were scored as plaque inhibition. Each compound was evaluated on five teeth, with percent inhibition values being reported. This method indicates the percentage of teeth that did not show plaque formation after the stated incubation period. The solvent served as the control.

## RESULTS AND CONCLUSIONS

In the group of compounds tested, I–III and VIII showed maximal activity at $10^{-4}$ *M* after 24 and 48 hr while IX showed a shorter duration of activity. Only III showed equivalent activity to that of 8-hydroxyquinoline at $10^{-5}$ *M* after 24 hr. At $10^{-5}$ *M*, all of the active, new agents showed decreased duration of action or decreased activity. The antiplaque studies showed that II and IX had 24-hr activity equal to 8-hydroxyquinoline at $10^{-1}$ *M* while I and III had only 80% of the activity of the parent compound. Compounds IV, XIV, XVI, XVII, and XVIII showed neither antibacterial nor antiplaque activity.

Analogs with ionizable functions in the 4- or 5-position of 8-hydroxyquinoline showed no antibacterial or antiplaque activity. The inactivity may be due to either the test compounds inability to cross the bacterial membrane or their inability to chelate properly as required for biological activity. These data, as well as previous studies in this laboratory (1), indicate that a single parameter such as log *P* is not adequate to predict antiplaque activity accurately.

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# Influence of Monovalent and Divalent Electrolytes on Sorption of Neomycin Sulfate to Attapulgite and Montmorillonite Clays


**JAMES W. McGINITY ˣ and JOHN A. HILL**



**Abstract** □ Langmuir isotherms for the adsorption of neomycin sulfate to clays such as attapulgite, bentonite, and magnesium aluminum silicate were constructed. Monovalent and divalent cations were investigated for their influence on the formation of neomycin–clay adsorbates and the resulting equilibrium concentration in a neomycin solution. Divalent magnesium ions were more effective in displacing the antibiotic from each clay than were monovalent sodium ions. Ions present in the GI fluid might increase the bioavailability of neomycin from such neomycin–clay adsorbates.



**Keyphrases** □ Neomycin sulfate—sorption to attapulgite and montmorillonite clays, influence of monovalent and divalent electrolytes, Langmuir isotherms □ Attapulgite clay—sorption of neomycin sulfate, influence of monovalent and divalent electrolytes, Langmuir isotherms □ Montmorillonite clay—sorption of neomycin sulfate, influence of monovalent and divalent electrolytes, Langmuir isotherms


The adsorptive capacities of magnesium aluminum silicate (1–3), bentonite (2–4), and attapulgite (5–8) for various medicinals have been studied. The ionic incompatibility of neomycin sulfate with these clays was well documented in studies that demonstrated the strong affinity of this cationic antibiotic for the negatively charged clays, with a resultant reduction in the bioactivity of neomycin *in vitro* (2, 3, 9).

Wai and Banker (10) studied the adsorption of cationic drugs to montmorillonite clays and concluded that the mechanism of binding was ion exchange. This mechanism was verified in a recent investigation[1]. These studies, which also encompassed the influence of mono- and divalent electrolytes on the adsorption of amphetamine sulfate to montmorillonite, showed that the electrolytic cations had a significant effect on displacing the drug from the clay.

One objective of the present studies was to evaluate the influence of sodium and magnesium ions on the desorption of neomycin from attapulgite and montmorillonite clays. Another objective was to pro-

[1] Unpublished data.

