

ABSORPTION OF PYRIMIDINES, PURINES, AND NUCLEOSIDES BY Co-, Ni-, Cu-, AND Fe(III)-MONTMORILLONITE (CLAY-ORGANIC STUDIES XIII)

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Abstract—Absorptions of purine and pyrimidine derivatives by Co- and Ni- montmorillonite at $\text{pH} < 6$ and by Cu-montmorillonite at $\text{pH} < 3$ are similar to their absorption by Ca-montmorillonite and take place primarily by a cation exchange process. In the weakly acidic to weakly alkaline range, absorption is due to complex formation with the inorganic cations, and decrease in the order $\text{Cu} \gg \text{Ni} > \text{Co} \gg \text{Ca}$. Adenine, 7-methyladenine, hypoxanthine, and purine are strongly absorbed, 9-methyladenine, 6-chloropurine, and cytosine are weakly absorbed, and thymine and uracil are not absorbed. At $\text{pH} < 5$, the nucleosides are absorbed by Co-, Ni-, and Cu-montmorillonite in approximately the same manner as by Ca-montmorillonite, but at $\text{pH} > 6$ their absorptions decrease in the order $\text{Cu} \gg \text{Ni} > \text{Co} > \text{Ca}$. Fe(III)-montmorillonite behaves quite differently from the other montmorillonites studied. With purines and pyrimidines, there is strong absorption from $\text{pH} 3$ to $\text{pH} 7-8$; with the nucleosides, the absorption varies considerably with the compounds considered decreasing in the order adenosine $>$ cytidine \gg guanosine \gg inosine.

INTRODUCTION

THE EXPERIMENTS reported here extend those previously described on the absorption of pyrimidines, purines, and nucleosides by Li-, Na-, Mg-, and Ca-montmorillonite (Clay-Organic Studies, Part XII, Lailach et al., 1968) to montmorillonites saturated with the transition metal cations, Co, Ni, Cu, and Fe(III). The previous work will be referred to as Part XII.

Attention has been given mainly to the absorption isotherms in the pH range 2-12, and the organic compounds have been selected in part to show the influence of substituent groups on the complexing behavior of the compounds with the inorganic cations.

The experimental methods for preparing the montmorillonites and measuring the organic absorptions are the same as those described in Part XII. Only for the alkaline samples of Fe(III)-montmorillonite was a change made in the procedure for u.v.-spectroscopic measurements. Since under alkaline conditions a Fe(III)-hydroxide sol was formed which was not removed from solutions by centrifugation, a $\text{pH} 2$ buffer (HCl-KCl) was added to the supernatant and sedimentation of a gel took place within a few minutes. The clear supernatant solutions were then used for the spectroscopic measurements.

X-ray data have been obtained for complexes of adenine with Cu- and Fe(III)-montmorillonite in the respective equilibrating solutions and these are summarized in an appendix. Infrared absorption data have been recorded, but, since the spectra of the investigated compounds are very complex and only a few bands have been assigned (Angell, 1961), these data have not helped significantly in the interpretation of the absorption isotherms and are omitted from the present publication. Interesting color changes have been observed which vary with the pH of the medium and the organic material and are related to the absorption processes; these are described briefly.

RESULTS

Absorption isotherms

Figures 1-4 show the percentage of each organic compound absorbed against the equilibrium pH of the medium. The concentrations of the montmorillonite and organic compounds were such (see Part XII) that 100 per cent absorption corresponds to about 25 per cent saturation of the exchange capacity. As in the previous work, thymine, uracil and their nucleosides were not absorbed under the experimental conditions.

