THIN-LAYER CHROMATOGRAPHY WITH PRECOATED ALUMINA SHEETS

I. GENERAL PROPERTIES*

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INTRODUCTION

The advent of thin-layer adsorbents precoated on flexible supports profoundly influenced the use of thin-layer chromatography as a tool in separations chemistry[1]. Simplifying the manual manipulations and eliminating some variables common to hand coating of glass plates encouraged the use of TLC in a wider range of areas. As a result of this, a number of articles have been published during the past year describing applications of flexible, precoated silica gel sheets[2-22].

The intent of this paper is to provide general technical data to help the analyst to select experimental conditions and to interpret results obtained when using flexible, precoated *alumina* sheets**. The sheets described consist of a 100 μ layer of alumina bound to a poly-(ethylene terephthalate) film support by the incorporation of a small amount of poly-(vinyl alcohol) as a binder. These sheets are commercially available from Distillation Products Industries, Division of Eastman Kodak Company, and can be obtained with or without an inorganic fluorescent indicator (lead manganese activated calcium silicate). In addition to the general information, the authors have compared data on the separation of various classes of compounds on coated glass plates with coated, flexible sheets.

EXPERIMENTAL

Adsorbent pH

The most significant single property of aluminum oxide as a thin-layer adsorbent is its inherent alkaline nature. Therefore, aluminum oxide complements silica gel (an acidic medium) as a chromatographic adsorbent. Table I compares the pH of a 25 % aqueous slurry of the adsorbent from a precoated alumina sheet with commercially available TLC grade aluminum oxide adsorbents.

While most literature separations utilize the basic properties of aluminum oxide, some very specialized applications can be found for acidic and neutral layers. It is possible to alter the nature and pH of the precoated adsorbent layer by dipping the sheet in an appropriate buffer solution. To obtain neutral layers of approximately

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* Communication No. 333, Distillation Products Industries.
** Eastman Chromagram sheet 6062 (alumina).

TABLE I

COMPARISON OF ADSORBENT pH

Material	pH
Chromagram sheet 6062 (alumina)	9.3
Supplier A (acidic grade)	4.3
Supplier A (neutral grade)	8.3
Supplier A (basic grade)	9.8
Supplier B (with CaSO$_4$ binder)	7.3
Supplier B (without CaSO$_4$ binder)	9.5

pH 7, the sheet was dipped in an alcoholic solution of acetic acid. The pretreatment solution was prepared by mixing 5 ml of 10 % aqueous acetic acid with 345 ml of ethyl alcohol. Acidic layers of pH between 5 and 6 were similarly prepared. In this case, the pretreatment solution was made by dissolved 23.6 g of sodium acetate in 70 ml of distilled water and adding 10.5 ml of glacial acetic acid and diluting to a total volume of 350 ml with ethyl alcohol. In both pretreatments, the sheets were dipped for 5 min without agitation. Once removed from the solution, the sheets were blotted dry with clean filter paper and activated for 30 min at 100°.

In preparing any pretreatment or buffer solution, it was found necessary to use as much ethyl alcohol or other polar organic solvent as possible. This reduces the chances for layer damage that may result when totally aqueous solutions are used. A further discussion can be found under "Solvent resistance".

Table II lists approximate R_F values obtained for the Eastman test dye mixture on sheets that were preadjusted to the pH values shown.

TABLE II

pH EFFECT ON TEST DYE SEPARATION

pH of Chromagram (alumina) layer	Approx. R_F values*		
	Yellow	Blue	Red
Basic (9.3 as supplied)	0.88	0.22	0.0
Neutral (6.8 after acid pretreatment)	0.96	0.47	0.0
Acid (5.5 after buffer pretreatment)	1.00	0.86	Tailed

* Yellow = N,N-dimethyl-p-phenylazoaniline; blue = N,N-dimethylindoaniline; red = 1-amino-4-hydroxyanthraquinone.

Purity of adsorbent

Adsorbents for TLC are usually supplied without specifications stating the nature and the amount of impurities present. In analyses involving the separation of compounds that complex with metal ions, the trace metal content, and in particular the iron content, of TLC grade adsorbents can adversely affect the chromatographic resolution. It is often possible to remove these interfering ions by premigrating the plate or sheet in an acidic solvent system and then thoroughly drying it to remove the excess acid prior to carrying out chromatography.

