism by which alginic acid was able to resolve the racemic amino acids, in addition to its biological importance.

Alginic acid impregnated in paper used for serum electrophoresis also produced a reversal of the direction of movement of albumin, which moved in the opposite direction from the starting line while all the globulins were separated and moved in the usual direction towards the anode (Fig. 2b). This effect requires further investigation.

## DISCUSSION

The chromatographic characteristics of alginic acid and its exchange mechanism with different eluents give rise to a hydrogen ion concentration gradient along the layer[5]. In resolving and separating the two forms of racemic amino acids, the difference in $R_F$ values depending on whether the amino group is attached to a primary,

secondary or tertiary carbon atom, can be attributed to the difference in the steric hindrance of the side-chains. The present results may be attributed to different degrees of steric hindrance in addition to other factors, which could be based on an interaction between the polar groups in sodium alginate by a mechanism similar to that by which alginic acid forms salts with divalent metal ions[11,12], where the amino acids may also behave as divalent ions, and in addition to a non-ionic interaction mechanism. The elution of amino acids with aqueous organic solvents is affected not only by ion exchange, but also by other factors such as liquid–liquid partition and the changes in the polarity of the eluent in the exchangers. The good resolution of amino acids on alginate suggests that ion exchange is not the only factor that affects the retention.

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