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THIN-LAYER CHROMATOGRAPHY OF DIAMINES, DICARBOXYLIC ACIDS AND  $\omega$ -AMINO ACIDS

## APPLICATION TO THE ANALYSIS OF COPOLYAMIDES

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SUMMARY

A method, which involves thin-layer chromatography and densitometry, was developed for the qualitative and quantitative analysis of the diamine dihydrochlorides, dicarboxylic acids, and  $\omega$ -amino acid hydrochlorides recovered from acid hydrolysed copolyamides prepared from diamine-diacid and/or  $\omega$ -amino acids (or their lactams). Two different solvent systems consisting of phenol-*n*-butanol-formic acid-water and phenol-formic acid-water were used. The qualitative analysis of the three homologous series was attained on the same chromatogram. For quantitative determination the method has an average relative error in accuracy of  $\pm 5\%$ .

A linear relationship between the  $R'_M$  values [ $R'_M = \log(1/1.1 R_F - 1)$ ] and the solute carbon numbers was observed. The deviation from linearity for the longer chain compounds might be caused by a gradient effect. The linearity between  $R'_M$  values and the number of carbon atoms depended upon the experimental conditions.

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## INTRODUCTION

Several techniques including gas chromatography, DTA, and paper chromatography have been reported for the analysis of copolyamides; however, they are either time consuming or only give quantitative or qualitative information, but not both. For example, gas chromatography<sup>1</sup> is an excellent technique for the quantitative analysis of copolyamides, but  $\omega$ -amino acids which are the hydrolysis products of  $\omega$ -amino acid type copolyamides cannot be resolved, and further treatment of the acid hydrolysate (esterification, neutralisation, etc.) is required.

The excellent resolving power, rapidity and simplicity of TLC makes it attractive for the analysis of copolyamides. Copolyamides are hydrolysed with hydrochloric acid, and diamine dihydrochlorides, dicarboxylic acids and  $\omega$ -amino carboxylic acid hydrochlorides are liberated according to the composition of the starting material.

Thin-layer chromatography methods have previously been described for the analysis of dicarboxylic acids by BRAUN AND GEENEN<sup>2</sup> who separated the dicarboxylic acids from oxalic to sebacic acid on silica gel plates using an alcohol-water-ammonia (100:12:16) solvent system. PETROWITZ AND PASTUSKA<sup>3</sup> used two different solvent systems, *viz.* benzene-methanol-acetic acid (45:8:4) and benzene-dioxane-acetic acid (90:25:4) and observed that the  $R_F$  values and the number of methylene groups had a curved relationship. KNAPPE AND PETERI<sup>4</sup> also used TLC for the separation of dicarboxylic acids with several solvent mixtures. No thin-layer chromatographic resolution of a homologous series of aliphatic diamines (or diamine dihydrochlorides) and  $\omega$ -amino acids (or their hydrochlorides) has been described previously.

Only the qualitative analysis of some polyamides (6, 66, 610, 11, 12, 6/66, and 6/66/DCHM) by TLC has been reported<sup>20</sup> where four solvent systems were used: benzene-methanol-acetic acid (90:16:8); benzene-dioxane-acetic acid (90:25:4); *n*-butanol-acetic acid-water (80:20:20); and chloroform-methanol-25% ammonia-water (45:45:7:3).

The quantitative determination of each spot separated on the thin-layer chromatogram can be carried out by either direct densitometry<sup>5,6</sup> on the plate or spectrophotometry<sup>6,7</sup> of a solution of the elute extracted from the spot. The first method needs special apparatus and the second is complicated.

The method described in this paper consists of the thin-layer chromatographic resolution of the polymer hydrolysates which are diamine dihydrochlorides, dicarboxylic acids and  $\omega$ -amino acid hydrochlorides, and their quantitative determination by densitometry on the thin-layer film scraped from the plate. The relation of the  $R_F$  values (or the derived values) and the number of carbon atoms for the homologous series was also reported here.

## EXPERIMENTAL

### *Standards*

All diamine dihydrochlorides, dicarboxylic acids, and  $\omega$ -amino alkanic acid hydrochlorides used in the experiment were commercial products of reagent grade or the corresponding polyamide hydrolysates. These were dissolved in 95% ethanol to give a 1% concentration.

