

ADSORPTION OF DIMETHYLANILINES ON MONTMORILLONITE IN HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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Abstract—High-pressure liquid chromatography columns were packed with quasi-spherical particles of bentonite (SP-bentonite) which were prepared from a predominantly Na-bentonite powder by spray drying. The strength of adsorption of ring-substituted dimethylanilines on bentonite was directly related to their basicity (pKa). The adsorption of the amino-substituted N, N-dimethylaniline was commonly much weaker than that predicted from its pKa, suggesting that the amino group was the dominant site of interaction between the substituted anilines and the clay.

A better separation between the dimethylaniline isomers was obtained on SP-bentonite than on a neutral silica under the same chromatographic conditions. A selectivity factor of 25 was achieved between the most strongly and the least strongly adsorbed ring substituted isomers (3, 5- and 2,6-dimethylaniline, respectively) on the SP-bentonite. The selectivity factor was 3.4 on the silica. This efficiency of separation demonstrated the potential advantage of the readily available bentonite in high-pressure liquid chromatography. The higher resolution achieved in column runs than in batch experiments may be utilized for the elucidation of mechanisms of interaction of organic molecules at clay surfaces by comparing the interaction of a number of closely related adsorbates.

Key Words—Adsorption, Bentonite, Dimethylaniline, Liquid chromatography, Silica.

INTRODUCTION

Clays are strong and versatile adsorbents and are potentially efficient solid phases for adsorption chromatography. George *et al.* (1946) and Lew *et al.* (1946) separated sugars on clays, Dimant *et al.* (1952) used clays for the separation of peptides and nucleotides, and Shirobokov *et al.* (1975) used clay columns for the separation of RNA from cells. In addition, Bickov *et al.* (1976) obtained adsorption isotherms and energies of adsorption of polar and non-polar hydrocarbons on montmorillonite and kaolinite by means of gas chromatography, thus demonstrating the potential use of chromatographic clay columns for the study of the interaction of various adsorbates with clays.

Yamagishi (1982), Yamagishi and Ohnishi (1982, 1983), Yamagishi and Soma (1981), and Yamagishi *et al.* (1982) used short montmorillonite packed columns with an optically active complex as the exchangeable cation for the partial optical resolution of different metal chelates by liquid chromatography. They obtained a partial (36%) resolution of Co(III) tris(acetylacetonato) on columns 2.5 cm long (1.5 cm O.D.) filled with Δ -tris(1,10-phenanthroline)Ni(II)-montmorillonite at flow rates of 0.2–0.3-ml/min and a working temperature of 2°C (Yamagishi, 1982; Yamagishi *et al.*, 1982). An approximately 70% resolution of Co(acac)₃ on Δ -tris(1,10-phenanthroline)ruthenium(II)-montmorillonite was achieved with a 0.03-ml/min flow rate through a shorter column (0.6 × 1.2 cm O.D.) by Ya-

magishi and Ohnishi (1982). In all these experiments the use of expandable clays in liquid chromatography was hindered by the low hydraulic conductivity of the clay columns resulting from the clay's laminar structure and high swelling capacity. Swelling may be reduced by using organic derivatives of the clay. Montmorillonite with dimethyldioctodecylammonium as the exchangeable cation has been used in HPLC either as an additive or as a coating on a large diameter support for the separation of the structural isomers of some flat aromatic molecules (e.g., Grant *et al.*, 1974; Kiselev *et al.*, 1972; Lafosse *et al.*, 1978). Various quaternary ammonium clays were also used by White (1957), Cowan and Hartwell (1961), Taramasso (1971), Berzkin and Gavrichev (1976), and McAtee and Robbins (1980) for the separation of various compounds by gas chromatography. Thelmann and McAtee (1975, 1978) used metal-tris (ethylenediamine) complexes with montmorillonites for the gas chromatographic separation of polarizable gases and light hydrocarbons.

Organic derivatization alters the physico-chemical properties of clay surfaces; when the exchangeable cation is dimethyldioctodecylammonium, the clay surface becomes hydrophobic because of the presence of the long hydrocarbon chains. The procedure which was developed in the present study for preparation of HPLC columns from bentonite overcame the need for drastic modifications in the surface properties of the clay before it could be used as a solid phase for HPLC. Quasi-spherical particles of bentonite (SP-bentonite) were

