Acid–Base Equilibria of Tetracycline in Sodium Montmorillonite Suspensions

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Abstract □ Changes in the partial molar free energy of the ionic species of tetracycline as a result of interaction with sodium-saturated montmorillonite were monitored by UV spectroscopic titrations. The stronger interaction between the clay and the protonated (H_3T^+) species relative to the zwitterionic (H_2T^0) species is responsible for the apparent displacement of pK_1 in clay suspensions. The displacement of the first equilibrium favors the H_3T^+ species due to its stabilization by the negative clay surface. Little effect of the clay was observed on pK_2 and pK_3, because the ionic species of tetracycline associated with these equilibria (H_2T^0, HT^-, and T^{2-}) did not interact strongly with the negative clay surface. Therefore, the ionic species distribution of tetracycline in a sodium montmorillonite suspension is significantly different than that expected based on solution equilibria. The degree of equilibrium displacement depended on the accessibility of the negative charge at the clay surface. Fraction bound curves support the hypothesis that the clay surface causes a change in tetracycline equilibria. The H_3T^+ form of tetracycline was highly bound to the sodium montmorillonite, but little adsorption was observed for the H_2T^0 species.

Keyphrases □ Tetracycline—acid-base equilibria in sodium montmorillonite suspensions □ Adsorption—bonding of tetracycline to sodium montmorillonite, acid-base equilibria of tetracycline □ Sodium montmorillonite—effect on acid-base equilibria of tetracycline

The mechanism of adsorption of tetracycline by montmorillonite was studied recently by X-ray diffraction and IR spectroscopy and was found to be highly dependent on the ionic form of tetracycline (1). Feldkamp and White (2) recently examined the effect of clay surfaces on the acid–base equilibria of weak bases in aqueous clay suspensions and showed that protonation was enhanced as a consequence of weak base–clay interactions. Since any changes in the distribution of ionic species present in a system have important consequences, the effect of sodium montmorillonite on the acid–base equilibria of tetracycline was studied.

THEORY

In the thermodynamic analysis given by Feldkamp and White (2), the behavior of the acid–base equilibrium for any weak base, B, was considered in clay suspensions (Scheme I).

$$BH^+ \rightleftarrows B + H^+$$

Scheme I

It was shown that the equilibrium can be displaced due to interactions between constituents participating in the equilibrium (B, BH^+, and H^+) and the clay surface. In particular, protonation in excess of that predicted based on the pK value of the compound and the bulk solution pH of the suspension (pH^b) results from a stronger BH^+–clay interaction than a B–clay interaction. In more concise terms, $\Delta\bar{G}_{BH^+}$ is less than $\Delta\bar{G}_B$, where $\Delta\bar{G}_i$ (i = BH^+, B) is the change in the partial molar Gibbs free energy for constituent i accompanying the addition of clay to the system at constant temperature, pressure, and composition. If $\Delta\bar{G}_{BH^+} = \Delta\bar{G}_B$, indicating that both BH^+ and B interact equally well with the clay, no enhanced protonation effect arises.

The $\Delta\bar{G}_{BH^+}$ and $\Delta\bar{G}_B$ values are related by:

$$\Delta\bar{G}_{BH^+} - \Delta\bar{G}_B = RT \ln \frac{K_{eff}}{K} \qquad \text{(Eq. 1)}$$

where:

$$K = a_{H^+} \frac{a_B}{a_{BH^+}} \qquad \text{(Eq. 2)}$$

and:

$$K_{eff} = a_{H^+} \frac{\bar{C}_B}{\bar{C}_{BH^+}} \qquad \text{(Eq. 3)}$$

The activities are defined by $\bar{G}_i = \bar{G}_i^* + RT \ln a_i$. Because the system is heterogeneous, external electric fields are present (due to the charged clay). As a consequence, $\Delta\bar{G}_i$ may be identified with the electrochemical potential (3), and the activity a_i is regarded as the total activity as described by Low (4). This activity is not to be confused with the activity commonly applied to homogeneous systems (*i.e.*, solutions, for which the activity is defined in terms of the chemical potential and relates to field-free systems). The K_{eff} value is simply an effective or apparent equilibrium constant, and $\bar{C}_i$ (i = B, BH^+, H^+) are average suspension concentrations.

If the ratio of K_{eff} to K is less than one (*i.e.*, $pK_{eff} - pK > 0$), then $\Delta\bar{G}_{BH^+} < \Delta\bar{G}_B$. In addition, when $K_{eff}/K = 1$, then $\Delta\bar{G}_{BH^+} = \Delta\bar{G}_B$. Consequently, K_{eff}/K and $pK_{eff} - pK$ serve as parameters indicating the degree of equilibrium displacement (or enhanced protonation) due to BH^+–clay and B–clay interactions.

