

The absorption maximum of protohaemin ME was found at 400 nm in chloroform (Fig. 2). Under other conditions the absorption maximum was found at 401–403 nm<sup>5</sup>. With the relatively flat maximum of the absorption curve (see Fig. 2), such a difference is not significant for the quantification of haemin; this was tested by comparing the height of the absorption at these maxima with various weights.

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### Calcium sulfate as an adsorbent for thin-layer chromatography

Calcium sulfate in the form of plaster of Paris ( $\text{CaSO}_4 \cdot 1/2\text{H}_2\text{O}$ ) is commonly used as a binder in thin-layer chromatography (TLC). As such, it is present in small amounts (5–13%) in commercially available adsorbents such as Silica Gel G, Aluminum Oxide G, and Cellulose Powder G.

As early as 1949, BROCKMAN<sup>1</sup>, in work on column chromatography, reported that  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  can be converted to an excellent adsorbent by heating it at temperatures between 150° and 200°. In 1962, KAUFMANN AND KHOE<sup>2</sup> used  $\text{CaSO}_4 \cdot 1/2\text{H}_2\text{O}$  as an adsorbent for the thin-layer chromatographic separation of fatty acids and synthetic and natural triglycerides. More recently, ALFONSO<sup>3</sup> described the use of self-supporting strips of plaster of Paris ( $\text{CaSO}_4 \cdot 1/2\text{H}_2\text{O}$ ), 1 to 5 mm thick, in preparative chromatography. Aside from these few references, the use of calcium sulfate by itself as a chromatographic adsorbent has been almost completely ignored. This may have been due to the difficulty in obtaining a plaster of Paris of suitable purity and uniformity.

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KAUFMANN AND KHOE<sup>2</sup>, who used a commercial  $\text{CaSO}_4 \cdot 1/2\text{H}_2\text{O}$ , pointed out the importance of its quality.

Calcium sulfate occurs in nature as gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) and as anhydrite ( $\text{CaSO}_4$ ), neither of which has adhesive properties suitable for TLC. But plaster of Paris, formed by calcining gypsum, does have such properties when properly treated. When slurried with water, plaster of Paris hardens, adheres to the glass plate used in TLC, and changes to gypsum. On activation, the gypsum thus formed is stable if it is not heated above  $60^\circ$ . In this form it is an excellent adsorbent for TLC and has definite advantages over many of the commonly used adsorbents. With  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  (Fisher Certified Reagent, Precipitate Calcium Sulfate, Cat. No. C-140) as our starting substance, we have been able to consistently prepare excellent TLC plates.

We prepared our plates by the following procedure:  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  was spread in a dish or tray and dried overnight at  $175^\circ$ . After it had cooled, 35 g of  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  was mixed with 55–60 ml of water to provide sufficient slurry to coat five  $8 \times 8$  in. plates. The slurry was applied in the usual manner. The plates were air-dried until set (10–15 min) and then activated by heating in an oven for one hour at  $60^\circ$ . After cooling, the plates are ready to be used.

Calcium sulfate TLC plates prepared as described have a number of advantages over the commonly used adsorbents. The adsorbent forms a very hard and non-flaking layer which can be easily coated with an immobile solvent, for example, by dipping the plate in an ether solution of mineral oil. The same solvent systems used to develop paper chromatograms can be used with the calcium sulfate TLC plates. Since the adsorbent is neutral or relatively inert, enzyme reactions may be carried out on the plate. The spot can be very easily removed from the developed plate by drawing a line around the spot with a hard pencil and then lifting up the intact spot. The compound of interest is then eluted from the removed adsorbent.

In our laboratories, we have used calcium sulfate TLC plates for the separation of organophosphate pesticide residues. The spots are located by enzyme inhibition reaction directly on the plate with a sensitivity as good as, or slightly better than, that attainable by paper chromatography<sup>4</sup>.

In general, most chromogenic reagents which can be used on silica gel or alumina plates or on paper chromatograms can also be used successfully on the calcium sulfate plates.

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