

MAGNESIUM HYDROXIDE AS A THIN-LAYER CHROMATOGRAPHIC ADSORBENT

A NEW SYSTEM FOR THE SEPARATION OF POLYNUCLEAR HYDROCARBONS

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Magnesium oxide (magnesia) in the past has been used to advantage as a column chromatographic adsorbent¹⁻⁵, but efforts in this laboratory and in others to adapt the material for use in thin-layer chromatography have not been entirely successful. RAGAZZI, VERONESE AND GIACOBAZZI⁵ were able to separate certain alkaloids on thin layers of "magnesium oxide heavy hydrated", but they emphasized the necessity of standardizing the adsorbent because of large differences in the suitability of the various commercially available samples. KIRCHNER, MILLER AND KELLER⁶ could effect no resolution of mixtures of terpene oils using magnesium oxide, and they described their layers as "soft."

Because of magnesium oxide's demonstrated ability to adsorb polynuclear hydrocarbons¹⁻³, we sought to employ thin layers of magnesia in the separation of these compounds. Our initial attempts were frustrated, however, by our inability to produce suitable layers with commercially available samples of magnesium oxide.

We eventually discovered that samples of magnesia could be used to prepare satisfactory layers only if they were first exposed to a moist environment for a prolonged period. Reasoning that gradual chemical hydration of the oxide⁷ had occurred during this preliminary period, we attempted to prepare thin layers of pure magnesium hydroxide. Our results not only offered support for the contention that success with magnesia depends upon prior chemical hydration of the oxide*, but also suggested that magnesium hydroxide holds considerable promise as a thin-layer chromatographic adsorbent. Some observations concerning the scope and limitations of this system are the subject of the present communication.

The new adsorbent bears a close resemblance in several respects to alumina, but the two systems are by no means equivalent; magnesium hydroxide/benzene has at least four important advantages over alumina/pentane-ether⁸. First, while relative R_F values are of the same order of magnitude in the two systems, the difference in R_F between two given compounds is usually significantly larger with magnesium hydroxide. For example, with alumina, the R_F difference between anthra-

* The similarity of our results to those of RAGAZZI, VERONESE AND GIACOBAZZI⁵ suggests that the "magnesium oxide heavy hydrated" used by these authors had in fact undergone at least partial conversion to the hydroxide before use. For a detailed discussion of the chemical hydration of magnesium oxide, see ref. 7.

cene and benz(*a*)anthracene (BA) is 0.24, and between BA and dibenz(*a,h*)anthracene, the difference is 0.10 (Table I); on the other hand, the corresponding values for the new adsorbent are roughly 50 % larger, *viz.* 0.34 and 0.15, respectively. It should be pointed out, however, that magnesium hydroxide is not invariably superior to alumina in this respect, since there are some particular pairs of hydrocarbons, such as 2,2'-binaphthyl and pyrene, for the separation of which alumina is better suited.

TABLE I

R_F (R_B) VALUES FOR VARIOUS HYDROCARBONS ON MAGNESIUM HYDROXIDE AND ALUMINA

Hydrocarbon	Magnesium hydroxide/ benzene*		Alumina/ pentane-ether (95:5, v/v)	
	R_F **		R_F **	R_B ***
Anthracene	0.53 ± 0.05 ₃	(6.6)	0.36 (6.0)	1.14
Phenanthrene	0.55 ± 0.05 ₇	(6.9)	0.37 (6.2)	1.13
1,1'-Binaphthyl	0.67 ± 0.07 ₈	(8.4)	0.43 (7.2)	—
2,2'-Binaphthyl	0.41 ± 0.05 ₂	(5.1)	0.18 (3.0)	—
Benz(<i>a</i>)anthracene	0.19 ± 0.03 ₂	(2.4)	0.12 (2.0)	1.03
Fluoranthene	0.33 ± 0.04 ₃	(4.1)	0.19 (3.2)	1.09
Benzo(<i>a</i>)pyrene	0.08 ± 0.01 ₇	(1.00)	0.06 (1.00)	1.00
Chrysene	0.16 ± 0.02 ₈	(2.0)	0.09 (1.5)	1.10
Dibenz(<i>a,h</i>)anthracene	0.04 ± 0.00 ₉	(0.5)	0.02 (0.3)	0.74
Perylene	0.06 ± 0.01 ₃	(0.8)	0.04 (0.7)	0.91
Pyrene	0.41 ± 0.04 ₅	(5.1)	0.24 (4.0)	1.25

* Values for this system represent the averages of 17 separate determinations, plus or minus the standard deviation.

