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Thin-layer chromatography of amino acids with agar–agar as binder

The chromatography of amino acids and dinitrophenylamino acids on thin-layer chromatographic (TLC) plates has been well studied in the past[1]. In a paper by MAINI[2], published in 1969, it was suggested that agar–agar could be used as a binder to give improved compactness and mechanical resistance to silica gel in thin layers. This has been tried successfully in this laboratory in the past. The best results were obtained with the following procedure and materials.

Amino acids

Silica Gel H suspended in a solution of agar–agar was used to prepare the thin layers on 20 × 20 cm glass plates with Shandon TLC equipment. The solution was prepared by dissolving 3.34 g of powdered agar–agar (Merck) in 1 l of distilled water, heating the solution in a boiling water-bath for about half an hour and finally filtering it through a Büchner funnel. This solution can be stored in a refrigerator for a long period. A 20-g amount of Silica Gel H was weighed into an erlenmeyer flask for each group of five TLC plates. The hot agar-agar solution (65 ml) was poured in and the flask was stoppered and vigorously agitated by hand for about 10 sec. The suspension was then poured into the spreader, which was immediately run down over the plates to give a 0.25-mm thick layer. After about 5 min the plates were taken out of the apparatus and allowed to dry for 24 h in a horizontal position. It has been found that it is not necessary to warm the plates before spreading, as MAINI[2] suggests, nor is it convenient to use Silica Gel G, which already has calcium sulphate mixed with it as binder.

A two-dimensional chromatogram was run twice with the system phenol–water (75:25, w/w; 20 mg of potassium cyanide were added for every 100 g of solvent) in the first direction and twice with *n*-butanol–acetic acid–water (4:1:1, v/v) in the second direction, to about 4 cm from the top of the plates[1]. After each run with the phenolic solvent, the plates were dried in a Baird and Tatlock chromatographic drying oven at 110° for 1 h. After each of the two runs with the second solvent, the plates were dried for 20 min in the same oven at the same temperature. After cooling, the layers were sprayed with a solution of 1 g of ninhydrin in 99 g of *n*-butanol until they were translucent. They were then placed in a small oven at 110° for 4 min. The atmosphere of the oven was saturated with water vapour. The separation obtained can be seen in Fig. 1. The compact, non-tailing spots of different colours permit good identification. Fig. 2 gives the key to the identification of the amino acids.

If Silicar TLC 7 (Mallinckrodt) was used instead of Silica Gel H with the same agar–agar solution as binder, the separation was not as satisfactory nor were the spots as compact. The same effect was obtained with Silicar TLC 4.

The use of Silica Gel G layers gave the same relative separation of amino acids as did layers of Silica Gel H with agar-agar as binder. The layers of the former adsorbent have a much lower mechanical strength. Furthermore, the layer of Silica Gel G tends to fall off the plate in the part submerged in the solvent. This happened for both solvents used. For the second run with the same solvent, the liquid level

Fig. 1. Two-dimensional chromatogram of a mixture of amino acids on a thin layer using Silica Gel H (Merck) with agar–agar as binder. The key to the identity of the amino acids is given in Fig. 2.