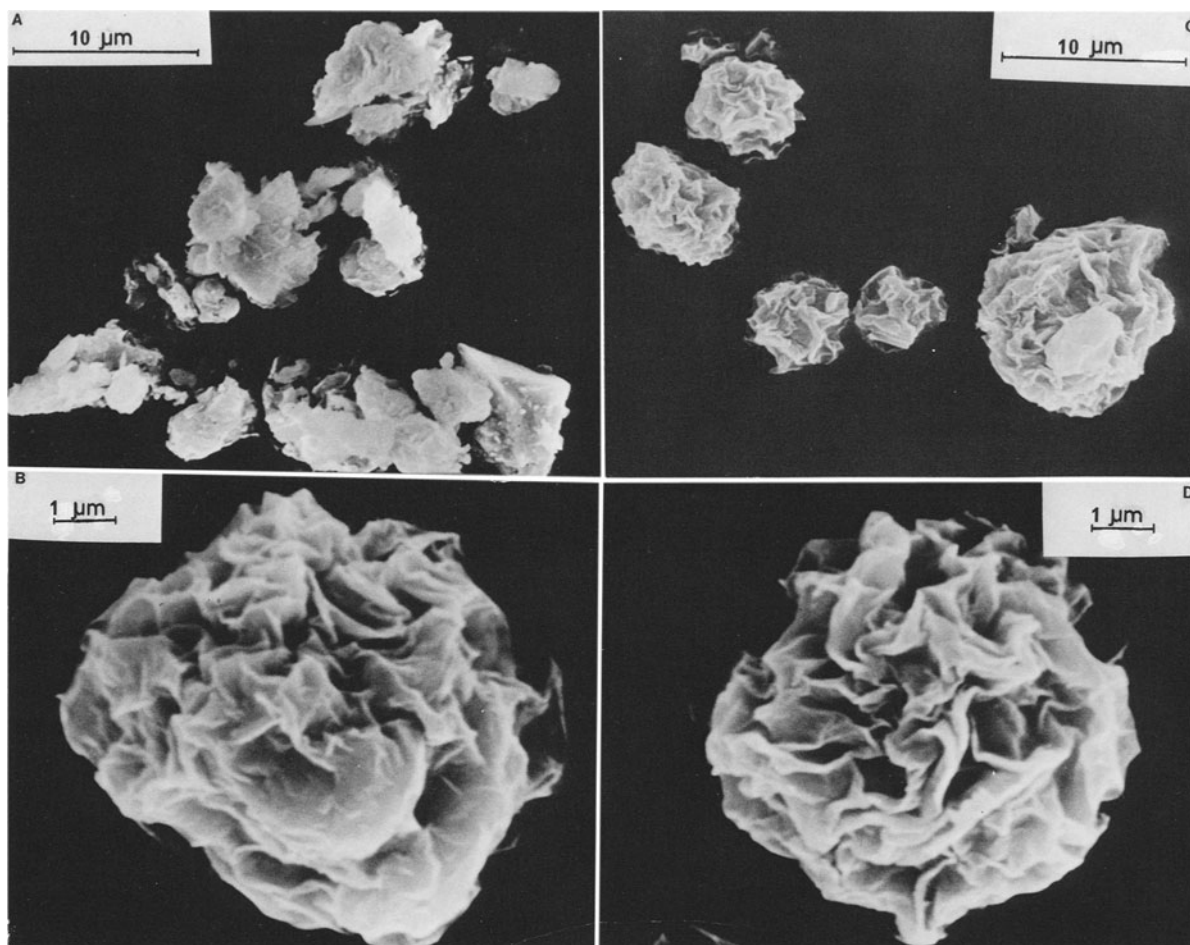


Figure 1. Scanning electron micrographs of (A) bentonite powder; (B, C) SP-bentonite; and (D) SP-bentonite preheated at 520°C.

prepared by spray drying in such a way that the surface properties of the clay were only slightly affected and the dominant arrangement of the plates within the particles was edge-to-face. Part of the prepared SP-bentonite was made less susceptible to swelling by a relatively mild thermal treatment which did not greatly alter the character of the clay surface. The flow rates used in the present study (0.5–1 ml/min) were achieved at moderate pressures (<1000 psi) with columns up to 20 cm long.

The preservation of the surface properties was attempted because the properties of bentonite, such as its high exchange capacity, large surface area, and ease of modifying reversibly the nature of its surface (e.g., by introducing different mineral or organic exchangeable cations) make it suitable for a wide range of chromatographic separations. This range may narrow considerably if specific modifications of the clay's surface are required for its use in HPLC. The small effect of the proposed procedure on the surface properties of

bentonite may make HPLC a powerful tool for the study of the interaction of many adsorbates with bentonite. The potential use of HPLC clay columns both for chromatographic separations and for elucidation of adsorption mechanisms has been exemplified in this study for dimethylanilines.

MATERIALS AND METHODS

Particle preparation and column packing

For the preparation of the solid particles, a 0.2% suspension of bentonite (Fisher B-235, Fisher Scientific Co., Fairlawn, New York) in deionized water was fed into a spray dryer (Anhydro Laboratory Spray Dryer, Copenhagen, Denmark). The inlet/outlet temperatures in the spray dryer were 250°/90°C, and the flow rate of the drying air was 120 liter/min. The main part of the spray dryer is a high-speed rotating nozzle atomizer. Drops of the suspension were sprayed out of the atomizer into the hot air flow, where the drops were evaporated immediately. The outlet temperature did