** Numbers in parentheses are the R_B values (*i.e.*, the R_F normalized to $R_F = 1.00$ for benzo(*a*)pyrene).

*** R_B values as given in ref. 8.

A second advantage of magnesium hydroxide is its high capacity. One plate of alumina was used to separate completely a mixture containing 1 mg of each of the five compounds listed in Table II, but a second alumina plate provided at best only a preliminary fractionation of a total of 50 mg of the same mixture. A single plate of magnesium hydroxide, on the other hand, easily resolved the 50 mg sample into five spectrophotometrically pure fractions.

Third, results with magnesium hydroxide appear to be far less sensitive to variations in experimental details, such as the extent of activation. SAWICKI, STANLEY, ELBERT AND PFAFF⁸ concluded that, of the three systems they studied, alumina gives the poorest separation of hydrocarbons; in our experience, it has been one of the best for this purpose (*cf.* Table I). BENDER, SAWICKI AND WILSON⁹ observed that results with alumina vary from one lot number to another, and BERTHOLD¹⁰ found that working conditions had to be very carefully controlled in order to achieve satisfactory reproducibility.

In marked contrast to these observations with alumina, an unactivated magnesium hydroxide plate used just 4 h after spreading gave results essentially identical to those obtained with a second plate that had been activated for 24 h at 105°. R_F values have appeared also to be independent of such factors as the amount of carbon

dioxide bound to the layer and the method of preparation of the aqueous slurry used to make the plates; useful results have routinely been obtained without meticulous attention to detail.

Finally, we have found plates of magnesium hydroxide to have excellent mechanical strength. Adsorbent layers can be handled directly without danger of chipping or cracking, and chromatograms may be marked with a pencil. By contrast, most other adsorbents require the addition of a binder to be rendered even minimally resistant to physical abuse.

In other respects, this new adsorbent is quite similar to alumina. For instance, inasmuch as the elution sequence for the compounds used in this investigation is nearly the same for magnesium hydroxide/benzene as for alumina/pentane-ether (Table I), it appears that R_F values in these two systems depend at least qualitatively upon the same factors. Either adsorbent may be used with any of several eluents, useful results having been obtained on magnesium hydroxide using such solvent systems as acetone, dioxane and chloroform-methanol (1:1). Chromatograms of either adsorbent can be visualized easily using short wave ultraviolet radiation, but the initially fluorescent spots of both fade quickly in air, giving way to colors visible in ordinary light¹¹. Preliminary work with magnesium hydroxide has suggested also that, like alumina, this adsorbent can be used satisfactorily in the chromatography of other classes of compounds, such as the aza aromatic derivatives⁹.

There are certain characteristics of magnesium hydroxide which make it less suitable than alumina for some applications. For instance, because magnesium hydroxide reacts with iodine¹², chromatograms cannot generally be visualized with this reagent, and thus alumina is to be preferred for compounds which cannot be located by fluorescence or spray techniques. In addition, elution times for the magnesium hydroxide/benzene system are longer than for alumina/pentane-ether; whereas the latter eluent travels 20 cm in 45 min, the former requires 90 min to migrate through the same distance.

Furthermore, slurries of magnesium hydroxide are rather basic. While this does not constitute a problem in the chromatography of most polynuclear compounds, the implications of this fact should be thoroughly investigated before the new system is used for separations involving base-sensitive materials.

Finally, the solubility of magnesium hydroxide in ethanol offers a disadvantage in quantitative recovery experiments, the results of which depend upon direct spectrophotometric assay of alcoholic extracts of the adsorbent. Dissolved or suspended inorganic material can contribute significantly to the observed absorbance at any wavelength studied in this investigation (400-220 $m\mu$), and in cases in which this background is large compared to the absorbance of the compound being determined, large uncertainties can result. For example, 20 μg of 1,1'-binaphthyl was applied to a magnesium hydroxide plate and recovered in an apparent yield of 160% (Table II). On the other hand, alumina, which is not appreciably soluble in ethanol, permitted an apparent recovery of 95% under the same conditions.

On the basis of the above considerations, we have concluded that magnesium hydroxide has much to recommend it as a thin-layer chromatographic adsorbent for the separation of polycyclic aromatic hydrocarbons. Despite several limitations of this new technique, we believe that its advantages over alumina render it worthy of consideration for use in at least some of the applications now served by that adsorbent.

