Mechanism of Adsorption of Clindamycin and Tetracycline by Montmorillonite

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Abstract □ IR and X-ray analyses of the interaction of clindamycin with montmorillonite indicate that clindamycin is adsorbed by a cation-exchange mechanism under pH conditions favoring the cationic form of the drug and by physical adsorption when the unionized drug is present. This physical adsorption is relatively weak since the drug is readily desorbed by alkaline washing. Tetracycline is adsorbed by cation exchange at low pH values where the +00 species predominates. Complexation with divalent interlayer cations contributes significantly to adsorption at higher pH values where the +−0 and +−− species exist. In a strongly alkaline solution, the 0−− species was not adsorbed in the interlayer space of montmorillonite but rather produced an external calcium–tetracycline complex. This study illustrates the utility of X-ray and IR analyses in elucidating the mechanisms responsible for clay–drug interactions.

Keyphrases □ Clindamycin—adsorption to montmorillonite, IR and X-ray analyses of mechanism □ Tetracycline—adsorption to montmorillonite, IR and X-ray analyses of mechanism □ Montmorillonite—adsorption of clindamycin and tetracycline, IR and X-ray analyses of mechanism □ Adsorption—clindamycin and tetracycline to montmorillonite, IR and X-ray analyses of mechanism □ Antibacterials—clindamycin and tetracycline, adsorption to montmorillonite, IR and X-ray analyses of mechanism □ Clays—montmorillonite, adsorption of clindamycin and tetracycline, IR and X-ray analyses of mechanism

Clays are widely used in pharmaceuticals, and the applications of their properties range from suspending agents (1) to carriers in sustained-release dosage forms (2). Therefore, the possible interactions of clays with drugs is important.

BACKGROUND

Clay–drug systems have been investigated by the use of adsorption isotherms. This classical approach indicates the quantity of compound adsorbed, the rate, and other physical parameters. However, only indirect evidence of the adsorption mechanism can be obtained. Swelling clays, especially montmorillonite, are unique in that their c-axis spacing adapts to the intercalated molecule when internal adsorption occurs. Changes in this parameter can be detected using X-ray diffractometry. In addition, the large internal surface in montmorillonite leads to a high degree of adsorption and permits direct observation of the adsorbate by IR spectroscopy. Perturbations of functional groups in the adsorbed molecules can be observed by IR spectroscopy and give direct information about the adsorption mechanism (3, 4).

Numerous organic molecules can be adsorbed by clays (5). For simple molecules, X-ray and IR methods have been used to elucidate the adsorption mechanisms and have defined the interlayer orientation of the molecule (6–8). Possible mechanisms include hydrogen bonding and van der Waals forces, which are means of physical adsorption, and cation exchange, protonation, and complexation, which are encompassed by chemisorption (9–11). For more complicated molecules, several adsorption mechanisms can operate simultaneously, depending on the pH, concentration, temperature, and interlayer cation (9).

Drug interactions with clays tend to be complex because of the size and structure of the drug molecules. Several papers reported the behavior of various drugs with montmorillonite but did not present direct evidence about the mechanisms (12, 13). To elucidate the adsorption mechanisms responsible for clay–drug interactions, the adsorption of the antibiotics clindamycin and tetracycline by montmorillonite was investigated.

Montmorillonite was chosen as the model clay because it exhibits both a high exchange capacity (80–150 mEq/100 g) (14) and a high surface area (700–800 m^2/g) (5). Other clays used in pharmaceutical systems include kaolin, a member of the kaolinite group, which has a low exchange capacity (3–15 mEq/100 g) (14) and a low surface area (20 m^2/g) (5), and attapulgite, a member of the fibrous pseudolayer clays, which in the pure state has no exchange capacity but possesses a high surface area (200 m^2/g) (15). Adsorption mechanisms of clays are related directly to surface area and/or exchange capacity. Results obtained with montmorillonite should be useful in predicting the adsorption behavior of clay minerals having a lower adsorptive capacity.

EXPERIMENTAL

Materials—All drugs and chemicals were either official or reagent grade. The source of clay was bentonite USP. X-ray diffraction indicated that montmorillonite was the major mineral present and that the bentonite also contained a small quantity of quartz. To study the clay component, which is responsible for the adsorptive properties in bentonite USP, the <2-μm clay fraction was separated by sedimentation and collected.

The clay fraction was concentrated to approximately 5% by evaporation of the water through dialysis tubing. Water removal was facilitated by placing the dialysis bags in an air stream. Bentonite USP is predominantly a sodium montmorillonite (1, 16); however, metal-ion analysis by atomic absorption spectrometry[2] showed that 10% of the exchange capacity is satisfied by divalent cations (7.1% Ca^{2+}, 2.7% Mg^{2+}).

Pure sodium montmorillonite was prepared by adding 1 *N* NaCl to the concentrated montmorillonite suspension until the clay suspension was approximately 2% (w/w). The suspension was stirred, centrifuged, and decanted; the clay was resuspended in 1 *N* NaCl to produce a 2% suspension. This washing procedure was repeated four additional times. Excess salt was removed by repeated washings with deionized water until the addition of silver nitrate to the supernate gave a negative chloride test.

Calcium montmorillonite was prepared in an analogous manner with 1 *N* $CaCl_2$ as the exchanging solution. All clays were stored as concentrated suspensions.

Sample Preparation—Clay suspensions and drug solutions were prepared separately and adjusted with hydrochloric acid or sodium hydroxide to the pH values at which each ionic species of the drug predominated. In very alkaline conditions, the exchangeable calcium in montmorillonite can be replaced to some extent by sodium from the sodium hydroxide solution used to adjust the pH. Lowered drug solubility was encountered at some pH values, resulting in the formation of drug suspensions.

After mixing and correcting for any pH change, the clay–drug system was equilibrated for 1 hr at 37°. The mixtures contained 0.6 mg of clindamycin or 1 mg of tetracycline and 10 mg of montmorillonite/ml of clay–drug suspension. After interaction, aliquots were washed one or five times with deionized water adjusted to the pH of the initial mixture and resuspended to yield a 1% suspension. In every case but that of clindamycin at pH 11.0, no difference was observed between the first and fifth wash.

For comparative purposes, each ionic species for the drugs was maximized by adjusting tetracycline solutions to pH 1.5, 5.0, 8.7, and 11.0 (17, 18) and clindamycin solutions to pH 2.0 and 11.0. Where precipitation occurred, the suspension was filtered to remove solid drug. Evaporation yielded solid drug in the appropriate ionic form.

