CHROM. 4417

THIN-LAYER CHROMATOGRAPHIC SEPARATION ON LAYERS HEAVILY LOADED WITH POWDERED SCINTILLATORS FOR LUMINESCENCE DETECTION OF RADIONUCLIDES

L. H. LANDMARK AND A. K. HOGNESTAD
Central Institute for Industrial Research, Blindern, Oslo 3 (Norway)
AND
S. PRYDZ
Institute of Physics, University of Oslo, Blindern, Oslo 3 (Norway)

(Received October 3rd, 1969)

SUMMARY

Experiments are reported demonstrating that interference is not necessarily caused in a chromatographic separation on standard thin layers by the admixture of powdered scintillators. The study is of importance in connection with a sensitive method of radionuclide detection by β-radioluminescence (either scintillation fluorography on photographic film material or by direct photoelectric detection). Kieselguhr-, silica gel-, and cellulose thin layers were prepared containing various proportions of either an inorganic or organic scintillator (zinc silicate or anthracene, respectively). Mixtures of carbohydrates, phospholipids, and amino acids were tested as representative systems with suitable layers and developing solvents.

INTRODUCTION

A successful chromatographic fractionation on thin layers of adsorbents containing powdered scintillators would be advantageous for the detection of radionuclides by means of β-radioluminescence (scintillation fluorography, or -autoradiography).

This method[1-7] is based on the conversion of the β-particle energy into light upon absorption in a suitable scintillator. The high reflection coefficients of Al_2O_3- and SiO_2-powders (standard thin-layer media) means that a large proportion of the emitted photons manage to escape from the chromatogram and can be used for localization of the radio-labelled spots and measurement of their activities.

The method is particularly useful for low-energy radionuclides such as tritium (^3H) and radiocarbon (^{14}C) (refs. 1, 4–7), the two most important radio labels. In the case of these nuclides only a small proportion of the electrons are capable of leaving the chromatogram and therefore direct electron detection of the spots is normally much slower than with the β-radioluminescence method.

There are several types of β-radioluminescence detection. They may be grouped into liquid scintillation, gel scintillation, and solid scintillation methods. In solid scintillation detection two different techniques may be employed for preparing the layer:

(a) The scintillator is added to the thin-layer plates (in the form of a solution or suspension) by a spraying technique following the completion of the separation procedure. After drying, either film- or photomultiplier detection is applied.

(b) The scintillator is added to the adsorbent before the preparation of the thin-layer plates. With this method much larger amounts of scintillators may be added[4–7]. Detection is as above.

The alternative (b) is normally preferred, provided the scintillator is chemically inert and the fractionation is obtained without difficulty. The advantages are less work in preparing the plates and a much better reproducibility in the nuclide quantization.

The purpose of these experiments has been to investigate whether the addition of scintillators (anthracene or zinc silicate) to some thin-layer media would hinder a successful separation. The separation properties of a thin-layer medium depend upon several parameters, such as grain size, pore diameter and volume, density, and surface area[8]. In addition, the chromatographic fractionation may be modified, for example, by mixing adsorbents, by using buffer solutions instead of water for dispersion, or, finally, by impregnation or heat activation[9]. Small variations in other parameters, such as those of the experiment itself, might give some degree of fluctuation in the results obtained[10,11].

It is now reported how some selected systems retained or modified their separation performances upon admixture with a scintillator.

EXPERIMENTAL

Kieselgel 7431 G Merck, Kieselguhr 8129 G Merck, and cellulose powder MN 300* were used as adsorbents. Zinc silicate (B.D.H.) or anthracene (purity 99%)**, were used as scintillator additives. Layer thickness was kept close to 0.25 mm, the plate dimension was 7.5 × 15 cm. The plates were coated with the layer by means of a Quickfit demonstration kit. Solute samples were applied in volumes from 1 to 10 μl. Ascending development for a distance of 10–12 cm was used. A reference plate was always developed together with the scintillator-loaded plate to avoid, as far as possible, any difficulty in establishing the reproducibility of thin-layer chromatography[8–11]. The selected chromatographic systems and visualization methods are presented in Table I.

