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## Note

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### An efficient chemically bonded reversed-phase tin-layer plate permitting the use of water-rich mobile phases\*

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Within the last few years, several new commercially available chemically bonded reversed-phase thin-layer chromatography (RPTLC) plates have led to a renaissance in TLC. Problems associated with the wettability of these RPTLC plates by the solvent, however, have prevented the use of water-rich mobile phases and detection techniques<sup>1</sup>.

In an effort to overcome these problems, we first silanized pre-coated TLC plates with dodecyltrichlorosilane according to Gilpin and Sisco<sup>2</sup>. We also tried to coat glass plates with dodecylsilanized silica gel prepared in our laboratories<sup>3</sup>, but the layer obtained was not stable enough. Finally, using our silanized silica gel, Antec AG (Bennwil, Switzerland) developed a pre-coated RPTLC plate, which is commercially available under the trade-name OPTI-UP C12\*\*.

During the last 3 years, we have compared their efficiency with those of (eight) other commercially available pre-coated RPTLC plates in systems containing more than 40% of water and using several classes of substances.

## EXPERIMENTAL

### *Materials*

The following commercially available TLC plates were tested: 20 × 20 cm plates pre-coated with OPTI-UP C12 containing a fluorescent indicator; 10 × 10 cm plates, pre-coated with LiChrosorb RP-2, RP-8 and RP-18, containing a fluorescent indicator (Merck, Darmstadt, G.F.R.); 10 × 10 cm plates pre-coated with NanoSil C18 (100%, 75% and 50%), containing a fluorescent indicator (Macherey, Nagel & Co., Düren, G.F.R.); 20 × 20 cm pre-coated KC 18 thin-layer plates (Whatman, Maidstone, Great Britain); and 20 × 20 cm RPS Uniplates, which have a support layer impregnated with long-chain hydrocarbons (Analtech, Newark, DE, U.S.A.).

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\* Dedicated to the late Professor R. B. Woodward, deceased July 8th, 1979, who shared with us his experience of using OPTI-UP C12 plates.

\*\* The plates are also available through Tridom Chemicals (Hauppauge, NY, U.S.A.), Fluka (Neu-Ulm, G.F.R.), Siccip-Emmop (Marseille, France) and Antec (Bennwil, Switzerland).

### Chemicals

All chemicals were of analytical-reagent grade and were purchased from Fluka (Buchs, Switzerland).

### RESULTS AND DISCUSSION

In our experience, only OPTI-UP C12 can be used conveniently with mobile phases containing more than 40% of water. They are, in fact, applicable irrespective of the water concentration and without sampling or detection limits.

Neither aqueous sprays nor aqueous test solutions can be applied to the Merck and Macherey, Nagel & Co. plates.

With RPS Uniplates we encountered the same problems as with all impregnated or silanized plates, namely that the solvents were limited to liquids that were relatively immiscible with the stationary phase, and the stability and reproducibility were poor.