The $\Delta\bar{G}_{BH^+}$ and $\Delta\bar{G}_B$ values can be measured individually under certain experimental conditions. If the system is sufficiently dilute with respect to clay and all other solutes, then $\Delta\bar{G}_{BH^+}$ and $\Delta\bar{G}_B$ are given by:

$$\Delta\bar{G}_{BH^+} = RT \ln (1 - f) \left(\frac{K_{eff} + a_{H^+}}{K + a_{H^+}}\right) \qquad \text{(Eq. 4)}$$

$$\Delta\bar{G}_B = RT \ln (1 - f) \left(\frac{K}{K_{eff}}\right) \left(\frac{K_{eff} + a_{H^+}}{K + a_{H^+}}\right) \qquad \text{(Eq. 5)}$$

where f is the fraction of base initially added to the system that is bound to the clay.

This approach may be applied to any acid–base equilibrium of tetracycline (Schemes II–IV) (5), and either K_j (j = 1, 2, 3) or $K_{j,eff}$ can be determined.

$$H_3T^+ \overset{K_1}{\rightleftarrows} H_2T^0 + H^+$$

Scheme II

$$H_2T^0 \overset{K_2}{\rightleftarrows} HT^- + H^+$$

Scheme III

$$HT^- \overset{K_3}{\rightleftarrows} T^{2-} + H^+$$

Scheme IV

Both $K_{j,eff}$ and $\Delta\bar{G}_i$ (i = H_3T^+, H_2T^0, HT^-, T^{2-}) depend on the composition of the system, *i.e.*, pH, ionic strength, temperature, pressure, other solutes, and $\bar{C}_i$. As $\bar{C}_i \to 0$, $K_{j,eff}$ and $\Delta\bar{G}_i$ approach a definite value that reflects an intrinsic interaction of species i with the clay surface, independent of $\bar{C}_i$.

EXPERIMENTAL

Materials—Tetracycline hydrochloride and all other chemicals were official or reagent grade. All water was double distilled. Wyoming bentonite[1] was the source of the montmorillonite used. Characterization by X-ray diffraction[2] and IR spectroscopy[3] showed the Wyoming bentonite

[1] Clay Mineral Repository, Department of Geology, University of Missouri, Columbia, Mo.

[2] Siemens A G Kristalloflex 4 generator and type F diffractometer, Karlsruhe, West Germany.

[3] Model 180, Perkin-Elmer Corp., Norwalk, Conn.

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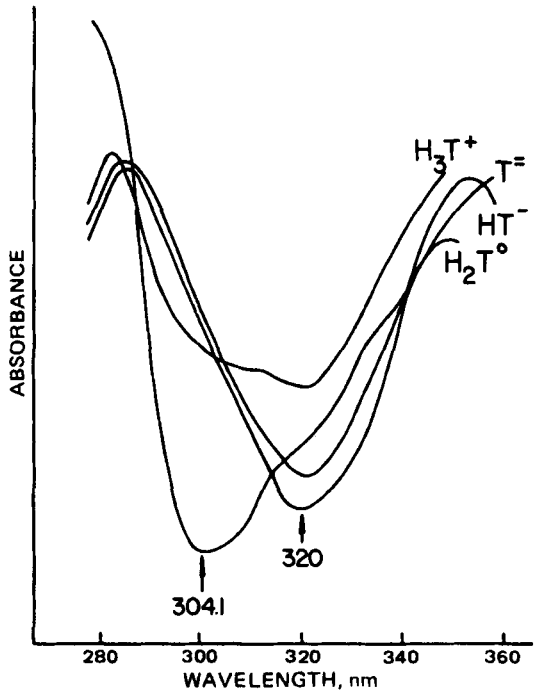

Figure 1—*UV spectra of the four ionic species of tetracycline in aqueous solution (20 mg/liter).*

to be montmorillonite containing a small amount of quartz. The Wyoming bentonite fraction (<2 μm) was washed five times with 1 *M* NaCl and then washed with water until it was salt free, as evidenced by the silver nitrate test to produce the sodium-saturated montmorillonite used. The suspension was passed through a 300-mesh screen to remove any extraneous matter and diluted to obtain a 1% sodium-saturated montmorillonite suspension.

Stock solutions of tetracycline, 200 mg/liter, were prepared fresh before each titration and passed through an ultrafine sintered-glass filter.

Determination of *K* and K_{eff}—UV spectroscopic titrations (6) were performed to determine $\overline{C}_B/\overline{C}_{BH^+}$ as required for Eq. 3. In very dilute solutions (clay free), $\overline{C}_B/\overline{C}_{BH^+} = a_B/a_{BH^+}$ in Eq. 2. The absorbance, A, of a tetracycline solution is related to $\overline{C}_B/\overline{C}_{BH^+}$ by:

$$\frac{\overline{C}_B}{\overline{C}_{BH^+}} = \frac{A - A^+}{A^- - A} \qquad \text{(Eq. 6)}$$

where A^+ is the absorbance when all of the tetracycline is present as BH^+ and A^- is the absorbance when all tetracycline is present as B.

