

## The purification of protoporphyrin IX and protoporphyrin IX dimethyl ester with Avicel-SF and Chromagram sheet

We have used cellulose thin-layer chromatography for the purification of commercial protoporphyrin IX (Calbiochem) and protoporphyrin IX dimethyl ester prepared by the method of GRINSTEIN<sup>1</sup>. Avicel-SF (microcrystalline cellulose)\* (ref. 2), which adheres firmly to glass without binders, needs little or no activation, and can be used for preparative work by increasing the thickness of the layers, was used for both compounds. Microscope slides can be coated very easily and used in small screw-cap jars for very rapid testing of solvent systems. Solvent systems which work on paper lend themselves with some modifications to this medium. For porphyrins, 2,6-lutidine-water-ammonia (10:3:vapor)<sup>3</sup> was used and for porphyrin esters, naphtha (or benzene)-*n*-propanol (52:2) was successful.

We have also used Eastman Chromagram Sheet\*\* for the purification of protoporphyrin IX dimethyl ester in benzene-ethyl acetate (9:2) in the Eastman Chromatoplate apparatus. Large samples of material can be purified by applying the sample as a streak along the origin.

### Experimental

Preparation of cellulose-coated plates: 100 g of Avicel-SF and 400 ml of water were mixed in a Waring blender for one minute. The resultant slurry could be spread or poured on glass plates and microscope slides and air-dried.

Chromagram sheets were heated at 100° for one hour at 15 mm.

Protoporphyrin IX was prepared at room temperature from hemin<sup>4,5</sup> and a sample was also purchased from Calbiochem.

Protoporphyrin IX dimethyl ester was prepared by the method of GRINSTEIN<sup>1</sup>.

Mesoporphyrin IX was generously supplied by Prof. DAVID SHEMIN.

Coproporphyrin III tetramethyl ester was purchased from Calbiochem.

Mesoporphyrin IX dimethyl ester was prepared from mesoporphyrin IX in 5% sulfuric acid in methanol. Coproporphyrin III was prepared by hydrolysis of the tetramethyl ester in 1% potassium hydroxide in aqueous ethanol.

The porphyrins were applied as solutions in ether-acetic acid or chloroform-acetic acid. Before development the plates were dried *in vacuo* in the dark at 80°. A mixture of 2,6-lutidine-water (10:3) was placed in the developing chamber and a tube of conc. ammonia was set on the bottom. For Avicel-SF the following  $R_F$  values were obtained: protoporphyrin IX, 0.54; coproporphyrin III, 0.023; mesoporphyrin IX, 0.53; protoporphyrin IX (Calbiochem), 0.55, 0.22, 0; for Chromagram sheet: protoporphyrin IX, 0.48; coproporphyrin III, 0.067; mesoporphyrin IX, 0.47; protoporphyrin IX (Calbiochem), 0.47, 0.0.

Porphyrin methyl esters were applied to Avicel-SF plates and to Chromagram sheet in chloroform solution. The developing solvent was benzene (naphtha)-*n*-propanol (52:2). The  $R_F$  values obtained were: protoporphyrin IX, 0.80; mesoporphyrin IX, 0.87; coproporphyrin III, 0.63.

The solvent system used with Chromagram sheet for porphyrin methyl esters was benzene-ethyl acetate (9:2). The following  $R_F$  values were obtained: protopor-

\* American Viscose Co., Marcus Hook, Pennsylvania, U.S.A.

\*\* Distillation Products Industries, Rochester, New York, U.S.A.

phyrin IX, 0.47; coproporphyrin III, 0.11; impure protoporphyrin IX prepared according to GRINSTEIN<sup>1</sup>, 0.47, 0.32, 0.17. When samples were applied in streaks along the origin, the developed chromatogram could be eluted in zones for the determination of spectra.

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## **Separation of some oxine derivatives on thin layers impregnated with ferric chloride**

The present methods available for the separation of oxine derivatives are inadequate. The method of CASTIGLIONI<sup>1</sup> will effectively separate oxine from iodochloroxine but does not distinguish between various halogenated derivatives. The method of KORZUN, BRODY AND TISHLER<sup>2</sup>, using polyamide layers does not separate 5,7-dichloroxine and iodochloroxine, or 5-monoiodo- and 5,7-diiodo-oxine.

Previous work in this laboratory, CLEGG AND CAWTHORNE<sup>3</sup> using Kieselgel G impregnated with phosphate buffer pH 5 (0.1 M) gave similar  $R_F$  values for all the halogenated derivatives examined. The author has used a reverse phase system of Kieselguhr—5% liquid paraffin in petroleum ether—acetone. Whilst separation could be achieved with this system, streaking could not be prevented. In connection with this work, it was found that spraying with a saturated solution of magnesium acetate in methanol and then viewing under 350 m $\mu$  light showed the oxine derivatives as green fluorescent spots against the blue background. This method of detection was found to be superior to one using diazotised reagents.

The separation of oxine derivatives was attempted by impregnating plates with ferric chloride. The choice of ferric chloride for chelation was made because of the known colour reaction of this material with oxine derivatives<sup>4</sup>.

### *Experimental*

*Preparation of plates.* The suspension for five plates (20 cm  $\times$  20 cm) was prepared from 30 g Kieselgel G (E. Merck A.G., Darmstadt) or 30 g Kieselguhr (E. Merck A.G., Darmstadt) by the addition of 60 ml water containing 6 g ferric chloride hexahy-