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ADVANCES IN THIN-LAYER CHROMATOGRAPHY

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INTRODUCTORY ADDRESS

Twenty years have passed since I and my colleagues first introduced (1951) the present-day system of thin-layer chromatography (TLC) in which adsorbent-coated plates are developed in a manner analogous to paper chromatography. During this time, the field has progressed with many modifications and new ideas. As time passes, it becomes increasingly difficult to find new ideas and the majority of papers that appear are based on applications of the methods to the separation of different compounds. Nevertheless, new ideas and equipment continue to appear as well as theories and explanations for the results that have been obtained. Some of these will be presented here today. In presenting an introduction to this symposium and especially to today's talks, I shall present one or two useful ideas snatched at random from the literature to illustrate the kinds of ideas that are needed to keep the techniques of TLC and electrophoresis advancing.

The slide from MULDER AND VEERSTRA (Fig. 1) shows the application to concen-

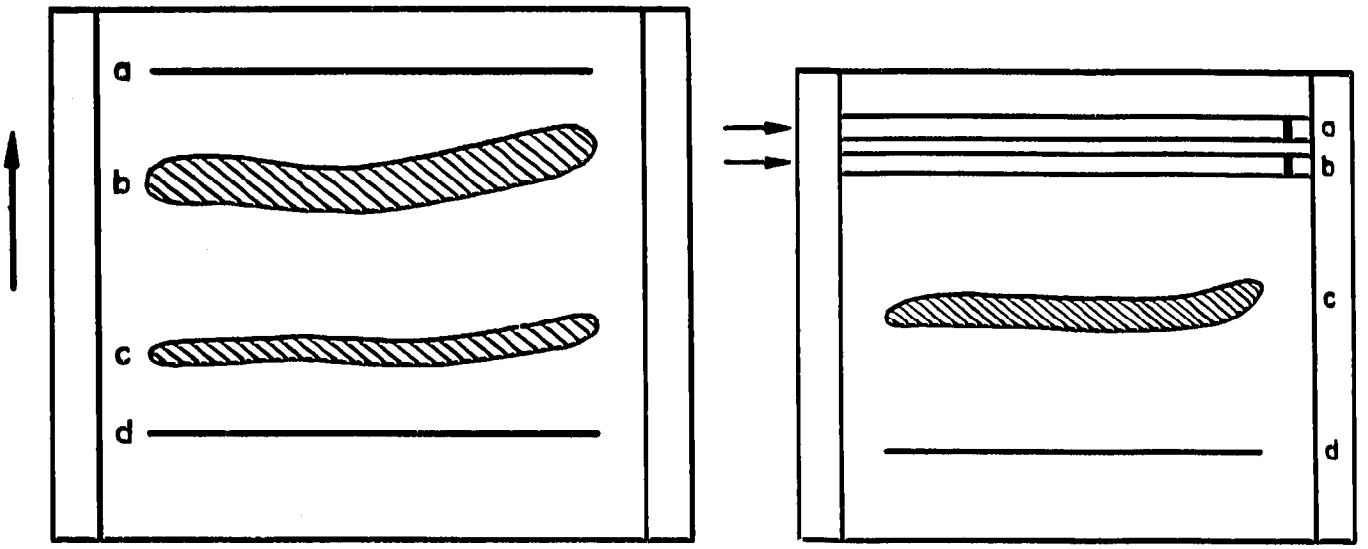

Fig. 1. Concentration in first direction. a = Concentrated in narrow band; b and c = not concentrated; d = origin. [From J. L. MULDER AND G. J. VEENSTRA, *J. Chromatogr.*, 24 (1966) 250].

Fig. 2. Concentration in second direction. a and b = Concentrated on sma surface areas; c = not concentrated; d = origin. [From J. L. MULDER AND G. J. VEENSTRA, *J. Chromatogr.*, 24 (1966) 250].

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trating the separated bands. Horizontal continuous chromatography is used to move the bands to the top for concentration by evaporation. A wick is used to transfer the solvent to the plate, the latter being open to the air at the far end so that the narrow band is concentrated at the exit as in (a). Additional bands can be concentrated by further exposing the plate so that the next band concentrates at a point below the first band. Then the plate is turned sideways and, using a narrow strip of paper to transfer solvent to the required area, the narrow bands are concentrated to spots as shown (Fig. 2). Thus, the desired material can be eluted from a much smaller quantity of adsorbent resulting in less contamination.