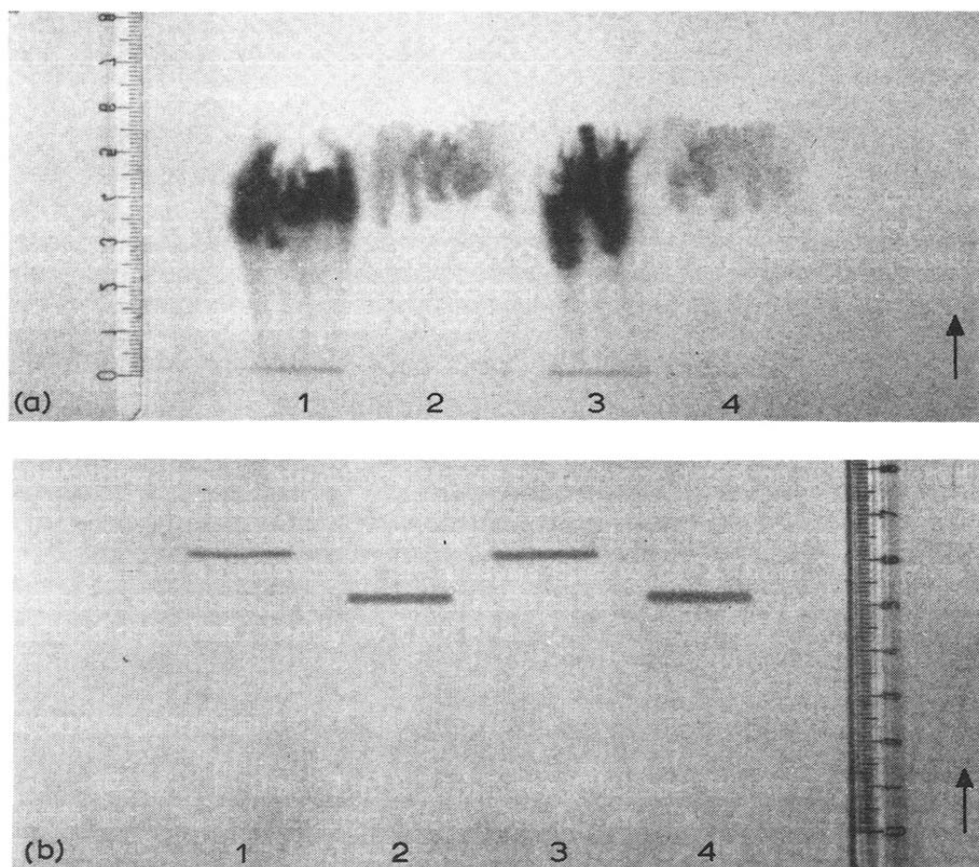


Fig. 1. Separation of methionine sulphoxide and methionine on KC 18 (a) and OPTI-UP C12 (b). Eluent: 3% NaCl in water. Development: (a) 180 min and (b) 45 min for 8.5 cm. Detection: ninhydrin. 1, 3 = Methionine sulphoxide; 2, 4 = methionine.

The Macherey, Nagel & Co. and Merck plates have disadvantages such as the application limits described by Siouffi *et al.*<sup>1</sup> and the Whatman KC 18 plate was useful only at water concentrations up to 38%. With the Whatman plate, the addition of 3% of sodium chloride to the solvent system prevented the dislodgement of the chemically bonded phase from the glass backing and a higher water concentration could be used, but other problems due to the salt arose (Fig. 1). Salt effects in RPTLC on silica gels have been described by Thijssen<sup>4</sup>.

Several examples of the application and performance of OPTI-UP C12 plates have recently been published, for the separation of diastereoisomers<sup>5</sup>, the quantitative radiometric assay of nucleosides<sup>6</sup>, the preparative separation of penems<sup>7,8</sup> and the bioautoradiography of antibiotics<sup>9,10</sup> using solvent systems containing 40–100% water.

In our laboratory, we have been able to separate five dansyl amino acids using 50% water (Fig. 2), strongly polar Cibacron dyes using 70% water (Fig. 3) and two catecholamines using 95% water (Fig. 4).

