A new apparatus for dynamic thin-layer chromatography

The technique of thin-layer chromatography, invented by JSMAILOW AND SCHRAIBER[1] and standardized by STAHL[2] has become an important tool in analytical procedures in many fields. This technique is also extensively applied to the separation of biological material because of its simplicity, rapidity and great resolving power.

In 1966 the author described an apparatus for dynamic thin-layer chromatography[3]. This apparatus makes it possible to observe the resolution of a mixture of substances which fluoresce or exert a quenching effect on a fluorescent adsorbent layer while the different components are actually migrating.

At the time of constructing this apparatus the Chance OX 7 filter was available only in dimensions of up to 5 × 18 cm, which was too narrow to observe more than one separation. As this filter is now being made in larger sizes a greatly improved apparatus has been constructed which makes the observation of the separations of up to eight different mixtures possible.

This new apparatus consists of a polished, colourless glass tank with lid, measuring 23 × 23 × 7 cm. A piece 14.5 × 14.5 cm is cut out of one of its 23 × 23 cm sides, 4.5 cm below the top of the tank. Over this window a Chance OX 7 glass filter measuring 18 × 18 cm and 3 mm thick is stuck with gelatin obtained from May & Baker. This gelatin has been tested for its resistance to the solvents used.

Two Philips 6 W mercury vapour lamps, 15 cm long, are fitted in front of the Chance filter. They have an output of 0.085 W at 2537 Å and their maximum radiation at this wavelength. Fig. 1 shows this apparatus.

On a Merck pre-Coated TLC plate (Silica Gel F 254), plasma extracts of steroid

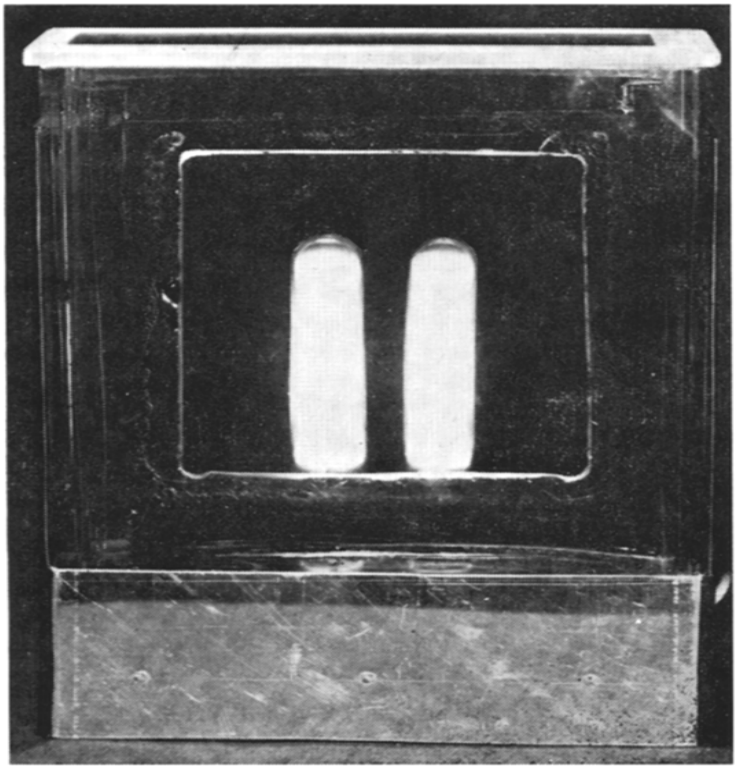

Fig. 1. Apparatus for dynamic thin-layer chromatography.

pyridinium sulphates (KAY AND WARREN[4], MCKENNA AND RIPPON[5]) were applied and developed in a solvent mixture of chloroform–methanol–ammonia with the fluorescent light shining on to the coated side of the plate.

Fig. 2 shows the plate fully developed and photographed *in situ*. The time of development was 25 min, when the solvent front had advanced to 10 cm from the starting point and the maximum resolution was reached.

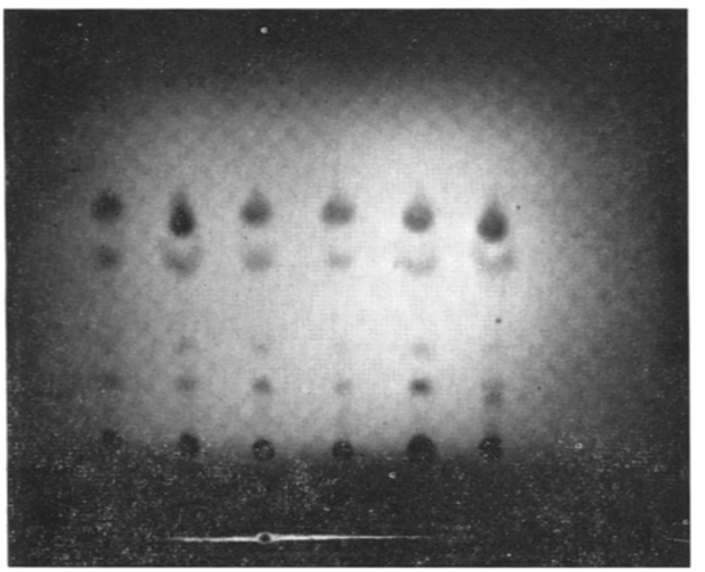

Fig. 2. Chromatogram showing the separation of plasma steroid pyridinium sulphates photographed in the apparatus under 2537 Å illumination.

As migration and separation are continuously visible in this apparatus the development can be stopped when the optimum resolution is attained.

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