Tetracycline calcium was obtained by adjusting a tetracycline solution to pH 11.0 and then adding sufficient aqueous calcium chloride to obtain

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[2] Model 290B, Perkin-Elmer Corp., Norwalk, Conn.

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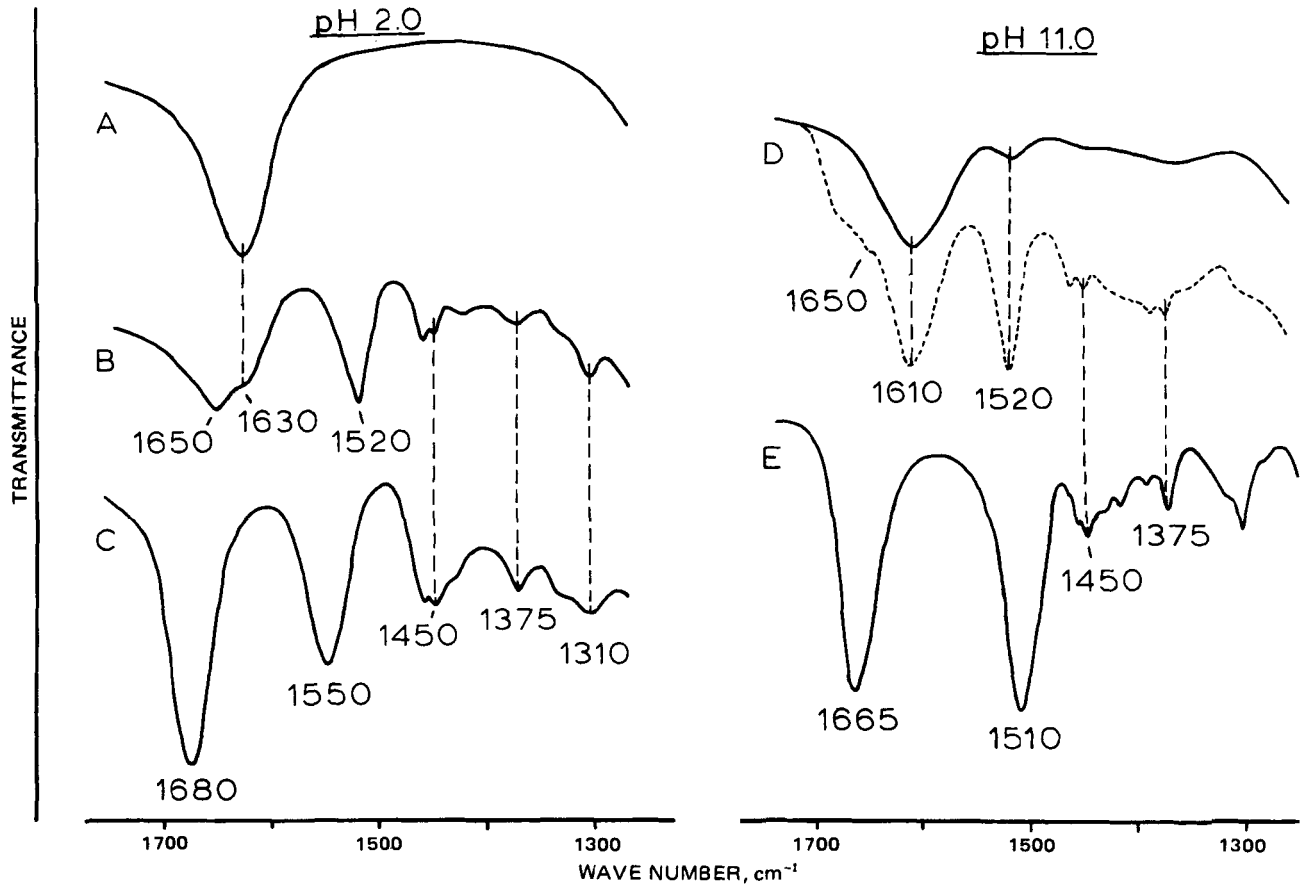


Figure 1—*IR spectra of montmorillonite interacted with clindamycin after five washings. Key: A, montmorillonite; B, clindamycin and montmorillonite, pH 2.0; C, clindamycin, pH 2.0; D, clindamycin and montmorillonite, pH 11.0 (one washing); and E, clindamycin, pH 11.0.*

a 1.5 *M* ratio of calcium to tetracycline. The resulting slurry was lyophilized in the absence of light and stored as a powder.

X-Ray Analysis—For X-ray diffraction[3], 2 ml of the 1% suspension was pipetted onto a glass slide and air dried. This technique produces a preferential orientation of the clay particles parallel to the 001 plane, allowing direct determination of the interlayer spacing. Tetracycline degradation was minimized by avoiding exposure to light and drying under vacuum. Prior to running X-ray diffractograms, all samples were dehydrated under vacuum for 30 min. The vacuum cell containing the samples was transferred to a controlled environment chamber of the X-ray diffractometer, which was constantly purged with a stream of dry nitrogen gas. This dry atmosphere maintained the dehydrated state of the sample.

To draw definite conclusions about the presence and size of the drug molecule in the interlayer space, water must be excluded from the clay–drug system when recording X-ray diffractograms. When drug molecules have been adsorbed onto interlayer clay surfaces, the observed increase in basal spacing compared to that of dehydrated clay will be due only to the dimensions of the adsorbed molecule without any contribution from water.

IR Analysis—Self-supporting clay films were prepared for IR analysis[4] by pipetting appropriate volumes of the 1% suspension onto polyethylene terephthalate[5] film. After air or vacuum drying (in the dark for tetracycline), the clay film was separated from the polymer film by bending both over a sharp 90° corner. The rigidity of the clay film caused it to be detached from the polymer sheet. Clay films of uniform thickness may be readily prepared by this technique.

The clay is free from IR absorption between 3000 and 1300 cm^{-1}, except for a strong water bending vibration at approximately 1630 cm^{-1}, providing a convenient window for the observation of drug absorption bands. The absorption near 1630 cm^{-1} is primarily due to water directly coordinated to the exchangeable cations of the clay (19). Therefore, its absence after drug interaction is a good indication of the replacement of this interlayer cation by the drug. For many drugs, the IR spectrum between 1800 and 1300 cm^{-1} is especially useful because most important absorption bands for potentially interacting functional groups are present in this region.

