Chromatoplate contamination removal

The presence of contaminating material in the silica gel powder used in preparing thin-layer chromatoplates has been pointed out and several procedures described to lessen or remove the problem[1-6]. A need for high quality plates becomes particularly acute when the thin-layer method of fractionation is followed by a complementary procedure capable of detecting low levels of impurity. Here one would like to have the assurance that of all components analyzed none originated in the thin layer.

In this communication a simple apparatus is described whereby thin-layer chromatoplates can be pre-cleaned. Silica gel purity was tested for the efficacy of the technique by gas–liquid chromatography of the residue extractable with diethyl ether.

Design of the apparatus

In Fig. 1 are shown the stand for supporting two 20 × 20 cm chromatoplates (left) and the complete cleaning system in operation (right).

Materials used to construct the apparatus were as follows: High quality stainless

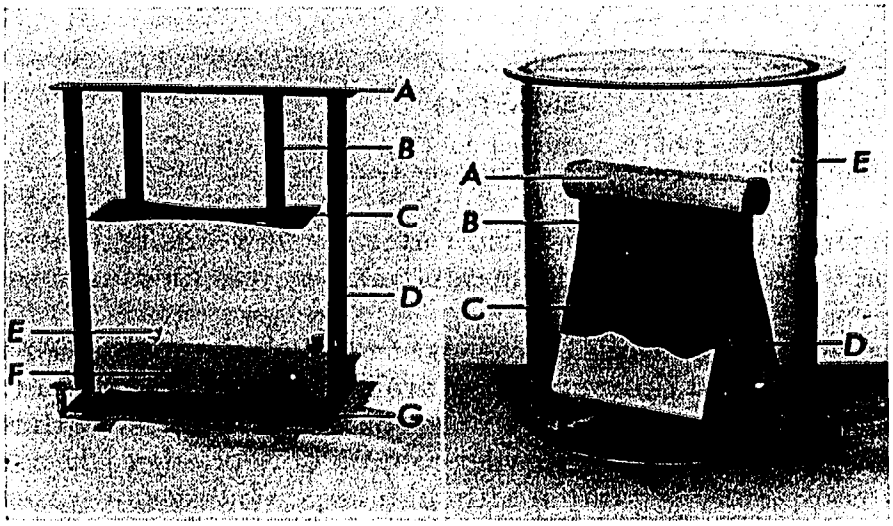

Fig. 1. Apparatus for cleaning thin-layer chromatoplates. Chromatoplate stand (left): A = cross bar (1.9 cm × 21.6 cm × 3.2 mm); B = hanging arm (8.9 cm × 12.7 mm diam.); C = center support (6.4 cm × 12.7 cm × 3.2 mm with curved indentation on long sides); D = side arm (25.4 cm × 12.7 mm diam.); E = projecting support arm (1.27 cm × 2.5 cm × 3.2 mm with 40° bend to hold plate; F = platform base (10.2 cm × 21.6 cm × 3.2 mm); G = leg (2.5 cm × 6.4 mm diam.). Complete system in operation (right): A = teflon boat containing cleaning solvent; B = paper wick; C = coated plate showing solvent front; D = chromatoplate stand; E = covered glass chamber.

steel was used in assembling the stand. The boat was fashioned from a round teflon rod 23 cm long and 5 cm in diameter available from the Crystal-X Corporation Plastics, Lenni Mills, Pa. The rod was hollowed out from a 2.5 cm × 21 cm opening to form a well of approximately 200 ml capacity. In the 1.9 cm wide flat bottom made to fit on the cross bar of the stand four holes of 0.35 mm diameter were drilled evenly distributed on each side. The boat rested on a 18 cm × 20 cm strip of Whatman No. 1 paper lying over the cross bar of the stand and folded to form two flaps (wicks) which were used for conducting the cleaning solvent to the chromatoplates.