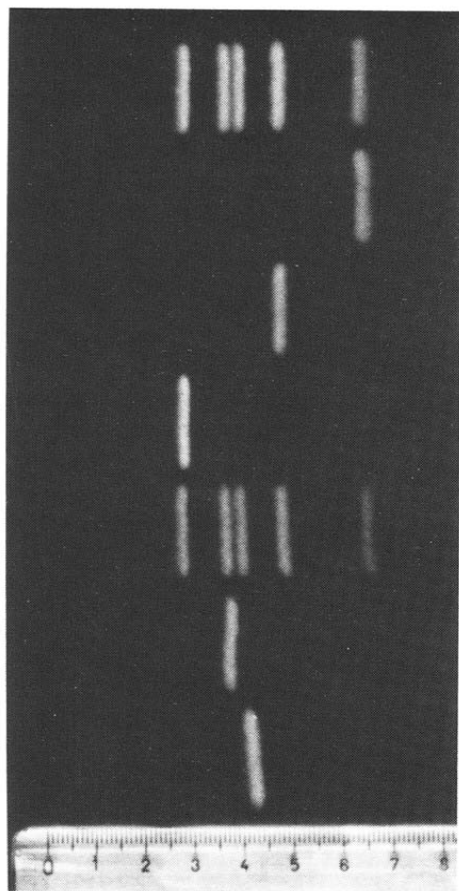


Fig. 2. Separation of five dansyl (Dns) amino acids on OPTI-UP C12. Eluent: 50% acetonitrile in water. Development: 45 min for 8.5 cm. Detection: UV, 366 nm. 1 = Mixture of 1–5; 2 = Dns-CySO<sub>3</sub>H (1 µg); 3 = Dns-Asn (1 µg); 4 = Dns-Arg (1 µg); 5 = mixture of 1–5; 6 = Dns-L- $\alpha$ -aminobutyric acid (1 µg); 7 = Dns-Ala (1 µg).

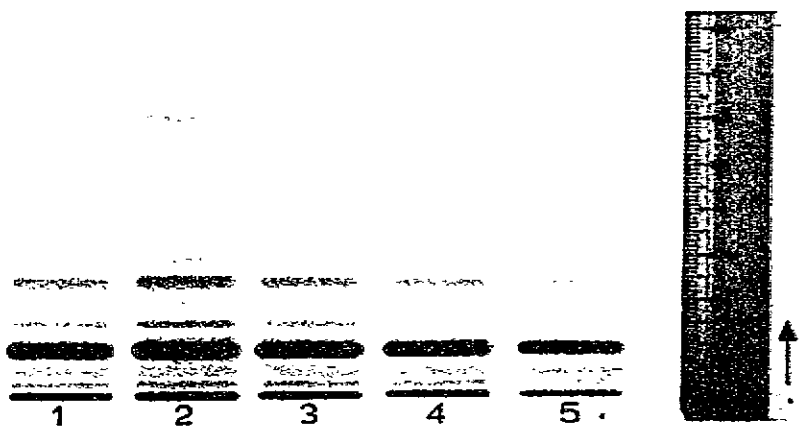


Fig. 3. Separation of a mixture of strongly polar Cibacron dyes on OPTI-UP C12. Eluent: 30% acetonitrile in water. Development: 60 min for 8.5 cm. Detection: daylight. 1 = 25  $\mu\text{g}$ ; 2 = 30  $\mu\text{g}$ ; 3 = 25  $\mu\text{g}$ ; 4 = 20  $\mu\text{g}$ ; 5 = 15  $\mu\text{g}$ .

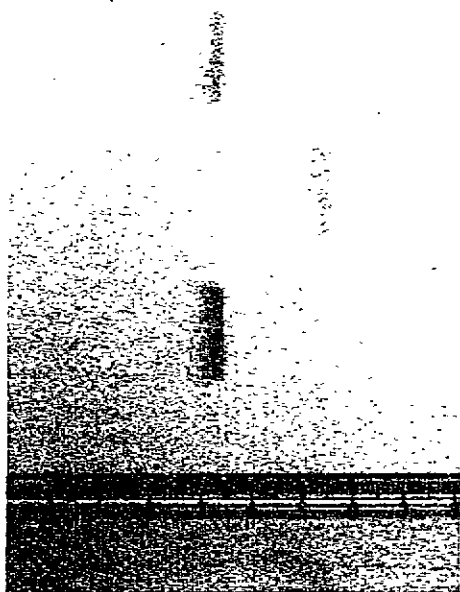


Fig. 4. Separation of noradrenaline and dopamine on OPTI-UP C12. Eluent: 5% formic acid in water. Development: 45 min for 8.5 cm. Detection: Pauly's reagent<sup>11</sup>. 1, 3 = Noradrenaline (30  $\mu\text{g}$ ); 2, 4 = dopamine (30  $\mu\text{g}$ ).

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