Tetracycline, tetracycline calcium, and clindamycin were prepared for IR analysis as potassium bromide pellets.

Adsorption Isotherms—Aqueous solutions of clindamycin (>2 mg/ml) were adjusted to pH 2.0 and 11.0. Tritium-labeled clindamycin[6] was added to these solutions, and final dilution adjustments were made to yield stock solutions with a specific concentration of approximately 160,000 dpm/ml. Two aliquots of the aqueous montmorillonite suspensions containing 0.096 g of montmorillonite were diluted (such that the combined volumes of clay and drug solutions would be approximately 90 ml), adjusted to pH 2.0 and 11.0, transferred to 100-ml volumetric flasks containing suitable aliquots of the stock drug solution, and diluted to volume with water of the proper pH.

After thorough shaking, the clindamycin–montmorillonite mixtures were placed in covered jacketed beakers (37°) and stirred continuously for 24 hr. Initial results indicated that equilibrium was attained after approximately 30 min. The initial pH was maintained by adding a minimum volume of hydrochloric acid or sodium hydroxide when necessary. Five-milliliter samples were taken periodically and centrifuged at 6000 rpm for 10 min. The equilibrium solution still containing small amounts of clay was decanted into another centrifuge tube to which a trace of sodium chloride crystals was added to facilitate separation of the clay. The tube was shaken and again centrifuged at 6000 rpm for 30 min.

The equilibrium concentration of the supernate was determined by liquid scintillation spectrometry[7] using a commercially prepared scintillation fluid[8]. Counts were recorded for 10 min or up to 10,000 (±2%), using an external barium-133 standard. In all cases, an appropriate clay blank was used to obtain a background count.

Tetracycline isotherms were obtained similarly. Aqueous stock solutions of tetracycline in the 2–4-mg/ml range were adjusted to pH 1.5, 8.7,

[3] Siemens AG Kristalloflex 4 generator, type F diffractometer, Karlsruhe, West Germany.
[4] Model 180, Perkin-Elmer Corp., Norwalk, Conn.
[5] Mylar.
[6] The Upjohn Co., Kalamazoo, Mich.
[7] Isocap/300 liquid scintillation system, Searle Analytical, Inc., Des Plaines, Ill.
[8] Riafluor, New England Nuclear, Boston, Mass.

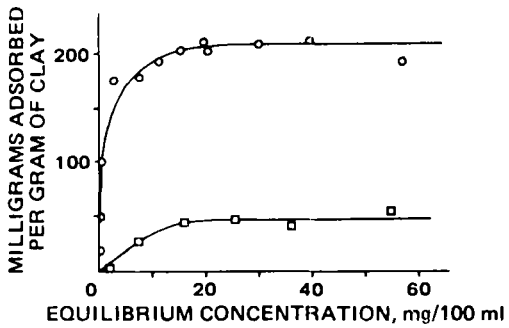


Figure 2—*Adsorption isotherm for clindamycin and montmorillonite. Key:* ○, *pH 2.0; and* □, *pH 11.0.*

and 11.0. Because of lowered tetracycline solubility at pH 5.0, a 0.5-mg/ml stock solution was prepared. Three aliquots of the montmorillonite suspension containing 0.096 g of the clay were diluted as with clindamycin, adjusted to pH 1.5, 8.7, and 11.0, transferred to flasks containing suitable aliquots of the stock tetracycline solution, and diluted to volume with water adjusted to the appropriate pH. For the pH 5.0 drug–clay mixture, 0.0256- and 0.0128-g portions of montmorillonite were treated similarly and added to a proportionately smaller amount of tetracycline.

Equilibrating conditions and sampling procedures were the same as those for clindamycin. Equilibrium concentrations were determined spectrophotometrically[9] with appropriate clay blanks in the reference beam. At pH 11.0, the equilibrium plateau concentrations obtained within the 1st hr were used to construct the adsorption isotherm. This approach was necessary since tetracycline degradation significantly affected the established plateau levels after that time.

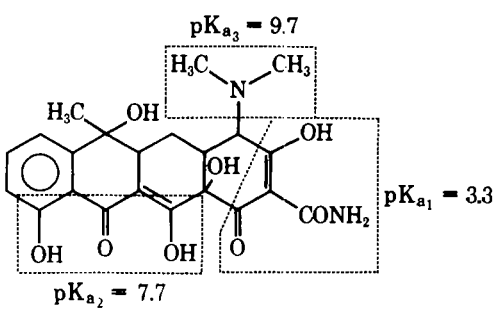
RESULTS AND DISCUSSION

Clindamycin—Clindamycin has a pKa of 7.6 and, therefore, exists predominantly as a cation in acidic solutions because of protonation of the tertiary amine and as a neutral species in basic solution.

When clindamycin interacts with montmorillonite at pH 2, interlaminar adsorption is suggested by an increase in the basal spacing of the clay from 9.5 to 13.4 Å, a difference of 3.9 Å. This interlayer spacing is somewhat smaller than the 4.8-Å minimum dimension of clindamycin, as determined by a Corey–Pauling–Kolton (CPK) molecular scale model. Green-Kelley (20) studied a number of organic molecules adsorbed onto the interlayer surface of montmorillonite and noted that the increase in interlayer space could be smaller than the dimension of the adsorbed molecule by as much as 1 Å. Further studies showed that keying or a different geometric packing can occur for the adsorbed molecule, resulting in smaller basal spacings than expected (21). The observed interlaminar spacing is then due to the adsorption of clindamycin and suggests that a single layer has been adsorbed in a parallel orientation.

At pH 11, where clindamycin is uncharged, the basal spacing for the montmorillonite interacted with drug was that of the dehydrated clay, 9.6 Å, indicating no adsorption in the interlayer space by the neutral molecule. The fact that clindamycin was adsorbed in the interlayer space of montmorillonite only as a cationic molecule suggests that the adsorption mechanism at low pH is cation exchange.

Figure 1A shows the IR spectrum of montmorillonite in the range of



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[9] Model 124, Perkin-Elmer Corp., Norwalk, Conn.