Thus, experimental values for $\overline{C}_{H_2T^0}/\overline{C}_{H_3T^+}$, $\overline{C}_{HT^-}/\overline{C}_{H_2T^0}$, and $\overline{C}_{T^{2-}}/\overline{C}_{HT^-}$ were obtained.

The titrations were performed at a wavelength where A^+ and A^- were significantly different. As shown in Fig. 1, the greatest absorbance difference between H_3T^+ and H_2T^0 occurs at 304.1 nm, while the maximum difference among H_2T^0, HT^-, and T^{2-} occurs at 320 nm. The bandwidth was set at 0.8 nm for all titrations.

Titration curves were constructed by preparing a series of solutions or suspensions at different pH^b values and determining the absorbance at 304.1 nm for the first ionization or 320 nm for the second and third ionizations of tetracycline. Equal volumes of 0.01 *N* HCl and NaOH were pipetted into two 100-ml volumetric flasks, depending on the pH^b desired. Ten milliliters of a 200-mg/liter tetracycline stock solution was added to the sample flask. If sodium chloride was to be added, a level of either 25 mg/liter or 5.844 g/liter was added to both the sample and reference flasks. The sample and reference flasks were diluted to ~95 ml with water. Two milliliters of the 1% sodium-saturated montmorillonite suspension was added to both flasks prior to dilution to 100 ml with water. This procedure minimized the change in pH^b while mixing the tetracycline with the clay. All sample suspensions contained 20 mg of tetracycline and 200 mg of sodium-saturated montmorillonite/liter.

The pH^b of a stirred 50-ml aliquot of the sample suspension was measured[4] after the pH^b had stabilized.

The difference in the absorbance[5] of the sample and reference suspensions, $A_s - A_r$, at the appropriate wavelength was taken as A in Eq. 6. The absorbance at different pH^b values was plotted to construct the titration curves.

[4] Model 801, Orion Research, Cambridge, Mass.

[5] Acta C-II UV-visible spectrophotometer with a light-scattering accessory, Beckman Instruments, Fullerton, Calif.

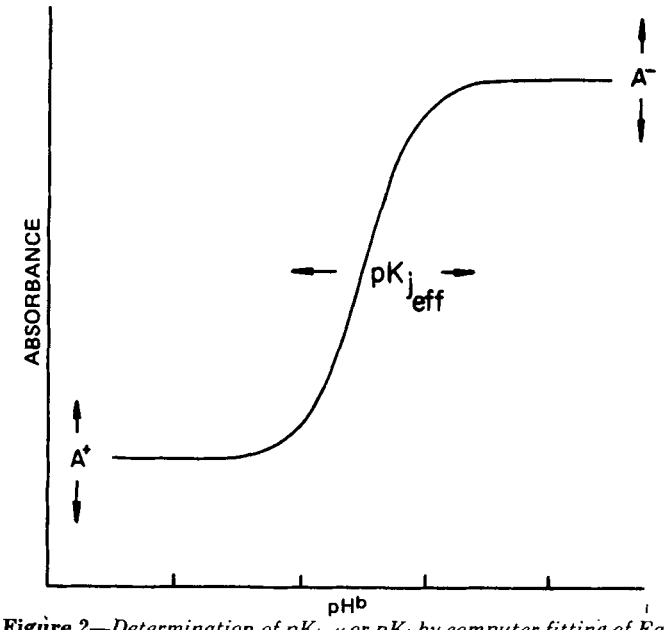

Figure 2—*Determination of $pK_{j,eff}$ or pK_j by computer fitting of Eq. 7 to pH^b versus A.*

The average concentration ratio was computed from the titration curve and used in Eq. 4 to obtain $pK_{j,eff}$. These calculations were restricted to values of $a^b_{H^+}$ such that $0.1K_{j,eff} \leq a^b_{H^+} \leq 10K_{j,eff}$.

In theory, the value of $pK_{j,eff}$ pertains only to the pH^b at which it is measured. However, $pK_{j,eff}$ usually is independent of pH^b over the range of interest, and the plot of pH^b *versus* A has a sigmoidal appearance. Based on this assumption, the Henderson–Hasselbalch equation (rewritten here in the terms of absorbance) describes the pH^b *versus* A graph:

$$A = \frac{A^+ + A^- 10^{(pH^b - pK_{j,eff})}}{1 + 10^{(pH^b - pK_{j,eff})}} \qquad \text{(Eq. 7)}$$

With a computer, the values for A^+, A^-, and $pK_{j,eff}$ were varied until Eq. 7 gave the best fit for the experimental values of pH^b and A (Fig. 2).

Determination of $\Delta\overline{G}_i$—Equations 4 and 5 may be used to calculate $\Delta\overline{G}_i$ following the determination of the fraction bound at a particular pH^b. This determination was accomplished following the pH^b and A measurements described in the preceding section by centrifuging ~30 ml of the remaining sample and reference suspensions and collecting the supernate. Sufficient acid was added to 15 ml of the supernate to ensure that all of the tetracycline was in the protonated form. The absorbance of the sample supernate, A_s, and the reference supernate, A_r, was measured at 304.1 nm.

