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Reduction and detection of aromatic nitro-compounds on thin layers of silica gel

Aromatic nitro-compounds are important derivatives for the chromatography of alcohols and carbonyl compounds as their 3,5-dinitrobenzoates (DNB) and 2,4-dinitrophenylhydrazones (DNPH) respectively. Unfortunately detection of these derivatives on thin layer chromatograms in very small amounts has proved difficult, despite the intense colour of DNPH derivatives. Therefore attempts were made to devise more sensitive methods of detection.

Attempted saponification of a model compound (1,2-dinitrobenzene) on thin layers of silica gel with alcoholic NaOH (4 %) at 105° gave no colouration on subsequent diazotisation and coupling according to FEIGL's method[1] for polynitroaromatic compounds. Similarly an attempt to reduce the nitro group to an amino group by spraying an ethereal solution of lithium aluminium hydride on to the spotted silica gel layer was unsuccessful. Visualisation of amine (by diazotisation with acidified 5 % $NaNO_2$ solution followed by coupling with 0.1 % N-1-naphthyl-ethylenediamine dihydrochloride (NEDDI) in water to develop the azo dye[2]) showed no colour. Presumably reduction had not taken place.

However a second attempt using tin and HCl to reduce the nitro group succeeded. As indicated below, the technique used did not interfere with the separation of a mixture of DNB derivatives. TLC plates were prepared by shaking silica gel (Whatman SG41) containing finely powdered tin (5 % w/w) with water for 1 min, spreading, drying and activation at 105° before spotting the layer with model compounds. The plate was placed in a developing tank containing HCl vapour, left overnight, by which time the specks of tin had disappeared, and sprayed with acidified $NaNO_2$ solution (5 % $NaNO_2$). After standing for about 1 min the plate was sprayed with NEDDI solution. An intense pink-purple colour developed on the model compound spots with very little discolouration of the background.

Comparison of the reducing power of different finely powdered metals incorporated into silica gel thin layers showed that tin and zinc gave rise to vivid spots on reduction and visualisation without disruption of the surface of the layer. Magnesium and iron gave less strongly coloured spots. The Fe/silica gel layer became yellow in the HCl tank, presumably due to the formation of ferric compounds. Incorporation of tin or zinc in cellulose thin layers (5 % w/w with Whatman CC41) failed to give consistent colour production on subsequent reduction and visualisation. Probably BARTON's procedure[3] of spraying a suspension of, or dusting with, zinc powder followed by reaction with HCl spray is more effective for thin layers of cellulose.

The sensitivity of the method for silica gel thin layers was determined by the application of aliquots of standard solutions of *iso*-butyl methyl ketone DNPH and of a mixture of the DNBs of ethanol, *n*-propanol, *n*-butanol and 3-methyl-butan-1-ol and development of the colours. Spots containing 0.1 μg of derivative on 0.25 mm thick layers of silica gel were too faint to be seen but spots containing 0.5 μg or more DNB or DNPH were readily visible. Chromatography of the mixture of DNBs in diethyl ether: 60/80° petroleum spirit (5:100) indicated that 0.3 μg was detectable

after development and visualisation while 1.0–5 μg quantities of the mixture were readily visualised as four distinct pink-purple spots.

This technique is currently being used to locate DNB and DNPH derivatives of compounds present in honey aroma after separation by TLC. It could have wider application as a general technique for reductions on thin layer chromatograms.

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1 F. FEIGL, *Spot Tests in Organic Analysis*, Elsevier, Amsterdam, 1960, p. 176
2 J. D. H. STRICKLAND AND T. R. PARSONS, *A manual of sea water analysis*, Fisheries Research Board of Canada, Ottawa, 1960, p. 63.
3 G. M. BARTON, *J. Chromatog.*, 34 (1968) 562.

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J. Chromatog., 38 (1968) 399–400

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Separation of cello-oligosaccharides by thin-layer chromatography*

While studying the initial products of hydrolysis of cellulose by rumen micro-organisms of the sheep it was found that separation of the cello-oligosaccharides by paper chromatography was time consuming and resulted in poor resolution. To achieve better separation of the oligosaccharides it was decided to make use of thin-layer chromatography (TLC).

The application of TLC for the separation and identification of malto-oligosaccharides has been reported by many workers[1–3]. On the other hand, little information is available on the resolution of cello-oligosaccharides by this method. BECKER *et al.*[4] reported on the separation of cellulose degradation products eluted from a charcoal–celite column by TLC on Kieselguhr G using *n*-butanol–ethanol–water (50:30:20, v/v). A suitable technique for the separation of cello-oligosaccharides by TLC is described in this communication.

Experimental

The chromatoplates (20 cm × 60 cm) were coated with Kieselgel G or Kieselguhr G (Merck & Co) to a thickness of 250 μ, according to the procedure described by STAHL[5]. Solutions of cello-oligosaccharides** (cellobiose, cellotriose, cellotetraose,

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