Trace metals in the precoated alumina layer, including the poly-(vinyl alcohol) binder, were determined by emission spectrographic analysis. These were compared with commercially available TLC grade aluminum oxides. Levels of most elements were minimal in the alumina layer removed from the flexible sheets. These data are shown in Table III and may serve as a guide in determining the need for prewashing.

TABLE III

METAL CONTENT OF ALUMINUM OXIDE ADSORBENTS

(Expressed as parts per millon)

Columns: 1 = adsorbent from Eastman Chromagram sheet 6062 (alumina)
Columns: 2 = Supplier A (acidic alumina)
Columns: 3 = supplier A (neutral alumina)
Columns: 4 = supplier A (basic alumina)
Columns: 5 = Supplier B (aluminum oxide G)
Columns: 6 = Supplier B (aluminum oxide H)

| | Column No. | | | | | |
	1	2	3	4	5	6
Boron	—*	—	100	—	—	—
Calcium	100	<100	3,000	<100	20,300	100
Chromium	3	3	3	3	3	3
Copper	10	10	10	10	10	10
Iron	100	100	100	100	100	100
Lead	10	—	—	—	10	10
Magnesium	50	300	10,000	50	1,000	50
Manganese	5	5	20	5	10	5
Silicon	1,000	1,000	10,000	300	300	100
Silver	—	—	—	—	—	—
Sodium	300	100	300	300	500	500
Titanium	10	3	3	3	3	100
Vanadium	—	3	3	—	—	—

* — = not detected.

Activation

Thin-layer chromatography on alumina adsorbent involves primarily an adsorption mechanism. Therefore, it is necessary to remove adsorbed water (activate) before use. Activation at 100° for 30 min produced uniformly good results with flexible sheets. Heating the sheets above 120° caused the support to curl. Temperatures above 130° caused permanent discoloration of the adsorbent.

Solvent resistance

The precoated alumina sheet may be used in essentially all of the solvent systems described in the thin-layer chromatographic literature. In the limited number of systems that are extremely polar, the following considerations may lead to improved results. Water, because it softens the binder, can be replaced in most cases with some very polar organic solvent. Acetonitrile, dimethylformamide, dioxane, and most alcohols are useful substitutes.

Methanol, particularly in combination with chlorinated solvents, dissolves a small fraction of the binder. This binder is insoluble in ethyl alcohol and higher alcohols and in other common organic solvents used in chromatography. Replacing methanol in a migrating solvent mixture with an equal, or slightly greater, proportion of ethyl alcohol minimized this effect.

A group of common migrating solvents tested to determine their effect on the adhesion of the coated layer to the poly-(ethylene terephthalate) film support showed that only ammonium hydroxide, hydrochloric acid and water used in concentrated form caused delamination of the coated layer. These solvents can be used, however, in preparing mixed migrating solutions.

Some migrating solvents produced a zone at the solvent front which appeared dark under U.V. light. This condition is also quite common in thin-layer chromatography on hand-prepared glass plates. The degree of front darkening obtained was visually compared for a group of common migrating solvents. These data are listed in Table IV. Solvents listed as "None" or "Trace" were successfully used to extract separated compounds from cut-outs from the flexible sheets for examination by U.V.

TABLE IV

DEGREE OF SOLVENT FRONT CONTAMINATION

Solvent tested	Appearance of solvent front under U.V. light
Hexane	None
Cyclohexane	None
2 2,4-Trimethylpentane	None
2-Butanone	None
Acetone	None
Methanol	None
Ethyl alcohol	None
Propyl alcohol	None
Isopropyl alcohol	None
Butyl alcohol	None
Acetonitirile	None
p-Dioxane	None
Acetic anhydride	None
1,2-Dichloroethane	Trace
Xylene	Trace
N,N-Dimethylformamide	Trace
Pyridine	Trace
Diethylamine	Trace
Acetic acid	Trace
Ligroine (B.P. 35–60°)	Moderate
Carbon tetrachloride	Moderate
Chloroform	Moderate
Dichloromethane	Moderate
Blacosolve (trichloroethylene)	Moderate
Benzene	Moderate
Toluene	Moderate
Ethyl acetate	Moderate
Ethyl ether	Moderate

and I.R. spectroscopy and by other instrumental techniques. Solvents listed as "Moderate" were avoided for purposes of zone elution since the simultaneous removal of the material responsible for dark solvent fronts might have interfered in subsequent analyses.