The fraction bound, f, was calculated from:

$$f = \frac{A_0 - (A_s - A_r)}{A_0} \qquad \text{(Eq. 8)}$$

where A_0 represents the absorbance for a solution containing only the protonated species having a concentration equal to C_0, the initial concentration.

Since $\Delta\overline{G}_i$ is in general a function of pH^b, its value pertains only to the pH^b at which it is calculated. In addition, Eqs. 4 and 5 were developed based on a single equilibrium where only two species are present at any $a^b_{H^+}$ value. These equations cannot be applied to compounds exhibiting multiple acid–base equilibria if significant quantities of a third species are present at the $a^b_{H^+}$ value of interest. Furthermore, $\Delta\overline{G}_i$ values should not be determined at $a^b_{H^+}$ values where very little of species i exists. In general, $\Delta\overline{G}_i$ values are most accurate when measured at pH^b conditions where a substantial amount of species i exists (which can be monitored readily) and where any interference from a second ionization is minimal. These restrictions were followed in all of the $\Delta\overline{G}_i$ determinations.

RESULTS AND DISCUSSION

The first acid–base equilibrium of tetracycline was strongly affected by sodium-saturated montmorillonite as seen in Fig. 3, where titration

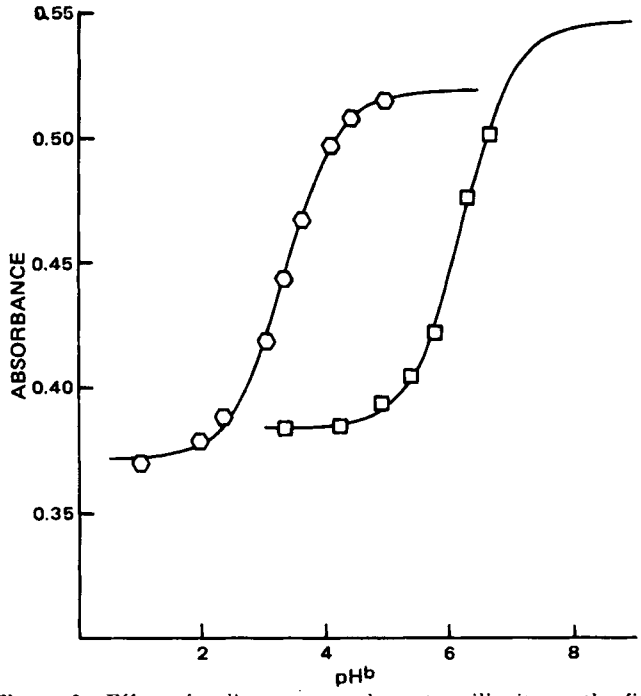

Figure 3—*Effect of sodium-saturated montmorillonite on the first acid–base equilibrium of tetracycline. Key:* ○, *aqueous solution; and* □, *sodium-saturated montmorillonite.*

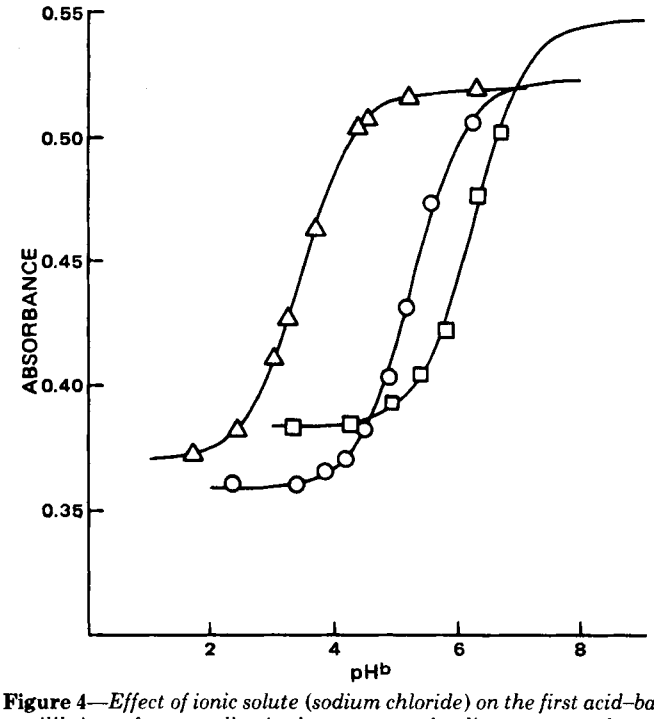

Figure 4—*Effect of ionic solute (sodium chloride) on the first acid–base equilibrium of tetracycline in the presence of sodium-saturated montmorillonite. Key:* □, *no sodium chloride;* ○, *25 mg of sodium chloride/liter; and* △, *5.844 g of sodium chloride/liter.*

curves that best fit the experimental pH^b *versus A* data are plotted. In aqueous solution, pK_1 was 3.34, which agrees well with previously reported values (5, 7). However, in the presence of sodium montmorillonite, $pK_{1,eff}$ was 6.23. The interaction of the protonated species of tetracycline, H_3T^+, with the negative clay surface was responsible for this shift of almost three pH^b units.

