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MAGNESIUM HYDROXIDE AS A THIN-LAYER CHROMATOGRAPHIC ADSORBENT

II. A UNIQUE SYSTEM FOR SEPARATING POLYNUCLEAR AZAAROMATIC COMPOUNDS

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SUMMARY

Mixtures of polynuclear azaaromatic compounds not separable on silica or alumina have been resolved using magnesium hydroxide, a versatile new thin-layer chromatographic adsorbent that is convenient to use, durable and cheap. Adsorbability depends upon the nature of the π -electronic system as well as the steric accessibility of the nitrogen atom, the former factor being relatively much more important than it is with silica and alumina. Preparative experiments involving two synthetic mixtures and the basic fraction of coal tar pitch are described. Unexpected increases in R_F values with activation and with decreased operating temperature are reported.

INTRODUCTION

A problem of increasing importance to analytical chemists is that of the detection and estimation of polynuclear azaaromatic compounds in the human environment. A substantial number of nitrogen aromatics have been identified among atmospheric pollutants^{1,2} and other environmental sources³, and although the biological action of most such compounds has not yet been investigated, several members of this series have been demonstrated to be potent carcinogens⁴.

Thin-layer chromatography has been used to great advantage in separations involving compounds of this sort. Silica^{3,5-10} and alumina^{1,9-14} have been the adsorbents most frequently employed, but cellulose and cellulose derivatives^{1,2,10,13,14}, Florisil^{10,14}, polyamide¹⁵, and Porapak¹⁶ have also received attention.

We wish to report that magnesium hydroxide¹⁷ is a powerful addition to the list of adsorbents that are suitable for the chromatography of polynuclear azaaromatic compounds. We believe further that the evidence presented below and elsewhere in this series of papers supports our conclusion that magnesium hydroxide is a novel adsorption medium, with potential applicability to a wide variety of chromatographic problems.

RESULTS AND DISCUSSION

Separations which are difficult or impossible using other systems can frequently be effected with ease on magnesium hydroxide. Examination of the R_F data summarized in Table I reveals that the benzologous phenazine derivatives VIII and X, for example, are virtually inseparable on silica and alumina ($|R_F(\text{VIII}) - R_F(\text{X})| \leq 0.02$), while the difference in R_F on magnesium hydroxide is 0.14. Other pairs of compounds more easily separable on magnesium hydroxide than on the other adsorbents include benz[*c*]acridine (IV) and dibenz[*a,h*]acridine (IX), the pyrazine benzologs III and VIII, and benzo[*h*]quinoline (I) and its naphtho derivative, IX.

TABLE I

R_F VALUES FOR NITROGEN AROMATIC COMPOUNDS

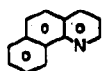
System A: Unactivated magnesium hydroxide plates (0.25 mm) developed in benzene at ambient temperature. Data are the averages of at least nine separate determinations, plus or minus the standard deviation; color of the adsorbed material under long-wave UV radiation is given in parentheses. System B: Silica plates (0.25 mm) activated at 110° for 10 min and developed in pentane-ether (7:3), *cf.* ref. 3. System C: Alumina plates (0.25 mm) activated 23 h at 110° and developed in pentane-ether (9:1); *cf.* ref. 1. System D: Alumina plates (0.25 mm) activated 23 h at 110° and developed in chloroform-benzene (1:1), *cf.* ref. 11.

Compound	$Mg(OH)_2$ (System A)	Silica (System B)	Alumina	
			(System C)	(System D)
(I) Benzo[<i>h</i>]quinoline	0.44 ± 0.04 ₂ (none)	0.57	0.46	0.78
(II) Phenazine	0.39 ± 0.02 ₈ (black)	0.35	0.20	0.71
(III) Dibenz[<i>f,h</i>]quinoxaline	0.35 ± 0.03 ₄ (blue)	0.64	0.46	0.85
(IV) Benz[<i>c</i>]acridine	0.30 ± 0.03 ₁ (blue)	0.73	0.52	0.86
(V) Acridine	0.29 ± 0.02 ₈ (blue)	0.32	0.18	0.60
(VI) Phenanthridine	0.24 ± 0.02 ₄ (none)	0.21	0.14	0.59
(VII) Carbazole	0.17 ± 0.02 ₅ (none)	0.51	0.16	0.75
(VIII) Dibenz[<i>a,c</i>]phenazine	0.16 ± 0.01 ₅ (blue)	0.73	0.58	0.89
(IX) Dibenz[<i>a,h</i>]acridine	0.05 ± 0.00 ₈ (blue)	0.70	0.47	0.87
(X) Tribenzo[<i>a,c,h</i>]phenazine	0.02 ± 0.00 ₀ (blue)	0.75	0.57	0.91