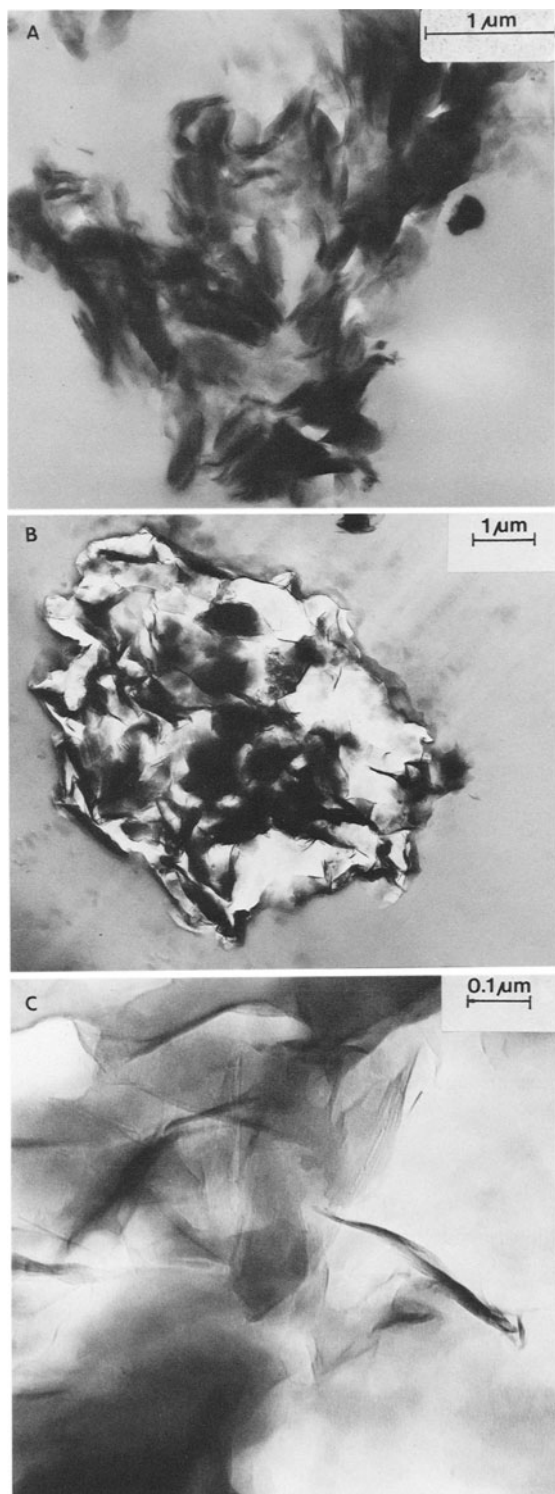


Figure 2. Transmission electron micrographs of (A) ultra-section of bentonite powder; (B, C) ultra-section of SP-bentonite.

not exceed 90°C; hence, the dried clay particles underwent no thermal modifications.

The particles used in this study had a mean diameter of 7 μm . The diameter of the particles was controlled by regulating the operating conditions of the spray dryer (e.g., suspension concentration and feed rate). The particles obtained were quasispherical and will be referred to here as SP-bentonite. A portion of the prepared SP-bentonite was heated at 520°C for 24 hr. Scanning electron micrographs of bentonite particles in the original powder (A), after drying in a spray dryer (B, C), and after heating at 520°C (D) are shown in Figure 1. The SP-bentonite was suspended in a 80% isopropanol–20% hexane mixture to sediment out the particles with diameters > 7 μm and then repeatedly sedimented in hexane to remove the smaller particles by decantation. For the preheated particles, a similar procedure was used; the coarse particles were removed in ethanol and the fine particles were removed in a 20% isopropanol–80% hexane mixture. HPLC columns were packed with unheated SP-bentonite suspended in hexane or with preheated SP-bentonite suspended in a 20% isopropanol–80% hexane mixture. The packing was performed using a high-pressure Tracor 955 LC pump at 4000 psi. The slurry was degassed before packing.

Chemicals

The 2,6-, 2,5-, 3,5-, and N,N-dimethylanilines were obtained from BDH Chemicals Ltd. (Broom Road, Parkstone, Poole BH12 4NN, England). Their purity was ascertained by examining them on TLC silica plates with 7% isopropanol in hexane as a developing solution. Solvents of HPLC grade (E. Merck, Darmstadt) were used in all experiments.

Analytical procedures

Chromatograms were obtained on a Tracor HPLC system equipped with a variable-wavelength UV detector model 970A, set at 237 or 254 nm. Columns packed with SP-bentonite, preheated SP-bentonite, and silica (Lichrosorb Si100, 5 μm ; Merck, Darmstadt) were used. Microscopic observations and photographs were made on a Cambridge Stereoscan 180 scanning electron microscope (SEM) and on a Philips-300 transmission electron microscope (TEM). The samples for TEM were prepared by the procedure of Shomer and Mingelgrin (1978). Ultrasections were prepared with an ultratome LKB-3. Cation-exchange capacity (CEC) was determined by the procedure of Richards (1954). Surface area was determined by the procedure of Ratner-Zohar *et al.* (1983) except that the samples were not ground before the determination. X-ray powder diffraction (XRD) patterns of the clay particles were taken on a Philips X-ray diffractometer type 1030 with

a Co target. Thermal gravimetric analyses (TGA and DTG) and differential thermal analysis (DTA) were performed on a Stanton Redcroft TG-DTA analyzer model STA-781. The DTA, TGA, and DTG patterns of SP-bentonite samples were observed while the samples were heated to 520°C at a rate of 5°C/min and then left for 24 hr at that temperature in order to determine whether changes in the structure of the clay occurred during the preheating procedure employed in the present study.

RESULTS AND DISCUSSION

From photomicrographs of thin sections of the original bentonite powder (Figure 2A), it is apparent that the clay particles were composed of stacks of many parallel plates. Photomicrographs of thin sections of SP-bentonite (Figures 2B, 2C), on the other hand, show a "card-house" structure with many cavities. The thin sections of the SP-bentonite particles displayed very thin longitudinal views of individual tactoids or of single plates, suggesting that the tactoids present consisted of a small number of plates. A small number of plates per tactoid was also indicated by the observed narrow cross-sections (Figures 2B, 2C). This arrangement exposed a large surface area and simulated the behavior of bentonite dispersed in a polar solvent. The "card-house" structure, composed of single plates and very thin tactoids, is shown clearly in the enlarged detail of the SP-bentonite section (Figure 2C) and in the scanning electron micrographs of Figure 1.