Procedure

To the boat was added 200 ml of methanol–diethyl ether (8:2, v/v), a highly polar cleaning solution for coated plates first described by BROWN AND BENJAMIN[3]. The solvent drained down the wick and across the chromatoplates at right angles to the direction of subsequent chromatogram development. Silica gel layers (250 μ thick) either with or without calcium sulfate binder were cleaned with this technique. Passage of 100 ml of solution down each plate required about 40 min. There was a

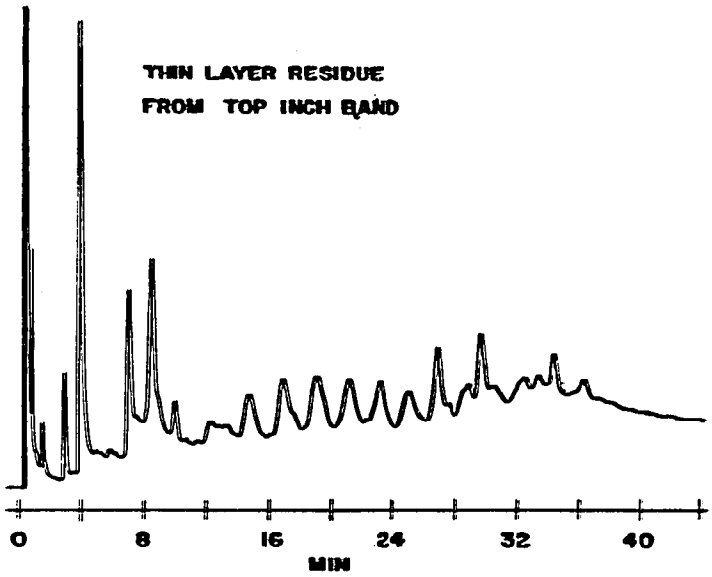

Fig. 2. Gas chromatographic analysis of impurities from developed thin-layer chromatoplate.

tendency on wetting for the silica gel to slip off those plates which had not been properly cleaned prior to application of the adsorbent layer.

The procedure for testing adsorbent cleanness after plate activation at 110° for 1.5 h in a forced draft oven was as follows: To the silica gel powder (0.20 g), scraped off one-half a 2.5 cm wide band, was added 5 ml of diethyl ether, and the mixture was swirled vigorously with a Vortex mixer. Following solvent removal the adsorbent was extracted twice more and the combined ether layers were evaporated to dryness.

For gas chromatographic analysis 10 μl of hexane was added to the residue and the whole volume (7–8 μl) able to be taken up with a microsyringe was injected directly on the column. This was a 1.2 m × 3 mm I.D. Pyrex glass tube packed with 5 % diethylene glycol succinate polyester (DEGS) coated on 80–100 mesh diatoport S. Helium was used as carrier gas at a flow rate of 75 ml/min with temperature programming from 100° to 210° at 3°/min. A flame ionization detector was used.

All solvents were checked before use for interfering impurities under the above-described gas chromatographic conditions.

Results

There is little doubt about the importance of purifying adsorbents used for TLC when considering the results obtained with a batch of silica gel G secured several years ago (Fig. 2).

That the problem of contamination in currently available powders still remains,

although at a reduced level, is evident from the gas chromatogram recordings shown in composite form in Fig. 3. In the top two sections, designated A and B, are given the results from an unwashed plate and one cleaned in the manner shown in Fig. 1, respectively. The former gave a more unstable, elevated base line. Rather than sharp, distinct peaks, the impurities were represented by a general broad rise above the base line. Various bands of the prewashed plate were analyzed for uniformity of results with the purifying procedure and found not to differ. The rise in base line in the latter region of all chromatograms was because of the increased column bleed of the polyester liquid phase at the elevated temperatures.