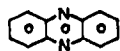
These favorable R_F differences can be rationalized by reference to Table I, the data of which permit an important generalization to be formulated regarding the relationship of structure to adsorbability. Unlike silica and alumina, for which the steric availability of the nitrogen atom^{9,11} is of overriding importance in determining adsorption energy, magnesium hydroxide is uniquely sensitive to the nature of the π -electronic system of the adsorbate molecule. This fact is most strikingly illustrated in the R_F values for the series of pyrazine benzologs II, III, VIII, and X. Successive increase in the number of aromatic rings gives rise to significant decreases in R_F on magnesium hydroxide. With silica and alumina, on the other hand, precisely the opposite order is observed, since with these adsorbents the primary effect of increasing the ring content is to render the nitrogen atom sterically less available for participation in chelation interactions at the surface of the adsorbent.

Obviously, however, adsorbability on magnesium hydroxide is influenced strongly also by the coordination capability of the nitrogen atom. Thus, while the differences in R_F among the five trinuclear aromatic compounds listed in Table II

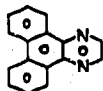
are not large ($R_{F(II)} - R_{F(I)} = 0.05 = R_{F(V)} - R_{F(VI)}$), a mixture of the five substances could, under suitable conditions, be separated into five distinct fractions, only one of which could be shown by UV spectrophotometry to be contaminated with the component of an adjacent fraction. Silica and alumina were adjudged unsuitable for the separation of this mixture, since the minimum R_F differences among the five components using systems B, C, and D (Table I) were 0.03, 0.02 and 0.01, respectively.



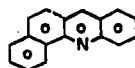
(I) Benzo[h]quinoline



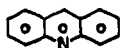
(II) Phenazine



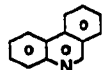
(III) Dibenzo[f,h]quinoxaline



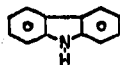
(IV) Benz[c]acridine



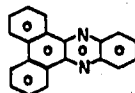
(V) Acridine



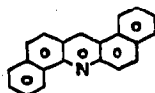
(VI) Phenanthridine



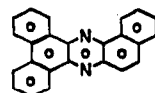
(VII) Carbazole



(VIII) Dibenzo[a,c]phenazine



(IX) Dibenzo[a,h]acridine



(X) Tribenzo[a,c,h]phenazine

TABLE II

SEPARATION OF A MIXTURE OF FIVE TRINUCLEAR AZAAROMATIC COMPOUNDS

Adsorbent, magnesium hydroxide (0.25 mm); developer, benzene; temperature, 10°.

Compound	R_F (Table I)	Amount applied to plate (μg)	Amount recovered (μg)
Benzo[h]quinoline (I)	0.44	40	45
Phenazine (II)	0.39	6	4.4
Acridine (V)	0.29	6	6.3
Phenanthridine (VI)	0.24	20	16 ^a
Carbazole (VII)	0.17	50	44

^a The phenanthridine fraction was found by UV spectrophotometry to contain approximately 0.5 μg of acridine.

The relative contributions of the two dominant structural features discussed above to the total solute adsorption energy can be crudely estimated by comparing the R_F values for acridine (V) and benz[*c*]acridine (IV). The near equivalence of these values on magnesium hydroxide suggests that the group adsorption energy of the additional aromatic ring in IV is nearly as large as that of the unhindered nitrogen atom, which is sterically much less available for coordination in IV than in V.

TABLE III

PREPARATIVE SEPARATION OF PYRAZINE BENZOLOGS

Adsorbent, magnesium hydroxide (1.0 mm); developer, benzene; temperature, ambient.

