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Thin-layer chromatography using a mixed silica gel–cellulose layer

A thin-layer chromatographic procedure has been developed that permits direct analysis of crude plant extracts plus separations generally obtained only by using two different stationary phases. In plant metabolism studies, it is best to chromatographically analyze crude extracts directly with no prior clean-up steps. Any additional step that is required can result in undetected losses of unknown metabolites. However, many times crude extracts chromatographed directly result in streaking and gross interferences. Also, in metabolism studies and many other areas of work, it is necessary to know that an observed spot is due to only one compound. This is generally accomplished by chromatography in two entirely different systems. In the procedure described, these analyses can be performed efficiently on one thin-layer plate. The procedure is based on the use of a mixed cellulose and silica gel thin layer. In aqueous solvent systems the silica gel is deactivated and remains inert resulting in chromatograms typical of cellulose systems. In organic solvent systems the cellulose is inert and chromatograms typical of silica gel systems are obtained. Using two-dimensional development, both cellulose and silica gel separations can be obtained using only one plate and requiring no tedious transfer of samples from one plate to another or from a plate to paper or vice versa.

A literature survey disclosed that a mixed cellulose–silica gel thin layer has been used by several workers[1,2]. In one instance[1], it was used in a two-dimensional manner with aqueous solvent systems for development in both directions, thus obtaining separations characteristic of only one layer and not two distinct layers. For amino acids it was reported that the tailing was decreased and the separations were increased. Its use for characterizing polynuclear air pollutants[2] has been described. A two-dimensional technique was employed with first an organic solvent system followed by an aqueous solvent system. It was reported that this procedure increased separation and aided characterization.

Experimental

The plates are prepared by mixing 15 g of Silica Gel G (E. Merck according to Stahl) and 15 g of cellulose without binder (Warner-Chilcott) in 100 ml of water in a Waring Blender or vigorously shaking by hand. A 250 μ layer is spread on 8 $\times$ 8 in. glass in the conventional manner. The plates are then allowed to air dry at ambient temperature for 1–2 h followed by oven drying at 110°.

Results and discussion

To illustrate the chromatographic characteristics of the mixed cellulose–silica gel layer, the separation of *m*-(1-methylbutyl)phenyl methylcarbamate (I), *m*-(1-methylbutyl)phenyl N-hydroxymethylcarbamate (II) and *m*-(1-hydroxy-1-methylbutyl)phenyl methylcarbamate (III) are discussed.

Using conventional organic solvent systems with a silica gel layer, II and III are not separated. However, I, II and III are separated using an aqueous solvent system with a cellulose layer. Unfortunately in crude plant extracts, the natural plant material does not tend to move in the cellulose system and tends to hold back

and mask I, II and III. However, in the silica gel system the natural plant material migrates and allows I, II and III to migrate.

One solution for obtaining clean-up with the silica gel system and separation with the cellulose system is the procedure described by IRVINE AND ANDERSON[3], who describe an apparatus and technique for transferring samples by elution from a thin-layer plate to a paper or from one thin-layer plate to another plate, etc.

Another solution is the technique described by GILMORE AND CORTES[4] and others which uses a reservoir divider in the thin-layer spreader enabling preparation of plates containing two bands each of a different adsorbent. This technique has been used in our laboratories with plates containing approximately a 1½ in. band of silica gel and a 6½ in. band of cellulose. Crude plant extracts were analyzed by spotting the sample on the silica gel band and developing in the first direction in an organic solvent system, then turning the plate and developing in an aqueous system on the cellulose layer. This procedure was not entirely satisfactory. The sample did not always migrate in a straight line on the silica gel band due to the irregular solvent front caused by the differing rates of migration of the developing solvent on the silica gel and cellulose adsorbents. Also, the preparation of the plates was somewhat of an art.

Using a mixed cellulose–silica gel thin layer in a two-dimensional manner, preliminary extract clean-up and final product separation can be easily obtained on one plate. To compare the chromatographic characteristics of the mixed silica gel–cellulose layer with a silica gel layer and a cellulose layer, R_F values of I, II and III are given in Table I. The solvent systems are ethyl acetate–toluene (2:1) and acetonitrile–water (1:3). Note that a cellulose layer developed in ethyl acetate–toluene results in compounds I, II and III migrating with the solvent front; however, diluting the cellulose 1:1 with silica gel gives separations typical of silica gel. Also, a silica gel layer developed in acetonitrile–water essentially does not separate compounds I, II and III, but results in a large diffuse spot. However, diluting the silica gel 1:1 with cellulose results in good separations in acetonitrile–water typical of a cellulose layer. Therefore, with a mixed cellulose–silica gel thin layer, the cellulose or the silica gel can be made predominant by judicious choice of developing solvent. Consequently, very difficult chromatographic separations and procedures can be accomplished on one thin layer using two-dimensional development.

TABLE I

COMPARISON OF R_F VALUES ON SILICA GEL, CELLULOSE AND SILICA GEL–CELLULOSE (1:1)

Compound	*Silica gel*		*Cellulose*		*Silica gel–cellulose (1:1)*	
	Ethyl acetate–toluene	*Acetonitrile–water*	*Ethyl acetate–toluene*	*Acetonitrile–water*	*Ethyl acetate–toluene*	*Acetonitrile–water*
I	0.67	0.73	1.00	0.44	0.84	0.56
II	0.48	0.79	1.00	0.68	0.69	0.71
III	0.48	0.79	1.00	0.91	0.69	0.89

The absolute R_F values on a mixed cellulose–silica gel layer differ from those on a plain silica gel or cellulose layer for a given solvent. This is because the active adsorbent has been diluted with an essentially inert support. Similar R_F values can be obtained by varying the solvent ratios. For example, ethyl acetate–toluene (1:4) on a mixed layer gives similar R_F values to ethyl acetate–toluene (2:1) on silica gel. Likewise, acetonitrile–water (1:9) gives similar R_F values to acetonitrile–water (1:3) on cellulose.

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Adaptation of Swinny filter holders for the collection and elution of samples from thin-layer plates

The collection of fractions from thin-layer chromatograms with a vacuum into Soxhlet thimbles in special holders[1] or onto the sintered glass plates of filter tubes[2] has been described. We present here an alternative procedure that utilizes the Swinny adapters which were designed for attachment to hypodermic syringes and are primarily used with membrane filters. The use of Swinny filter holders for collection of samples fractionated by gas–liquid chromatography has been reported[3].

Method and materials

Filter paper (Whatman No. 42) or glass fiber filter (Reeve Angel No. 934AH) discs were mounted on the side of the support grid of the holder indicated in Fig. 1(A). The male leur fitting of the holder was attached to a vacuum line with a No. 13 hypodermic needle inserted into a length of tubing (Fig. 1(B)). The sample was aspirated up with the female leur fitting of the holder being used as a nozzle. After the fraction was collected, the assembly was inverted with the vacuum still attached, and the barrel of a syringe was connected to the female leur fitting of the holder. The vacuum was then detached and the adsorbed compound eluted directly by pouring a suitable solvent into the syringe (Fig. 1(C)). If an adequate flow rate was not obtained, the plunger of the syringe was inserted and pressure applied.