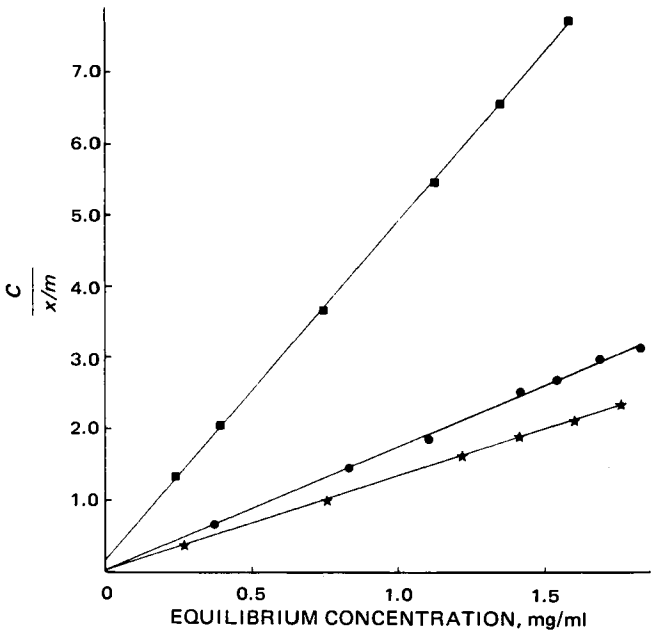


**Figure 1**—*Langmuir adsorption isotherms for neomycin sulfate and various clays. Key: ■, attapulgite; ●, bentonite; and ★, magnesium aluminum silicate.*

vide data as a basis for speculating on the probable bioavailability of neomycin sulfate in antidiarrheal suspensions containing these clays.

## EXPERIMENTAL

**Reagents**—The following were used: magnesium aluminum silicate[2], bentonite[3], attapulgite[4], amaranth[5], potassium hydrogen phthalate[6], neomycin sulfate[7], sodium chloride[6], and magnesium chloride[6].

**Neomycin Sulfate Assay**—The assay[8] for the antibiotic is based on a colorimetric reaction between amaranth and neomycin sulfate, buffered to pH 4.0 with potassium hydrogen phthalate. The absorbance of the uncomplexed dye was read spectrophotometrically[9] at 520 nm. A linear relationship existed between the absorbance of the dye and the concentration of the antibiotic; the amount of free amaranth was inversely related to the concentration of neomycin sulfate present. Excellent agreement exists between the results of this chemical assay and those obtained by an assay of microbiological activity.

**Sample Preparation for Langmuir Studies**—Neomycin sulfate, 200 mg, was dissolved in 10 ml of purified water and added to weighed amounts of each clay. Clay samples had previously been passed through a 100-mesh screen and hydrated in 80 ml of 60° water. Each suspension was adjusted to 100 ml with room temperature purified water and then agitated in a constant-temperature water bath. After centrifugation, the supernatant liquid was assayed colorimetrically for neomycin content.

**Sample Preparation for Electrolyte Studies**—The quantity of each clay to adsorb 80% of the neomycin was calculated from the Langmuir studies. These concentrations (w/v) were: magnesium aluminum silicate, 0.8%; bentonite, 1.2%; and attapulgite, 3.2%. Neomycin sulfate, 200 mg, was dissolved in 10 ml of purified water and added to the hydrated clay (prepared as described) with stirring.

After agitation and equilibration for 24 hr at 24°, the electrolyte solution was added with stirring and the resulting mixture was adjusted to 100 ml. After an additional 4 hr of equilibration, the mixture was centrifuged and the supernatant liquid was assayed for neomycin.

[2] Veegum Regular, R. T. Vanderbilt Co., New York, N.Y.
[3] White Bentonite, Whittaker, Clark and Daniels, Inc., South Plainfield, N.J.
[4] Pharmasorb Colloidal, Engelhard, Edison, N.J.
[5] H. Kohnstamm & Co., New York, N.Y.
[6] Mallinckrodt, St. Louis, Mo.
[7] E. R. Squibb & Sons, Princeton, N.J.
[8] Developed by J. Hill and coworkers, unpublished data.
[9] Spectronic 20.

**Table I—Adsorptive Capacities of Attapulgite and Montmorillonite Clays for Neomycin Sulfate**

| Clay | Limiting Adsorptive Capacity, $b^a$, mg/g |
|---|---|
| Attapulgite | 215 |
| Bentonite | 604 |
| Magnesium aluminum silicate | 772 |

[a] $b$ appears in the Langmuir equation and denotes the theoretical maximum adsorptive capacity of an adsorbent for a particular drug.