<i>Compound</i>	R_F (Table I)	<i>Amount applied to plate (mg)</i>	<i>Amount recovered (mg)</i>
(II) Phenazine	0.39	3.3	3.5
(III) Dibenzo[<i>f,h</i>]quinoxaline	0.35	8.3	7.7
(VIII) Dibenzo[<i>a,c</i>]phenazine	0.16	3.3	3.2
(X) Tribenzo[<i>a,c,h</i>]phenazine	0.02	0.22	0.22

A second important advantage of magnesium hydroxide is its high capacity¹⁷. A total of more than 14 mg of a mixture of the four pyrazine benzologs mentioned above was completely separated (Table III) on a single plate of magnesium hydroxide, layer thickness 1 mm; elution of the individual fractions with ethanol or ethanol-benzene permitted essentially complete recovery of the pure components. Repetition of this experiment using 1 mm plates of alumina or silica with pentane-ether (9:1 and 7:3, respectively) produced in both cases a fluorescent continuum, showing that the layer's capacity had been exceeded at this sample load.

Magnesium hydroxide has many other advantages as a thin-layer chromatographic adsorbent. It is inexpensive; the price per unit weight of the least expensive grade of silica or alumina is several times that of the new adsorbent. Layers of magnesium hydroxide are particularly resistant to chipping and abrasion; we have judged them to be at least as durable as the pre-coated sheets now commercially available, and addition of binder only detracts from its performance. Finally, the new adsorbent is relatively insensitive to variations in working conditions; while there is some batch-to-batch variation in R_F and/or development time, the problem has not thus far been as serious as has the variation among different sources of alumina and silica¹⁸.

We have assumed that chromatography on magnesium hydroxide is an adsorption phenomenon. There are, however, certain interesting differences between this adsorbent and the two currently most widely used media for adsorption chromatography. Firstly, the R_F value generally* drops as the water content of alumina or silica is decreased¹⁸. The opposite effect is observed with magnesium hydroxide,

* GEISS¹⁸ has described two special circumstances in which increased water content of the adsorbent may lead to decreased R_F value, *viz.*, in the separation of inorganic ions or other very hydrophilic compounds, or when excess water promotes appropriate patterns of selective demixing in polyzonal chromatography. Neither consideration seems pertinent, however, to the question of the origin of increased R_F values of azaaromatic compounds on activated magnesium hydroxide/benzene.

TABLE IV

EFFECT OF ACTIVATION AND OF INCREASED DEVELOPMENT TEMPERATURE ON MAGNESIUM HYDROXIDE R_F VALUES

Layer, magnesium hydroxide (0.25 mm); developer, benzene.

Compound	R_F at 25° (Table I)	R_F after activation ^a	R_F at 35°
I	0.44	0.67	0.08
II	0.39	0.65	0.06
III	0.35	0.54	0.06
IV	0.30	0.50	0.06
V	0.29	0.53	0.05
VI	0.24	0.50	0.05
VII	0.17	0.26	0.03
VIII	0.16	0.33	0.03
IX	0.05	0.13	0.02
X	0.02	0.05	0.01

^a Activated at 105° for 20 h before use.

TABLE V

SEPARATION ON MAGNESIUM HYDROXIDE OF THE BASIC FRACTION OF COAL TAR PITCH

Layer thickness, 1.0 mm; 3.4 mg of the basic fraction of coal tar pitch (benzene-soluble portion) was applied in a streak and developed in benzene at ambient temperature.

Fraction	R_F	Mass spectral peaks ^a (<i>m/e</i>)	Components identified
8	0.48	179, 193, 207	benzo[<i>h</i>]quinoline; methylbenzo[<i>h</i>]quinoline(s); dimethyl- (or ethyl-) benzo[<i>h</i>]quinoline(s)
7	0.36	203, 179	acridine (trace); azapyrene or azafluoranthene (?)
6	0.30	179, 229, 203, 167, 193	acridine; benz[<i>c</i>]acridine; methylacridine(s)
5	0.23	179, 203	azapyrene or azafluoranthene (?)
4	0.17	203, 179, 229	azapyrene or azafluoranthene (?)
3	0.13	253, 229, 267	
2	0.07	253, 279, 267	
1	0.03	168, 253	carboline (?)

