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Note

Novel methods for the detection of polystyrene used as an internal standard in thin-layer gel chromatography

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In thin-layer gel chromatography, there is no solvent front to which the migration distance of a sample component is referred, so that the R_F of the component is expressed in terms of the distance travelled by a standard substance; materials that are completely excluded from the network of the gel are usually incorporated as internal standards. In organic-solvent media, polystyrene is often used as internal standard because polystyrene species with various mean mol. wt. are commercially available. Thin-layer gel chromatography of polystyrene has so far been conducted only on silica gel, and the detection has been effected by spraying with corrosive reagents, such as permanganate-concentrated sulphuric acid¹, or thymol blue-concentrated sulphuric acid². Otsuka *et al.*³ tried to incorporate a fluorescent indicator ($ZnSiO_3$) in the silica gel layer in order to detect polystyrene as a dark spot under UV radiation. The methods cited, however, cannot be used with very fragile organic gel layers such as polystyrene-divinylbenzene copolymer or poly(vinyl acetate). In this connection, we have devised two novel detection methods for standard polystyrenes; one is conducted *in situ*, with no chromogenic reagents or apparatus, and the other involves use of a non-corrosive reagent (antimony pentachloride) on a paper replica.

EXPERIMENTAL

Standard polystyrene (mol. wt. 2×10^5) obtained from Pressure Chemical Co. (Pittsburgh, Pa., U.S.A.) was used, and the layers were prepared from Styragel 60A, a polystyrene-divinylbenzene copolymer of particle size $< 37 \mu m$ (Waters Assoc., Milford, Mass., U.S.A.), and Merckogel OR-PVA 2000, a poly(vinyl acetate) (E. Merck, Darmstadt, G.F.R.).

A small amount of the swollen gel slurry was spread on standard glass plates (20 × 20 cm) with an ordinary spreading device (Yamato Scientific, Tokyo, Japan); two uniform layers (250 μm thick) were made from each batch of slurry, and a strip (*ca.* 5 mm wide) of the gel layer was scraped from each side. Before the gel became dry, the plate was placed in a developing apparatus⁴ for development as described previously⁴.

Stock solutions (2-10 mg/ml) of standard polystyrene were prepared by dissolving appropriate amounts of the polystyrene in the organic solvents to be used

for development, and a 1- μ l portion of such a solution was applied to the gel with a Drummond micro-pipette. After development, the polystyrene was detected directly on the plate, or on a paper replica, as described below.

In situ detection

We found that, when the plate was taken out of the developing apparatus after development and then dried in air, tapping a side of the plate caused Styragel 60A to fall easily from the glass except in the position occupied by the polystyrene, at which the gel grains adhered tightly. In this way, we could locate the polystyrene without any chromogenic reagent or apparatus. This method was particularly suitable when using volatile developing solvents such as chloroform, ethyl methyl ketone or benzene.

Merckogel OR-PVA 2000 grains, on the other hand, tended to adhere tightly to the glass after development, even when the cited solvents had been used. Thus, it was necessary to remove the Merckogel grains from the plate (except from the position occupied by the polystyrene) by careful washing with an appropriate organic solvent, *e.g.*, ethanol. The washing stage was critical, so that, for Merckogel OR-PVA 2000 layers, we recommend detection by use of a paper replica.

Detection on paper replica

The wet gel layer is very fragile and difficult to handle, so that sample detection with chromogenic reagents must usually be carried out on a paper replica.

After development, the plate is taken out of the developing apparatus and a sheet of silicone-treated paper (Whatman ST 81, W. & R. Balston Ltd., Springfield Mill, Maidstone, Great Britain) that has been impregnated with the developing solvent is quickly placed on the gel layer and left in contact with it for several minutes. In this step, use of a dry filter-paper, as is usual with aqueous systems, resulted in unsatisfactory "printing" of the polystyrene (due to rapid evaporation of solvent from the gel layer). As the solvent evaporated from the filter-paper, most of the polystyrene was transferred from the gel to the paper. The use of hydrophobic silicone-treated filter-paper is therefore preferable and ensures better "printing" of the polystyrene. The polystyrene on the paper replica is located as a red spot by spraying with a 20% (w/w) solution of antimony pentachloride in carbon tetrachloride and heating gently over a hot-plate.

DISCUSSION

In this method, preparation of a paper replica was successfully accomplished with solvents of rather low volatility, such as dioxan and isobutyl methyl ketone. Both the *in situ* and replica methods were equally applicable when an ethyl methyl ketone developer was used. When more volatile solvents (*e.g.*, benzene or chloroform) were used for development, preparation of reproducible replicas was difficult. For these solvents, when used in conjunction with Styragel 60A, the *in situ* method is recommended. Paper replicas can be prepared more easily from layers of Merckogel OR-PVA 2000 than from those of Styragel 60A.

A survey of the literature indicated that BDH universal indicator as used by Langford and Vaughan⁵ was a possible reagent for the detection of polystyrene and

other long-chain macromolecular substances [including poly(vinyl chloride) and poly(vinyl acetate)] in paper chromatography. We tested many reagents and finally succeeded in detecting polystyrene on paper replicas by using antimony pentachloride, which has been used for the detection of D vitamins⁶ and sterols^{6,7} in paper chromatography. Antimony pentachloride is a common and inexpensive reagent, and should find many uses in thin-layer gel chromatography.

The proposed *in situ* method and the paper replica-antimony pentachloride method permit as little as 1 μg of polystyrene to be detected.

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