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Note

Combination of infrared spectroscopy and thin-layer chromatography for the identification of slightly soluble substances

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In thin-layer chromatographic (TLC) investigations the R_F values obtained are often used for identification of substances. However, these values are not sufficiently specific for the identification of unknown compounds, although selective colour reactions may be applied.

Substances can be identified with a high degree of reliability by a combination of chromatographic separation and spectroscopic characterization methods, *e.g.*, the Wick-Stick method of Garner and Parker¹. Thus, a wide range of substances amenable to direct infrared (IR) spectroscopic investigation have been separated with a suitable TLC system, purified and concentrated on potassium bromide which serves as a carrier material for IR spectroscopy. However, this method can be used only for substances with solubilities sufficiently high for chromatography.

Recently Székely and Baumgartner² reported that substances with low solubilities can be chromatographed at temperatures up to 170°C using solvents of low volatility. We have combined this method with the Wick-Stick technique mentioned above and obtained good results with substances thermally stable over the range of temperature employed.

EXPERIMENTAL

Small 10 × 10 cm thin-layer plates were obtained by cutting commercially available 20 × 20 cm silica gel pre-coated plates³. The TLC separation was performed with high boiling eluents at temperatures of *ca.* 150°C (according to ref. 2). The Wick-Stick, a pre-pressed KBr triangle¹ (purchased from Harshaw, Cleveland, OH, U.S.A.), was inserted into a metal holder and placed in a cylindrical glass vial (Fig. 1). The spot to be analyzed was scraped off the TLC plate and transferred into this vial. The spot should contain at least 10 µg of material. Better results were obtained if the corresponding by-spots* of several chromatograms were collected, combined and rechromatographed to yield a single spot.

About 200 µl of high boiling solvent, *e.g.*, 1-bromonaphthalene, were added to the Wick-Stick and the vial heated in an oven to 170°C. The solution rose in the KBr triangle because of capillary forces. The solvent evaporated in the upper part

* "By-spot" = chromatographic zone with lower concentration and intensity than the main zone (see Fig. 3).

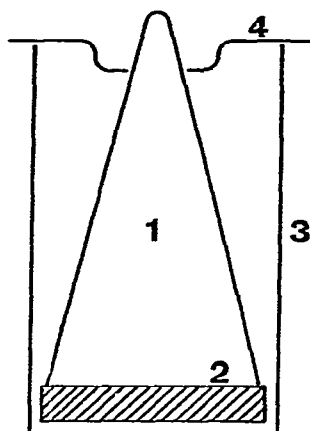


Fig. 1. Wick-Stick. 1 = KBr triangle; 2 = triangle holder; 3 = glass vial; 4 = metal cap. The length of the vial has been somewhat reduced in order to allow the top of the pyramid to extend beyond the upper end of the vial. This greatly facilitates the evaporation of the solvent.

of the triangle and the component to be analyzed was accumulated in the tip. It was necessary to repeat this procedure two or three times. After a few hours, even colourless compounds could be detected on the tip of the Wick-Stick as light yellow spots. The tip of the triangle corresponding to about 20 mg of KBr was cut off, dried in a vacuum at 50°C for several hours and used to make a micro-pellet.

The tip was ground in an agate mortar and placed in the 5×1 mm opening of a lead mask (see Fig. 2). The mask was pressed in a conventional press for KBr pellets (diameter, 13 mm; applied pressure, $p = \approx 15$ tons). Then the resulting embedded micro-pellet was focused in the beam of the IR spectrophotometer. It should be noted that only older models of spectrophotometers need a beam condenser accessory. The lead mask also obviates very delicate handling of a micropress.

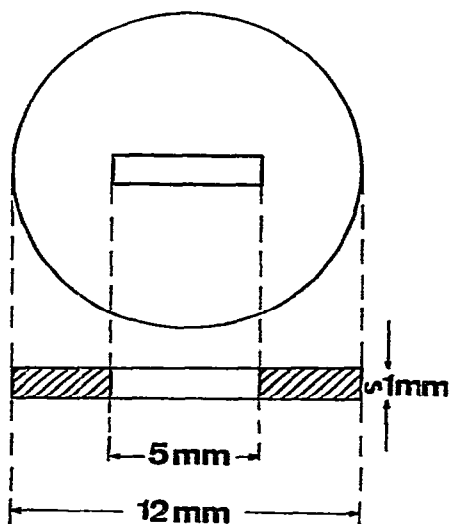


Fig. 2. Lead mask pressed in a conventional press for KBr pellets (diameter, 13 mm; applied pressure, $p = 15$ tons). The resulting embedded micro-pellet was then focused in the beam of the IR spectrophotometer.

RESULTS

The method described was tested on some dyestuffs and their intermediates which are virtually insoluble at room temperature. The TLC separation of indigo (Fig. 3) at 150°C serves as an example: a blue main spot (R_F 0.70) and a reddish by-spot (R_F 0.60) were observed². The main-spot was purified directly by the Wick-Stick method using 1-bromonaphthalene as eluent, whereas the by-spots from several chromatograms had to be collected first and then purified using the same method.

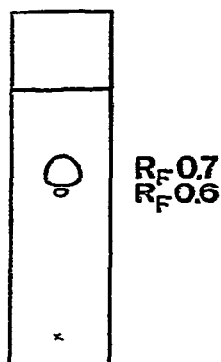
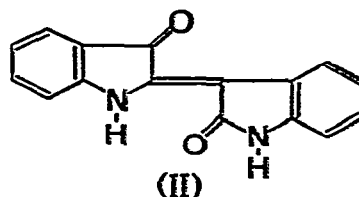
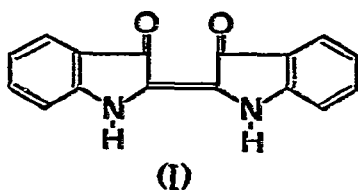


Fig. 3. Separation of indigo at 150°C Layer: Merck silica gel F₂₅₄ pre-coated plate. Solvent: di-*n*-butyl phthalate-N-methylpyrrolidone (9:1). Application: 5 μ l of 0.1% sample solutions in 1-bromonaphthalene. Separation time: 15 min for a separation distance of 7 cm Wick-Stick: 200 μ l 1-bromonaphthalene at 170°C, followed by two 100- μ l portions. Time: ca. 5 h.

IR spectra

The IR spectrum of the main spot exhibits specific absorption bands at 1628, 1615, 1483, 1463, 1316, 1298, 1200, 1172, 1127, 1070, 879, 858, 763, 754, 745, 712, 698 and 668 cm^{-1} . With the aid of these absorptions the substance has been identified as indigotin (I).

The chemical structure of the by-spot could be determined as indirubin (II) by its characteristic absorptions at 1667, 1618, 1595, 1482, 1464, 1321, 1300, 1258, 1208, 1178, 1146, 1098, 1019, 1002, 962, 802, 783, 754/749 and 709 cm^{-1} .



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REFERENCES

- 1 H. R. Garner and H. Packer, *Appl. Spectrosc.*, **22** (1967) 122.
- 2 G. Székely and P. Baumgartner, *J. Chromatogr.*, **186** (1979) 575.
- 3 R. Delley and G. Székely, *Chimia*, **32** (1978) 261.