### *Acid hydrolysis*

About 50 mg of the copolyamide or polyamide sample were weighed into a glass tube, and 5 ml of 6 *N* HCl were added. The tube was then sealed and placed in an oven at 130° for 24 h. After cooling, the seal was broken and the hydrolysate was evaporated on a steam bath and dried *in vacuo*. The dried hydrolysate was dissolved in 95% ethanol, with or without the internal standards, to give a 1% concentration.

### *Thin-layer chromatography*

Thin-layer plates (250 micron thick) were prepared according to the usual method, using "Wakogel" B-5 (silica gel for TLC, which contains about 5% calcium sulphate) on 20 × 20 cm glass plates. The plates were activated at 120° for 1 h and stored over silica gel desiccant until used. The TLC plates were marked off in channels

5–10 mm in width every 20–25 mm to prevent the spots from spreading<sup>6</sup> before development.

For qualitative analysis about 5  $\mu$ l of the solution were applied for each spot. After spotting the thin-layer plates were chromatographed until the solvent front ascended 10 cm from the spotting point. System (A) phenol-*n*-butanol-formic acid-water (5:2:1:2), and system (B) phenol-formic acid-water (74:1:25) were employed.

After chromatography the plates were sprayed with a 0.2% solution of ninhydrin in ethanol and heated at 90° for 15 min. After positioning the diamine dihydrochlorides and  $\omega$ -amino acid hydrochlorides, the plates were sprayed again with a solution of BCG for detection of dicarboxylic acids. Duplicate runs were made for all  $R_F$  values recorded in this study.  $R_F$  value deviations were within  $\pm 0.02$ .

### Densitometry

Densitometry was used for the quantitative analysis. After checking the components of diamine dihydrochlorides and  $\omega$ -amino acid hydrochlorides in the copolyamide, the appropriate internal standards of homologues (*e.g.*, for copolyamide 6/66: hexamethylenediamine dihydrochloride and  $\epsilon$ -amino caproic acid hydrochloride were the hydrolysis products, and octamethylenediamine dihydrochloride and  $\gamma$ -amino-*n*-butyric acid hydrochloride were selected as standards) were blended with the copolyamide hydrolysate in ethanol and TLC was performed with system A or B.

The dried plate was sprayed with 0.2% ninhydrin solution in ethanol and heated at 90° for 15 min. In order to ensure accuracy and reproducibility in this method, the spraying procedure must be standardised by using the same volume of spray for each plate, spraying evenly and thoroughly. The plate was then sprayed with a 5% solution of polyvinyl chloride in tetrahydrofuran and dried under an infrared light. This silica gel layer reinforced with polyvinyl chloride film was scraped from the plate and cut into long, narrow strips of 2 cm width along the channel in the direction of the solvent flow. The strips were suspended in a vessel saturated with water vapour at 50° for 15 min. Densitometric scanning was started after this conditioning. A Shimadzu spectrophotometer, Model QR-50, equipped with paper chromatographic stage<sup>8</sup> was used at a wavelength of 560 m $\mu$ . The absorbance of each spot was read at intervals of 1 mm of the strip and plotted as absorbance *vs.* the distance from the starting point. The standard solutions were prepared by dissolving 1–10 mg of the diamine dihydrochloride and/or the  $\omega$ -amino acid hydrochloride analysed and 5 mg of the internal standards of them in 10 ml of ethanol. A calibration graph was constructed by plotting the ratio of the peak areas *vs.* the ratio of the amount of the component and the internal standard. For analysis of copolyamide 10 mg of the acid hydrolysate and 5 mg of the internal standards were dissolved in 10 ml of ethanol. About 5  $\mu$ l of each solution were spotted on the plate.

## RESULTS AND DISCUSSION

### Qualitative analysis

Representative  $R_F$  values and  $R_S$  values of standard diamine dihydrochlorides, dicarboxylic acids and  $\omega$ -amino acid hydrochlorides are shown in Tables I and II. The identification of the components in the copolyamide should be possible from the  $R_F$  values, or by the thin-layer chromatography of the acid hydrolysate together