## RESULTS AND DISCUSSION

Data obtained from adsorption experiments fit the linear form of the Langmuir equation (11):

$$\frac{C}{x/m} = \frac{1}{ab} + \frac{1}{b}(C) \qquad \text{(Eq. 1)}$$

where $x/m$ is the weight of neomycin in milligrams adsorbed per gram of adsorbent, $C$ is the concentration of neomycin in milligrams per milliliter at equilibrium, and $a$ and $b$ are constants. Constant $a$ is related to the forces involved in binding the drug to the clay, and constant $b$ is the maximum amount of neomycin that can be adsorbed per gram of clay.

Langmuir isotherms for the adsorption of neomycin sulfate to magnesium aluminum silicate, bentonite, and attapulgite appear in Fig. 1. From the reciprocal of the slope for each linear curve, the limiting adsorptive capacity of each clay for neomycin was calculated (Table I). These experimental values were calculated from the regression lines in Fig. 1, using the method of least squares. The $b$ values, particularly for the montmorillonite clays, demonstrated the strong binding capacity of these clays for neomycin.

The influence of sodium chloride and magnesium chloride on the adsorption of neomycin to magnesium aluminum silicate, bentonite, and attapulgite is demonstrated in Figs. 2–4. These studies showed that divalent magnesium cations were more efficient than

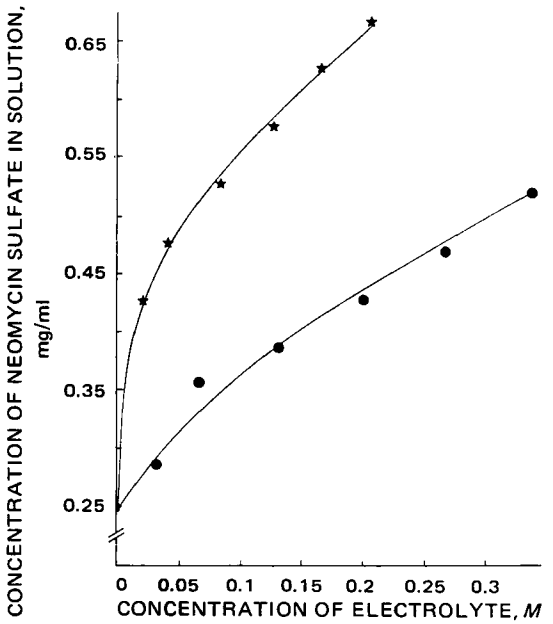


**Figure 2**—*Effect of electrolyte concentration and valency on the desorption of neomycin from a neomycin–magnesium aluminum silicate adsorbate. Key: ●, sodium chloride; and ★, magnesium chloride.*

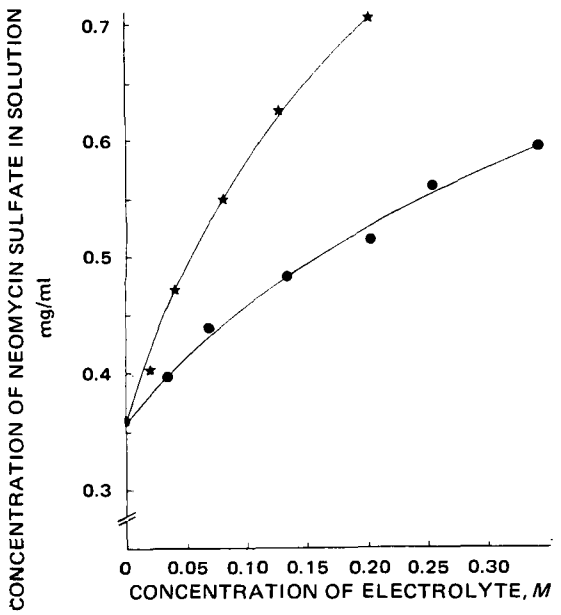


**Figure 3**—*Effect of electrolyte concentration and valency on the desorption of neomycin from a neomycin–bentonite adsorbate. Key: ●, sodium chloride; and ★, magnesium chloride.*