1800–1300 cm^{-1}; only one absorption band appears at 1630 cm^{-1} because of the bending vibration of water. (In the presence of basic drug solutions, this deformation band shifts to 1610 cm^{-1}, indicating less hydrogen bonding between water molecules.) The IR spectrum of the montmorillonite–clindamycin system at pH 2 shows relatively intense drug absorption bands (Fig. 1B) and confirms that the drug has been adsorbed in the interlaminar space of the clay.

Comparison between absorption bands for the drug alone and those of the adsorbed drug (Figs. 1C and 1B) shows a shift in the position of the amide I band from 1680 to 1650 cm^{-1}. This shift to lower frequency might be interpreted as carbonyl participation in hydrogen bonding with hydroxyl groups of the water coordinated to the interlayer cation or as coordination with the exchangeable ions on the interlayer clay surface (22). However, the interaction is not due to hydrogen bonding because of the irreversible nature of the reaction. On the other hand, coordination can be supported by the decrease in frequency of the amide II band (NH deformation vibration) from 1550 to 1520 cm^{-1}, indicating less restricted NH bending as is the case in the resonance structure favored by a carbonyl coordination. The amide CN and NH stretching vibrations are obscured by the OH stretching vibrations of the clay and adsorbed water and cannot contribute to elucidation of the adsorption mechanism.

Although the amide II shift to lower frequency could support a coordination mechanism, the difference in environment between an adsorbed molecule and a molecule in the pure state might also cause such a shift. Generally, secondary amides in the solid state display an amide II band at higher frequency than when in dilute solution. This effect would correspond to the change in environment and configuration of the drug molecules from an intermolecularly bonded aggregate to almost totally isolated molecules on the clay surface, as indicated by X-ray diffraction data. Thus, the observed perturbations of the amide II and amide I bands may be explained without a covalent interaction occurring between the drug molecule and the clay.

Hydrogen bonding other than with the carbonyl is not an additional stabilizing factor, as evidenced by both irreversible adsorption and the presence of absorption bands at 2960, 2920, and 2870 cm^{-1} due to CH stretching vibrations and at 3370 cm^{-1} due to OH stretching vibrations (not shown in Fig. 1), which are unperturbed compared to their position in the spectrum of the solid drug at pH 2.0.

Both coordination and cation-exchange mechanisms can be supported by the shifts in amide I and II band frequencies and the irreversible nature of the adsorption (as evidenced by identical spectra after one or five washings). However, direct evidence for an exchange mechanism is obtained by noting the low intensity of the water absorption band at 1630 cm^{-1} (Fig. 1B). Since the exchangeable cations are hydrated to a considerable extent, the observed decrease of the associated water absorption band, as compared to that in Fig. 1D (one wash), indicates replacement of the inorganic cation by clindamycin cations.

At pH 11, clindamycin is a neutral species; after several washings, it does not interact significantly with montmorillonite *via* a cation-exchange mechanism, as evidenced by the virtual absence of IR drug absorption bands (Fig. 1E). This result is further supported by the presence of the water absorption band at 1610 cm^{-1} after one washing. The decrease in absorption band intensity with washing indicates that reversible physical adsorption is responsible for drug adsorption on the external clay surface.

Adsorption isotherms of clindamycin adsorbed onto montmorillonite are presented in Fig. 2. The amounts that could theoretically be adsorbed by montmorillonite, calculated on the basis of cation-exchange capacity and external surface area, are listed in Table I. Under acidic conditions, the magnitude of adsorption suggests cation exchange as the adsorption mechanism. The experimental adsorptive capacity greatly exceeds that of the external surface but satisfies 49% of the calculated cation-exchange capacity. The fact that approximately one-half of the theoretical adsorption occurred and the parallel orientation of the adsorbed monolayer, as indicated from X-ray diffraction data, suggest that clindamycin cations are too large to neutralize completely the charge on the internal clay surface. This conclusion is verified by comparing the area of a clindamycin molecule to the area per exchange position on each basal plane surface of the clay. Based on a 190-$Å^2$ planar surface area for clindamycin and 80 $Å^2$ for a montmorillonite exchange site (23), 42% of the interlayer cation-exchange capacity can be satisfied by the cationic drug if it is assumed that the montmorillonite is in the expanded state in the adsorption isotherm experiments.

The data obtained in this study were applied to both the empirical Freundlich equation and the theoretical Langmuir isotherm equation. Conformity to the Freundlich equation was found at pH 2.0 and 11.0. Clindamycin adsorption at pH 2.0 also followed a linear isothermal plot

Table I—Comparison of Observed and Theoretical Adsorption Capacity of Montmorillonite

Adsorbate (Ionic Form)	Maximum Adsorption Capacity, mg of Adsorbate/g of Montmorillonite	Theoretical Adsorption Capacity, mg of Adsorbate/g of Montmorillonite	
		Based on Cation Exchange Capacity	Based on External Surface Area
Clindamycin			
pH 2.0 (+)	210	425	33
pH 11.0 (0)	50	425	33
Tetracycline			
pH 1.5 (+00)	375	481	44
pH 5.0 (+−0)	240	481	44
pH 8.7 (+−−)	80	481	44
pH 11.0 (0−−)	<10	481	44

of the Langmuir equation. At pH 11.0, however, the data did not yield a straight line. Mechanistic interpretations of the Langmuir adsorption equation are difficult and are not entirely agreed upon; however, conformity of the data to the equation usually implies a chemisorption mechanism. Physical adsorption might then be indicated for the neutral molecule since chemisorption is not an apparent mechanism at pH 11.0.

