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## The use of butanol in the production of cellulose thin layers

In spite of the commercial availability of ready-made layers for chromatography, laboratory production of such layers is still necessary for convenience and economic reasons. Usually, a high-speed propeller blade stirrer is employed to make a homogeneous suspension of cellulose in water or aqueous ethanol<sup>1-3</sup>, since otherwise the sorbent is likely to form aggregates which cannot be prevented or removed by simple stirring or shaking of the slurry.

In our laboratory it was necessary to produce Whatman CC-41 microcrystalline cellulose thin layers on supports of limited area and in small batches. Because a stirrer for small volumes of liquid (about 10 ml) was not available, we decided to try some organic solvents for the preparation of thin layers without mechanical stirring of the slurry. Acetone<sup>4,5</sup>, methanol<sup>6,7</sup>, ethanol\*, isopropyl alcohol, ethyl acetate, chloroform, benzene and xylene were found unsuitable since the layers formed on the glass plates contained lumps and/or their surface was not smooth. In the case of ethylene glycol monoethyl ether and glacial acetic acid, the surface gradually became pitted during storage. *n*-Butanol, however, was found to be suitable. Amyl acetate formed a smooth lump-free layer by the method to be described for *n*-butanol, but the suspension had to be diluted twice in order to prevent premature evaporation of the solvent.

The production of the layer is very simple. *n*-Butanol (8 ml) is added to cellulose (3 g Whatman CC-41) and slurried by stirring with a glass rod. The slurry is poured onto the glass plate to form a 16 × 13 cm layer. By careful slanting of the plate a macroscopically uniform layer is obtained after several seconds. The layer becomes dry within 2 h and is then left for 24 h at room temperature to equilibrate with the laboratory atmosphere. The layers adhere well to the support even if the glass, though thoroughly cleaned by detergent solutions, had not been rigorously de-fatted before use. In the case of aqueous suspensions however, it was sometimes necessary to roughen the glass surface with fine emery paper beforehand to ensure firm adherence.

It is thus possible to prepare readily small batches of plates of different sizes. The method was satisfactory with a number of solvent systems, including isopropyl alcohol-ammonia (25 %)-water (4:1:1) and benzene-propionic acid-methanol-water (20:14:1:1), which we use routinely in the chromatography of urinary indolylacryloylglycine. Mild swelling of the cellulose occurs at the site of application when aqueous solutions exceeding 20  $\mu$ l are used for spotting, but the swelling does not adversely affect the subsequent separation of the substances.

\* LEHMAN *et al.*<sup>8</sup> have described layering of cellulose (MN 300 UV) in 90% ethanol (denatured with petroleum ether) in the way we describe (without the use of a mixer). In our hands such layers, though applicable, have a surface whose regularity is definitely inferior to that made in butanol, and lumps do sometimes occur.

This method of layering was equally successful for Macherey & Nagel Co. cellulose MN-300 and a microcrystalline cellulose supplied by Messrs Lachema.

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