Anthracene layers

The anthracene available was in large crystals which required grinding before preparing the layers. The adsorbent and the anthracene were thoroughly mixed before and after addition of the suspension liquid. 50% amounts of anthracene only were used. Table II shows the proportions used for mixing the layer suspension (sufficient for approximately 2 plates 7.5 × 15 cm).

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* Macherey, Nagel & Co., Düren, G.F.R.
** Schuchardt, Munich, G.F.R.

TABLE I

SELECTED CHROMATOGRAPHIC TEST SYSTEMS AND VISUALIZATION

	Carbohydrates	*Phospholipids*	*Amino acids*
Test systems			
Compound	Rhamnose, xylose, arabinose, mannose, glucose, and galactose (Eastman Organic Chemicals)	Lecithin, unpurified (A/S Norsk Soyamelfabrik)	Cystine, glycine, leucine, lysine (Shandon standard solutions)
Adsorbent	Kieselguhr G[a]	Silica Gel G	Cellulose and Silica Gel G
Developing solvent	Butanol–acetone–water (40:50:10)	Chloroform–methanol–water (65:25:4)	Chloroform–methanol–17% ammonia (40:40:20)
Color reagent and reaction			
Reagent	Phthalic acid–aniline in acetone	Molybdenum reagent	Ninhydrin reagent
Adsorbent only	Brownish	Bluish	Violet
Adsorbent + anthracene	Brownish	Bluish with blue-grey background	Red-grey
Adsorbent + zinc silicate	Pale brownish	Bluish	Orange

[a] Pre-treated with 0.2 *M* NaH_2PO_4.

TABLE II

ANTHRACENE LAYER COMPONENTS

Anthracene (%)		*Anthracene* (g)	*Adsorbent*	*(g)*	*Suspension liquid*	*(ml)*
0	Reference	0	Kieselguhr G	4	0.2 *M* NaH_2PO_4	7
50		1.5	Kieselguhr G	1.5	100% ethanol	8[b]
0	Reference	0	Silica gel G	3	50% ethanol	7
50		1	Silica gel G	1	96% ethanol	6
0	Reference	0	Cellulose	1.5	50% ethanol	8
50		1	Cellulose	1	96% ethanol	7

[b] 0.2 *M* NaH_2PO_4 was applied to the layer by spraying *ca.* 3 ml 0.2 *M* NaH_2PO_4 per plate.

Zinc silicate layers

The zinc silicate available seemed to be of variable grain size. We tried to get it more homogeneous by grinding. The zinc silicate was used in amounts of 25, 50, 75 and 100%. Table III shows what proportions were used when mixing the layer suspensions (for approximately 2 plates 7.5 × 15 cm).

RESULTS AND DISCUSSION

The effect of the addition of anthracene and zinc silicate to the selected systems was examined; the experimental results are presented in Tables IV and V, where the

TABLE III

ZINC SILICATE LAYER COMPONENTS

Zinc silicate			*Adsorbent*	*(g)*	*Suspension liquid*	*(ml)*
(%)		*(g)*				
0	Reference	0	Kieselguhr G	4	0.2 *M* NaH_2PO_4	7
50		2	Kieselguhr G	2	0.2 *M* NaH_2PO_4	10
25		1	Kieselguhr G	3	0.2 *M* NaH_2PO_4	9.5
0	Reference	0	Silica Gel G	3	50% ethanol	7
50		1	Silica Gel G	1	50% ethanol	6
75		2	Silica Gel G	0.7	50% ethanol	8.7
100		3	Silica Gel G	0	50% ethanol	10
0	Reference	0	Cellulose	1.5	50% ethanol	8
50		0.8	Cellulose	0.8	50% ethanol	8

TABLE IV

R_F VALUES WITH AND WITHOUT ANTHRACENE IN THE LAYER

Brace shows unseparated compounds.