The surface area of the SP-bentonite was practically identical to that of the bentonite powder—634 and 644 m²/g, respectively, calculated on the basis of 0.286 m²/mg EGME (ethylene glycol monoethyl ether) adsorbed. Similarly, no discernible difference was observed between the CEC of the original bentonite powder and the SP-bentonite (0.79–0.81 meq/g), nor was there a significant difference in the moisture content or in the c-spacing of the two systems at equilibrium with ambient conditions.

Dimethyl-substituted anilines may serve as model compounds for the study of the interactions of aniline and its derivatives, in particular pesticides and their decomposition products, with clays (Theng, 1974). In a less direct way they are also models for various compounds containing amino groups, because the amino groups displays a strong affinity with clays (see e.g., Yariv *et al.*, 1969) and may dominate the interaction of many molecules which contain an amino group with clays. In the present study HPLC columns of SP-bentonite were used to gain insight into the interaction of the dimethylanilines with bentonite. A chromatogram of four isomers of dimethylaniline obtained using a SP-bentonite column with 1.2% isopropanol in hexane as an eluent is shown in Figure 3. The order of elution

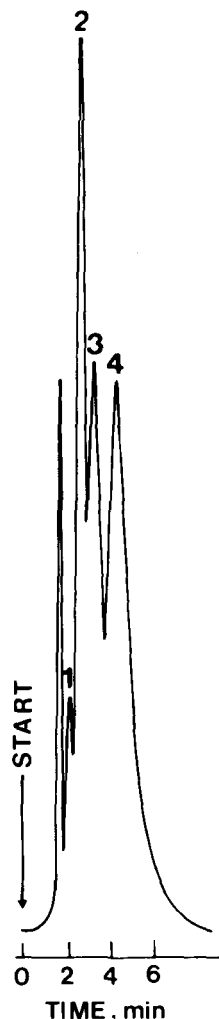


Figure 3. Separation of (1) N,N-, (2) 2,6-, (3) 2,5-, and (4) 3,5-dimethylaniline by an SP-bentonite column (17 × 0.46 cm). Eluent, 1.2% isopropanol in hexane; flow, 1 ml/min; detection UV, $\lambda = 254$ nm.

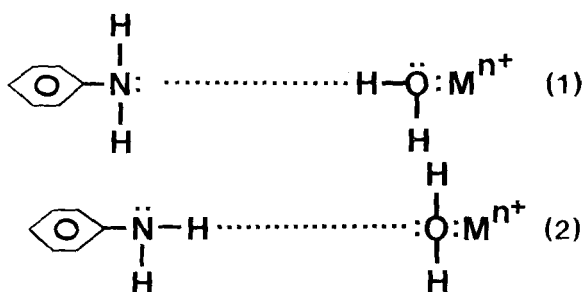
for the ring-substituted dimethylanilines correlated with their basicity (pK_a): as the basicity increased, so did the retention time (Figure 3 and Table 1). These results are in agreement with the findings of Yariv *et al.* (1969), who used powder Na-bentonite as opposed to the quasispherical particles used in the present study. These authors employed infrared spectroscopy to demonstrate that 2,5-dimethylaniline adsorbs more strongly on Na-montmorillonite than does 2,6-dimethylaniline, and stated that the adsorption mechanism is a hydrogen bond between the hydration water of the exchangeable cation and the amino group of the anilines. Two modes of interaction between the amino group of the anilines and the hydration water of the exchangeable cations are thus possible:

Table 1. Separation factors of ring-substituted dimethylanilines on different solid supports.¹

Compound	Eluent:		1.2% isopropanol in hexane						10% isopropanol in hexane				Acidity constant pKa ²
	Solid support		5- μ m Silica 100 (25 \times 0.46) cm		Unheated SP-bentonite (17 \times 0.46) cm		Preheated SP-bentonite (12 \times 0.46) cm		5- μ m Silica 100 (25 \times 0.46) cm		Preheated SP-bentonite (12 \times 0.46) cm		
			k'	α	k'	α	k'	α	k'	α	k'	α	
2,6-dimethylaniline			1.8	1.0	0.5	1.0	0.4	1.0	0.3	1.0	0.4	1.0	3.9
2,5-dimethylaniline			3.1	1.7	0.9	1.9	2.0	5.0	0.6	2.0	0.9	2.2	4.6
3,5-dimethylaniline			6.2	3.4	1.6	3.1	10.0	25.0	1.0	3.3	2.2	5.5	4.9

¹ k' = capacity; α = selectivity factor taken relative to 2,6-dimethylaniline.

² From Smith (1968).



Yariv *et al.* (1969) claimed that both interactions may take place.

If the adsorption is indeed through the hydration shell of the exchangeable cations, the increase in the retention time with the basicity of the ring-substituted dimethylanilines suggests the predominance of interaction (1) in the adsorption of dimethylanilines. This arrangement is likely because the adsorbate molecule serves in interaction (1) as a proton acceptor (base), whereas in interaction (2) it serves as a proton donor. Despite its highest basicity (pKa = 5.1; Smith, 1968), N,N-dimethylaniline when eluted with 1.2% isopropanol in hexane, passed through the column faster than any of the ring-substituted dimethylanilines (Figure 3). Interaction (2) could not have existed for N-N-dimethylaniline because both hydrogen atoms in the amino group were substituted by methyl groups and interaction (1) was sterically hindered by the two bulky and hydrophobic methyl groups attached to the nitrogen atom. The strong effect of the substitution of the two amino hydrogens by methyl groups on the adsorption is, in any case, consistent with the proposed adsorption mechanism (Yariv *et al.*, 1969).