As seen in Table I, $\Delta\overline{G}_{H_3T^+}$ was −3.99 kcal/mole, indicating a much greater stabilization of the H_3T^+ species than the H_2T^0 species ($\Delta\overline{G}_{H_2T^0}$ = −0.03 kcal/mole). This stabilization of the H_3T^+ species caused a shift in the acid–base equilibrium in favor of the H_3T^+ species.

The interaction of H_3T^+ with sodium-saturated montmorillonite was affected by other ionic species in the system. As seen in Fig. 4, the shift in the titration curve was reduced in the presence of 25 mg of sodium chloride/liter and approached the solution titration curve when large amounts of sodium chloride (5.844 g/liter) were present.

The value for $pK_{1,eff}$ calculated from Fig. 4 approached pK_1, and $\Delta\overline{G}_{H_3T^+}$ approached zero as large sodium chloride concentrations were added to the tetracycline–sodium-saturated montmorillonite suspension (Table I). The interaction of the H_2T^0 species remained negligible ($\Delta\overline{G}_{H_2T^0}$ = −0.04 kcal/mole) in the presence of sodium chloride.

The titration curve for the second ionization was displaced slightly in the presence of sodium montmorillonite (Fig. 5). As was observed in the first ionization, sodium chloride reduced the displacement of the titration curve.

Calculation of $pK_{2,eff}$, $\Delta\overline{G}_{H_2T^0}$, and $\Delta\overline{G}_{HT^-}$ based on the titration curves in Fig. 5 is tenuous due to the weak interaction of the H_2T^0 and HT^- species with the clay. In addition, the close proximity of pK_2 and pK_3 (7.86 and 9.51, respectively) means that more than two ionic species of tetracycline probably are present in the system in the pH range of interest. However, Eq. 7 was applied to the titration data in Fig. 5, and the resulting increase in $pK_{2,eff}$ was only 0.15 in the presence of the sodium-saturated montmorillonite. This slight increase in $pK_{2,eff}$ may suggest that the zwitterionic form of tetracycline, H_2T^0, interacts slightly with the clay surface. However, this conclusion is made cautiously because of the cited limitations. Furthermore, the values obtained for $\Delta\overline{G}_{H_2T^0}$ and $\Delta\overline{G}_{HT^-}$ were too small to be considered significant.

Virtually no change in the titration curve for the third ionization of tetracycline was observed in the presence of sodium-saturated montmorillonite (Fig. 6), and it was concluded that the clay does not interact significantly with the HT^- and T^{2-} species.

The data in Figs. 3–6 and Table I indicate that the negatively charged sites on montmorillonite are responsible for the strong interaction with the protonated species of tetracycline, H_3T^+. However, this interaction

Table I—Effects of Sodium-Saturated Montmorillonite on Acid–Base Equilibria of Tetracycline

Sample	$pK_{1,eff}$	$pK_{2,eff}$	$\Delta\overline{G}_{H_3T^+}$, kcal/mole	$\Delta\overline{G}_{H_2T^0}$, kcal/mole
Aqueous solution	3.34	7.86	—	—
Sodium-saturated montmorillonite suspension	6.23	8.01	−3.99	−0.03
Sodium-saturated montmorillonite suspension containing 25 mg of sodium chloride/liter	5.25	7.98	−2.72	−0.03
Sodium-saturated montmorillonite suspension containing 5.844 g of sodium chloride/liter	3.45	7.92	−0.27	−0.04

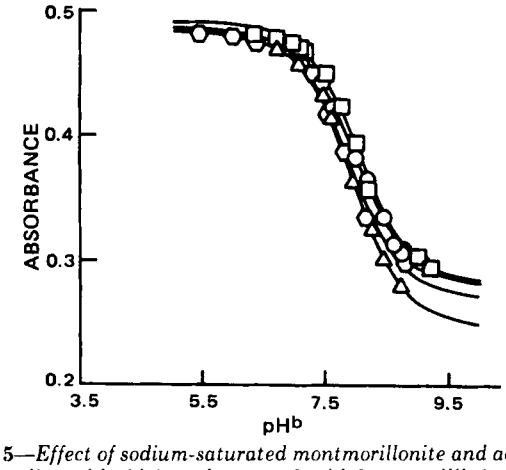

Figure 5—*Effect of sodium-saturated montmorillonite and added ionic solute (sodium chloride) on the second acid–base equilibrium of tetracycline. Key:* ○, *aqueous solution;* □, *sodium-saturated montmorillonite suspension;* ○, *sodium-saturated montmorillonite suspension containing 25 mg of sodium chloride/liter; and* △, *sodium-saturated montmorillonite suspension containing 5.844 g of sodium chloride/liter.*