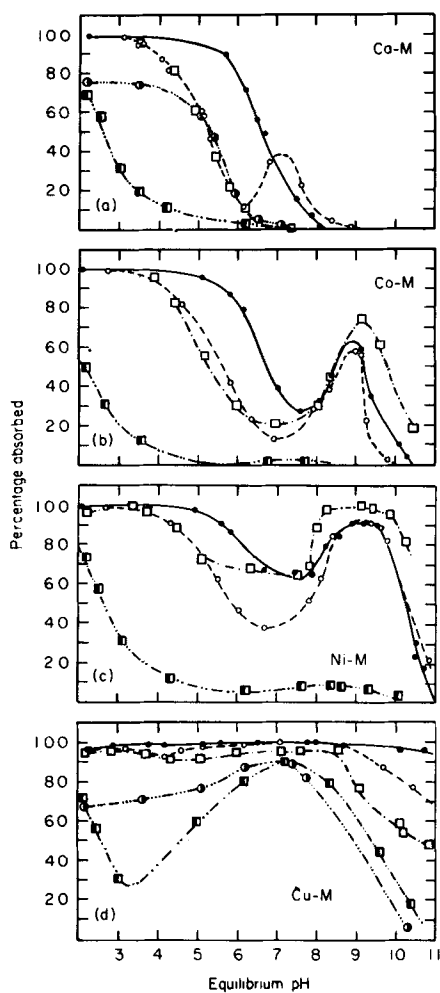


Fig. 1. Absorption by (a) Ca-; (b) Co-; (c) Ni-; (d) Cu-montmorillonite. Absorbed compounds: adenine ●; hypoxanthine ○; purine □; pyridine ◐; 6-chloropurine ■.

Color changes

Co-, Cu-, and Fe(III)-montmorillonite exhibited color changes related to the equilibrium pH and, in some cases, to the particular organic compounds.

The pale pink color of the Co-clay increased in intensity from pH 2 to pH 7 corresponding to the increasing amount of Co^{2+} ions remaining in the montmorillonite. Above those alkaline pH's where the absorption isotherms fall rapidly, the color changed through pale blue to a dirty yellow. With 9-methyladenine, 6-chloropurine, cytosine and the nucleosides, this color change appeared between pH 8 and pH 8.5. With 7-methyladenine, purine, adenine, and hypoxanthine, the color changed to a reddish-pink above pH 7 before passing through blue to yellow.

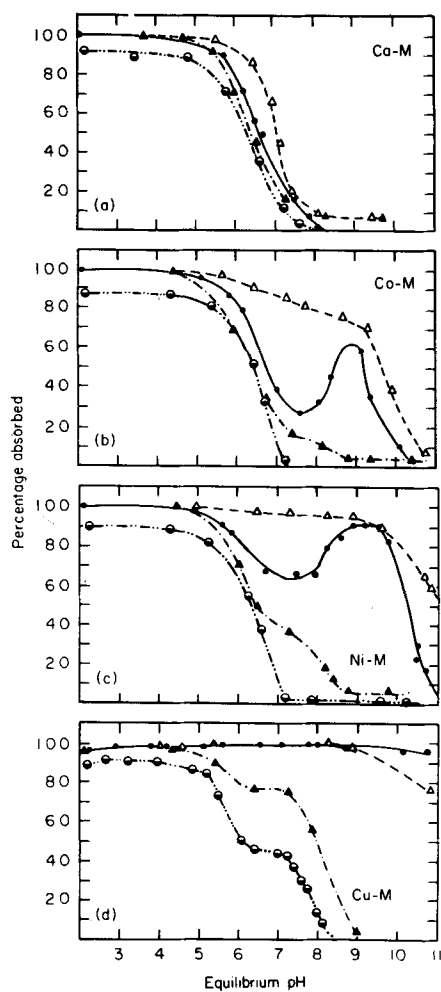


Fig. 2. Absorption by (a) Ca-; (b) Co-; (c) Ni-; (d) Cu-montmorillonite. Absorbed compounds: adenine ●; 7-methyladenine Δ; 9-methyladenine ▲; cytosine ◐.