TABLE I

TLC  $R_F$  VALUES OF DIAMINE DIHYDROCHLORIDES, DICARBOXYLIC ACIDS AND  $\omega$ -AMINO ACID HYDROCHLORIDES

Diamine dihydrochlorides: ethylene-; trimethylene-; pentamethylene-; hexamethylene-; heptamethylene-; octamethylene-; nonamethylene-; decamethylene-; dodecamethylene-; *o*-phenylene-; *m*-phenylene-; and *p*-phenylene diamine dihydrochlorides. Dicarboxylic acids: oxalic-; malonic-; succinic-; glutaric-; adipic-; pimelic-; suberic-; azelaic-; sebacic-; dodecadionic-; *o*-phthalic-; isophthalic-; and terephthalic acids.  $\omega$ -Amino acid hydrochlorides: glycine;  $\beta$ -alanine;  $\gamma$ -amino-*n*-butyric-;  $\delta$ -amino-*n*-valeric-;  $\epsilon$ -amino-*n*-caproic-;  $\eta$ -amino-*n*-caprylic-;  $\kappa$ -amino-*n*-undecanoic-;  $\lambda$ -amino-*n*-lauric-; *o*-amino-benzoic-; and *p*-amino-benzoic acid hydrochlorides.

Compound/ carbon number	System A			System B		
	Amine	Acid	Amino acid	Amine	Acid	Amino acid
<i>Aliphatics</i>						
C <sub>2</sub>	0.38	0.29	0.18	0.21	0.45	0.40
C <sub>3</sub>	0.44	0.44	0.26	0.26	0.50	0.45
C <sub>4</sub>	0.50	0.55	0.35	0.31	0.59	0.52
C <sub>5</sub>	0.56	0.67	0.45	0.37	0.64	0.57
C <sub>6</sub>	0.62	0.74	0.53	0.41	0.69	0.62
C <sub>7</sub>	0.66	0.81	—	0.47	0.74	—
C <sub>8</sub>	0.70	0.84	0.67	0.55	0.77	0.71
C <sub>9</sub>	0.74	0.86	—	0.63	0.83	—
C <sub>10</sub>	0.77	0.88	—	0.74	0.88	—
C <sub>11</sub>	—	—	0.80	—	—	0.81
C <sub>12</sub>	0.80	0.91	0.82	0.86	0.92	0.84
<i>Aromatics</i>						
<i>o</i> -	0.78	0.60	0.70	0.94	0.56	0.70
<i>m</i> -	0.60	0.78	—	0.95	0.68	—
<i>p</i> -	0.45	0	0.65	0.87	0	0.73

TABLE II

TLC  $R_S$  VALUES OF DIAMINE DIHYDROCHLORIDES, DICARBOXYLIC ACIDS AND  $\omega$ -AMINO ACID HYDROCHLORIDES

Only aliphatic compounds tested.

Compound/ carbon number	System A			System B		
	Amine	Acid	Amino acid	Amine	Acid	Amino acid
C <sub>2</sub>	0.61	0.39	0.34	0.51	0.65	0.65
C <sub>3</sub>	0.71	0.59	0.49	0.63	0.72	0.73
C <sub>4</sub>	0.81	0.74	0.66	0.76	0.85	0.84
C <sub>5</sub>	0.90	0.91	0.85	0.90	0.93	0.92
C <sub>6</sub>	1.00	1.00	1.00	1.00	1.00	1.00
C <sub>7</sub>	1.07	1.09	—	1.15	1.07	—
C <sub>8</sub>	1.13	1.14	1.26	1.34	1.12	1.15
C <sub>9</sub>	1.19	1.16	—	1.54	1.20	—
C <sub>10</sub>	1.24	1.19	—	1.80	1.28	—
C <sub>11</sub>	—	—	1.51	—	—	1.31
C <sub>12</sub>	1.29	1.23	1.55	2.10	1.33	1.36

with the internal standards on one plate simultaneously. If two spots overlapped in one solvent system, another system was used for complete separation. The  $R_F$  values, however, are bound to deviate to some extent under uncontrolled conditions<sup>9</sup>. Provided that serious mistakes have been avoided the  $R_F$  values could vary regularly<sup>10</sup>, consequently the  $R_S$  values (*i.e.* the ratio of the  $R_F$  values of the sample and the internal standard) are used to eliminate the deviation of the  $R_F$  values.

#### Relationship of the $R_F$ values to the number of carbon atoms

A theoretical consideration of linear dependence of  $R_F$  values on the number of solute carbon atoms has been given by SNYDER<sup>11</sup>. The  $R'_M$  function ( $R'_M = \log \frac{1}{1-R_F} - 1$ ) was defined for  $R_F$  values in systems where solvent gradients exist by analogy with the previously used  $R_M$  function, defined as  $\log (\frac{1}{R_F} - 1)$ .