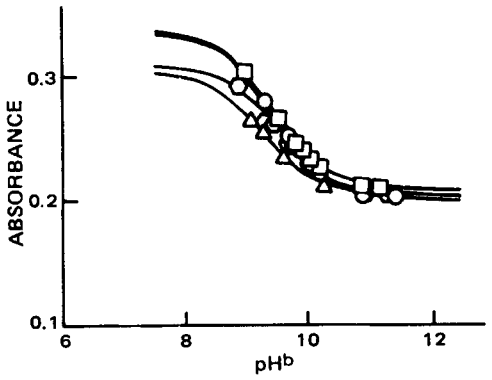

Figure 6—*Effect of sodium-saturated montmorillonite and added ionic solute (sodium chloride) on the third acid–base equilibrium of tetracycline. Key: ○, aqueous solution; □, sodium-saturated montmorillonite suspension; ○, sodium-saturated montmorillonite suspension containing 25 mg of sodium chloride/liter; and △, sodium-saturated montmorillonite suspension containing 5.844 g of sodium chloride/liter.*

is reduced in the presence of other ions such as sodium chloride. It is hypothesized that sodium ions compete with H_3T^+ for the negative sites on the clay surface or, equivalently, that sodium ions screen the negatively charged clay surface, thereby reducing the apparent negative charge of the clay surface. Sodium chloride also may affect the interaction by altering the state of dispersion of the clay so that negative sites are less accessible to the H_3T^+ ions.

Figure 7 shows that $\Delta\overline{G}_{H_3T^+}$ is affected by pH^b and the presence of an additional ionic solute such as sodium chloride. The $\Delta\overline{G}_{H_3T^+}$ value was calculated at each pH^b using Eq. 4 when $(A - A^+)$ and $(A^- - A)$ in Eq. 6 were large enough to determine $pK_{1,eff}$ accurately; if $a^b_{H^+} \gg K_{1,eff}$, $\Delta\overline{G}_{H_3T^+}$ was calculated from:

$$\Delta\overline{G}_{H_3T^+} = RT \ln (1 - f) \frac{a_{H^+}}{K + a_{H^+}} \qquad \text{(Eq. 9)}$$

The change in $\Delta\overline{G}_{H_3T^+}$ seen in the salt-free sodium-saturated montmorillonite suspension and the clay suspension containing a small amount of sodium chloride (25 mg/liter) indicate that increased interaction between H_3T^+ and the clay occurred as the pH^b was increased. At low pH^b values, protons apparently compete with H_3T^+ for the negative clay sites. At pH 4–6, $\Delta\overline{G}_{H_3T^+}$ reached a constant value, indicating an interaction free of competition with H^+. The plateau value of $\Delta\overline{G}_{H_3T^+}$ probably represents an intrinsic interaction of H_3T^+ with the clay since large changes in $\overline{C}_{H_3T^+}$ have little effect on $\Delta\overline{G}_{H_3T^+}$.

The $\Delta\overline{G}_{H_3T^+}$ values seen in Fig. 7 clearly show the reduced interaction of H_3T^+ and the sodium-saturated montmorillonite due to the competitive effect of the added ionic solutes. The effect of pH on $\Delta\overline{G}_{H_3T^+}$ was diminished in the presence of a low level of sodium chloride (25 mg/liter) and could not be observed in a suspension containing 5.844 g of sodium chloride/liter.

The fraction bound curves in Fig. 8 support the conclusions reached based on the titration curves. The fraction of tetracycline bound to the sodium-saturated montmorillonite increased with pH^b until a maximum

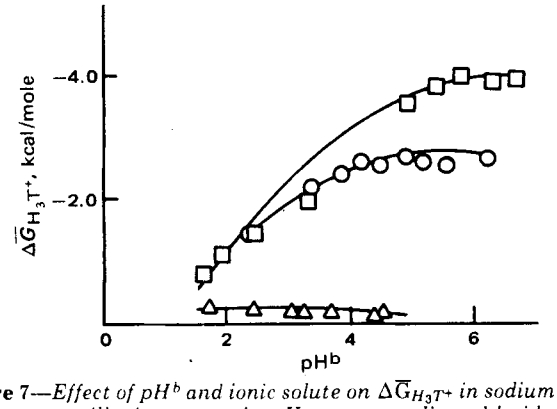

Figure 7—*Effect of pH^b and ionic solute on $\Delta\overline{G}_{H_3T^+}$ in sodium-saturated montmorillonite suspension. Key: □, no sodium chloride; ○, 25 mg of sodium chloride/liter; and △, 5.844 g of sodium chloride/liter.*