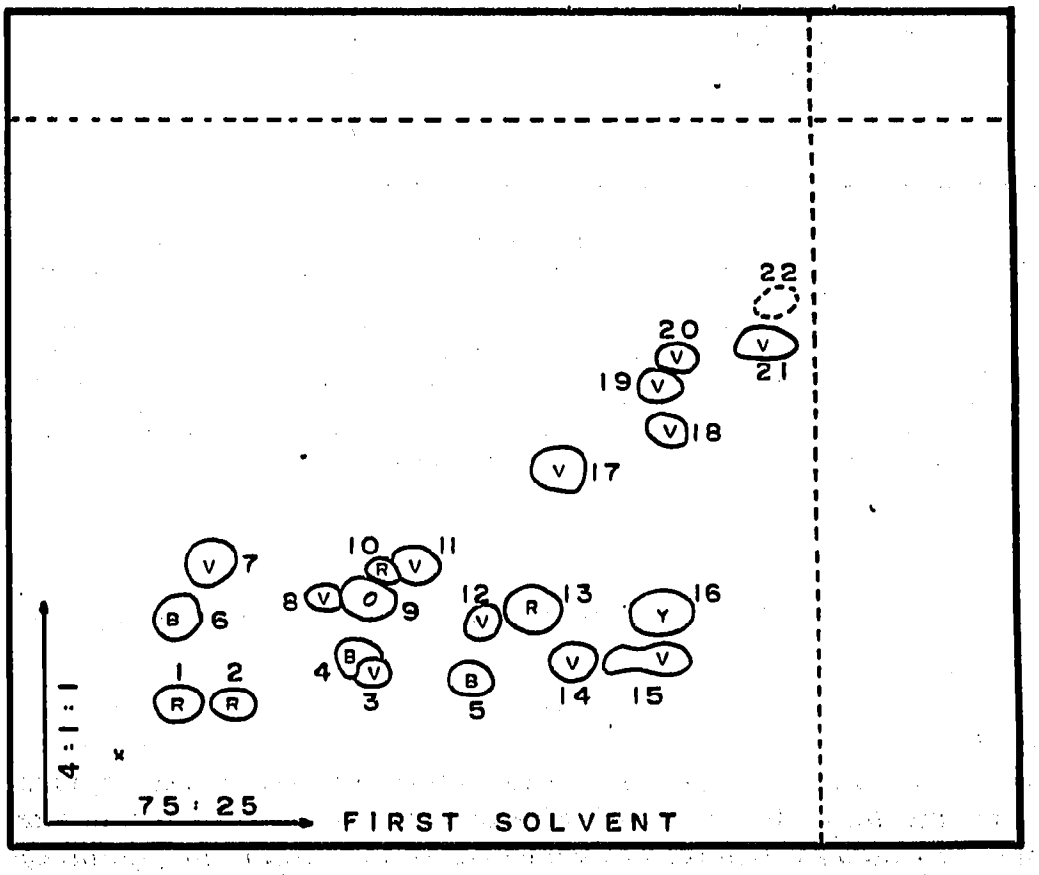

Fig. 2. Two-dimensional chromatogram of a mixture of amino acids on a thin layer, using Silica Gel H (Merck) with agar–agar as binder. Colours: V = violet; R = red; B = brown; O = orange; Y = yellow. Spots: 1 = hydroxylysine; 2 = lysine; 3 = arginine; 4 = cannavanine; 5 = histidine; 6 = aspartic acid; 7 = glutamic acid; 8 = serine; 9 = glycine; 10 = threonine; 11 = alanine; 12 = glutamine; 13 = 4-hydroxyproline; 14 = methionine sulphoxide; 15 = methionine sulphone; 16 = proline; 17 = valine; 18 = methionine; 19 = isoleucine; 20 = leucine; 21 = phenylalanine; 22 = tryptophan.