In evaluating the potential use of bentonite as a solid phase in HPLC it should be emphasized that considerably sharper chromatograms than those presented in Figures 3–5 could be achieved if a narrower particle size distribution (e.g., Schram, 1980) and better column packing had been obtained. Efficient methods (e.g., Jewett and Lawless, 1980; Larsen and Schou, 1981) are available for a nearly complete particle size separation. Because an optimal size distribution was not a

goal of this work, the size selection by decantation was discontinued as soon as the columns prepared from the fractionated material enabled chromatographic separations under convenient flow-rates and pressures.

Preheated SP-bentonite

The unheated Na-saturated SP-bentonite displayed some swelling in media more polar than 3% isopropanol in hexane. This swelling was inhibited by exchanging the Na with a polyvalent cation. Although such an exchange was successful in reducing the swelling capacity, preheating of SP-bentonite particles was preferred for this purpose. Preheating maintained the universality of the chromatographic systems, i.e., it allowed the use of any exchangeable cation desired. The exchange of one cation for another may considerably modify the adsorption of many organic molecules on clays (e.g., Heller and Yariv, 1969; Yariv *et al.*, 1969; Mingelgrin and Saltzman, 1979). The ability to employ a wide range of exchangeable cations is therefore important in the use of SP-bentonite both for chromatographic separations and as a tool for the study of the interaction of many adsorbates with clays.

To be able to use more polar eluents such as methylene chloride, methanol, or water, the SP-bentonite was preheated at 520°C. This temperature was chosen so as to achieve a stronger binding at points of contact between contiguous plates, with a minimum of damage to the surface properties of the clay. In the DTA of montmorillonite, the peak corresponding to the loss of structural water occurred at 630°C (e.g., van Olphen, 1977). At 520°C, the loss of structural water had already occurred, but at a very slow rate. The CEC of the preheated SP-bentonite decreased by only 15% as compared with that of the unheated SP-bentonite (Table 2), suggesting only minimal damage to the structure. The XRD and thermal analyses confirmed the small effect of the preheating on the structural properties of the SP-bentonite. There was no observable difference in the XRD pattern in the interplanar-spacing range of 1.4 to 34 Å between the unheated and preheated SP-bentonite except for a difference in the

Table 2. Cation-exchange capacity (CEC) and moisture content of the bentonite and silica supports.

Support	CEC (meq/g)	Moisture content ¹ (mM water/g)
Bentonite powder (Fisher)	0.81	5.9
SP-bentonite	0.79	3.0
SP-bentonite dried at 105°C	0.81	—
SP-bentonite preheated at 520°C	0.69	1.2
Silica (5- μ m Lichrosorb, Si 100, Merck)	0.22 ²	4.2

¹ Moisture content determined immediately before column packing. The unheated and preheated SP-bentonite samples underwent size selection by sedimentation (see text), followed by storage in sealed containers for ~30 days before column packing. Values are relative to oven-dried (105°C for 24 hr) samples.

² CEC at pH 8.2.

reflection corresponding to the c-spacing. Thus, the XRD pattern suggested a possible collapse of parallel plates but no change in the crystal structure itself.

The thermal analyses (DTA, TGA, and DTG) were made under conditions which reproduced the preheating procedure employed in the present study. They revealed no significant weight loss (or transformations which did not involve weight loss) except for a peak below 110°C, which was assigned to the loss of hydration water (e.g., Borhardt, 1977). After it was kept at 520°C for 24 hr, the SP-bentonite weight loss was 10.80%, whereas after 24 hr at 110°C the loss was $10.55 \pm 0.15\%$. Borhardt (1977) reported for montmorillonite a 4.88% weight loss due to dehydroxylation associated with the peak at 630°C. It is evident that the difference between the weight loss due to dehydration of the surface (heating to 110°C) and that due to preheating at 520°C constituted only a very small part of the water loss that may have resulted from dehydroxylation. Thus, significant dehydroxylation did not take place. Preheating at lower temperatures (320° and 420°C) did not cause the desired reduction in swelling.

Figures 4 and 5 present the separation of the ring-substituted dimethylanilines on preheated SP-bentonite. The order of adsorption of the ring-substituted anilines was the same for preheated and unheated SP-bentonite. Other work in this laboratory has demonstrated further the similarity in the adsorptive properties of the bentonite powder, the SP-bentonite, and the preheated SP-bentonite. Batch measurements of the adsorption of parathion (O,O-diethyl-O-p-nitrophenyl phosphorothioate), methyl parathion (O,O-dimethyl-O-p-nitrophenyl phosphorothioate), and paraoxon (O,O-diethyl-O-p-nitrophenyl phosphate) from hexane showed the same order of adsorption on all three adsorbents: parathion < methyl parathion < paraoxon.