A somewhat similar technique is shown on the next slide (Fig. 3). Here, after

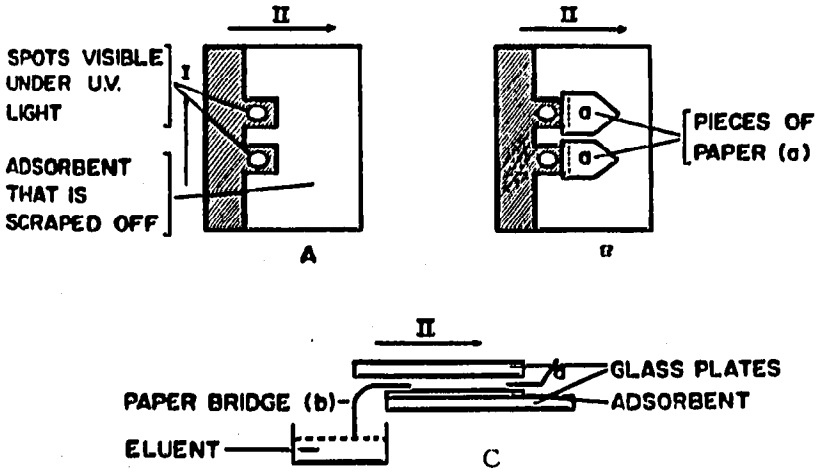

Fig. 3. (A) After development in direction I the adsorbent layer is partly scraped off. (B) On the remaining parts of the adsorbent, small pieces of paper are placed. (C) The whole is eluted in direction II with a horizontal elution technique. [From V. J. R. DEDEYNE AND A. F. VETTERS, *J. Chromatogr.*, 31 (1967) 261].

the usual development, the plate is dried and the adsorbent is then removed from the plate in the manner shown here. As you can see, development was in the direction of I, resulting in these two spots. From here on, the procedure can be handled in one of two ways. After scraping away the adsorbent as shown, small pieces of ash-free filter paper are cut to a point and placed on the remaining strips of adsorbent. The tips of the paper are bent up and the whole set-up is covered with a glass plate, leaving the points of the paper strip exposed. Using horizontal development (direction II), the compounds in the spots are moved to the tips of the paper. It is then a simple matter to extract them from the paper.

As a second alternative, the adsorbent itself is shaped to a point where the products can be concentrated. In some cases, it is possible to develop sufficiently so that the product is eluted from the adsorbent and crystallized out on the glass plate.

There is another technique that can be used to advantage; this is the case of multiple layers of different adsorbents. This idea can be applied in a number of ways. Fig. 4 shows one way that this can be done. A strip of one adsorbent is coated along one edge of the plate and the balance of the plate is covered with a second adsorbent. In this case, magnesium silicate and silicic acid were used. The adsorbent in this hatched area has been removed to prevent contact with the solvent and the sample

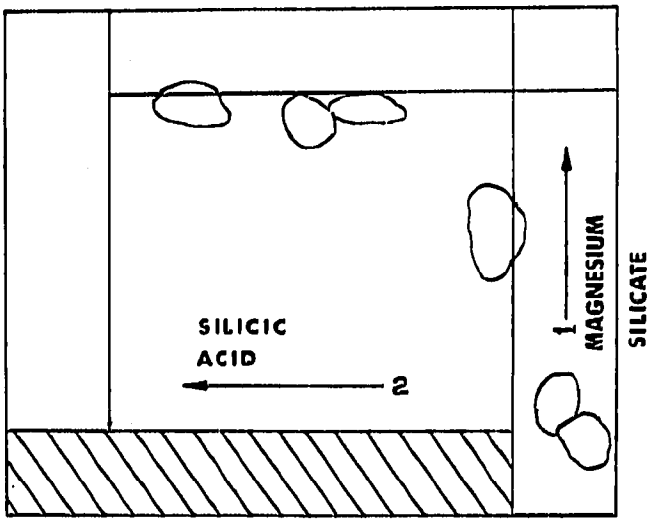

Fig. 4. Two-dimensional separation of Bergamot oil on a dual-layer plate of magnesium silicate and silicic acid, both adsorbents bound with 2½% starch. Solvent: benzene in both directions. Visualizing agent: 0.05% fluorescein in water, then bromine vapor. Cross-hatched area: Plate cleared of adsorbent. (From J. G. KIRCHNER, *Thin-Layer Chromatography*, Interscience, New York, 1967, p. 121 with permission of the publishers).