Solvent migration rate

Speed is a major advantage of thin-layer over other forms of chromatography. Table V compares the solvent travel rate of the precoated alumina sheets with that of standard aluminum oxide layers coated to a wet thickness of 250 μ on glass plates. Solvents chosen were of widely different polarities. The development methods were employed using activated layers. A battery jar lined with filter paper was allowed to stand for several hours to establish vapor equilibrium. Eastman Chromagram and A. H. Thomas sandwich chambers required no pre-equilibration since they equilibrate during migration. Experiments were carried out at 25 % relative humidity.

TABLE V

COMPARISON OF SOLVENT MIGRATION RATES

Coating	Time to travel 10 cm (min)			
	Battery jar development		Sandwich development	
	CHCl₃	MeOH	CHCl₃	MeOH
Chromagram Sheet 6062 (alumina) (pH 9.3)	34	36	39	53
Supplier B basic (alumina powder) (pH 9.5)	44	43	35	40

These data are in general agreement with the conclusions of PORGES AND PORGESOVA[23] who studied the effect of chamber width. They prefer increasing the width of the developing chamber as the polarity of the migrating solvent is increased. Due to differences in vapor pressure of mixed solvents, the choice of developing chamber used in our laboratory is still determined by empirical methods rather than polarity considerations.

Visualization

Visualization reagents compatible with these sheets are typical of those employed with glass TLC plates. Iodine vapor, U.V. light and the specific spray reagents (listed in Table VI) were used. Charring techniques involving corrosive reagents were, of course, precluded due to the presence of the organic binder.

It was often found advantageous to dip the flexible sheets in reagent solutions, particularly when quantitation of the separated zones was anticipated. Dipping allowed a more uniform reagent application and provided greater penetration of the reagent than did spraying. Precautions outlined under "Solvent resistance" were considered when preparing visualization reagents. The least polar solvent in which the reagent was soluble was selected to minimize possible diffusion of separated zones.

TABLE VI

EXPERIMENTAL CONDITIONS FOR APPLICATION STUDIES

Compounds	Migrating solvent	Visualization reagent	Comments	Approx. R_F value	
				Chromagram	Glass plate
Chlorinated pesticides	Hexane	Rhodamine B	5 µg/spot		
2,4-D Acid				0.00	0.00
Dieldrin				0.05	0.05
Lindane				0.10	0.10
DDT				0.20	0.25
Heptachlor				0.30	0.40
Aldrin				0.60	0.50
2,4-Dinitrophenylhydrazine derivatives	Hexane–benzene (1:1)	None	5 µg spot; 2 multiple elutions		
Glyoxal DNP				0.00	0.00
2-Furaldehyde DNPH				0.15	0.10
Acetaldehyde DNPH				0.25	0.20
Acetone DNPH				0.30	0.20
2-Butanone DNPH				0.40	0.30
β-Ionone DNPH				0.60	0.45
Fat-soluble vitamins	Cyclohexane–diethyl ether (100:25)	Antimony pentachloride	1–5 µg/spot		
all-trans-Retinol				0.00	0.00
Vitamin D_2				0.10	0.05
d-α-Tocopherol				0.25	0.10
all-trans-Retinyl acetate				0.60	0.45
Vitamin K				0.75	0.60
all-trans-Retinyl palmitate				0.85	0.70
β-Carotene				0.90	0.90

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Alkaloids	Chloroform–ethyl acetate–diethylamine (60:60 plus 4 drops)	U.V. light, fresh iodoplatinate	20 μg/spot		
Morphine sulfate				0.00	0.00
Codeine				0.11	0.08
Papaverine				0.71	0.68
French's food colors	Acetonitrile–water–ammonia (100:10:10)	None	Diluted 10 times for spotting; two multiple elutions		
French's Blue dye				0.56	0.52
French's Green dye				0.52, 0.02	0.50, 0.02
French's Red dye				0.10, 0.03	0.34, 0.13
French's Yellow dye				0.00, 0.21	0.15, 0.38
Plasticizers	Hexane–ethyl acetate (100:10)	Iodine vapors	50 μg/spot		
N-n-Butylacetanilide				0.25	
Ethyl N-n-butylcarbamate				0.30	
Ethyl lactate				0.05	
Di-(tetradrofurfuryl) adipate				0.10	
Triacetin				0.15	
Triethyl citrate				0.05	
Triphenyl phosphate				0.15	
Tricresyl phosphate				0.05–0.30 streak	
Diethyl phthalate				0.35	
Dibutyl phthalate				0.45	
Diethyl succinate				0.40	
Diethyl sebacate				0.45	
Butyl sebacate				0.50	

