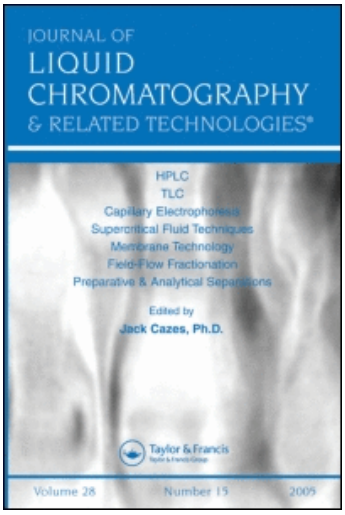

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HIGH EFFICIENCY LIGAND EXCHANGE CHROMATOGRAPHY OF AMINO ACID ENANTIOMERS

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SUMMARY

We obtained resolution of α-amino acids with an efficiency in the range of 10 000-25 000 plates/m and a selectivity factor in the range of 1.12-2.5 with packings easily prepared by adsorption of chiral polymers on a chromatographic silica.

INTRODUCTION

Ligand exchange chromatography appears to be a powerful technique for enantiomer resolution (1-2). The selectivity parameter, α, (ratio of the capacity factors of the two enantiomers) can usually be better than 2 (3-4). However, with chiral packings, the final chromatographic resolution is generally poor : for example, typically 500 plates/m for our packings obtained by single adsorption of a copper complexed chiral polymer on a 5μm silica.

This is not due to poor filling of the column, nor to a slow physical mass transfer to the solute in the stationary phase since,

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for non-complexed species we observed reasonable resolution (for instance about 16 000 plates/m for D_2O).

In such a systems, the ligand in the stationary phase mainly exists in the form of a complex G Cu S according to the equilibrium

$$G\ Cu\ G + S \rightleftharpoons G\ Cu\ S + G \qquad [1]$$

Where S is a solute and G a chiral moiety that is grafted onto a polymer. G and S ensure multidentate complexation towards copper. Consequently, the mean lifetime of the stereocomplex G Cu S is generally large (6) in comparison with the mean residence time of a non-complexed solute molecule when it enters into the stationary phase. This explains the observed poor efficiency and the tailing of the peaks.

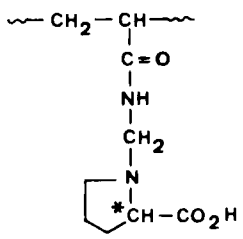
To improve the speed of decomplexation of S, we have previously shown that an increase in temperature was effective when the packing was a gel (5). We tested this approach with silica-supported polymers. But a better way was to introduce into the mobile phase a compound which is a monodentate complexant of copper. We used ammonia, which competes with the solute, reducing the lifetime of G Cu S. But, at the same time, the solute concentration in the stationary phase is diminished. This latter effect is counterbalanced by decreasing the solubility of S in the mobile phase. And finally, we proposed a ternary mixture water/acetonitrile/ammonia.

EXPERIMENTAL

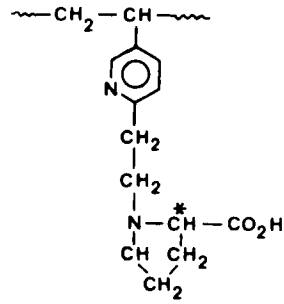
We used classical chromatographic conditions and apparatus which have previously been described (3,4). However as there is a risk of silica dissolution we added a precolumn, filled with pure silica.

As stationary phases, we used linear polymers based on acrylamide (A) and a copolymer of acrylamide and of a vinylpyridine derivative (B) (4,5,8).

In type A polymer, the L-proline capacity was 0.86meq/g (11% of original units of the polyacrylamide were grafted by L-proline). For type B, the chiral units were 48% in the copolymer.



(A)



(B)

The packings were not systematically saturated with copper ; we studied the influence of the parameter r (r = number of copper atoms/number of L-proline moities). The composition of the mobile phase is adjusted by mixing the appropriate volumes of deionized water (Millipore "Milli-Q" system), acetonitrile (analytical grade) and ammonia (analytical grade). The elution of about 20-50ml of eluent is necessary to equilibrate the column. Under normal conditions, practically no copper is eluted ; however, a little part of the copper is complexed by solutes (complexes S Cu S) and can be detected by U.V. spectroscopy (254nm). If the rate in acetonitrile in the eluent is large enough (> 50%) the system is very stable, even for an eluent containing up to 0.4mole/l of ammonia.