IR and X-ray analyses together show that clindamycin is adsorbed by

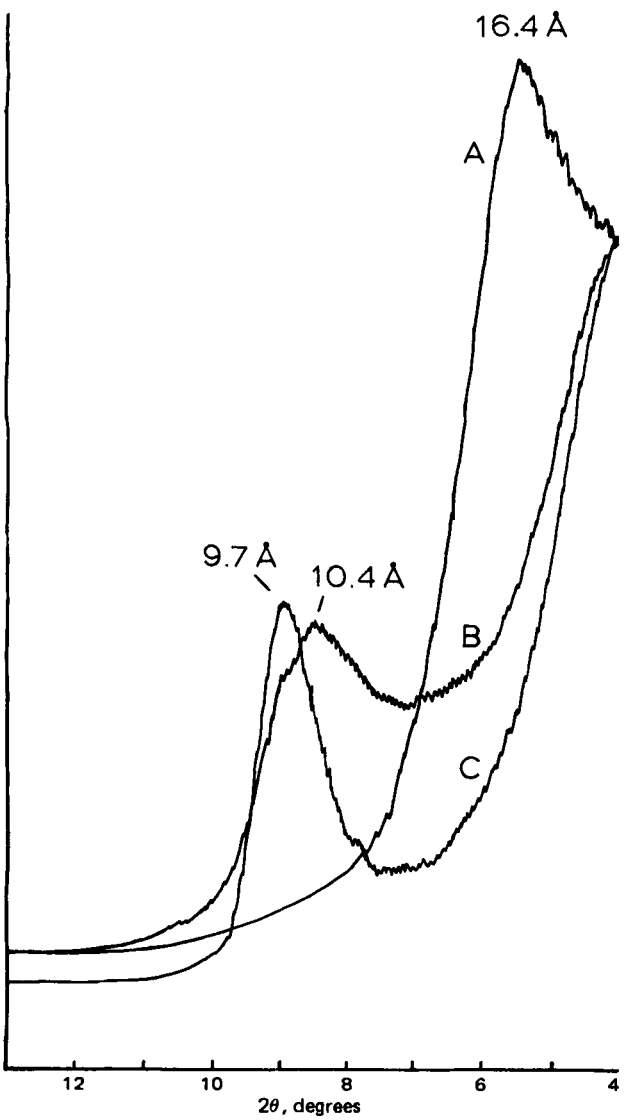

Figure 3—*X-ray diffractograms of montmorillonite interacted with tetracycline at pH 1.5 and 5.0 (A), pH 8.7 (B), and pH 11.0 (C). Curve C is also the diffraction pattern for untreated montmorillonite.*

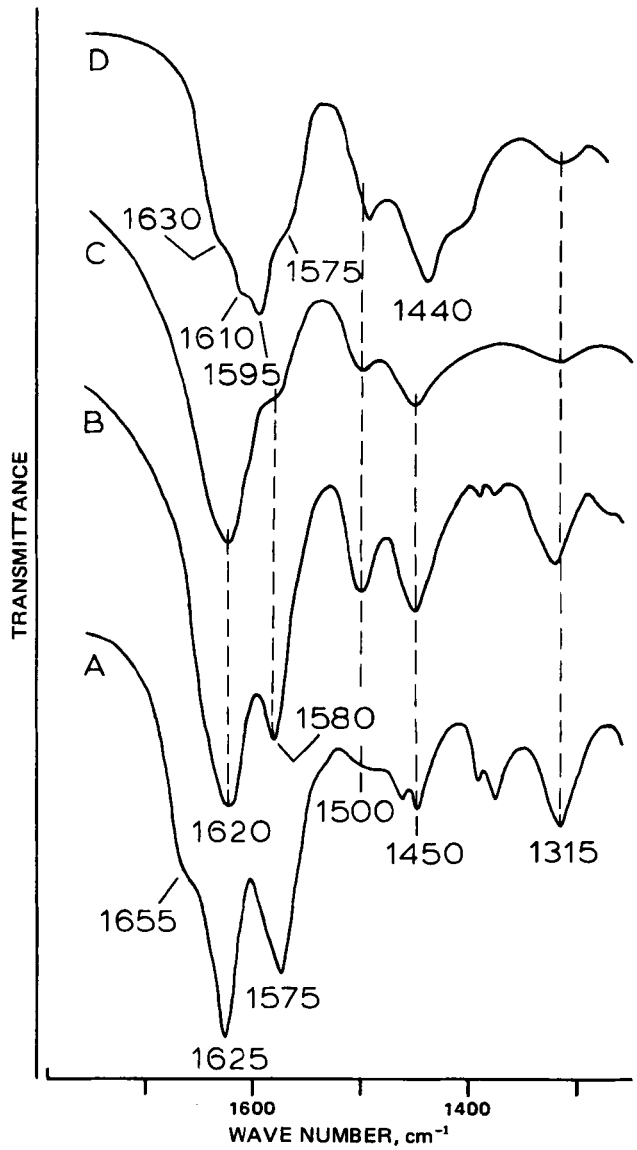

Figure 4—*IR spectra of montmorillonite interacted with tetracycline. Key: A, pH 1.5; B, pH 5.0; C, pH 8.7; and D, pH 11.0.*

a cation-exchange mechanism under pH conditions favoring the cationic form of the drug and by physical adsorption when the nonionized drug is present. Since physical adsorption is relatively weak, the drug is desorbed readily by alkaline washing.

Tetracycline—Tetracycline is ionized throughout the pH range and, as illustrated in Structure I, exists predominantly as a cation, +00, below pH 3.3, a zwitterion, +−0, between pH 3.3 and 7.7, and an anion, +−− or 0−−, above pH 7.7 (19).

The X-ray patterns of montmorillonite interacted with tetracycline at pH 1.5, 5.0, 8.7, and 11.0 are shown in Fig. 3. At pH 1.5 and 5.0, the increase in interlaminar space of 6.8 Å indicates that tetracycline is adsorbed. This dimension is in good agreement with the smallest dimension of tetracycline, 6.3 Å, based on a molecular model and suggests a parallel or slightly tilted orientation of the tetracycline molecules between the clay layers.

At pH 8.7, the X-ray diffractogram shows a diffuse reflection, indicating irregular interlayer spacing. This pattern is characteristic of partial interlayer adsorption (15) and is consistent with the difficulty expected in adsorbing a predominantly negative molecule (+−−) onto the negative clay surface. At pH 11, no increase in interlayer spacing is observed, indicating that the 0−− form of tetracycline is not adsorbed by montmorillonite.