A useful general relationship for the prediction and correlation of  $R_F$  in TLC was defined as"

$$R'_M = \log (VaWa/Vs) + \alpha (S^\circ - \varepsilon^\circ As)$$

The parameter  $\alpha$  is the adsorbent activity function, a dimensionless quantity that is proportional to adsorbent surface energy. The parameter  $\varepsilon^\circ$  is the eluent strength of the solvent, equal to the adsorption energy of the solvent per unit area of adsorbent surface. For a particular adsorbent (constant  $\alpha$ ) and solvent (constant  $\varepsilon^\circ$ ), a linear relationship of  $R'_M$  to the number of solute carbon atoms can be predicted. This prediction is demonstrated in Fig. 1 for the systems A and B calculating  $R'_M$  values from the  $R_F$  values in Table I. Somewhat large deviations from the linearity of the  $R'_M$  plot are to be noted for the longer chain compounds and it is easily assumed that the deviations beyond experimental error are caused by a gradient effect.

Other relations have been cited by several authors. HROMATKA AND AVE<sup>12</sup>

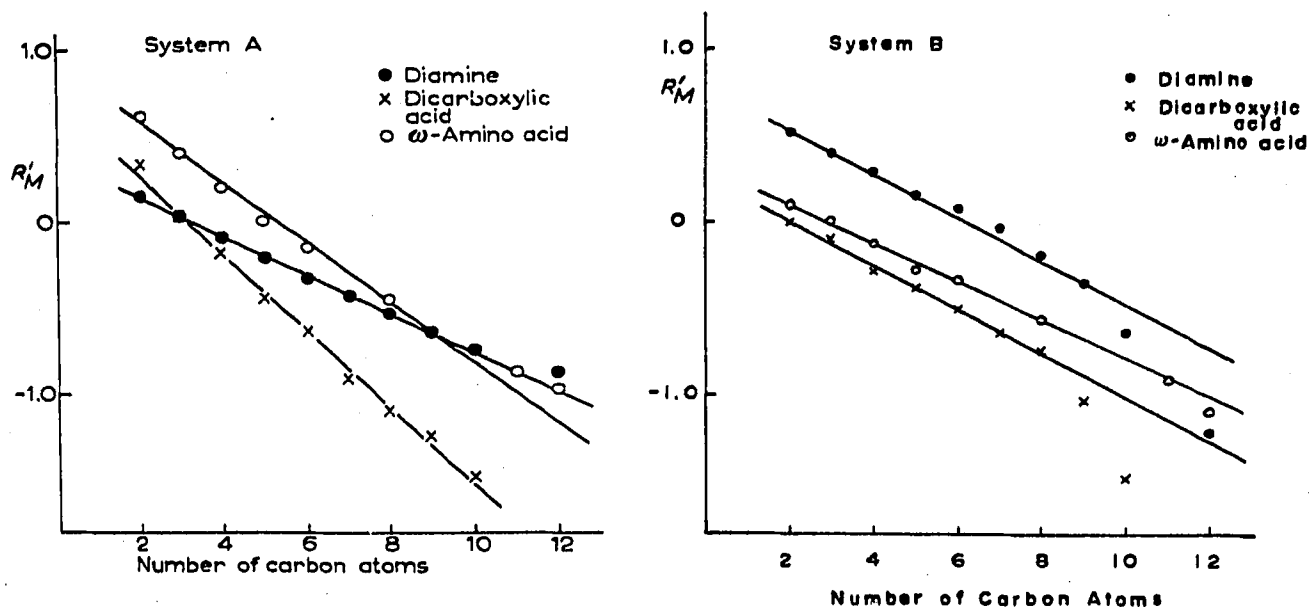


Fig. 1. Linear relationship between  $R'_M$  values and the number of solute carbon atoms.