is then applied in this lower corner. After development in the first direction, the solvent is allowed to evaporate before developing at right angles on the second adsorbent. This is a sample of Bergamot oil developed in benzene in both directions. Both adsorbents are bound by 2½% starch.

Another example is shown in Fig. 5. In this case, charcoal and silicic acid layers are used for the separation of a group of ketones. The charcoal layer is bound with

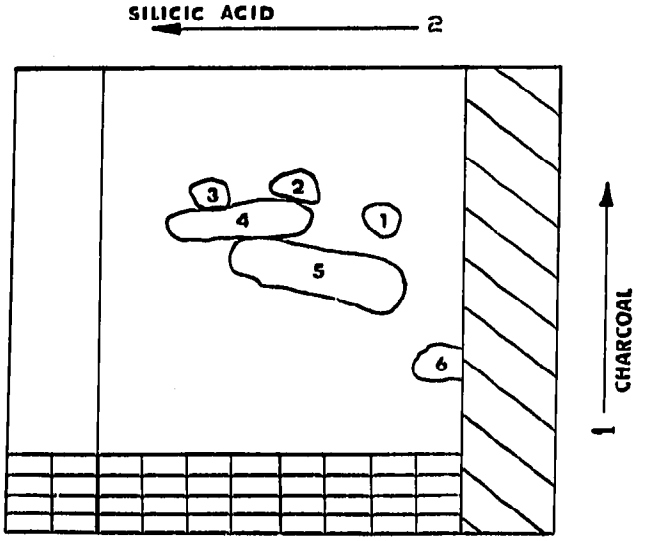

Fig. 5. Two-dimensional separation of some ketones on a dual-layer chromatoplate. First development with benzene–ether–acetic acid (82:9:9); second development with benzene–ether (85:15). Visualizing agent: 2,4-dinitrophenylhydrazine in 2 *N* HCl. Diagonal-hatching: charcoal with 10% starch binder; cross-hatching: adsorbent-free area; balance of plate: silicic acid with 2½% starch binder. Development distance: 10 cm. 1 = Angelica lactone; 2 = acetophenone; 3 = 7-tridecanone; 4 = bromoacetophenone; 5 = 2-methylcyclohexanone; 6 = 2-hydroxyacetophenone. (From J. G. KIRCHNER, *Thin-Layer Chromatography*, Interscience, New York, 1967, p. 120 with permission of the publishers).

10% starch and the silicic acid layer with 2½% starch. The solvent used for the first development is benzene–ether–acetic acid (82:9:9) and for the second benzene–ether (85:15). There is one problem here, however, because, as the solvent travels in the first direction, it also tends to spread over into the second adsorbent, thus pulling the compounds in the same direction and slowing the rate of development. This can be overcome by first removing the adsorbent from a narrow slit along the line where the two adsorbents meet. Then after developing the chromatogram in the first direction, the slit can be closed by tamping in dry dicalite. This technique of using two adsorbents as illustrated here can be varied. For example, the layer for the second development could be a gradient layer so that substances near the starting point, those which were strongly adsorbed in the first direction, would now move into a less adsorbing layer whereas those compounds which were less strongly adsorbed and moved rapidly, would move into a stronger adsorbing field.

There are two fairly recent techniques which I will mention briefly as I believe they hold a good deal of promise. The first is vapor programming in which the thin-layer plate is pretreated with solvent vapors to condition the plate prior to development. This can yield separations not possible by regular procedures. The second is coupled TLC–GLC. Since GLC separates according to relative volatility and TLC according to the functional groups present, the combination of the two chromatographic methods supplement one another very nicely. With commercial equipment now available for TLC–GLC coupling, it is to be hoped that this technique will find wider acceptance.

These few examples, I believe, serve to introduce our topic for today: Advances in thin-layer chromatography.