INFLUENCE OF TEMPERATURE

By using a polyacrylamide based gel as packing we saw that column efficiency strongly increases with temperature (5), probably in connection with the improvement of the segment mobility inside the gel. Figure 1 shows that this result cannot be extended

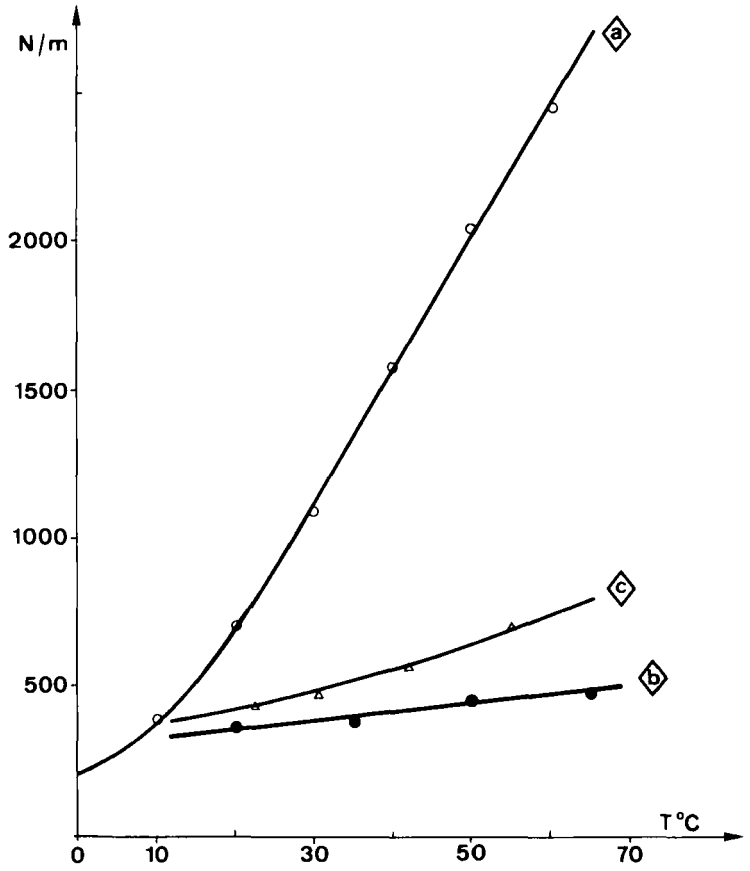


Figure 1 : Influence of temperature on the efficiency (N/m in plates/metre) of various packings.

a) Polyacrylamide gel (Ø = 10µm) grafted by L-proline (5)

b) Polymer A adsorbed on silica (partisil 5µm)

c) Polymer B adsorbed on silica (partisil 5µm)

