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Note

Simplified procedure for the quantitation of radioactive phosphoinositides by thin-layer chromatography

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While establishing a system in this laboratory for the quantitation of [^{32}P]phosphoinositides, we made the fortuitous observation that silica gel thin-layer chromatography (TLC) plates which had been chromatographed in strongly polar solvents can be slightly wetted and then easily scraped with a razor blade resulting in the silica gel scrolling off of the glass plate into a single, easily managed piece of silica gel. This method is far superior to the standard procedure for recovery of compounds chromatographed on TLC which involves scraping a dry silica gel plate and quantitatively recovering the "chips" of silica which are difficult to handle and easily lost[1–3]. This report describes in detail the procedure for scrolling of these chromatographed lipid spots.

MATERIALS AND METHODS

Silica gel 60 thin-layer plates, 0.25-mm thickness with preadsorbent spotting area were purchased from Merck (Darmstadt, F.R.G.) and J. T. Baker (Phillipsburg, NJ, U.S.A.). Aerosol power cans (Chromist) were from Gelman (Ann Arbor, MI, U.S.A.). All solvents were of reagent grade.

^{32}P-Labeled lipids from human erythrocytes were prepared and isolated according to Ferrell and Huestis[4]. Lipids were separated by chromatography in chloroform–methanol–3.3 *N* ammonium hydroxide (75:60:19)[4].

RESULTS AND DISCUSSION

The thrust of this technique is that after chromatography, silica gel thin-layer plates can be wetted just enough to interfere with the silica's adherence to the plate but not enough to turn the silica into an unmanageable "mush". Specifically, after TLC, [^{32}P]phospholipids are localized by autoradiography and outlined in pencil on the plate. The glass-backed silica gel plate is then held vertical with backlighting, and the plate is sprayed with de-ionized water utilizing an aerosol power can (Fig. 1A). Only experience with the technique will allow the investigator to know what is a sufficient amount of water to spray, but in our experience the appropriate amount is when the silica gel just begins to turn translucent to the backlighting. An alter-

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native, but technically more difficult procedure, is to press a damp paper towel to the plate area to be scraped.

After wetting, one edge of the spot to be scraped is scored with a razor blade. The spot is then "shaved" from the glass by starting opposite the scored edge of the spot and using a clean, sharp safety razor blade with the blade angled away from the direction of movement and the razor pressed firmly to the glass. The angle of the blade should be 30 to 45 degrees above horizontal (see Fig. 1B). Each band is shaved from the glass in one smooth stroke. When the method is done properly, the silica will roll into a scroll (see Fig. 1C) which will pop loose from the plate when the blade reaches the scored edge of the spot. If the plate is too wet, the silica will come off in a crumbly slab; if the plate is too dry, the silica will come off in chips. Both of these extremes are easily managed by either waiting a few minutes for the plate to dry to the right consistency or respraying the plate, respectively. When done properly the silica from a spot as small as one half square centimeter will form a scroll that can be picked up from the plate using a hypodermic needle (Fig. 1D).

While refining this technique, it became apparent that the procedure does not work if the TLC plate has not been developed with the chromatography solvent; *i.e.*, a brand new plate cannot be scraped with this technique. The critical parameter was determined to be water exposure during chromatography. If the chromatography solvent does not contain at least 8% water, then the procedure does not work. With this limitation in mind, we have not proposed this scraping procedure as a general technique; however, it is clearly applicable to a variety of systems that use very polar

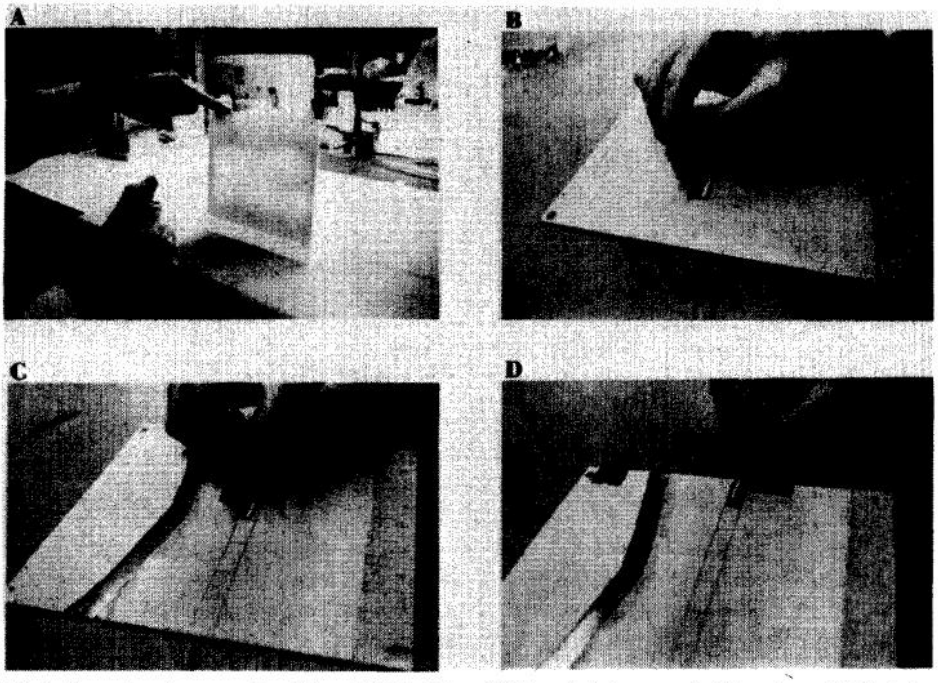

Fig. 1. Procedure for scrapping silica gel TLC plates. (A) The plate is sprayed with water until it just starts to turn translucent to the backlighting. (B, C) The silica is scrolled off the plate with a clean razor blade held at a 45° angle. (D) The final scroll is rigid enough to be lifted with a hypodermic needle.

solvents for chromatography. For example, we have successfully used this scraping technique for the quantitation of [^{32}P]nucleotides which are separated in a water–dioxane–ammonium hydroxide system[5].

One concern with this technique is whether spraying water on the plate will cause diffusion and band spreading of the chromatographed compounds. We have examined this with red blood cell lipids which were separated in two dimensions. The lipid spots were stained in an iodine chamber and photographed. The iodine was then allowed to evaporate from the plate, and the plate was sprayed with water as outlined above. After drying, the plate was again placed in an iodine chamber and photographed; the lipid spots remained unchanged (data not shown). The fact that a water spray does not affect the lipid spots is further supported by a previous observation that water spraying can be used to localize lipid spots on thin-layer chromatograms[1].

In our experiments, the scrolls of scraped silica produced with this technique are placed directly into scintillation vials for quantitation of radioactivity; however, the scrolls are quite suitable for transfer to a standard extraction apparatus if the investigator wishes to isolate a chromatographically purified compound. The advantages of this extremely simple technique are its speed and ease of performance. In addition, it avoids some of the inherent problems of working with dry silica, such as poor reproducibility resulting from loss of chips and the health hazards associated with silica dust.

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REFERENCES

1 H. Gänshirt, in E. Stahl (Editor), *Thin-layer Chromatography*, Springer-Verlag, New York, 2nd ed., 1969, p. 133.
2 O. Sudilovsky and P. H. Hinderaker, *Anal. Biochem.*, 45 (1972) 525.
3 H. Falk and K. Krummen, *J. Chromatogr.*, 103 (1975) 279.
4 J. E. Ferrell, Jr. and W. H. Huestis, *J. Cell. Biol.*, 98 (1984) 1992.
5 G. E. Bronnikov and S. D. Zakharov, *Anal. Biochem.*, 131 (1983) 69.