

Acknowledgements

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Sample application to silica gel layers via kieselguhr layers

Manually applying a large sample to a thin layer, as a narrow straight line, is both difficult and tedious. However, the problem may sometimes be alleviated by a simple means. If for example, a proposed separation takes place on a layer of silica gel, which adsorbs strongly, it is expedient to apply the sample quickly and with little care to a band of weakly adsorbing kieselguhr spread next to the silica gel (*i.e.* a silica gel-kieselguhr twin layer) whence the sample is eluted into the silica gel by the developing solvent. Since the sample is adsorbed only weakly by kieselguhr it may well be eluted as a narrow line at the solvent front. Irrespectively, it will condense as a narrow line at the start of the silica gel because it is eluted faster through the kieselguhr than through the silica gel. The sample may be applied quickly and without care to the entire area of kieselguhr (*i.e.* approx. 3×20 cm) and yet the line of sample semi-automatically applied to the silica gel is narrower and more even than can ever be achieved by manual application.

The technique depends on the contrast in adsorbing powers of the two media, and therefore to achieve maximum effect the adsorption by commercial kieselguhr was minimized through treatment with hydrochloric acid to remove iron and calcium sulphate binder. The method is illustrated by the separation of Stahl's dye mixture on kieselguhr-silica gel twin layers.

Experimental

To remove the iron (about 0.05 % by weight) and binder from Merck Kieselguhr G 500 g were mixed with 1 l of 18% HCl and the mixture was allowed to stand for a few hours. The residue was separated from the yellow-green supernatant liquid in a Buchner funnel and washed with distilled water until neutral. It was then washed successively with 300 ml ethanol and 300 ml benzene and dried at 120° (ref. 1).

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Thin layers, 300 μ thick, were made on 5 \times 20 cm plates as described before². The width of the kieselguhr layer was 4.5 cm and the silica gel layer was accordingly 15.5 cm wide. The layers were activated at 120° for 1 h.

Stahl's dye mixture (Desaga, Heidelberg) was diluted (three times) with benzene and applied as two rows of spots to the washed kieselguhr of each of three twin layers (see Fig. 1). The chromatograms were then developed in petroleum ether-diethyl ether (7:3) such that the dyes were eluted through the kieselguhr into the silica gel. The development of one chromatogram was stopped as the solvent front reached the silica gel, while another was left until the front was 3 cm into the silica gel (see Fig. 1).

Discussion

It is self evident from Fig. 1 that most of the dye mixture was eluted into the silica gel as a very narrow band which as a result was separated into its components on a short chromatogram. Each of the dyes was eluted through the kieselguhr at the solvent front, but Sudan Red G trailed to the points of application (see Fig. 1). Nonetheless it all condensed into a narrow band before any appreciable separation had occurred because the trailings were eluted through the kieselguhr faster than the rest was eluted into the silica gel.

Equivalent success has been achieved with more complex mixtures using similar twin layers (750 μ thick) to separate some of the components of plant extracts. At