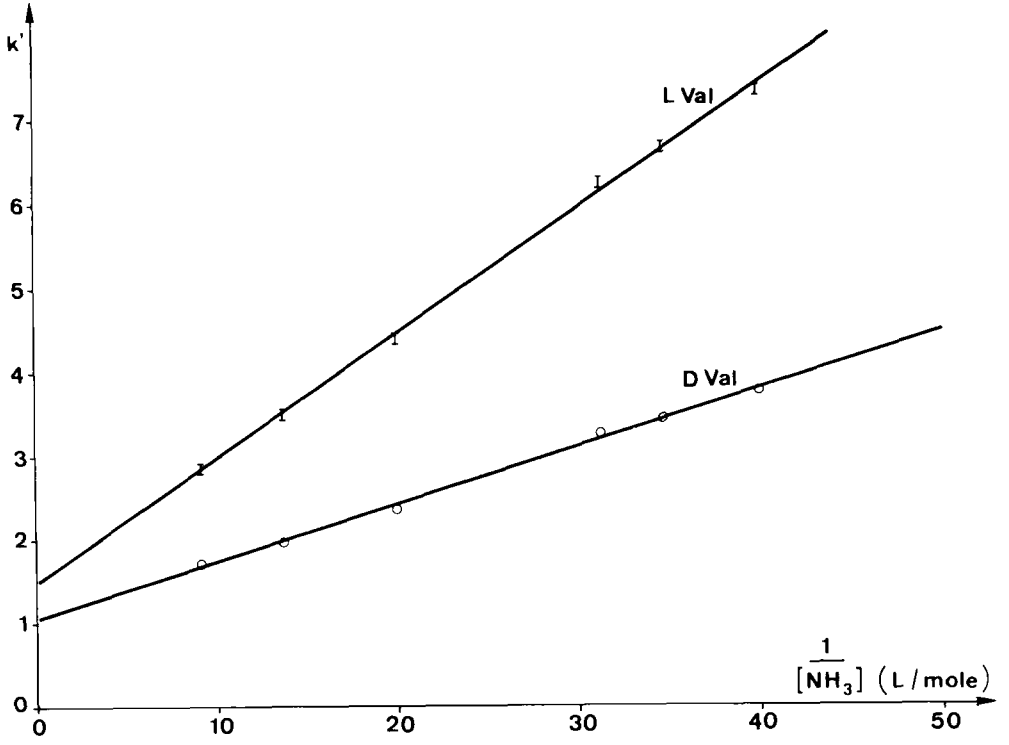


Figure 2 : Separation of D,L-valine. Variation of capacity factors versus the inverse of ammonia concentration.

- Polymer A adsorbed on silica (partisil 5µm) 16% W/W, r = 0.4.
- Eluent water/acetonitrile (47/53) with ammonium chloride (0.002M). Room temperature.
- Column 15cm long, 0.46cm I.D.

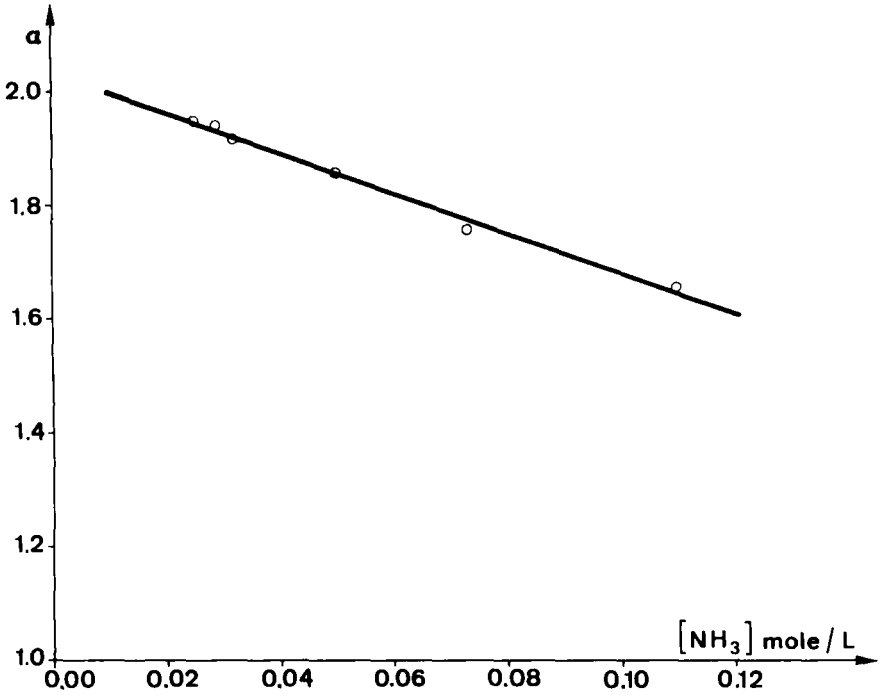


Figure 3 : Separation of D,L-valine. Evolution of the selectivity factor versus the ammonia concentration. Other chromatographic conditions : see Fig.2.

to supported phases. Even when the chemical structure of the linear polymer is very close to the one of the gel (a and b Fig.1), the evolution of N/m is very different. The adsorption of linear polymers hinders movements of the chains and the efficiency stays low even at elevated temperatures.

