## CHROM. 4074

## Agar-agar as a binder for improved compactness and mechanical resistance of silica gel and alumina thin layers

Work in our laboratories on foliar absorption of some amino acids by plants, requires a particular kind of thin-layer chromatography on silica gel. On the layer is placed a small disk of agar-agar containing the amino acids to be separated. However, this small disk does not adhere to the normal silica gel layers and removes the top layer of silica gel particles.

In order to improve the compactness of the layer and the adherence of the agar disk we decided to prepare silica gel layers which contained agar-agar. The results were excellent. The layers obtained by this procedure are more compact and resistant to mechanical action. For instance, the possibility of layer breakage while using glass spotting pipettes is minimized, and touching does not harm the surface either. Furthermore the  $R_F$  values do not undergo detectable variations.

The procedure for preparing ten  $20 \times 20$  cm plates, 0.25 mm thick, with the use of an automatic coating apparatus (e.g. Camag, Chemetron or an equivalent), is quite simple. In a 500 ml, ground-necked erlenmeyer flask, prepare a 0.4 g agaragar solution in 120 ml distilled water. Allow to boil in a water bath until the contents are completely dissolved. Quickly pour 50 g of silica gel (Kieselgel G, Merck, or equivalent) into the solution before it cools. Shake vigorously for 45 sec, then coat the plates using the automatic applicator.

To avoid the rapid coagulation of the agar-agar, carry out the procedure described above as quickly as possible, previously heating the glass plates in an oven at  $60-70^{\circ}$ . After the coating, the plates should be air dried and then activated at higher temperature in an oven (30 min at 110°).

In addition to the advantages mentioned above, these layers exhibit better absorption than normal layers. They allow the spotting of larger amounts of solution (including aqueous solutions), while reducing the possibility of layer removal.

Results as good as those found with this procedure are also obtained with alumina layers. With regard to our work, the small agar-agar disk placed on the layer and air dried on it may elute slowly with the solvent mixtures normally used for the amino acids<sup>1,2</sup>. This is avoided by a preliminary elution with a mixture of water-isopropanol (9:1), up to a 3.0 cm front. The amino acids are then completely extracted from the agar-agar disk, and all of them move to the solvent front. The layer is then dried in an oven, and a further elution using the proper solvent mixture is performed with a resulting good separation of the amino acids.

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