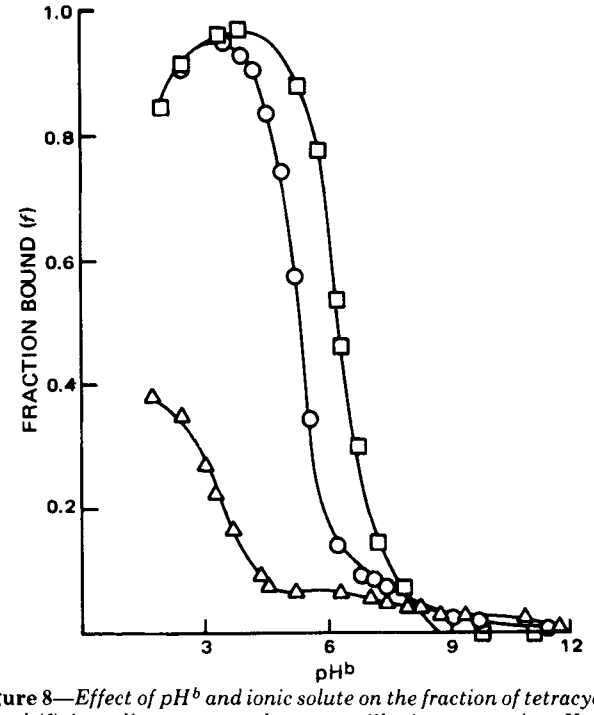

Figure 8—*Effect of pH^b and ionic solute on the fraction of tetracycline bound (f) in sodium-saturated montmorillonite suspension. Key: □, no sodium chloride; ○, 25 mg of sodium chloride/liter; and △, 5.844 g of sodium chloride/liter.*

was observed at pH^b 3.8. This behavior agrees with the reduced interaction of H_3T^+ and clay in the presence of high proton concentrations (Fig. 7). A maximum in the adsorption of tetracycline occurred under pH^b conditions at which the competitive effect of protons was low and the H_3T^+ concentration was still high. This situation occurred at pH 3.8 when no added salt was present. The concentration of H_3T^+ was very high at this pH^b because the $pK_{1,eff}$ under these conditions was 6.23 (Table I).

In the presence of low concentrations of sodium chloride (25 mg/liter), a high fraction of tetracycline was bound by the clay. However, the maximum adsorption occurred at pH^b 3.4, probably reflecting the smaller displacement of $pK_{1,eff}$ in the presence of sodium chloride (Table I).

At high added salt concentrations (5.844 g/liter), only 38% of the tetracycline was adsorbed by the clay (Fig. 8). Under these conditions, H_3T^+ and H^+ cannot compete effectively with sodium ions for negative clay adsorption sites. As a result, $\Delta\overline{G}_{H_3T^+}$ was −0.27 kcal/mole and $pK_{1,eff}$ approached pK_1 (Table I). Thus, a greatly diminished fraction bound curve is seen.

The fraction of tetracycline bound to the sodium-saturated montmorillonite decreased rapidly in all three conditions shown in Fig. 8 because the pH^b conditions allowed the zwitterionic form of tetracycline, H_2T^0, to appear in the system. The $\Delta\overline{G}_{H_2T^0}$ values ranging from −0.03 to −0.04 kcal/mole (Table I) indicate little interaction of this ionic species with the clay.

In the pH^b region above 8.5, the fraction of tetracycline bound to the clay approached zero (Fig. 8) since the pH^b was above $pK_{2,eff}$ (Table I) and only the anionic forms of tetracycline (HT^- and T^{2-}), which cannot interact with the negative clay surface, were present.

Figure 8 shows that a small fraction of tetracycline was bound to the sodium-saturated montmorillonite in the pH region where the anionic forms of tetracycline were present in suspensions containing excess sodium chloride. The added sodium ions are believed to screen the anion–clay repulsive forces by collapsing the electrical double layer associated with the clay and thus allow physical adsorption of the HT^- and T^{2-} species.

The behavior of the fraction bound curves at higher pH^b conditions provides further understanding of the small shift in the titration curve for the second acid–base equilibrium of tetracycline shown in Fig. 5. The interaction of the zwitterionic form of tetracycline with the clay probably is a physical adsorption indicated by the low $\Delta\overline{G}_{H_2T^0}$ value associated with the interaction. This weak interaction led to the displacement of pK_2 from 7.86 to a $pK_{2,eff}$ of 8.02 in the presence of clay. The addition of sodium chloride to the system allows the anionic conjugate base, HT^-, to interact with the clay by collapsing the electrical double layer, and $pK_{2,eff}$ decreased to 7.98 and 7.92 with 25 mg and 5.844 g of sodium chloride/liter,

respectively. This result suggests that H_2T^0 and HT^- interact to approximately the same degree with the clay surface so that $\Delta\bar{G}_{H_2T^0}$ is approximately equal to $\Delta\bar{G}_{HT^-}$, as would be expected if the mechanism of adsorption for each species is physical adsorption.