TABLE III

LINEAR RELATIONS HIPS BETWEEN  $R_F$  VALUE AND CARBON NUMBER IN THIN-LAYER CHROMATOGRAPHY OF ALIPHATIC DIAMINE DIHYDROCHLORIDES, DICARBOXYLIC ACIDS AND  $\omega$ -AMINO ACID HYDROCHLORIDES

$$R_M = \log(1/R_F - 1); R'_M = \log(1/1.1 R_F - 1).$$

Function	Diamine $\cdot 2HCl$		Dicarboxylic acid		$\omega$ -Amino acid $\cdot HCl$	
	A	B	A	B	A	B
$\log R_F$ -C.N. <sup>a</sup>	Curve <sup>b</sup> (C <sub>2</sub> -C <sub>12</sub> )	C <sub>2</sub> -C <sub>5</sub> <sup>c</sup> , C <sub>4</sub> -C <sub>10</sub>	Curve (C <sub>2</sub> -C <sub>12</sub> )	C <sub>2</sub> -C <sub>5</sub> , C <sub>4</sub> -C <sub>10</sub>	Curve (C <sub>2</sub> -C <sub>12</sub> )	C <sub>2</sub> -C <sub>5</sub> , C <sub>4</sub> -C <sub>8</sub>
$R_F$ -log C.N.	C <sub>3</sub> -C <sub>10</sub> <sup>d</sup>	Curve (C <sub>2</sub> -C <sub>12</sub> )	C <sub>3</sub> -C <sub>7</sub> , C <sub>7</sub> -C <sub>12</sub>	C <sub>3</sub> -C <sub>8</sub>	C <sub>4</sub> -C <sub>8</sub>	C <sub>3</sub> -C <sub>6</sub> , C <sub>5</sub> -C <sub>12</sub>
$R_F$ -C.N.	C <sub>2</sub> -C <sub>6</sub> , C <sub>6</sub> -C <sub>10</sub>	C <sub>2</sub> -C <sub>6</sub>	C <sub>8</sub> -C <sub>12</sub>	C <sub>4</sub> -C <sub>10</sub>	C <sub>2</sub> -C <sub>8</sub>	C <sub>4</sub> -C <sub>8</sub> , C <sub>8</sub> -C <sub>12</sub>
$R_F$ -log (C.N. of methylene group)	—	—	C <sub>4</sub> -C <sub>7</sub> , C <sub>7</sub> -C <sub>12</sub>	C <sub>3</sub> -C <sub>6</sub>	C <sub>4</sub> -C <sub>11</sub>	C <sub>3</sub> -C <sub>6</sub>
$R_M$ -C.N.	C <sub>2</sub> -C <sub>6</sub> , C <sub>5</sub> -C <sub>9</sub>	C <sub>2</sub> -C <sub>5</sub> , C <sub>3</sub> -C <sub>9</sub>	C <sub>3</sub> -C <sub>7</sub>	C <sub>2</sub> -C <sub>6</sub>	C <sub>2</sub> -C <sub>5</sub>	C <sub>2</sub> -C <sub>6</sub> , C <sub>5</sub> -C <sub>12</sub>
$R'_M$ -C.N.	C <sub>2</sub> -C <sub>10</sub>	C <sub>2</sub> -C <sub>9</sub>	C <sub>2</sub> -C <sub>10</sub>	C <sub>2</sub> -C <sub>8</sub>	C <sub>2</sub> -C <sub>8</sub>	C <sub>2</sub> -C <sub>11</sub>

<sup>a</sup> C.N. refers to the number of carbon atoms.

<sup>b</sup> There is a curved relation between C<sub>2</sub> and C<sub>12</sub>.

<sup>c</sup> There are two linear relationships; the first between C<sub>2</sub> and C<sub>5</sub>, and the second between C<sub>4</sub> and C<sub>10</sub>.

<sup>d</sup> There is a linear relationship between C<sub>3</sub> and C<sub>10</sub>.

TABLE IV

## COPOLYAMIDE ANALYSIS

## Accuracy

Synthetic mixture	Concentration (mg)		Rel. error (%)
	Added	Found	
Nylon 6	10.0	11.1	+ 11.0
Nylon 66	40.0	41.0	+ 2.5
Nylon 6	20.0	20.8	+ 4.0
Nylon 66	30.0	29.0	- 3.3
Nylon 6	30.0	28.5	- 5.0
Nylon 66	20.0	20.6	+ 3.0