TERNARY ELUENTS - INFLUENCE OF AMMONIA CONCENTRATION

For a given ration H_2O/CH_3CN = 47/53 (vol/vol) we studied, as a test, the resolution of D,L-valine for various levels of ammonia concentration.

k' obeys a law in the form $k' = a + b/[NH_3]$ (Fig.2). α linearly decreases with $[NH_3]$ (Fig.3) but this diminution is balanced by the strong increase in the number of theoretical plates and, finally, the chromatographic efficiency increases linearly with $[NH_3]$ (Fig.4). However, when the ammonia concentration was increased, the chromatographic peaks became stepped back and ghost peaks appeared. Such drawbacks can be avoided by addition of electrolytes (NH_4Cl 0.01 to 0.7M) to be eluent, but this method is no longer effective if $[NH_3] > 0.4N$. We performed experiments with $0.04 < [NH_3] < 0.4N$. The deformation of the peaks is related to the electrostatic repulsion between the solutes (amino acids are in an anionic form in basic media) and the negative charges of the packing surface (prolinate and silicate groups). The effect is less marked for the polymer based upon vinylpyridine than for those based on acyrlamide, which agrees with the pK_1 value of the subsituted L-proline (respectively 10.0 and 8.5). The addition of salt screems these repulsions and leads to the solute penetrating all the pores.

INFLUENCE OF THE ACETONITRILE RATIO

Figure 5 shows the rapid increase of k' with the acetonitrile ratio in the eluent for a given ammonia concentration. At the same time, the selectivity practically does not evolve which confirms the permanence of ligand exchange as the mechanism in the setereoselection.

The capacity factor is proportional to the partition coefficient of the solute in its free form (not complexed) between the stationary phase and the mobile phase (7). Consequently, the more important the hydrophilic behaviour of the solute is, the larger is the increase of k'.

INFLUENCE OF THE COPPER CONTENT

With an aqueous eluent we showed that, for polyacrylamide based polymer (type A), k' obeys a law in the form $k' = r/(1-2r)$ which is