The distribution of the ionic species of tetracycline in an aqueous solution and in an aqueous sodium-saturated montmorillonite suspension, based on the respective values of pK_1, pK_2, and pK_3 and $pK_{1,eff}$, $pK_{2,eff}$, and $pK_{3,eff}$ found in this study, is shown in Fig. 9. The changes in the acid–base equilibria of tetracycline due to the stabilizing effect of the montmorillonite surface caused the H_3T^+ species to be present over a wider pH range than expected based on acid–base equilibria in solution. In addition, the H_2T^0 form was present over a much smaller pH region when in a clay suspension.

Previous investigators concluded that the zwitterion, H_2T^0, was adsorbed significantly by montmorillonite (1). This conclusion was based on the pK_j (j = 1, 2, 3) values for tetracycline in aqueous solution rather than the $pK_{j,eff}$ (j = 1, 2, 3) values appropriate for clay systems. Feldkamp and White (8) suggested that the ability of a clay to interact with weak bases may increase in concentrated mixtures. Thus, dramatic shifts in species distributions may occur in concentrated suspensions of tetracycline and montmorillonite.

The changes in the distribution of the ionic species of a drug due to interactions with a clay surface may have important consequences in drug therapy. For example, the antimicrobial activity of tetracycline and other antibiotics was reported to be diminished in the presence of clays (9). Tetracycline exhibits its maximum antimicrobial activity between pH 5.5 and 6.0 (10), the region where the zwitterionic form predominates in aqueous solution (Fig. 9). The H_2T^0 species appears to be absorbed preferentially across the GI membranes due to its lipophilic nature (7). In the presence of montmorillonite, the zwitterionic form of tetracycline exists over a dramatically reduced pH region. As a consequence, the most readily absorbable form of tetracycline may be present over a smaller region of the GI tract.

The distribution changes for the ionic forms of a drug also may provide insights into the catalytic effects of a clay on drug degradation. For example, the sequence of steps illustrated in Scheme V is associated with acid-catalyzed hydrolysis of drugs.

$$\text{drug} + H^+ \rightleftarrows \text{drug-}H^+ + H_2O \rightarrow \text{degradation products}$$

Scheme V

The first step of the hydrolysis involves protonation of the drug, followed by nucleophilic attack by water. The degradation rate depends on the concentration of protonated drug. Interaction of the drug with a clay surface may cause a shift in the acid–base equilibrium of the drug that favors the protonated form and thus leads to an accelerated hydrolysis rate in the presence of a clay.

Along with the effect of an increased concentration of reactant (protonated drug), there is the possibility that the activation energy for the hydrolysis reaction might be increased due to the drug–clay interaction. The net effect on the reaction rate will vary for each system, depending on the relative magnitudes of each factor.

Clays have been suggested as a vehicle for controlled-release dosage forms (11). An understanding of the effect of drug–clay interactions on the acid–base equilibria of the drug should provide the information needed to select the proper drug and clay to obtain the desired *in vivo* release pattern.

In summary, the acid–base equilibria of drugs can be affected greatly by interaction with clay surfaces. The thermodynamic quantities, $K_{j,eff}$ and $\Delta\bar{G}_i$, are useful for quantifying and monitoring drug–clay interactions and should be used in the formulation of clay-containing drug products as well as in the evaluation of the effects of the coadministration of drugs and clays.

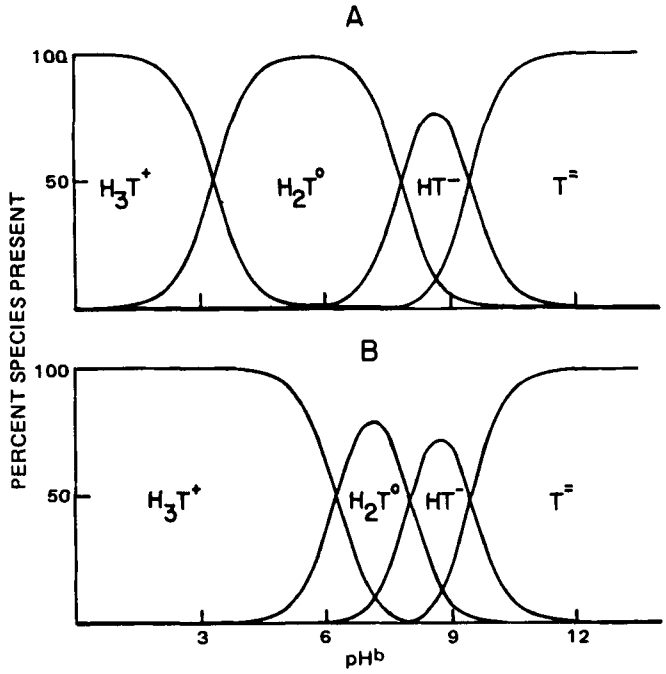

Figure 9—*Distribution of the ionic species of tetracycline. Key: A, aqueous solution; and B, sodium-saturated montmorillonite suspension.*

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