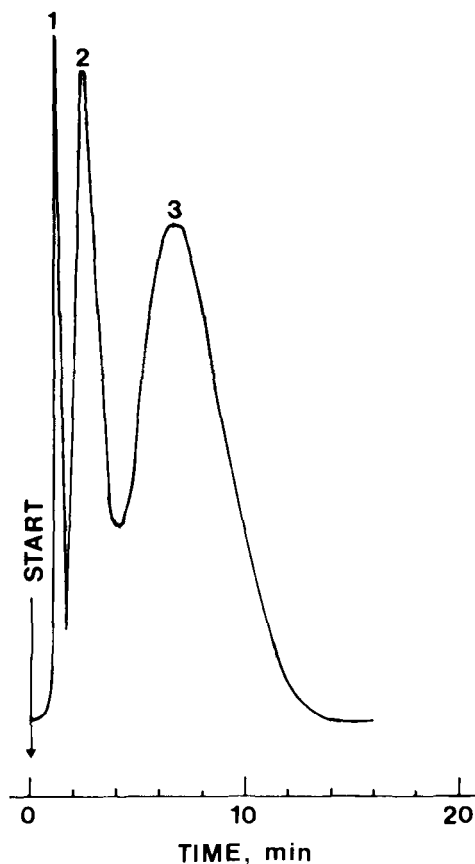


Figure 4. Separation of (1) 2,6-, (2) 2,5-, and (3) 3,5-dimethylaniline by a preheated SP-bentonite column (12 × 0.46 cm). Eluent, 3% isopropanol in hexane; flow, 1 ml/min; detection UV, $\lambda = 237$ nm.

The preheating caused collapse of whatever tactoids that were present. Although the exchange capacity was kept relatively high, the preheated SP-bentonite lost much of its swelling capacity. The XRD data indicated that the c-spacing remained at 9.6 Å after 30 days of exposure to air. Equilibration with hexane supersaturated with water caused no swelling either. Under similar conditions, the unheated SP-bentonite swelled. In the batch measurements of the adsorption of parathion and methyl parathion, the c-spacing of the preheated SP-bentonite remained at 9.6 Å. This value indicates that the phosphate esters failed to penetrate into the interlayer spaces, whereas such penetration did occur in the unheated SP-bentonite. The surface area of the preheated SP-bentonite was 454 m²/g, calculated on the basis of 0.286 m²/mg adsorbed EGME, 28% less than the surface area of unheated SP-bentonite. The loss of swelling capacity implies that the reduction in surface area arose from the aforementioned collapse of whatever tactoids that were present.

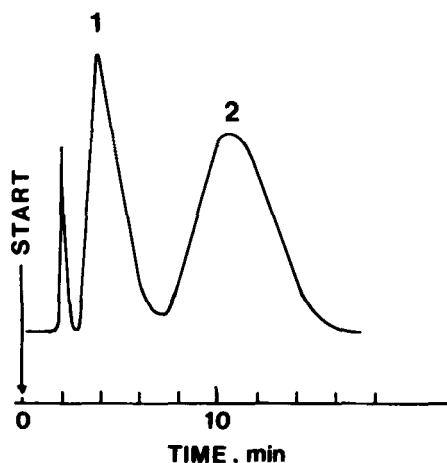


Figure 5. Separation of (1) 2,6- and (2) 2,5-dimethylaniline by a preheated SP-bentonite column (12×0.46 cm). Programmed elution was used from pure hexane to 5% isopropanol in hexane; programming was completed in 20 min; flow, 0.5 ml/min; detection UV, $\lambda = 237$ nm.

The apparent number of plates per tactoid (n) is equal to

$$S_t/S_0, \quad (3)$$

where S_t and S_0 are the total and outer surface areas of the bentonite, respectively. If, as the XRD data suggest, there was practically no penetration of EGME into the interlayer spaces of the preheated clay, the surface area measured after preheating was equal to the S_0 of the SP-bentonite. Thus, the loss of 28% of the surface area implies an average of 1.4 plates per tactoid. Equation (3) neglects the broken edges, but at low n values this is acceptable. Sodium was the dominant exchangeable cation in the bentonite used in the present study, and the apparent tactoid size computed above is close to that proposed for Na-montmorillonite in an aqueous suspension (Banin and Lahav, 1968), suggesting that the arrangement of the plates in the SP-bentonite particles simulated their arrangement in aqueous montmorillonite suspensions. An average of 1.4 plates per tactoid implies that the effect of the inaccessibility of the interlayer spaces due to the collapse on the total adsorption by SP-bentonite was small. This small value of n also agrees with the loss of only 15% of the CEC, despite the collapse of whatever tactoids were present.

The similarity of the thin sections of preheated SP-bentonite (Figure 6) to those of unheated SP-bentonite (Figure 2C), as well as the similarity of the SEMs of the preheated and unheated samples (Figure 1), were clear. The "card-house" structure and the prevalence of isolated plates or very thin (2–3 plates) tactoids (e.g., Figure 6a) are in agreement with the average number of plates per tactoid calculated from the surface area