The IR spectrum at pH 1.5, in which the cationic species of tetracycline interacts with montmorillonite (Fig. 4A), shows the presence of tetracycline in the interlaminar space. The IR spectrum of the adsorbed tetracycline does not show any significant shifts compared to the spectrum

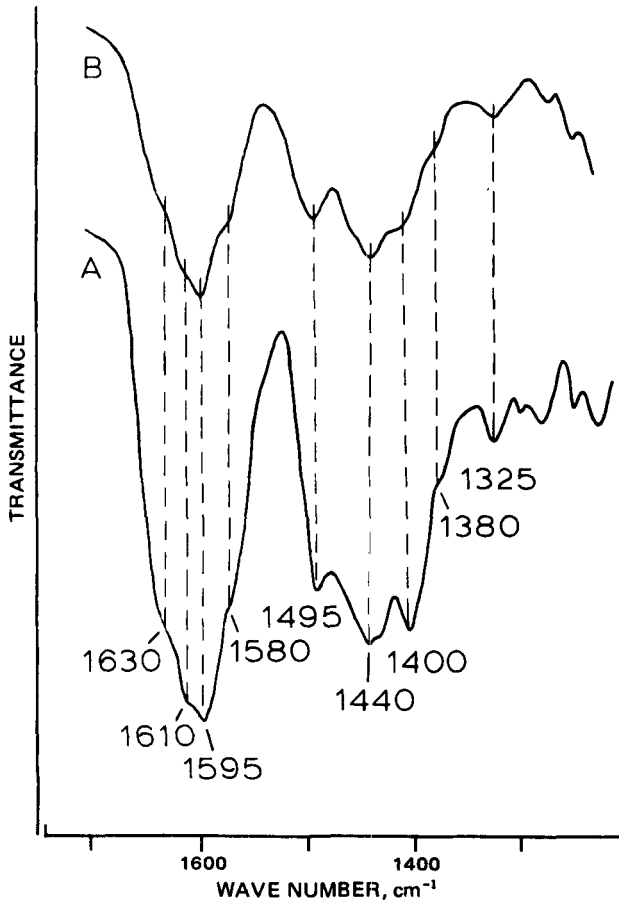

Figure 5—*Role of complexation when tetracycline interacts with montmorillonite. Key: A, calcium–tetracycline complex at pH 11.0; and B, tetracycline interacted with montmorillonite at pH 11.0.*

of tetracycline at pH 1.5, thus indicating no covalent interaction between drug and clay. This result suggests that adsorption occurs by exchange with the interlayer cations. Relative water band intensities are not useful in supporting this mechanism, as with clindamycin, since the water band is obscured by strong drug absorption bands. The higher concentration of tetracycline within the clay layers may also account for the similarity between spectra of the drug and drug interacted with clay. A more crowded drug environment would resemble the solid state more closely and produce an equivalent spectrum.

The IR spectra of tetracycline–montmorillonite equilibrated at pH 5.0 and 8.7 (Figs. 4B and 4C), where the +−0 and +−− forms, respectively, predominate, indicate that tetracycline is adsorbed in the interlayer space. However, spectral shifts suggest that a second mechanism in addition to cation exchange is responsible for adsorption. The carbonyl vibration associated with the ketone and amide I bands occurring at 1660 and 1640 cm^{-1} for tetracycline at pH 5.0 and 8.7, respectively, shifts to a lower frequency and is hidden under the amide II bands when tetracycline interacts with montmorillonite.

A shift of ketone carbonyl vibrations to lower frequency when organic compounds are adsorbed by clay was observed in cases where the carbonyl group was directly coordinated with the interlayer cations or when the carbonyl group hydrogen bonded with hydroxyl groups of the water coordinated to the interlayer cation (24). A shift of the amide I band to lower frequency was discussed previously. Hydrogen bonding of the ketone or amide carbonyl was not appreciable, as evidenced by irreversible adsorption on the montmorillonite. Since calcium comprises 10% of the exchangeable cations, the spectral shifts observed at pH 5.0 and 8.7 can be attributed to drug complexation with the interlayer divalent cations.

Drug absorption bands are observed when tetracycline is interacted with montmorillonite at pH 11 (Fig. 4D). However, the spectrum is significantly different from the spectrum of tetracycline at pH 11. Notably, a shift in the amide I band from 1640 to 1630 cm^{-1} and splitting of the amide II band occur to give absorption bands at 1610 and 1595 cm^{-1}, which suggests a coordination type of interaction (22). This participation of the amide function in the formation of a complex with divalent cations

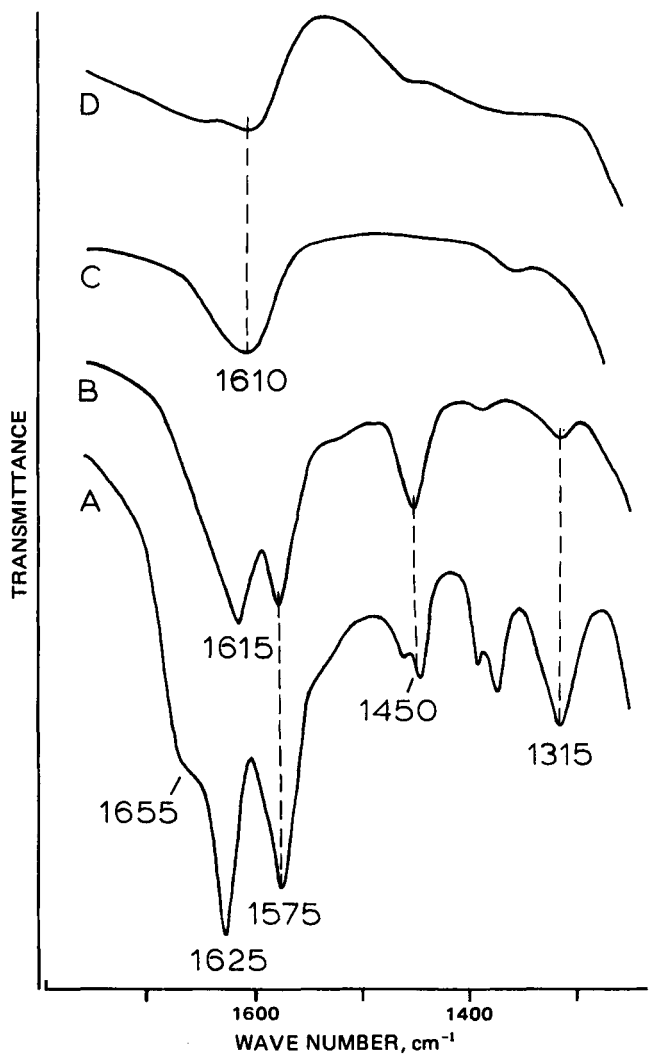

Figure 6—*IR spectra of sodium montmorillonite interacted with tetracycline. Key: A, pH 1.5; B, pH 5.0; C, pH 8.7; and D, pH 11.0.*

at high pH values also was demonstrated in a recent NMR study of tetracycline complexation sites (25).