The color of Cu-montmorillonite changed with pH as follows:

| | | | | |
|-------|-------|-----------|---------------|-------------|
| pH | 2 | 3 | 4-5 | 6-9 |
| color | white | pale-blue | blue | darker blue |
| | | | 9-11 | >11 |
| | | | greenish-blue | dark brown |

The intensity of the blue color in the range pH 4-9 depended on the absorbed organic compounds; with weaker absorption, the blue color was lighter and changed to greenish-blue and dark brown, respectively, at lower pHs.

With Fe(III)-montmorillonite, the color changes were determined by the pH. From pH 2 to 4 the yellow color of the clay became more intense as

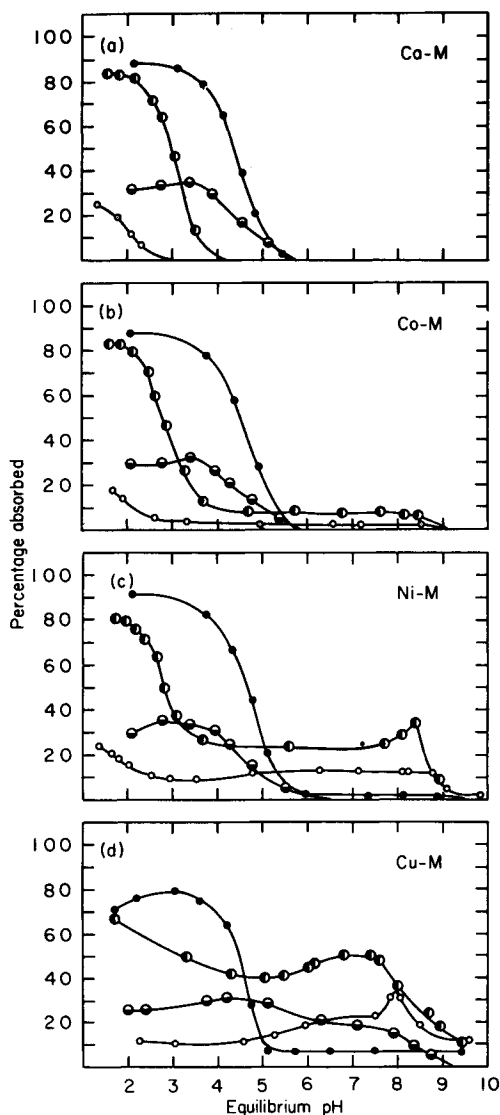


Fig. 3. Absorption of nucleosides by (a) Ca-; (b) Co-; (c) Ni-; (d) Cu-montmorillonite. Adenosine ●; inosine ○; guanosine ◐; cytidine ◑.

the concentration of Fe(III) in solution decreased. The more organic cations were absorbed, the lighter appeared the clay. From pH 4 to the pH's where the absorption isotherms begin to descend the color changed from yellow to yellowish brown; in this pH range, very few Fe(III) ions were in the supernatant solutions. In the following pH range the suspensions appeared brown and after centrifugation there remained a brown sol as supernatant.

All color changes, except that of the blue color

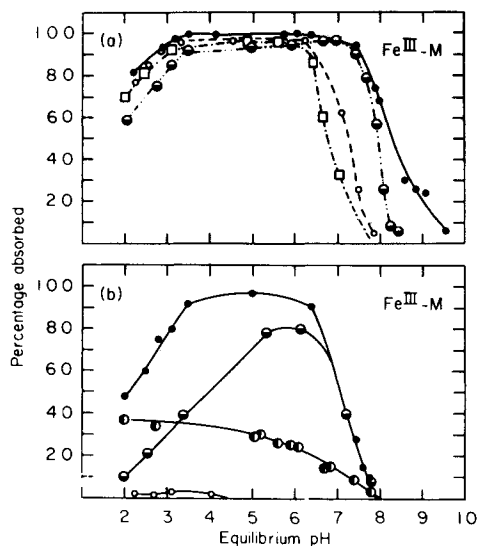


Fig. 4. Absorption by Fe(III)-montmorillonite. (a) adenine ●; hypoxanthine ○; purine ◻; cytosine ◕; (b) adenosine ●; inosine ○; cytidine ◑; guanosine ◐.

of Cu-montmorillonite to dark brown, took place within a few minutes after adding the clay suspensions to the organic solutions. This indicates that these reactions proceeded rapidly.