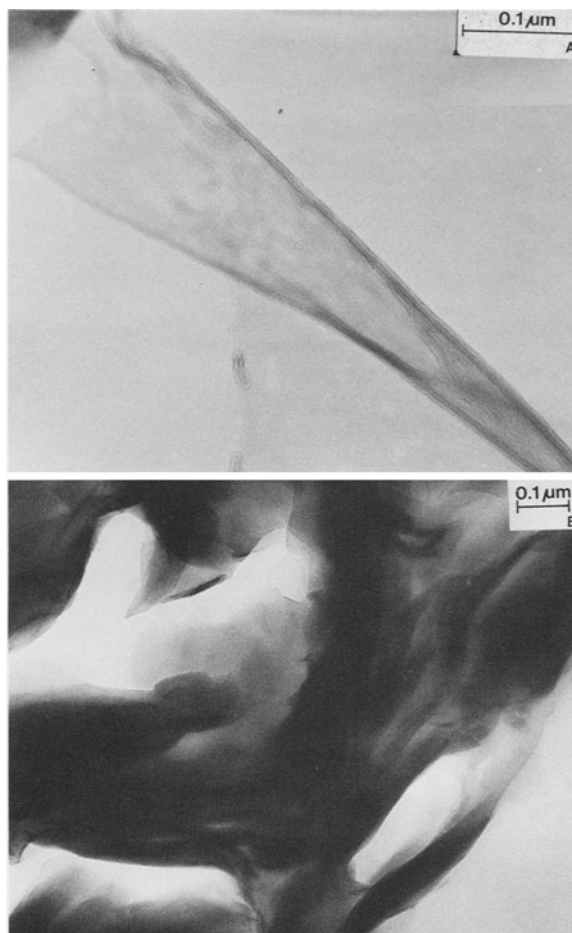


Figure 6. Transmission electron micrographs of ultrasections of preheated SP-bentonite.

measurements. This arrangement of plates within the SP-bentonite particles minimized the effect of the non-expandibility of the preheated clay on its surface activity. Yet, as other work in this laboratory has demonstrated, the stabilization achieved by preheating enabled the separation of optical isomers of amino acids in an aqueous solution using preheated SP-bentonite.

The collapse observed in the preheated SP-bentonite was reversible to some extent, but the rate of recovery was very low. The c -spacing of the preheated clay, as revealed by XRD, was 9.6 Å after 30 days of exposure to air. The reflection corresponding to the c -spacing became broader with time and shifted toward a larger spacing, reaching 13.8 Å after 24 months of exposure. The air-dried, unheated SP-bentonite produced a somewhat narrower reflection centered at 13.8 Å both after a month and after a 24-month exposure to air. These data demonstrate again that whereas preheating enabled the use of SP-bentonite with polar eluents, it produced minimal changes in the properties of the clay.

The capacity (k') of a substance in a given chromatographic system is defined as:

$$(t_R - t_0)/t_0, \quad (4)$$

where t_R is the retention time of the substance and t_0 is the retention time of non-interacting molecules. The selectivity constant (α) is defined as:

$$k'/k'_0, \quad (5)$$

where k'_0 is the capacity factor of a reference substance. The separation of the ring substituted dimethylanilines was greater on the preheated SP-bentonite than on the unheated one (Figures 3 and 4; Table 1). This improved separation following preheating is in agreement with the proposed mechanism of adsorption (e.g., Yariv *et al.*, 1969). The mechanism involves hydrogen bonding between the amino group of the dimethylanilines and hydration water on the clay surface or, when the clay is sufficiently dry, an interaction between the lone pair of electrons on the amino-nitrogen and an anhydrous exchangeable cation.

Preheating may have resulted in partial dehydration of the exchangeable cations, as suggested by the molar moisture contents of the various clay prepreparates (Table 2). This decrease in the number of water molecules in the hydration shell of the cation increased the polarity of the hydration water (e.g., Mortland, 1970). When the surface was dehydrated to the point where the polarity of the remaining hydration water became high enough, the water molecules likely served as a Bronsted acid. The hydrogen bond described by interaction (1) may then have been replaced by the actual protonation of the amino group, resulting in a strong ionic bond between the protonated dimethylaniline and the surface. Further drying produced anhydrous exchangeable cations, which were strong Lewis acids. Thus, the drier the clay surface, the stronger was its acidity (e.g., Mortland, 1970).

The k' values of the ring-substituted dimethylanilines increased with the increase in their basicity (Table 1). This fact and the suggested dominance of interaction (1) in the adsorption on the clay imply that the more acidic the surface, the stronger was the adsorption. The effect of preheating (and hence drying) on k' , which increased with the increasing basicity of the dimethylanilines (Table 1), is in agreement with the above discussion. As expected, the α values relative to 2,6-dimethylaniline (the least basic isomer) of the other dimethylanilines increased upon preheating (and hence drying) of the clay (Table 1). The k' value of the least basic isomer (2,6-dimethylaniline) was actually slightly larger on the unheated SP-bentonite than on the preheated clay (Table 1), possibly due to the somewhat larger specific surface area of the former.

N,N-dimethylaniline

N,N-dimethylaniline is the most basic of the di-

Table 3. The effect of the chromatographic conditions on the behavior of *N,N*-dimethylaniline.

Solid support	Eluent % isopropanol in hexane	<i>N,N</i> -dimethylaniline	
		Capacity ¹ k'	Selectivity ¹ α
SP-bentonite	1.2	0.20	0.13
Preheated SP-bentonite	1.2	6.50	0.65
	5	1.55	0.67
	10	2.63	1.20
	30	1.45	2.41
Silica	1.2	0.40	0.06
	10	0.10	0.10

¹ k' = capacity; α = selectivity factor relative to 3,5 dimethylaniline.

methylanilines studied ($pK_a = 5.1$). Table 3 presents k' and selectivity factors which were measured for this substance with 3,5-dimethylaniline (the next most basic isomer) as the reference under different chromatographic conditions. The data in Table 1 and Figures 3 and 4 indicate that the order of elution of the three ring-substituted dimethylanilines did not change with the composition of the eluent or with the preheating (drying) of the solid phase. The *N,N*-isomer, however, changed its relative elution rate from the slowest to the fastest of all the isomers depending on the eluent and on the preheating (drying) of the solid phase (Tables 1 and 3; Figure 3).

