Source of error in preparative thick-layer chromatography

Because preparative thin-layer chromatography ("thick-layer" chromatography) allows the facile separation of useful quantities of materials from complex mixtures, this technique has become increasingly popular[1–5]. This paper describes techniques developed in this laboratory for the preparation of plates of reproducible properties, a device for the efficient elution of samples, and the characterization of a troublesome contaminant of several commercial adsorbents.

Preparation of the plates

Table I lists the proportion of adsorbents and liquids used in the preparation of plates from several commercial adsorbents. A well-stirred slurry so formulated is spread on the plate, which is then tapped until a uniform layer is formed. Accurate

TABLE I

COMPARISON OF COMMERCIAL SILICIC ACIDS

Commercial products (Lot No.)	*Thickness (mm)*	*Weight/plate (g)*	*H_2O req. (ml)*	*MeOH req. (ml)*	*Drying temp. (°C)*	*Drying time (initial)*	*Package*	*Aliphatic impurity*
SilicAR TLC 7 GF	1.9	50	72	5	105°	45 min	Glass	trace
(Mallinckrodt PI-1760)	1.9	50	80	—	room	1 day	bottle	
Kieselgel DF-5	1.4	20	48	3	105°	45 min	Plastic	+ + + +
(Camag)	1.4	20	45	15	room	2 days	bottle	
Silica Gel HF_{254}	0.9	30	78	—	105°	45 min	Yellow	+ +
(Merck T 61247)	0.9	30	80	—	room	1 day	plastic	
Kieselguhr G (Merck 6462)	2.2	50	81	9	105°	80 min	Plastic bottle	?
Silica Gel TLC (Woelm 474)	0.9	30	35	—	room	1 day	Al bottle and rubber	+
Silica Gel G (R.S.C. 8076)							Glass bottle	+
Bio-Sil A (Bio-RAD B-2070)							Glass bottle	trace
Silicic Acid (Fisher A-228)							Plastic bag	+ +

adjustment of the amount of water used is important to prevent cracking of the surface during drying. Adsorbent stocks which have been exposed to the air may require 1 to 3 ml less water per plate. The addition of methanol to the slurry may help prevent cracking[2], but absorbents containing a fluorescent indicator may show an uneven background after such treatment. Plates are conveniently air-dried overnight; they may then be activated in an oven without danger of cracking. A cracked plate

may be salvaged by rubbing the cracks gently with dry silica gel. Useful separations of 50 to 300 mg of material can be achieved on a 20 × 20 cm plate so prepared.

Sample elution

Fig. 1 shows an all-glass device which allows efficient elution of samples from the collected bands. A high-speed stirrer (500 r.p.m.) is used to assure that the calcium sulphate binder is pulverized during the extraction. The rapid elution minimized the possibility of decomposition of samples on silica gel, which may result if a Soxhlet apparatus is used.

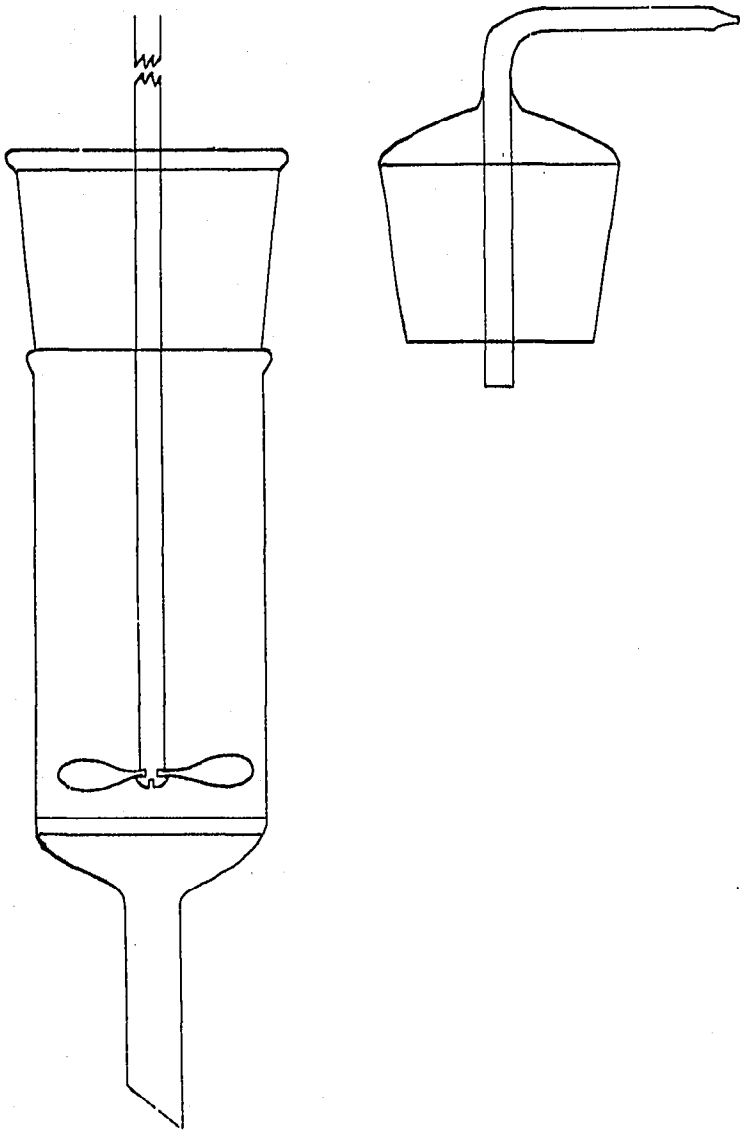

Fig. 1. Extraction apparatus and collection adapter.