^a Listed in order of decreasing relative abundance.

increases in R_F value as large as 0.2 unit (*cf.* Table IV) being the result of heating the plate at 105° prior to development*.

A second apparent anomaly was noted in the variable temperature studies. As shown in Table IV, a large decrease in R_F value on magnesium hydroxide resulted when the development temperature was raised by 10°, while small increases are the rule with silica and alumina¹⁸.

* It should be mentioned in this connection that our earlier report on the chromatography of polynuclear hydrocarbons¹⁷ contains the statement that results before and after activation were "essentially identical". Re-examination of our data has revealed that a small increase (averaging about 0.03 R_F unit) was probably encountered in that case also, but that it was disregarded because it was smaller than experimental error.

APPLICATION

A brief investigation into the applicability of this new system to the separation of naturally occurring mixtures of polynuclear azaaromatics was undertaken. When 3.4 mg of the basic fraction of coal tar pitch was applied to a 1 mm plate, eight different fractions (Table V) were selected on the basis of the fluorescence properties of the resulting chromatogram. The fastest moving band showed a UV absorption spectrum which was nearly identical with that of pure benzo[*h*]quinoline; this assignment was confirmed by the mass spectrum, the largest peak of which was found at $m/e = 179$. The two next most abundant ions were found at m/e 193 and 207, respectively, demonstrating the presence of substantial amounts of methyl and dimethyl (or ethyl) benzo[*h*]quinoline derivatives among the coal tar bases. Identification of other constituents was hampered by a lack of reference compounds and their absorption spectra, but acridine (together with its alkyl derivatives) was apparently the major component of fraction 6 ($\lambda_{\text{max.}} = 250 \text{ m}\mu^*$; $R_F = 0.30$; $m/e = 179$); this band also contained some benz(*c*)acridine ($\lambda_{\text{max.}} = 275, 285, 365$ and $385 \text{ m}\mu^*$; $m/e = 229$).

While our data on the constitution of the basic fraction of coal tar are far from complete, we feel that the results presented above clearly demonstrate the utility of magnesium hydroxide as an adsorbent for separating complex mixtures of naturally occurring polynuclear azaaromatic compounds.

EXPERIMENTAL

UV spectra were recorded on a Cary 15 instrument, and mass spectral data were collected on an AEI Model MS-9 mass spectrometer, at an ionization potential of 70 eV.

Dibenz[*a,h*]acridine was obtained from Schuchardt (Munich, G.F.R.), benz[*c*]acridine from Koch-Light Laboratories, Ltd. (Colnbrook, Great Britain), acridine, carbazole, phenanthridine, and benzo[*h*]quinoline from Rütgerswerke-A.G. (Dortmund, G.F.R.), and the remaining compounds from Aldrich Chemical Company (Milwaukee, Wisc., U.S.A.). Compounds found by chromatographic or spectral methods to be impure were recrystallized before use.

Data reported for silica were obtained using plates of Kieselgel G or H which had been activated at 110° for 10 min before use. Data for magnesium hydroxide and alumina systems were collected essentially as previously described¹⁷. All three adsorbents were routinely mixed with 1% lead-manganese activated calcium silicate short-wave phosphor (Kensington Scientific Corp., Oakland, Calif., U.S.A.) to aid in the location of non-fluorescent spots.

Isolation of basic fraction of coal tar

A sample of coal tar pitch (56 g) was dissolved in 400 ml of benzene. After warming until apparently homogeneous, the mixture was filtered twice through a sintered glass funnel. The filtrate was placed in contact with 600 ml of 15% hydrochloric acid overnight in the dark. The aqueous layer was removed and extracted twice

* Solvent, 95% ethanol.

with a total of 400 ml of benzene, basified with sodium hydroxide, and again extracted with a total of 300 ml of benzene. The organic phase from the second benzene extraction was evaporated, leaving 2.7 g of residue which was treated with 30 ml of benzene. A considerable portion of the solid would not redissolve. The concentration of the supernate, 0.2 ml of which was subsequently applied to a magnesium hydroxide plate (Table V), was therefore estimated by evaporating a 1 ml aliquot, the residue of which weighed 17 mg.

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