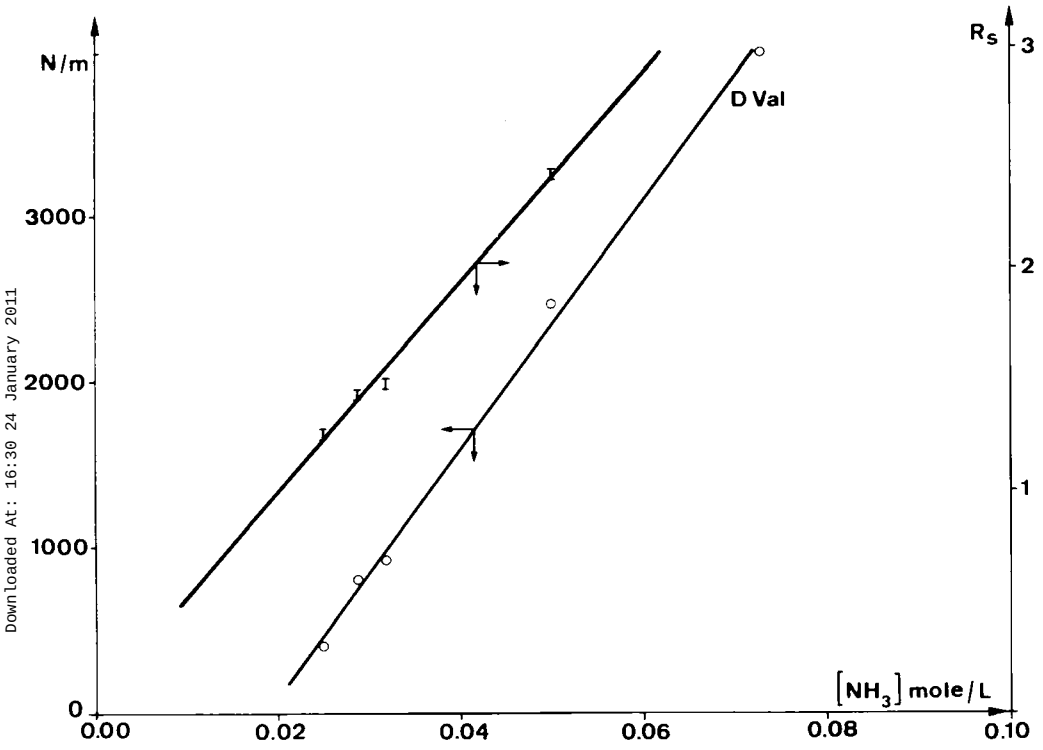


Figure 4 : Separation of D,L-valine. Variation of the efficiency (N/m) and the resolution factor (R) versus the ammonia concentration. Other chromatographic conditions : see Fig.2.

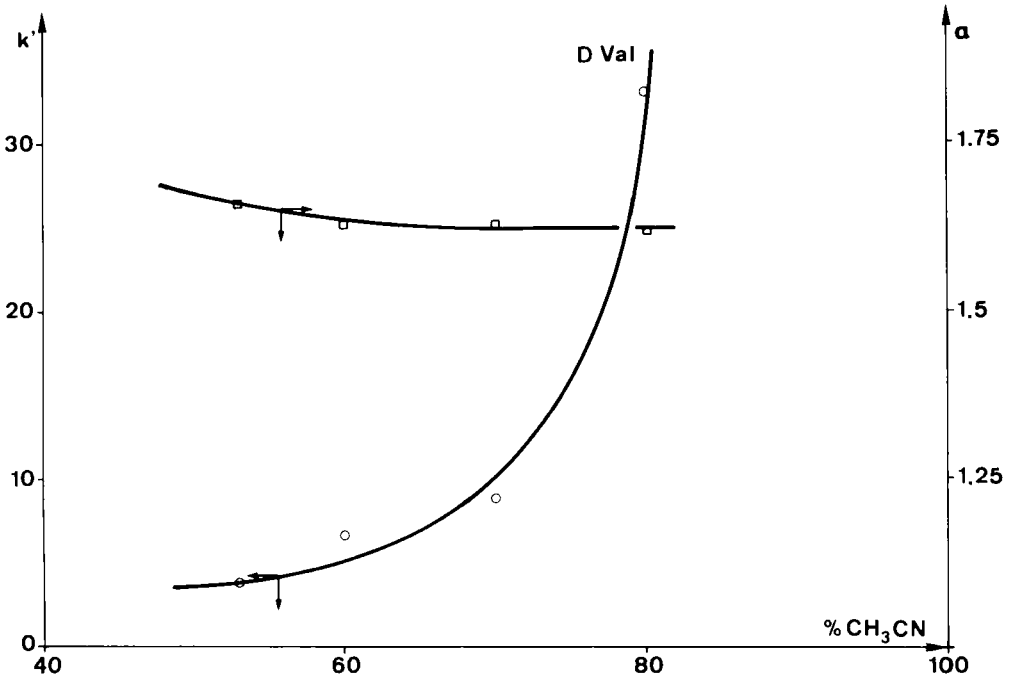


Figure 5 : Separation of D,L-valine. Variation of the selectivity (α) and the capacity factor (k' for D valine) versus the acetonitrile content. $[NH_3]$ = 0.072N, other chromatographic conditions : see Fig.2.

justified from the equilibrium [1] (6). With the ternary eluent, k' varies linearly with r (Fig.6). This suggests the disappearance of the G Cu G type of complexes but supports the idea of the formation of G Cu $(NH_3)_n$ species. They are now the active centers for the stereoselection. This is in accordance with the decrease of k' when the ammonia concentration increases (Fig.2), while α varies in the inverse way (Fig.3).

EXAMPLES OF SEPARATION

The best chromatographic conditions are obtained for the highest values of ammonia concentration compatible with the absence of electrostatic gel repulsion effects.