The presence of calcium in the montmorillonite suggested a comparison to tetracycline calcium. As seen in Fig. 5, the tetracycline in the clay mixture has the same IR spectrum as tetracycline calcium. Since X-ray diffraction showed no increase in interlayer space at pH 11, it was hypothesized that the interaction of the 0−− species leads to the formation of an external calcium–tetracycline complex.

This hypothesis was confirmed by determining the concentration of calcium ion in calcium montmorillonite before and after interaction with tetracycline at pH 11. Only 32% of the original exchangeable calcium was found by atomic absorption spectroscopy after interaction with tetracycline. Therefore, 68% of the calcium must be attributed to the formation of external tetracycline calcium.

To demonstrate that cation exchange is one mechanism responsible for tetracycline adsorption and to determine which cationic species can be adsorbed by cation exchange, the interaction of tetracycline and pure sodium montmorillonite was studied at pH 1.5, 5.0, 8.7, and 11.0. Since the monovalent sodium ion is the only exchangeable cation, the only possible chemisorption mechanism for tetracycline is cation exchange. X-ray diffraction showed an increase in the interlayer space of 7.3 Å at pH 1.5 and an irregular spacing at pH 5.0 (Table II). No increase was observed at pH 8.7 and 11.0.

Adsorption of the drug within the clay at pH 1.5 and 5.0 was confirmed by IR absorption bands corresponding to those of tetracycline at pH 1.5 and 5.0 (Figs. 6A and 6B). Although tetracycline is a neutral molecule at pH 5.0, some degree of adsorption by cation exchange can occur due to the higher acidity of the clay surface (26). No drug absorption bands were seen at pH 8.7 or 11 (Figs. 6C and 6D). The increase in interlayer spacing and the presence of drug absorption bands at pH 1.5 and 5.0 in-

Table II—Interlayer Spacing, Å, of Montmorillonite Interacted with Tetracycline

pH	Montmorillonite	Sodium Montmorillonite	Calcium Montmorillonite
1.5	6.8	7.3	6.7
5.0	6.8	Irregular	5.9
8.7	Irregular	—[a]	Irregular
11.0	—[a]	—[a]	—[a]

[a] Less than 0.35 Å, signifying no interlayer adsorption.

dicate that, in the absence of complexing interlayer cations, adsorption occurs by a cation-exchange mechanism. At higher pH conditions, the anionic species, +−− or 0−−, cannot be adsorbed because of the negative surface of the clay.

The role of complexation in tetracycline adsorption was studied by examining the interaction of tetracycline and calcium montmorillonite, a clay that can interact with tetracycline by both cation exchange and complexation with divalent calcium cation. As seen in Table II, interlayer adsorption occurred at pH 1.5, 5.0, and 8.7, as evidenced by the increase in interlayer spacing. No interlayer adsorption occurred at pH 11. The IR spectrum of tetracycline–calcium montmorillonite at pH 1.5 (Fig. 7A) corresponds to the IR spectrum of tetracycline at pH 1.5, indicating adsorption by cation exchange. The IR spectra at pH 5.0, 8.7, and 11.0 are identical (Fig. 7B) and correspond to the spectrum of tetracycline calcium. Thus, the +−0, +−−, and 0−− species readily form complexes with divalent exchangeable cations. Complexation appears to predominate over a cation-exchange mechanism for the +−0 species.

Adsorption isotherms of the four tetracycline species (Fig. 8) show that the adsorptive capacity of the clay increases as a function of increasing acidity. Since the tetracycline ion develops a stronger cationic character with increasing acidity, a chemisorption mechanism is suggested as responsible for tetracycline–montmorillonite interaction. This hypothesis is further supported at pH 1.5 and 5.0 by the magnitude of adsorption, which considerably surpasses the capacity of the external surface (Table I). The unusual behavior of the pH 11.0 isotherm is probably due to negative adsorption resulting from the repulsion of the doubly charged anion, 0−−, from the negative surface of the clay.

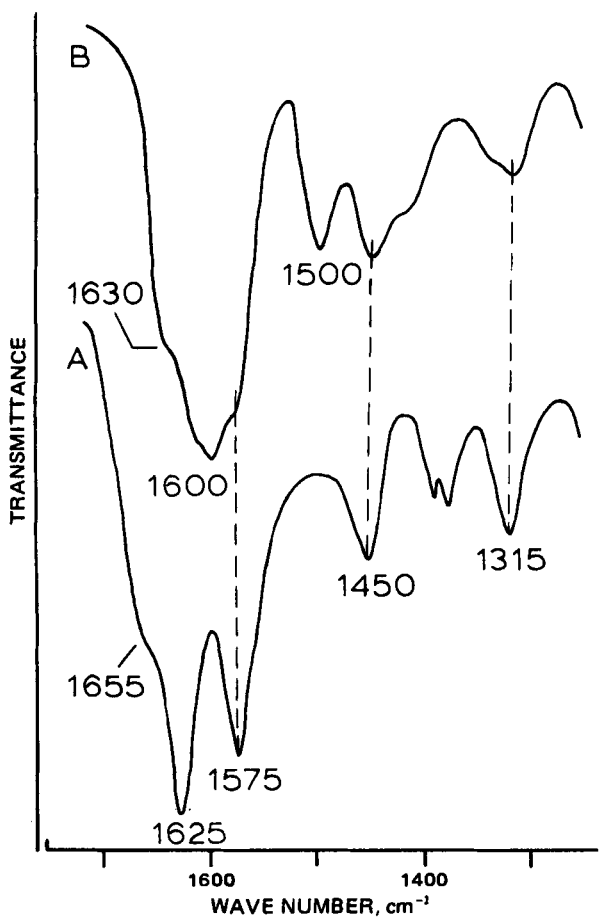

Figure 7—*IR spectra of calcium montmorillonite interacted with tetracycline. Key: A, pH 1.5; and B, pH 5.0, 8.7, and 11.0.*