DISCUSSION

Absorption of purines and pyrimidines

Consideration will be given first to the absorption data for the pyrimidines and purines, summarized in Figs. 1, 2, and 4a. To facilitate comparison with the results given previously (see Part XII), the absorption isotherms for Ca-montmorillonite are included in Figs. 1 and 2. At pH < 6, the results for Co- and Ni-montmorillonite are very similar to those for Ca-montmorillonite, and at pH < 3, there is some similarity between the results for Cu- and Ca-montmorillonite. Under these acid conditions, it appears that absorption by Co-, Ni-, and Cu-montmorillonite takes place primarily by cation exchange processes, as discussed in Part XII. The data for Fe(III)-montmorillonite, Fig. 4a, are considerably different and are discussed later.

At pH > 7 for Co- and Ni-montmorillonite and > 4 for Cu-montmorillonite, well-marked absorption maxima are observed. Only Mg- and Ca-montmorillonite with hypoxanthine showed similar absorptions in the previous study. Color changes accompany these absorptions for Co- and Cu-montmorillonite and suggest that they are related to complex formation between the inorganic cations and the organic molecules.

At still higher pH values, hydroxides form and this complicates still further the interpretation of the absorption isotherms.

Purine and pyrimidine complexes formed by Co-, Ni-, and Cu-montmorillonite

Various authors have considered the formation of bidentate complexes between certain of the organic molecules studied here and the transition metal cations. Some consider (see, for example, Harkins and Freiser, 1958, and Cheney *et al.*, 1959) that the amino or hydroxyl group attached to the 6-position, and N-7 are involved, while others (Weiss and Venner, 1963, 1965; and Sletten, 1967) favor the N-3 and N-9 positions. (These positions are marked in the formulae given in Part XII.) With a view to differentiating between these possibilities, the absorptions of 7- and 9-methyladenine were measured. The blocking of the N-9 position in the second of these compounds requires that a bidentate complex, if formed, will involve the amino group and the N-7 position, while the blocking of the N-7 position in the first compound would require the operating of the N-3 and N-9 positions. The much stronger absorption of the 7-methyladenine than of the 9-methyladenine (see Fig. 2) suggests that the N-3 and N-9 positions give rise to stronger complexes, and this is in agreement with the views of Weiss and Venner (1963, 1965). The strong complexes of adenine, hypoxanthine and purine are interpreted in the same way. The noticeably greater absorption of 7-methyladenine than of adenine may be related to the electron-releasing effect of the methyl group which influences the basicity of the atoms involved in the complex formation.

The compounds adenine, hypoxanthine, purine, and 6-chloropurine (see Fig. 1) differ with respect to the substituents in the 6-position, respectively NH_2 , OH, H and Cl, and only the last named has an electron-withdrawing functional group. The first three compounds are comparably and strongly absorbed, but the last is very weakly absorbed by Ca-, Co-, and Ni-montmorillonite except under strongly acid conditions where the mechanism is cationic. Only with Cu-montmorillonite is 6-chloropurine strongly absorbed in the pH range 5-9. This difference in absorption behavior indicates that the basicity of the coordinating positions is important in determining the strength of a complex.

Cytosine does not form complexes with Co- and Ni-montmorillonite, and only a weak complex with Cu-montmorillonite. Cytosine complexes with Cu(II) ions have been considered by Wang and Li (1966) and are believed to be monodentate; the preferred site for binding is the N-3 position.