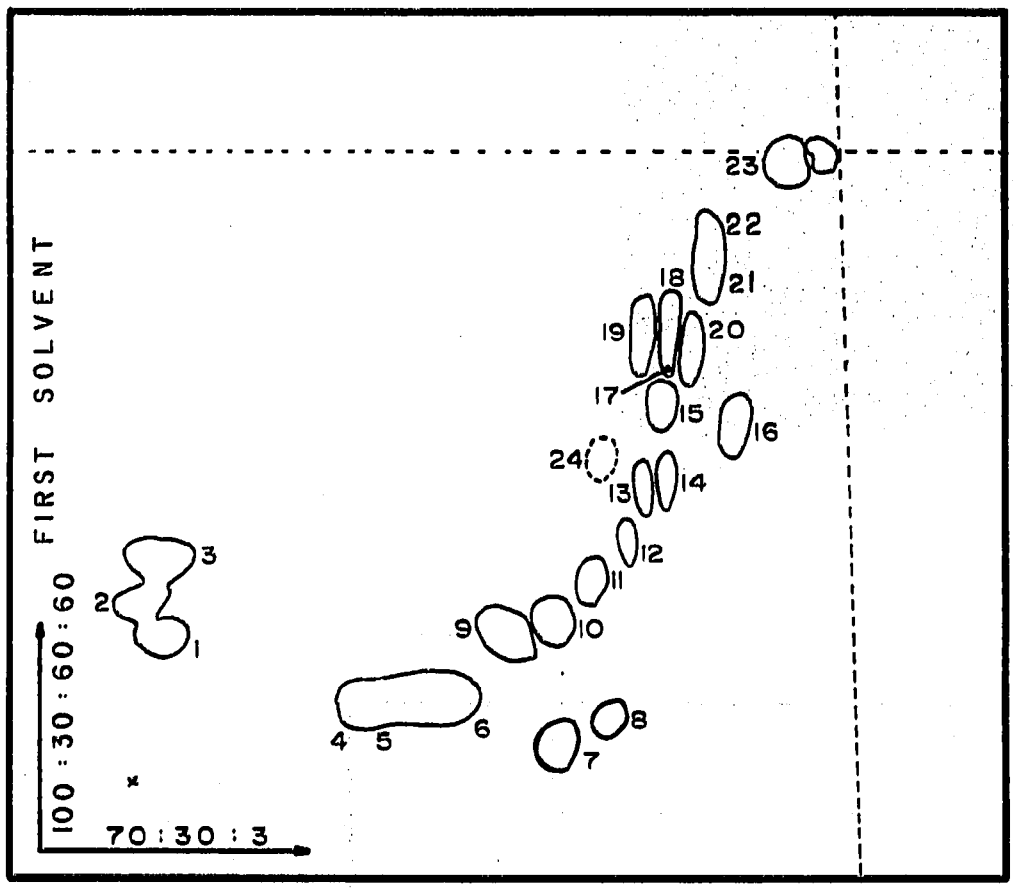

Fig. 3. Two-dimensional chromatogram of a mixture of dinitrophenylamino acids on a thin layer, using Silicar TLC 7 (Mallinckrodt) with agar–agar as binder. Spots (DNP = dinitrophenyl): 1 = di-DNP-histidine; 2 = DNP-arginine; 3 = ε-DNP-lysine; 4 = DNP-asparagine; 5 = DNP-methionine sulphoxide; 6 = DNP-glutamine; 7 = DNP-aspartic acid; 8 = DNP-glutamine; 9 = DNP-methionine sulphone; 10 = DNP-serine; 11 = DNP-threonine; 12 = DNP-glycine; 13 = DNP-proline; 14 = DNP-alanine; 15 = DNP-methionine; 16 = 2,4-dinitrophenol; 17 = DNP-valine; 18 = DNP-phenylalanine; 19 = di-DNP-lysine; 20 = DNP-tryptophan; 21 = DNP-isoleucine; 22 = DNP-leucine; 23 = 2,4-dinitroaniline (the spot to the right of 23 is an accumulation of yellow decomposition products); 24 = not identified.

had to be elevated either by tilting the chromatographic chamber or by adding solvent.

Dinitrophenylamino acids

The preparation of the plates was carried out in exactly the same way as with amino acids. The best results were obtained with Silicar TLC 7 as adsorbent and the same agar–agar solution as was used before. For each 20 g of this adsorbent, 55 ml of the hot agar–agar solution were used.

The two-dimensional separation of a mixture of dinitrophenylamino acids gave good results (Fig. 3). In the first direction the run was made with toluene–pyridine–2-chloroethanol–0.8 *M* ammonium hydroxide solution (100:30:60:60, v/v) to about 4 cm from the upper end of the plate[1]. The plate was then dried for 20 min