The contaminant of commercial adsorbents

In the course of the preparation of various natural materials in this laboratory, it was found that many samples were contaminated by a material revealed by spurious aliphatic peaks in the n.m.r. spectrum. This was ultimately shown to arise from a hydrocarbon constituent of various commercial adsorbents[6].

Twenty-five gram samples of the various adsorbents listed in Table I were packed in a chromatographic column and washed with chloroform thoroughly, and the amount of contaminant was estimated by the intensity of the methylene peak (δ = 1.26 p.p.m.; Fig. 2). As those adsorbents packed in plastic containers yield much more of the contaminant than those from glass bottles, it seems likely that the containers are a chief source of this impurity. Support for this inference was found in the following experiment: Pre-washed Kieselgel GF-5 (Camag) was replaced in its original clean plastic bottle for three weeks with occasional shakings. The same type of contaminant was found again in similar yield. A chloroform extract of the bottle or the rubber stopper produced a similar n.m.r. spectrum, characterized by the methyl-to-methylene ratio. The materials are high-boiling hydrocarbons with molecular weights shown by mass spectrometry to be about 550. Such a contaminant is particularly troublesome in the separation of constituents which are to be characterized by weight, n.m.r., infrared, or mass spectra, and will interfere with subsequent purification by crystallization or distillation. It is to be hoped that suppliers will shortly adopt shipping procedures which obviate such contamination.

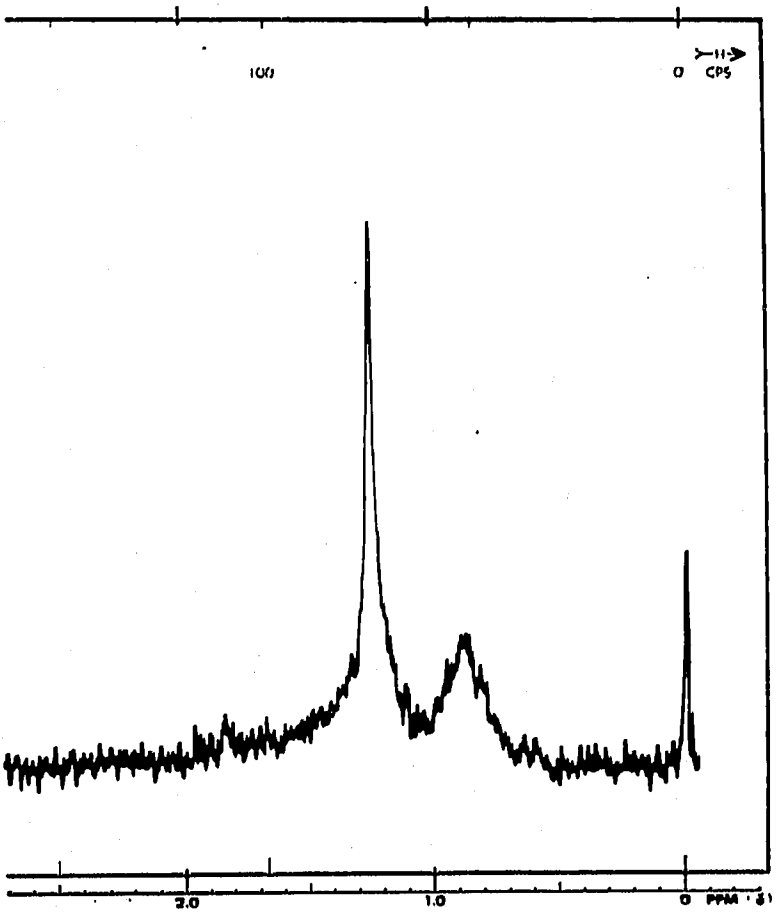

Fig. 2. N.M.R. spectrum of the contaminant from commercial silica gel.

At the present time, adsorbents containing binders are used in this laboratory without purification, and the plates are partially purified by pre-developing with chloroform a few times. An adsorbent without binder is pre-washed with chloroform

in a large chromatographic column, dried at 110° and stored in screw-cap glass bottles with teflon liners, and 12% anhydrous calcium sulfate is added just before use. Formation of thick-layer plates with buffer is listed in Table II.

TABLE II

FORMULATION FOR THICK-LAYER PLATES

	Non-buffer	*pH 2.2*	*pH 4*	*pH 8*	*pH 9.2*	*pH 11*
Kieselgel DF-O ($CHCl_3$-washed)	18 g	18 g	18 g	18 g	18 g	18 g
Calcium sulfate (dried at 200°)	2.4 g	2.4 g	2.4 g	2.4 g	2.4 g	2.4 g
Water	34 ml	10 ml	12 ml	12 ml	12 ml	11 ml
McIlvaine's buffer solution		20 ml	24 ml	24 ml		
Sodium borate, 0.2 *M*					24 ml	11 ml
Sodium borate, 0.2 *M*						11 ml
Thickness	1.1 mm	1.0 mm	1.0 mm	1.2 mm	1.2 mm	0.7 mm

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