Absorption of nucleosides by Co-, Ni-, and Cu-montmorillonites

Figure 3 shows similarities between the absorptions by these montmorillonites and by Ca-montmorillonite at $\text{pH} < 5$, when cation exchange is the principle absorption mechanism. At $\text{pH} > 5$ absorption increases in the order $\text{Cu} > \text{Ni} > \text{Co}$. For different nucleosides the absorption is in the order guanosine $>$ inosine $>$ cytidine $>$ adenosine. Fiskens *et al.* (1965), studying complex formation between certain nucleosides and Cu(II) ions, also found that guanosine forms the strongest and adenosine the weakest complexes, which points to stronger complexing by the OH group and N-7, than by NH_2 and N-7. That inosine is the second most strongly absorbed compound is consistent with this view. The weak complexing of adenosine in relation to the strong complexing of adenine is to be attributed to the role of the available N-3 and N-9 positions in adenine and the non-availability of the N-9 position in adenosine, or, in other words, the behavior of adenosine is to be compared with that of 9-methyladenine, which has been shown to be weakly absorbed.

Absorption by Fe(III)-montmorillonite

The isotherms in Fig. 4 differ considerably from those of the other transition metal-montmorillonites. The pyrimidine and purine isotherms, Fig. 4a, show strong absorption plateaux ranging from pH 3 to pH 6-7 which fall rapidly without the formation of separate absorption peaks at the higher pH values. A few of the isotherms for Cu-montmorillonite are somewhat similar. With Fe(III)-montmorillonite it is possible that the absorption under acid conditions, which is generally attributable mainly to cation exchange processes, merges with complex formation at nearly neutral pH values so that no break is seen between the two processes such as appears generally in Figs. 1 and 2. It is observed that the sequence in which the isotherms fall to zero in Fig. 4a is similar to that observed in Fig. 1b, though at smaller pH values.

The nucleoside isotherms in Fig. 4b differ considerably from those shown in Fig. 3. The shape of the curves for guanosine and inosine suggests that their absorption is primarily by cation exchange. The much larger absorptions of adenosine and cytidine may indicate complex formation, possibly involving the NH_2 group and/or the N-1 position of adenosine and the N-3 position of cytidine. The possibility of the N-7 position of adenosine being involved appears to be small because of the non-complexing behavior of guanosine and inosine. Further evidence that the N-7 position is not involved comes from the isotherms

of 7- and 9-methyladenine which are identical with that of adenine and therefore were not explicitly shown in Fig. 4a; evidently the blocking of the N-7 and N-9 positions does not affect the absorption isotherm.

SUMMARY AND CONCLUSIONS

The absorption isotherms of pyrimidines, purines, and nucleosides by Co-, Ni-, and Cu-montmorillonite in the acidic pH range are similar to those observed with Mg- and Ca-montmorillonite discussed previously (Part XII), and the absorption depends primarily on cation exchange processes. With rising pH, complex formation with the inorganic cations becomes increasingly important and the strength of the complexes is mainly in the order $\text{Cu} \gg \text{Ni} > \text{Co} \gg \text{Ca}$. Complex formation occurs most probably with the N-3 and N-9 positions of adenine, hypoxanthine, purine, 6-chloropurine, and 7-methyladenine for Co-, Ni-, and Cu-montmorillonite in agreement with the conclusions of Weiss and Venner (1963, 1965), and Sletter (1967). Much weaker complexes are formed by 9-methyladenine, guanosine, and inosine in which bonding is probably to the substituent in position 6 and N-7, or possibly to N-1.

Complexes of adenosine are too weak to cause any measurable absorption by montmorillonite. Cytosine and cytidine form very weak complexes with Cu-montmorillonite.

The behavior of Fe(III)-montmorillonite is considerably different. Complexing may occur in the weakly alkaline pH range, but the isotherms show no clear differentiation between cationic absorption and that due to complexing. The binding sites favoring absorption of nucleosides appear to be the NH_2 and/or the N-1 position of adenosine and the similar N-3 position of cytidine.