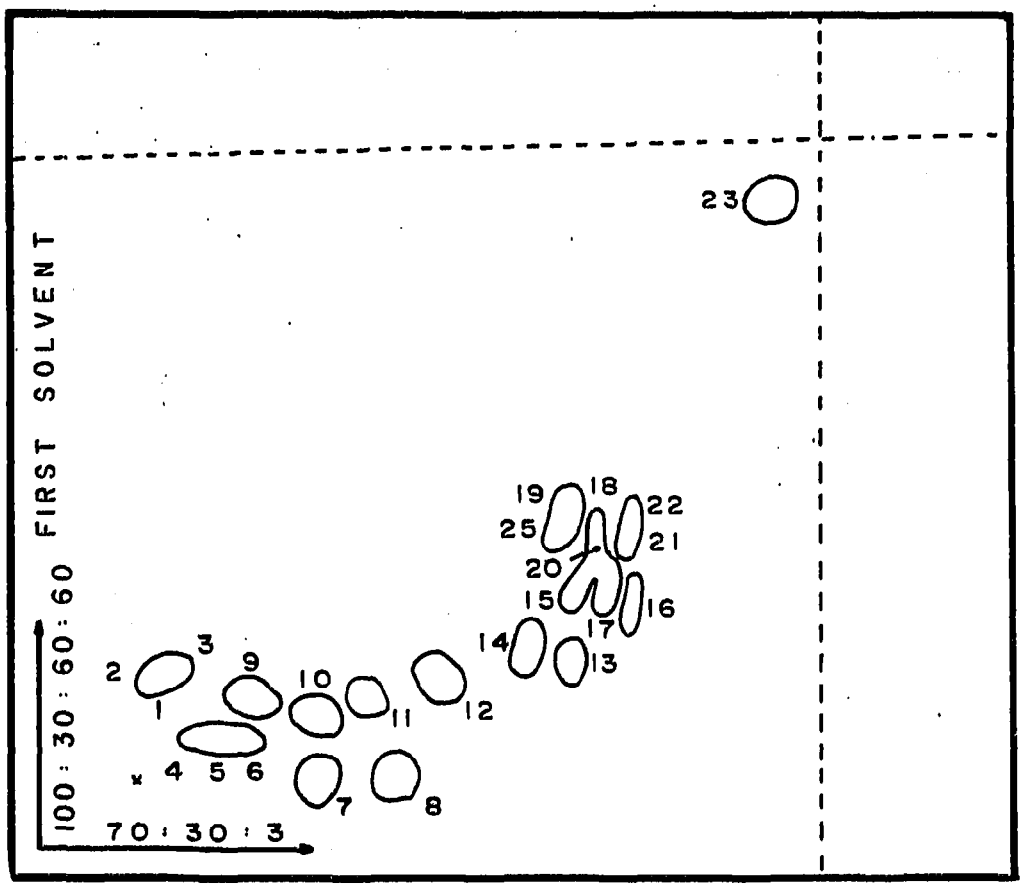

Fig. 4. Two-dimensional chromatogram of a mixture of dinitrophenylamino acids on a thin layer, using Silica Gel H (Merck) with agar–agar as binder. The key to the identity of the spots is given in Fig. 3.

at 110°, cooled and run in the second direction in chloroform–benzyl alcohol–acetic acid (70:30:3, v/v)[1]. After a second drying at 110° for 20 min and cooling, it was viewed with UV light by transmittance. The light source was a Philips UV lamp (93123E Hg). At 360 nm the transmittance of the glass plate is 87% and at 340 nm it is 57%. This is sufficient for the dinitrophenylamino acid spots to stand out clearly from the background when illuminated through the glass.

When the thin layer was prepared with Silica Gel H (Merck) using agar–agar as binder, the separation of dinitrophenylamino acids is not quite so good. Although the spots are more compact, the relative separation in both solvents is less than with Silicar TLC 7. The separation can be seen in Fig. 4. Silicar TLC 4 produces the same relative separation as does the former adsorbent, but all R_F values are much greater. There is a massing of spots towards the front of the solvent.

With layers of Silicar TLC 7GF, the separation of the dinitrophenylamino acids is somewhat less good than with Silicar TLC 7 using agar–agar as binder. As already stated, the former layers have far less mechanical resistance than the latter and, furthermore, both solvents used ascend with about 30% less speed.

It should be noted that the spot for 2,4-dinitrophenol is quite faint. If this spot is exposed to ammonia vapour the yellow colour intensifies markedly.

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