TABLE II

QUANTITATIVE RECOVERY OF POLYCYCLIC AROMATIC HYDROCARBONS FROM THIN-LAYER CHROMATOGRAMS

Abbreviations: DBA = dibenz(*a,h*)anthracene; BP = benzo(*a*)pyrene; BA = benz(*a*)anthracene; P = pyrene; Bin = 1,1'-binaphthyl.

<i>System</i>	<i>Fraction</i>	<i>Component</i>	<i>Amount applied (μg)</i>	<i>Recovery (%)</i>	<i>Other detectable components</i>
Magnesium hydroxide/benzene	1	DBA	20	100	None
	2	BP	20	105	None
	3	BA	20	100	None
	4	P	20	90	None
	5	Bin	20	160*	None
Alumina/pentane-ether (95:5, v/v)	1	DBA	20	100	None
	2	BP	20	90	None
	3	BA	20	90	None
	4	P	20	105	None
	5	Bin	20	95	None

* Dissolved magnesium hydroxide interferes with spectral analysis in the case of 1,1'-binaphthyl because of the latter compound's relatively small absorptivity (see Experimental).

EXPERIMENTAL

Preparation of plates

Magnesium hydroxide. A slurry of 100 g of this reagent (Fisher Chemical Co.) in 150 ml of water was prepared and used to spread five 20 × 20 cm glass plates. A layer thickness of 1.0 mm was used in preparative scale experiments, while diagnostic work was performed on layers 0.25 mm thick. Because addition of a binder was not necessary, plates could be spread completely satisfactorily several hours after preparing the slurry. Organic solvents could also be used for the preparation of slurries, but the resulting layers were more susceptible to physical damage, and R_F values were much different from those obtained from plates prepared from aqueous slurries. Plates could be activated at 105° before use if so desired, but this did not appear to affect the results appreciably.

Alumina. Neutral Alumina AG-7 (Calbiochem) or Aluminium Oxide G (Brinkmann Instruments, Inc.) was shaken thoroughly with water (8–10 ml per 10 g of adsorbent) for 10 sec before spreading. Plates were dried in the atmosphere for several hours before use; activation at 105° was not employed, since this procedure greatly diminished R_F values and gave rise to extensive tailing in pentane-ether.

Application of sample

Solutions of the hydrocarbons listed in Table I were prepared by dissolving sufficient sample in benzene such that 1 μl of the solution produced a visibly fluorescent spot. Aliquots of 1 μl of each were applied to the plates with the aid of a spotting template.

For quantitative experiments, a stock solution was prepared by dissolving 100 mg portions of each of the hydrocarbons listed in Table II in 100 ml of benzene.

An aliquot of 1.0 to 10.0 ml of this solution or a suitable dilution thereof was applied in a band 5-7 mm wide by means of a mechanical streaking device¹³.

Elution and visualization

Elution chambers were lined on all four sides with filter paper which was saturated with eluent. Spots and bands were located with the aid of a Chromato-Vue fluorescence visualization apparatus (Ultra-Violet Products, Inc.) while the plates were still slightly wet with the eluent.

Recovery of separated hydrocarbons

In small scale runs, such as those described in Table II, the fluorescent zones were removed from the plate, crushed to a powder, and placed in small columns equipped with tight cotton plugs. Sufficient 95 % ethanol was filtered through each column with suction to provide 5.0 ml (10.0 ml in the case of dibenz(*a,h*)anthracene) of solution for the spectrophotometric assay, which was performed on a Cary 15 instrument. Alternatively, solvents such as benzene-ethanol could be used to remove the hydrocarbon more efficiently from the adsorbent; this technique appears to be preferable if the extract is to be subjected immediately to a second chromatographic separation, but we have found that losses incurred during the required evaporation of solvent prior to spectral analysis can be quite large, particularly for the lower-boiling compounds.

On several occasions, attempts were made to recover the hydrocarbons by dissolving the magnesium hydroxide in hydrochloric acid and extracting the aqueous phase with benzene. This procedure resulted, however, in chemical alteration of several of the polycyclic compounds, and it is not to be recommended, except perhaps in cases of extremely tenacious binding to the adsorbent.

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SUMMARY

We have found that magnesium hydroxide can be used successfully as a thin-layer chromatographic adsorbent in the separation of mixtures of polynuclear hydrocarbons. The characteristics of this system include high capacity, experimental simplicity, and the ability to effect a wide variety of specific separations, and it is suggested that this technique might be used to advantage in several of the applications for which alumina is presently employed.

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