Compounds in mixture applied		*Amount (µg)*	*R_F values on adsorbent only (reference)*	*R_F values with 50% anthracene in the layer*
Carbohydrates			*Kieselguhr G*[a]	
Rhamnose		2	0.93	0.92
Xylose		2	0.70	0.55
Arabinose		2	{ 0.50	{ 0.35
Mannose		2		
Glucose		2	0.36	0.27
Galactose		2	0.24	0.18
Phospholipids			*Silica Gel G*	
Impure lecithin		150		
Unknown component	I		0.98	0.87
Unknown component	II		0.84	0.78
Unknown component	III		0.66	0.67
Unknown component	IV		0.51	0.47
Unknown component	V		0.41	—
Unknown component	VI		{ 0.30	{ 0.27
Unknown component	VII		{ 0.25	{ 0.23
Unknown component	VIII		0.13	0.12
Amino acids			*Cellulose*	
Leucine		1.3	0.85	0.91
Glycine		0.8	0.50	0.54
Cystine		2.4	0.32	0.35

[a] Pre-treated with NaH_2PO_4.

R_F values of the same components separated on layers with and without scintillator are shown. Figs. 1 and 2 illustrate the separation.

Anthracene

The separation properties (and the developing rate) were not influenced by the addition of anthracene to the layer. The R_F values however, were displaced a little

TABLE V

R_F VALUES WITH AND WITHOUT ZINC SILICATE IN THE LAYER

Brace shows unseparated components.

Compounds in mixture (applied)	*Amount (μg)*	*R_F values on adsorbent only (references)*	*R_F values on layers with 50% zinc silicate*	*R_F values on layers with 25-, 75- and 100% zinc silicate*	
Carbohydrates		*Kieselguhr G[a]*		*25%*	
Rhamnose	4	0.90	0.68	0.82	
Xylose	4	0.61	0.58	0.72	
Arabinose	4	0.42	0.45	0.62	
Mannose	4				
Glucose	4	0.31	0.30	0.56	
Galactose	4	0.22		0.47	
Phospholipids		*Silica Gel G*			
Impure lecithin	100				
Unknown component I		0.98	0.82		
Unknown component II		0.85	—		
Unknown component III		0.67	0.65		
Unknown component IV		0.36	0.45		
Unknown component V		0.17	(0.27–0.18)		
Unknown component VI		0.06	0.03		
Amino acids				*75%*	*100%*
Leucine	2.6	0.50	0.68	0.63	0.78
Glycine	2.1	0.26	0.36	0.44	0.59
Cystine	4.8		—	0.38	
Lysine	3.6	0.10	0.25	—	
Amino acids		*Cellulose*			
Leucine	2.6	0.79	0.69		
Glycine	2.1	0.37	0.33		
Cystine	0.21	0.21	0.09		

[a] Pre-treated with 0.2 *M* NaH_2PO_4.

as Table IV and Fig. 1 show. This generally minor influence of anthracene on the separation properties of silica gel has been reported earlier by LÜTHI AND WASER[4].

Zinc silicate

Admixtures of zinc silicate caused some changes in the separation properties as Table V and Fig. 2 show. Developing time increased considerably (*e.g.* 2 h per 10 cm for phospholipids on the silica gel layer with 50% zinc silicate).

Zinc silicate in Kieselguhr G layers resulted in a smaller spread in the R_F values of the carbohydrates, as shown in Fig. 2, and a fainter coloring of the spots when sprayed with the color reagent.

Zinc silicate had no effect on the separation of the impure lecithin on the Silica Gel G layers, but the R_F values were displaced. Of the amino acids tried on this layer, cystine and glycine did not separate though they had different R_F values. Glycine formed a two-edged tail.