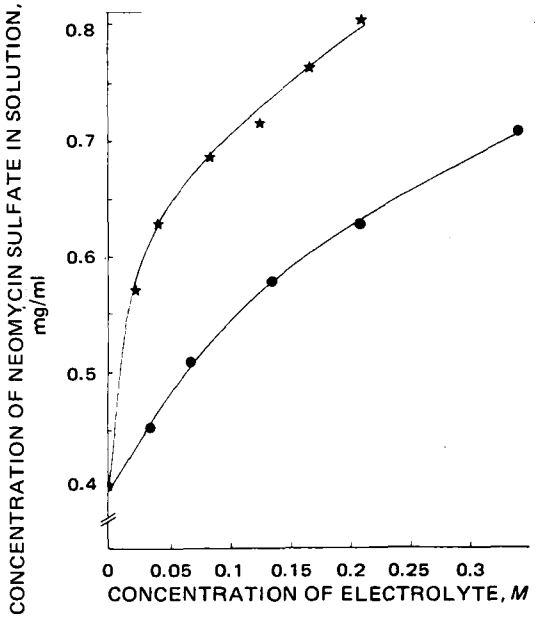


**Figure 4**—*Effect of electrolyte concentration and valency on the desorption of neomycin from a neomycin–attapulgite adsorbate. Key: ●, sodium chloride; and ★, magnesium chloride.*

monovalent sodium ions in displacing neomycin from negatively charged clays. These data suggest that any cations secreted in the GI fluids could influence the displacement of the antibiotic from these clays.

Sorby (5) suggested earlier that the presence of electrolytes and dilution by various fluids within the GI tract could hasten the release of promazine from activated attapulgite. The test doses that Sorby administered to human subjects had 75% of the drug adsorbed to the clay. However, under the conditions of the Sorby study, the clay slowed the rate of adsorption but had no significant effect on the total availability of promazine.

The average concentrations for sodium ions and potassium ions in GI fluids were reported (12) as 140 and 15 mEq/liter, respectively, but the concentrations of these ions are greater in the lower portion of the intestinal tract, precisely where the local action of neomycin is desired. On the other hand, divalent calcium and magnesium ions appear in negligible quantities in the luminal fluids. However, calcium from an external source (*e.g.*, milk), administered along with the antidiarrheal suspension, may act synergistically with secreted monovalent cations in releasing more free neomycin from the adsorbate. Supplemental oral therapy to replace sodium or potassium ions lost by the patient suffering diarrhea should also increase the desorption of neomycin and, consequently, improve the bioavailability of the antibiotic. During passage through the GI tract, the neomycin–clay adsorbate is continually exposed to freshly secreted fluids, so the desorption process is continuous.

Data presented in Figs. 2–4 were generated in a closed system in an attempt to establish rapidly an equilibrium between adsorbed drug and drug in solution. This equilibrium prevented excessive desorption of neomycin by the electrolyte *in vitro*. However, since equilibrium conditions are never established in the body, it can be argued that electrolytes would exert a greater influence *in vivo* than is suggested by the present *in vitro* studies.

Results of these studies also imply that the poor bioavailability of neomycin from similar adsorbates seen in *in vitro* studies (2, 3, 9) may not have accurately reflected the actual concentration of drug in the luminal fluids under the sink conditions of the body. Besides the electrolytic effect, other factors, such as the presence of bile salts, may influence the process of desorption, making it more difficult still to extrapolate from data for these neomycin–clay systems obtained *in vitro* to the actual situation *in vivo*.

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