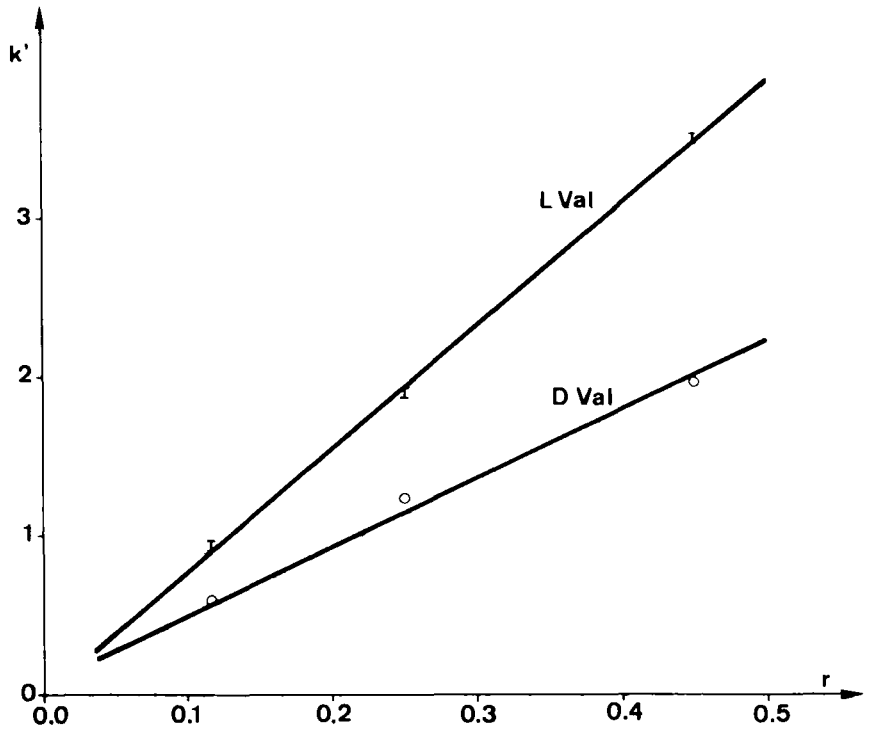


Figure 6 : Separation of D,L-valine. Evolution of the selectivity factor (k') versus the copper content r. $[NH_3]$ = 0.072N, other chromatographic conditions : see Fig.2.

Table 1 : Retention data and efficiency of the packing.

Solutes	k'_2	α	N_2/m
Alanine	13.9	1.12	13 300
Valine	6.66	1.30	10 600
Leucine	6.12	1.29	14 000
Isoleucine	6.58	1.16	14 000
Proline	25.5	1/2.4	13 200
Pipecolic acid	4.11	1.27	25 000
Phenylalanine	2.61	1.39	10 000
Tryptophan	7.11	1.49	13 300