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APPENDIX

X-ray data for adenine-water-Cu-montmorillonite and adenine-water-Fe(III)-montmorillonite equilibrium system

The X-ray data are summarized in Table 1 for the absorption of adenine by Cu- and Fe(III)-montmorillonite under various pH conditions.

Table 1. X-ray data on complexes of adenine with Cu- and Fe(III)-montmorillonite in aqueous solutions

| (1) Cu-montmorillonite | | | | |
|-----------------------------|------------------|------------------------------------|---------------------------------|------|
| Solutions | Equilibrium pH | Basal Spacings, Å, and Intensities | Molecules adenine per unit cell | |
| | HCl | 2.0 | 19.2 (vs) | — |
| adenine, | HCl | 1.9 ₅ | 12.5 (ms), 10.2 (mw) | 0.21 |
| | H ₂ O | 5.1 | 19.2 (vs) | — |
| adenine, | H ₂ O | 5.2 ₅ | 15.8 (s), ~18(w,d) | 0.22 |
| adenine*, | H ₂ O | 5.0 | 15.7 (vs) | 0.44 |
| | NaOH | 9.0 | >40 (s,d) | — |
| adenine, | NaOH | 9.4 ₅ | 29.2† (vs), 10(vw) | 0.35 |
| (2) Fe(III)-montmorillonite | | | | |
| | HCl | 2.1 | 21.0 (vs, b) | — |
| adenine, | HCl | 2.2 ₅ | 21.0 (s), ~11(w,d) | 0.10 |
| adenine*, | HCl | 2.4 | 12.6 (ms), 20(vw) | 0.20 |
| | H ₂ O | 4.8 | 21.0 (vs,b) | — |
| adenine, | H ₂ O | 4.9 | 20.0 (s), 12.4(w,d) | 0.12 |
| adenine*, | H ₂ O | 5.6 | 12.5 (s), >28(w,d) | 0.24 |
| | NaOH | 6.9 | >28 (vs,d) | — |
| adenine, | NaOH | 7.2 ₅ | >28 (s,d), >13(m,d) | 0.09 |
| adenine*, | NaOH | 7.6 ₅ | >28 (s,d), ~13(ms,d) | 0.13 |

*Signifies adenine solution of twice the usual concentration.

†At least three orders of this reflection were obtained, 29.2 (vs), 14.6(w) and 9.7 (vw).

Intensities: vs = very strong, s = strong, ms = medium strong, mw = medium weak, w = weak, vw = very weak. Also, b = broad, d = diffuse.

The table lists also the observed spacings in the absence of adenine, and also with adenine of twice the usual concentration.

Cu-montmorillonite expands to a basal spacing of 19.2 Å in acid and neutral media (like Ca-montmorillonite) but under basic conditions expands to >40 Å (like Na-montmorillonite). Under acid conditions, adenine is absorbed principally in cationic form and gives a 12.5 Å spacing (as it does with Ca- and Na-montmorillonite), corresponding to a single sheet of flat lying molecules. Under neutral conditions, with the same amount of adenine absorbed and also with double this amount, a 15.8 Å spacing is observed which is almost certainly the result of a different orientation of the molecules. Under basic conditions, the spacing expands to 29.2 Å which has no counterpart with the results for Na- and Ca-montmorillonite. Further study of this long-spacing arrangement is required.

Fe(III)-montmorillonite expands to about 21 Å in acid and neutral media and to >28 Å in basic media and these results are somewhat similar to those for Cu-montmorillonite. With the usually used concentration of adenine and under acid or near neutral conditions, there is no change in the 21 Å spacing, but with twice the adenine concentration the spacing diminishes to the value 12.5 Å which corresponds to that normally observed for cationic absorption. Under basic conditions, the spacing increases to >28 Å, but is not sharply defined. There is a close similarity between the X-ray data for Cu- and Fe(III)-montmorillonite, and the only noteworthy difference is the forma-

tion of the 15.8 Å spacing with Cu-montmorillonite under more-or-less neutral conditions.