## Precision

% 66 in the synthetic mixture of Nylon 6 and Nylon 66  
(50/50, w/w)

a.	51.5
b.	47.0
c.	45.4
d.	50.6
e.	48.0
Av.	48.5
S.D.	2.3

observed a linear relationship between the log of the  $R_F$  value and the number of carbon atoms of saturated  $C_3$ - $C_{11}$  aliphatic fatty acids for even numbers and odd numbers, separately, in an ether solvent. SINGH AND GERSHBEIN<sup>13</sup> reported a linear dependence between the  $R_F$  values and even numbers of carbon atoms of saturated  $C_6$ - $C_{18}$  aliphatic fatty acids in *n*-butanol. An  $R_M$  study of the homologous series of aliphatic  $\alpha$ -amino acids was reported by TRZASKA AND KOWKABANY<sup>14</sup>, and they pointed out that the deviations from linearity of the  $R_M$  plot for the higher amino acids might be due to adsorption effects competing with the liquid-liquid distribution process.

Several functions between  $R_F$  values and carbon numbers for the diamine dihydrochlorides, dicarboxylic acids and  $\omega$ -amino acid hydrochlorides studied in this paper are listed in Table III. The best linear relationship was that between the  $R'_M$  values and the carbon number. The results for the  $R_M$  study in Table III showed two straight-line relationships or the deviation from the linear dependence between  $R_M$  values and the number of carbon atoms or its logarithm. These results might be due to the difference of distribution coefficient<sup>15</sup> or chemical potential<sup>16</sup> or adsorption effects<sup>14</sup> affected by the gradient effect of the solvent mixtures (mobile phase).

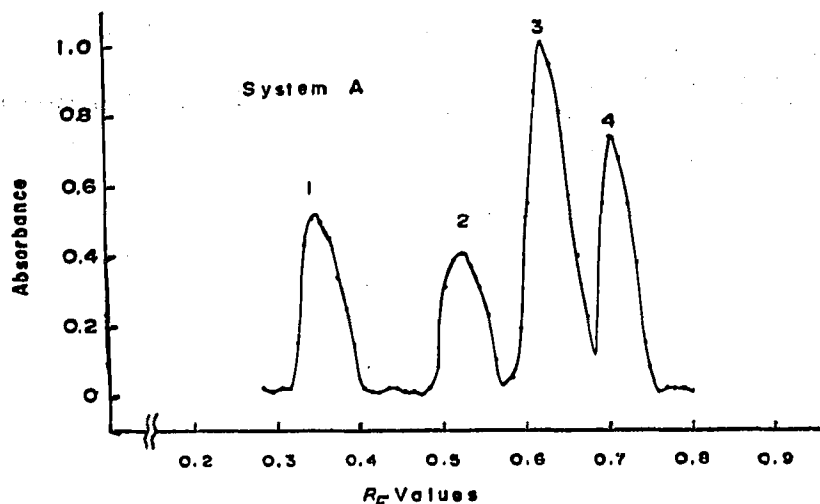


Fig. 2. Densitometric profile of diamines and  $\omega$ -amino acids. 1 =  $\gamma$ -amino-*n*-butyric acid HCl; 2 =  $\epsilon$ -amino-*n*-caproic acid HCl; 3 = hexamethylene diamine 2HCl; 4 = octamethylene diamine 2HCl.

With the exception of the  $R'_M$  function it could not be predicted whether there was a linear relationship; it depended upon the solvent, the solutes and other experimental conditions.

#### Quantitative analysis

A typical densitometric profile of the diamine dihydrochlorides and  $\omega$ -amino acid hydrochlorides is shown in Fig. 2. For quantitative analysis of copolyamide 6/66, the peak areas of hexamethylenediamine dihydrochloride and  $\epsilon$ -aminocaproic acid hydrochloride in densitometric units were ratioed with the internal standards, and calibration curves were prepared. A similar treatment was carried out for other types of copolyamide.

The data presented in Table IV indicate that the method had a maximum relative error of about 11%, and a standard deviation  $s$  of  $\pm 2.3\%$  for the analysis of Nylon 66 in the 50% copolymer range.

The reaction between the amino group in an amino acid and ninhydrin in paper chromatography is not quantitative and the stability of the coloured spots on the chromatograms is affected by the humidity in atmosphere<sup>17</sup>, the temperature and the time of heating<sup>18</sup>, and the pH of the solute solution<sup>17</sup>. The coloured spots have been stabilised by steam conditioning<sup>19</sup>. The stabilisation of the coloured spots in our experiment was attained by the uniform heating at 90° for 15 min and the water vapour conditioning.

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