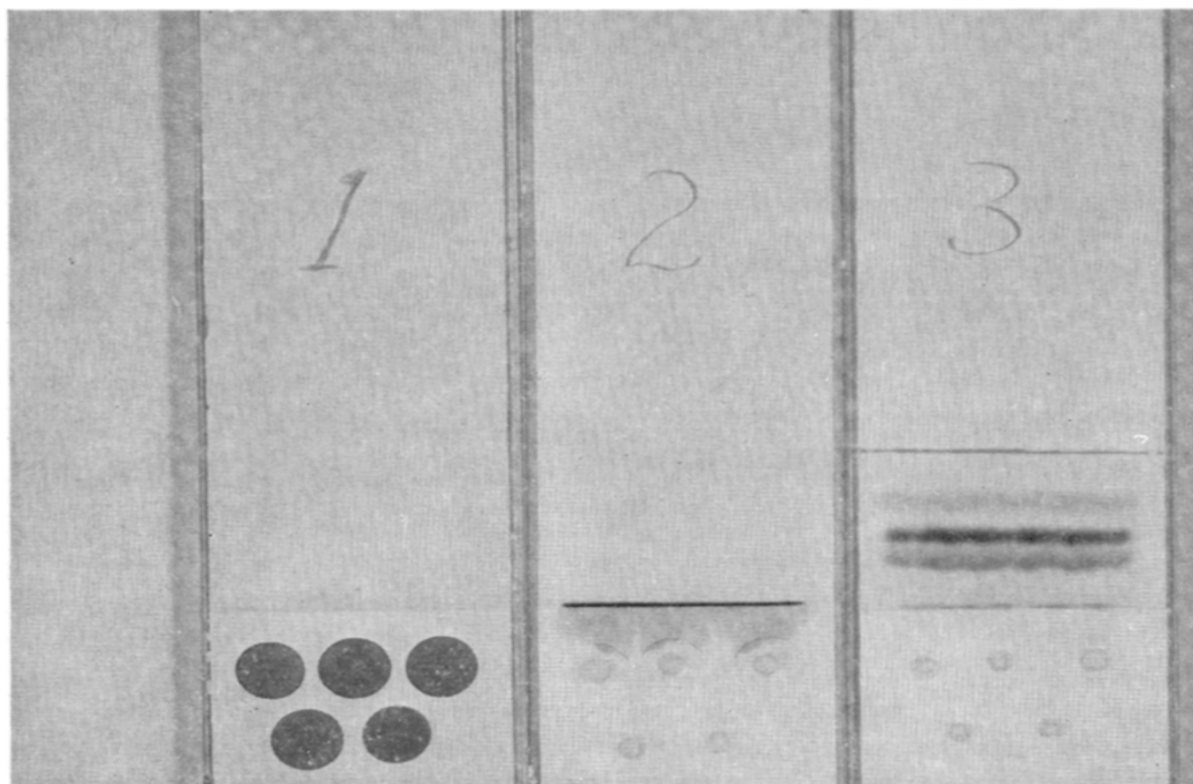


Fig. 1. Chromatography of Stahl's dye mixture on a silica gel layer after elution from a layer of kieselguhr. R_F values: Butter Yellow, 0.65; Indophenol Blue, 0.45; Sudan Red G, 0.35. The compounds at the origin and at the silica gel-kieselguhr interface are impurities in the dye mixture. (1) Sample applied to kieselguhr. (2) Solvent front reaches layer of silica gel. (3). Solvent front 3 cm into the silica gel.

times the entire area of the kieselguhr has been covered by the plant extract by quickly running it out of a 1 ml pipette, yet the mobile components all entered the silica gel as straight narrow bands.

The removal of iron and calcium sulphate from kieselguhr markedly reduced the adsorption (*e.g.* the R_F value of gibberellin A₁ in benzene-acetic acid-water (8:3:5) on untreated kieselguhr was 0.1 and on washed kieselguhr was 0.5) but in addition reduced the stability of the kieselguhr layer. As a result, small holes were sometimes made in the layer when a sample was pipetted quickly onto it. Fortunately though, kieselguhr is very malleable and the layers were easily remolded with a spatula.

The idea of applying a sample over a large area and then condensing it into a narrow band prior to separating its constituents was first put into practice by BUSH³, using paper chromatography, and by FESSLER AND GALLEY⁴, using thin-layer chromatography. Both groups initially chromatographed their samples over a short distance using a highly polar solvent to elute all the components into a narrow band at the solvent front. The development was then stopped, the layer or paper dried and a second chromatogram developed in the normal manner. In comparison, the twin-layer technique appears to have certain advantages providing the separation takes place on a high-adsorption medium like silica gel. Primarily, concentrating the sample in the kieselguhr at the solvent front is equivalent to the result from using FESSLER AND GALLEY's method without the need to stop development and free the layer of a polar solvent. Secondly, an additional condensation takes place at the start of the silica gel layer because of the different rates of elution in the two media. This applies to all mobile compounds including those not eluted or incompletely eluted through kieselguhr at the solvent front *e.g.* Sudan Red G in the above system.

In conclusion it should be appreciated that although the method has so far been limited to silica gel-kieselguhr twin layers it should work equally well using other combinations of low- and high-adsorption media.

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