Methyl groups substituted both hydrogen atoms of the amino group of the *N,N*-dimethylaniline. If the active site of an adsorbate was shielded by bulky groups, small changes at the surface of the adsorbent or in the mobile phase may have strongly affected the adsorption process. Accordingly, steric hindrance caused by the apolar methyl groups of *N,N*-dimethylaniline may have necessitated some specific orientation of the amino group relative to the adsorption site ("keying") for an interaction to have occurred. When several water molecules were present in the hydration shell of the exchangeable cation, every hydrogen atom of the hydration water was located close to a few water molecules. These water molecules may have blocked the approach of the amino-nitrogen to the hydrogen atom by repelling the hydrophobic methyl groups attached to that nitrogen. Thus, no conformation may have existed, which enabled the approach of the lone pair electrons of the N close enough to a hydrogen atom of the hydration water for the formation of a stable hydrogen bond. When only one water molecule was present in the hydration shell of the cation, the two methyl groups might have found an orientation relative to the surface which enabled the nitrogen's lone pair electrons to approach close enough to a hydrogen atom of the hydration water to form a strong hydrogen bond. The high sensitivity of the adsorption of *N,N*-dimethylaniline to the properties of the surface was also reported by

Engelhardt and Muller (1981), who found that the retention of N,N-dimethylaniline on silica is strongly dependent on the pH of the silica in an aqueous suspension ($k' = 1.1$ on a silica with pH = 9.0; $k' = 5.2$ on a silica with pH = 7.2; eluent: dichloromethane containing 100 ppm water). The retention of aniline (Engelhardt and Muller, 1981) is appreciably less dependent on the pH ($k' = 3.2$ on the silica with pH = 9.0; $k' = 4.6$ on the silica with pH = 7.2).

Comparison with silica columns

The order of adsorption of the ring-substituted dimethylanilines was the same on the silica as on the clay (Table 1). The fact that the k' values of these substances on silica columns were also a function of their basicity was observed by Schmeltz *et al.* (1982). Inasmuch as both the bentonite and the silica possessed a CEC (Table 2) and the clay's surface consists almost entirely of silica sheets, a similar mechanism could have controlled the separation of the ring-substituted dimethylanilines on the clay and on the silica columns. If interaction (1) dominated the adsorption of the dimethylanilines on the silica as it apparently did on the SP-bentonite, the retention of the anilines by the surface should have been a function of the CEC, of the hydration status of the surface, and of its polarizing strength (or acidity).

The silica used here had a CEC of 0.22 meq/g (at pH 8.2), as compared with 0.69 meq/g for the preheated SP-bentonite (Table 2). As opposed to the montmorillonitic clay studied, most of the CEC in the silica originated from the acidic silanol groups and was therefore pH-dependent (Unger, 1979). The pH of the investigated silica in an aqueous suspension was 7.0, but the CEC was determined at pH 8.2. The CEC included, therefore, the more active protons which were possibly capable of protonating the dimethylanilines. The dimethylanilines may have been protonated either by dissociation of hydration water or by protons donated by the surface itself. Protonation, which generates a strong affinity between the surface and the adsorbate, may have also contributed, as suggested above, to the relatively high k' values (Table 1) of the more basic ring-substituted dimethylanilines on the preheated SP-bentonite. The silica sample studied was more hydrated than the SP-bentonite (Table 2). The higher hydration level brought about a smaller polarization of the hydration water (e.g., Mortland, 1970). On the other hand, hydrogen, which may have held much of the hydration water on the silica, has a greater electronegativity (and hence polarizing strength) than sodium which was the dominant exchangeable cation on the SP-bentonite. Engelhardt and Muller (1981) reported that in the pH range 6.5 to 9.0 the retention of anilines on silica increases as the pH of the silica in aqueous suspension decreases. Thus, they found that an acidic silica (pH 6.5) retained anilines from di-

chloromethane containing 100 ppm of water so strongly that they could not be eluted within a reasonable time. Their findings are in agreement with interaction (1), or with a similar interaction between the lone electron pair of the amino nitrogen and an acidic species on the surface.

The order of the efficiency of resolution between the ring-substituted isomers was: preheated SP-bentonite > silica \approx unheated SP-bentonite (Table 1). This order was consistent with the differences in the dominant adsorption site (sodium, hydrogen, and their hydration water), the hydration status, and the CEC (Table 2) among these three phases.

CONCLUSIONS

Spray-dried quasispherical particles of bentonite (SP-bentonite) were utilized as packing material for HPLC columns. These columns offer new possibilities in the investigation of the interactions of clay-organic molecules because the chromatographic procedure was faster, more accurate, sensitive, and convenient than the classical batch adsorption technique. The short contact time required eliminated interference by surface degradation reactions which may occur in the slower batch procedures. Preheated SP-bentonite may be utilized in chromatography and specifically in HPLC with eluents of any polarity. It is an adsorbent with a large active surface area and an ability to retain different cations on its surface. This solid phase may be used for otherwise difficult separations, such as the resolution of optical isomers of amino acids and the separation of peptides and many structural isomers.

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