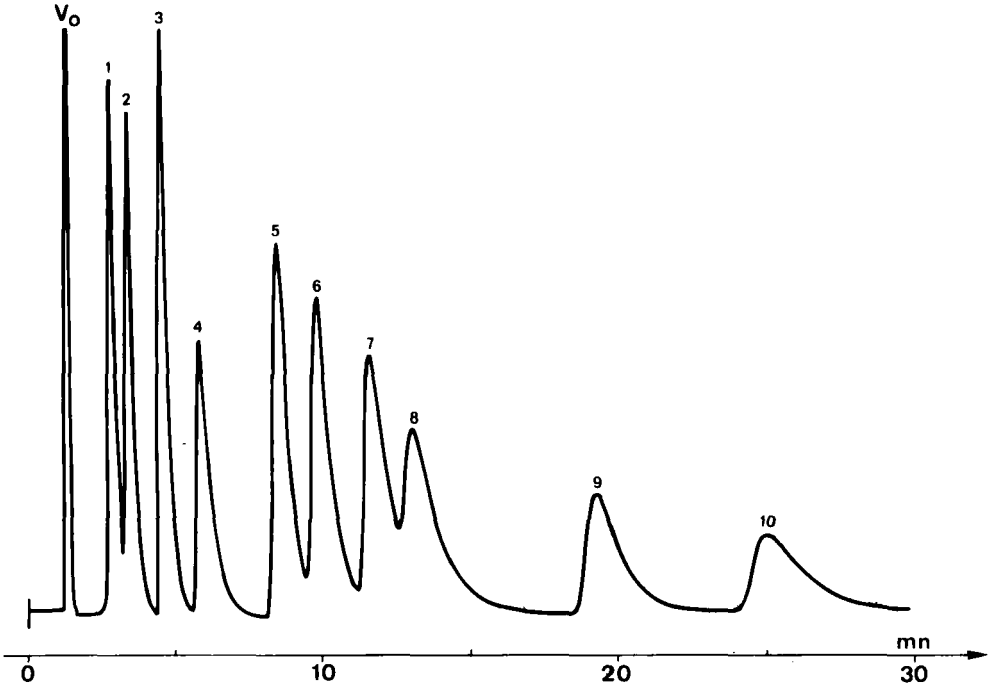


Figure 7 : Resolution of 5 amino acids.

- Support : Polymer A adsorbed on silica (partisil 5µm) 16% (W/W), r = 0.4.
- Column : 15cm long ; 0.46 cm I.D.
- Mobile phase : H_2O/CH_3CN 30/70 ; $[NH_3]$ = 0.11N ; $[NH_4Cl]$ = 0.02N 1,5ml/ml. 25°C.
- Solutes : 1. D-phe.gly ; 2. L-phe.gly ; 3. L-pip.acid ; 4. D-pip.acid ; 5. D-val ; 6. D-nor.val ; 7. L-nor.val ; 8. L-val ; 9. D-ser ; 10. L-ser.

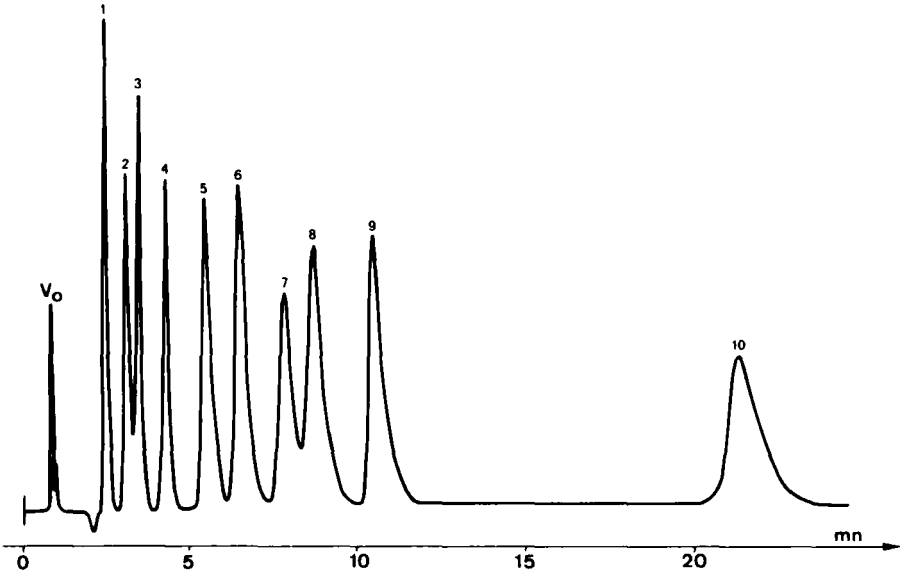


Figure 8 : Resolution of 5 amino acids.

- Support : Polymer B adsorbed on silica (partisil 5µm) ≈ 10% (W/W) ; r = 0.8
- Column : 15cm long ; 0.46 cm I.D.
- Mobile phase : H_2O/CH_3CN 30/70 ; $[NH_3]$ = 0.4N ; $[NH_4Cl]$ = 0.002N 2ml/mn ; 25°C.
- Solutes : 1. D-phe.ala ; 2. L-phe.ala ; 3. L-pip.acid : 4. D-pip.acid ; 5. D-trp ; 6. D-tyr ; 7. L-trp ; 8. L-tyr ; 9. L-pro ; 10. D-pro.

As expected (Fig.7 and 8) the system is powerful. Table I gives the selectivity and the efficiency in the separation of some amino acids (calculated from the most retained isomer).

Theses results were obtained with a type B packing (x = 0.48 ; r = 0.8), H_2O/CH_3CN 30/70, Ammonia 0.4N, NH_4Cl 0.002N.

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