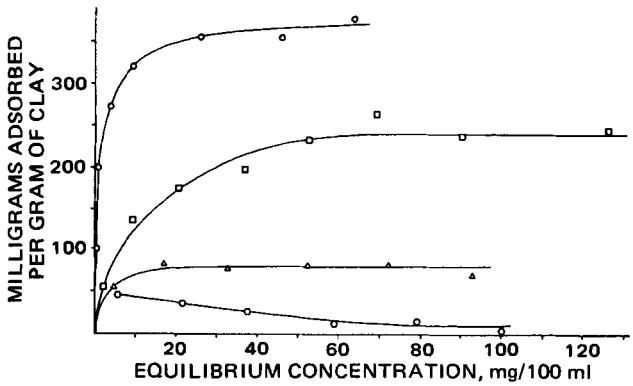

Figure 8—*Adsorption isotherm for tetracycline and montmorillonite. Key: ○, pH 1.5; □, pH 5.0; △, pH 8.7; and ○, pH 11.0.*

Under pH conditions favoring the cationic form of tetracycline, about 375 mg/g of montmorillonite is adsorbed. Clindamycin is adsorbed to a maximum of only 160 mg/g of adsorbent when it exists as a cation. Tetracycline and clindamycin have virtually identical volumes, 944 and 947 Å^3, and very similar planar surface areas, 190 and 150 Å^2, respectively. The greater adsorptive capacity suggests that tetracycline more fully occupies adsorption sites within the interlayer spaces of montmorillonite. This result could be accounted for by assuming a tilted orientation, which permits closer packing than does the parallel orientation of clindamycin. X-ray diffraction evidence supports this orientation since slightly larger c-axis spacings were observed than would be required for a parallel orientation. This orientation would be even more likely if foreshortening of tetracycline bond lengths occurs in the intercalated molecule.

Adsorption isotherms for tetracycline adsorbed onto montmorillonite followed the Freundlich equation for pH 1.5, 5.0, and 8.7. The data obtained at pH 11.0 also yielded a straight line but with a negative slope. Conformity to the Langmuir adsorption equation was observed for the tetracycline isotherms obtained at pH 1.5, 5.0, and 8.7 but not for that at pH 11.0. The linear relationships suggest chemisorption as the mechanism of adsorption for the +00, +−0, and +−− species of tetracycline. A suitable explanation for the adsorption at pH 11.0 is not apparent from the isotherm.

IR spectroscopy and X-ray diffraction show that tetracycline can be adsorbed onto montmorillonite by two mechanisms, cation exchange and complexation, depending upon the ionic species of the drug and the nature of the interlayer cation. At low pH values, where the +00 species exists, cation exchange is the mechanism of adsorption. At slightly acidic pH values, the zwitterion is adsorbed by both cation exchange and complexation. The 0−− species is not adsorbed but rather produces an external calcium–tetracycline complex.

Physical adsorption is not an important factor in tetracycline adsorption since washing does not change the IR spectra of the tetracycline–montmorillonite mixtures.

SUMMARY

The utility of X-ray diffraction and IR spectroscopy in elucidating the mechanisms responsible for clindamycin and tetracycline adsorption by montmorillonite was demonstrated in this study. Montmorillonite is suggested as a model clay for screening clay–drug interactions because it possesses a higher exchange capacity and surface area than other clays used in pharmaceutical systems, thereby undergoing any adsorption reactions to a greater degree than other pharmaceutical clays. In addition, its physical properties such as swelling and formation of self-supporting films make montmorillonite especially suitable for X-ray and IR examination.

The adsorption behavior of sodium montmorillonite showed that adsorption due to complexation with multivalent cations, as occurred with tetracycline, can be eliminated by prior treatment of the clay to replace all multivalent exchangeable cations with monovalent cations.

A full understanding of the adsorption mechanisms involved in clay–drug interactions should permit rational predictions of potential clay–drug interactions based on an understanding of clay properties and drug structures.

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Physical Characterization of Erythromycin: Anhydrate, Monohydrate, and Dihydrate Crystalline Solids

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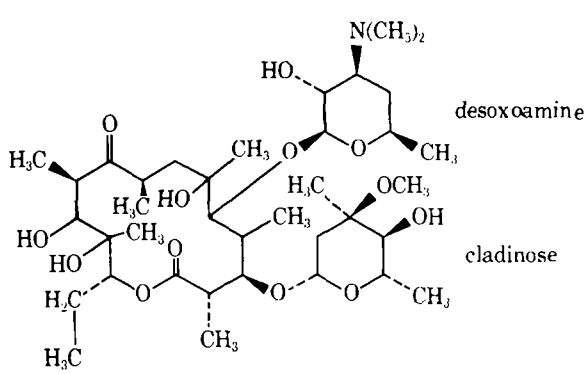
Abstract □ Hot-stage microscopy, thermoanalytical methods, and X-ray powder diffraction were used to demonstrate that crystalline erythromycin dihydrate converts to the crystalline anhydrate *via* a noncrystalline intermediate. X-ray powder diffraction, IR spectral, thermogravimetric, and differential thermal analyses were used to characterize the monohydrate material. The flow interrupt technique, a procedure recently developed to deal with low surface area samples, was employed successfully in obtaining isotherms and specific surface areas for the monohydrate and anhydrate. The relative dissolution rates of the various hydrates were determined in an aqueous solution (0.01 *M* phosphate buffer, pH 7.5) at 37°. The results showed a significant difference in the dissolution rate of the dihydrate compared to the monohydrate and anhydrate.

Keyphrases □ Erythromycin—physical characterization of anhydrate, monohydrate, and dihydrate crystalline solids, dissolution rates in aqueous solution □ Hydrated forms—of erythromycin crystalline solids, physical characterization, dissolution rates in aqueous solution □ Dissolution rates—erythromycin anhydrate, monohydrate, and dihydrate crystalline solids in aqueous solution □ Antibacterials—erythromycin, physical characterization of anhydrate, monohydrate, and dihydrate crystalline solids, dissolution rates in aqueous solution

Erythromycin is a potent antibiotic effective against various microorganisms. Commercial erythromycin is a mixture of several active components. Erythromycin A (I), the major component, is unstable in acidic media. To prevent deactivation of the drug by gastric acid and to facilitate absorption in the small intestine, erythromycin can be administered as enteric-coated tablets, which are stable in acid media but dissolve in intestinal fluids. Once the enteric coating has dissolved, the absorption and therapeutic efficacy of this material may be affected by the physical characteristics of the remaining solid. It has been postulated (1) and demonstrated (2–6) that the bioavailability of various drugs can be influenced by their dissolution rate, particle size, solubility, wettability, and extent of hydration.

Erythromycin exists in many different forms, including at least two hydrates (7, 8) and an anhydrate (7), which can



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