Comparison of separations

A number of compounds were separated on flexible alumina sheets (adsorbent layer thickness nominally 100 μ) and on hand-prepared glass plates coated to a wet thickness of 250 μ using TLC grade alumina (pH 9.5) without calcium sulfate binder. The dry thickness of the coated plates averaged 165 μ after activation. All layers were activated under the same conditions and developed in sandwich-type chambers at a relative humidity below 30 %. The flexible sheets were placed in the Eastman Chromagram developing apparatus while coated glass plates were placed in the A. H. Thomas sandwich-type chamber.
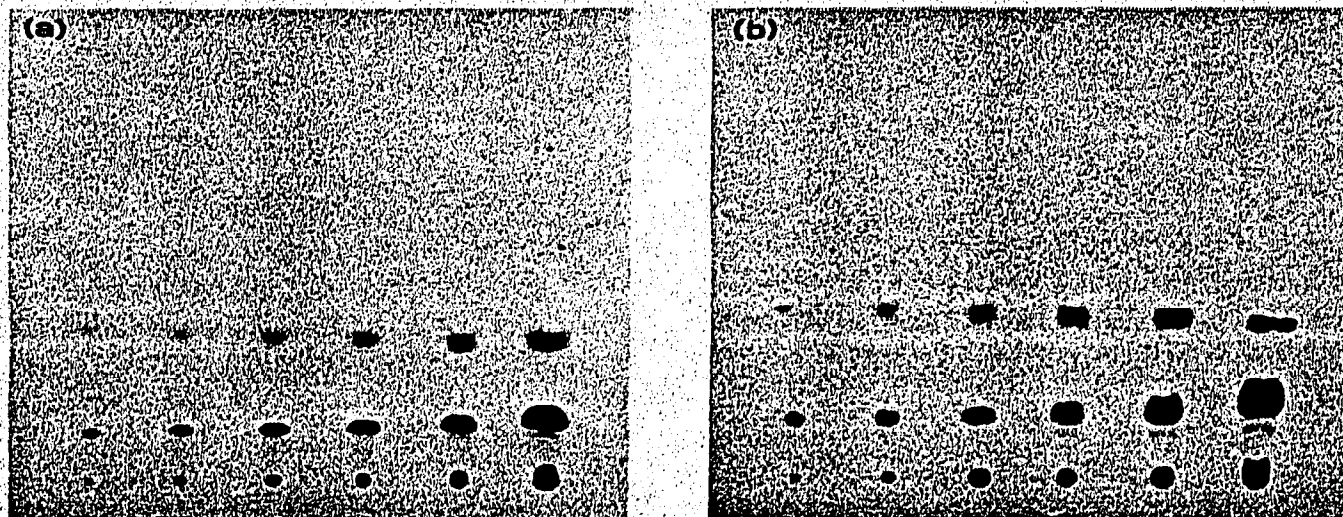

Fig. 1. Load carrying capacity of alumina coated glass plate (a) and alumina precoated sheet (b). Standards, on each, left to right: 1, 2, 5, 10, 20, 50 μl of Eastman test dye mixture (supplied as 1 μg/μl solution of each dye).

Fig. 1 shows the relative loading capacity of the flexible sheet *versus* a glass plate coated with alumina adsorbent. A three dye test mixture was spotted in increasing concentrations across both the plate and sheet. Results indicated the load carrying capacity of the precoated sheet to be approximately 50 % greater than glass plates coated to an equivalent dry thickness. The precoated alumina sheet will therefore accomodate the same load as a glass plate coated with a 250 μ blade setting. This increase in load carrying capacity is thought to be due to the nature of the alumina particles that are used for the preparation of this product.

Table VI lists the experimental conditions used to separate various classes of compounds. Separations obtained on these sheets were essentially equivalent to those obtained on the aluminum oxide coated glass plates.

SUMMARY

This paper describes the general properties of a new aluminum oxide adsorbent medium for thin-layer chromatography. Characteristics and quality of the alumina adsorbent layer from precoated sheets are compared with those of commercially available TLC grade aluminum oxide powders. Specific performance data of this precoated flexible sheet are related to those obtained on glass plates manually coated with alumina adsorbent.

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