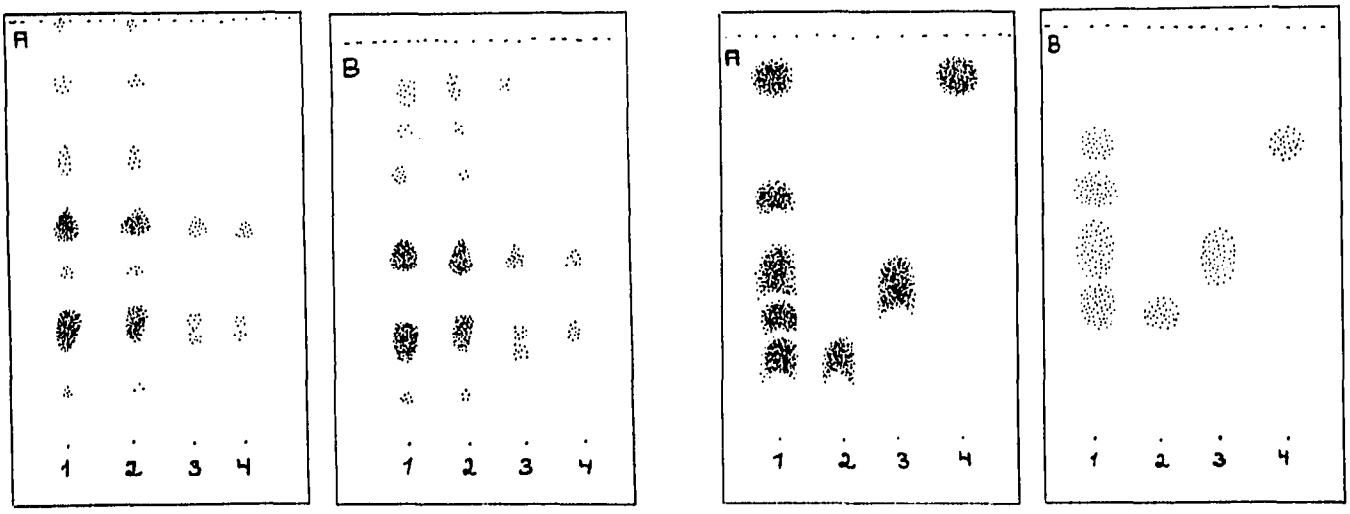

Fig. 1. Comparison between separation of phospholipids (impure lecithin) on Silica Gel G layers with and without anthracene addition. A = 100% Silica Gel G (reference); B = 50% Silica Gel G, 50% anthracene. Developing solvent: chloroform–methanol–water (65:25:4). Amounts of lecithin applied: (1) 150 μg, (2) 100 μg, (3) 10 μg, (4) 5 μg.

Fig. 2. Comparison between separation of carbohydrates on Kieselguhr G layers with and without zinc silicate addition. A = 100% Kieselguhr G pre-treated with 0.2 *M* NaH_2PO_4 (reference); B = 50% Kieselguhr G, 50% zinc silicate pre-treated with 0.2 *M* NaH_2PO_4. Developing solvent: *n*-butanol–acetone–water (40:50:10). (1) A mixture (from top to bottom) of: rhamnose 4 μg, xylose 4 μg, arabinose 4 μg, mannose 4 μg, glucose 4 μg and galactose 4 μg; (2) galactose 4 μg; (3) mannose 4 μg, arabinose 4 μg; (4) rhamnose 4 μg.

The effect of adding zinc silicate to the cellulose layers was studied using the same amino acids and developing solvent as was used in the Silica Gel G layers. Cystine and glycine did not separate in this case either, and glycine formed the same sort of tail as on silica gel plus zinc silicate layers.

CONCLUSION

Addition of anthracene to the layers leads to only small displacements of the R_F values of the compounds separated. Zinc silicate addition, however, resulted in changed separation properties for some of the selected systems.

The systems studied were chosen on the basis of common chromatographic practice and no special adjustments were tried. The experiments are somewhat preliminary. One may suppose that more accurate adjustments in selected systems will result in improved performances as compared to those demonstrated so far.

ACKNOWLEDGEMENTS

L. H. LANDMARK and A. K. HOGNESTAD thank the Central Institute for Industrial Research for the necessary financial support.

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