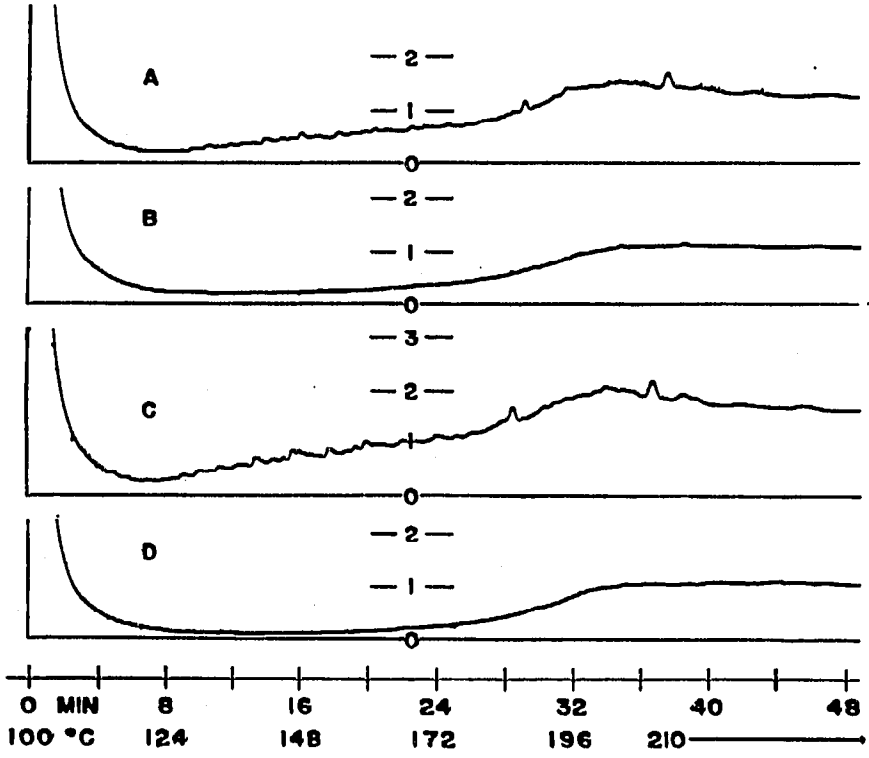

Fig. 3. Gas chromatograms of diethyl ether-extractable residue from bands of thin-layer adsorbent scraped off chromatoplates treated differently. A = Unwashed plate; B = plate washed with apparatus shown in Fig. 1; C = developed plate not previously washed; D = developed plate forewashed according to the procedure described here. The developing solvent was hexane–diethyl ether–acetic acid (80:20:1, v/v/v); the cleaning solvent, methanol–diethyl ether (8:2, v/v). The numbers in the center of the figure refer to the recorder response.

The more exacting test of purity required that prior to GLC analysis the chromatoplate be developed to concentrate any impurities remaining in the adsorbent subsequent to the cleaning treatment. Normally the region demonstrating impurity accumulation best is that just beneath the finish line. Fig. 3C shows that the contamination level in this band from an unwashed plate developed with a solvent mixture commonly used in lipid class separations[7] was indeed clearly increased over that found with an undeveloped plate (Fig. 3A). However, when the thin-layer was precleaned with the apparatus described here and then developed, this upper band was essentially free of impurities measured by the gas chromatograph (Fig. 3D).

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Washing of adsorbents for thin-layer chromatography

As the technique of thin-layer chromatography (TLC) has diversified, there has been an increasing need for adsorbent layers which, for one reason or another, have been prewashed with some special solvent. In this laboratory, for example, we have been removing divalent cations before phosphate ester chromatography; removing ninhydrin-positive substances before quantitative amino acid assay or peptide mapping; and removing oily substances which otherwise interfere when TLC spots or bands are eluted and rechromatographed. Originally we eluted each TLC plate individually in the washing solvent prior to chromatography. We have now found that we can achieve faster and better results by washing the adsorbent in bulk on a Buchner funnel. Though we have applied the technique only to cellulose powders (M & N 300 and Whatman No. CC 41 both require treatment), other adsorbents can probably be treated in the same way. Besides removing specific impurities, the washing has two other desirable results. The subsequent flow rate of relatively viscous solvents is increased, up to 50 %, and virtually no residue remains at the dried solvent front after chromatography in the first dimension. Trimming the dried front from the plate is not needed, and running in the second dimension is much more uniform. Details of a typical washing procedure are given.

Cellulose powder, 50 g, was homogenized with 250 ml 80 % methanol (or methanol–formic acid–water, 80:12:8), the slurry was poured into a 9 cm diameter Buchner funnel over two filter papers, and filtered slowly under low vacuum (400 mm Hg). When the surface of the pad became free of liquid, but before it cracked, a further 250 ml methanol–formic acid–water (60:10:30) was poured on (if the cellulose