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Résumé—L'absorption de dérivatifs de purine et de pyrimidine par la montmorillonite Co et Ni avec pH < 6 et par la montmorillonite Cu avec pH < 3 se compare à leur absorption par la montmorillonite Ca; il s'agit essentiellement d'un procédé d'échange de cations. A l'intérieur d'une gamme qui va de la faible acidité à la faible alcalinité, l'absorption résulte d'une formation complexe avec des cations inorganiques, et décroît dans l'ordre Cu ≫ Ni > Co ≫ Ca. Il y a une forte absorption d'adénine, de 7-méthyladénine, d'hypoxanthine et de purine, une faible absorption de 9-méthyladénine, de 6-chloropurine et de cytosine, et la thymine et l'uracile ne sont pas absorbés du tout. Avec pH < 5, les nucléosides se font absorber par la montmorillonite Co, Ni et Cu à peu près de la même manière que par la montmorillonite Ca, mais avec pH > 6, leur absorption décroît dans l'ordre Cu ≫ Ni > Co > Ca. La montmorillonite Fe(III) réagit d'une manière toute différente des autres montmorillonites étudiées. Avec les purines et les pyrimidines, il y a absorption importante de pH 3 à pH 7-8; avec les nucléosides, l'absorption varie de manière considérable; les composés en question décroissent dans l'ordre adénosine > cytidine ≫ guanosine ≫ inosine.

Kurzreferat—Die Absorption von Purin- und Pyrimidinderivaten durch Co- und Ni-Montmorillonit bei pH < 6 und durch Cu-Montmorillonit bei pH < 3 ist ähnlich der Absorption dieser Verbindungen durch Ca-Montmorillonit und erfolgt in erster Linie durch Kationenaustauschprozesse. Im schwach sauren bis schwach alkalischen Bereich beruht die Absorption auf Komplexbildung mit den anorganischen Kationen und nimmt in der Reihenfolge Cu ≫ Ni > Co ≫ Ca ab. Adenin, 7-Methyladenin, Hypoxanthin und Purin werden stark absorbiert, 9-Methyladenin, 6-Chlorpurin und Cytosin werden schwach, und Thymin sowie Uracil werden überhaupt nicht absorbiert. Bei Werten unterhalb pH 5 werden die Nucleoside durch Co-, Ni- und Cu-Montmorillonit in ungefähr der gleichen Art wie durch Ca-Montmorillonit absorbiert, während bei pH > 6 ihre Absorptionen in der Reihenfolge Cu ≫ Ni > Co > Ca abnehmen. Fe(III)-Montmorillonit verhält sich ganz anders als die anderen untersuchten

Montmorillonite. Bei Purinen und Pyrimidinen erfolgt starke Absorption im Bereich von pH 3 bis pH 7–8; bei den Nucleosiden variiert die Absorption beträchtlich je nach dem zur Verwendung gelangenden Material und nimmt in der Reihenfolge Adenosin > Cytidin » Guanosin » Inosin ab.

Резюме—Абсорбция производных пурина и пиримидина Со- и Ni- монтмориллонитом при pH < 6 и Cu-монтмориллонитом при pH < 3 сходна с поглощением их Са-монтмориллонитом и производится преимущественно катионообменным процессом. В слабокислом или слабощелочном диапазоне абсорбция происходит благодаря сложному образованию с неорганическими катионами и уменьшается в порядке Cu » Ni > Со > Са. Аденин, 7-метиладенин, гипоксантин и пурин сильно абсорбируемы; 9-метиладенин, 6-хлоропурин и цитозин слабо абсорбируемы; а тимин и урацил не абсорбируемы. При pH < 5 нуклеозиды поглощаются Со-, Ni- и Cu- монтмориллонитом приблизительно так же, как и Са-монтмориллонитом, но при pH > 6 поглощения уменьшаются в порядке Cu» Ni > Со > Са. Fe (III)-монтмориллонит ведет себя иначе, чем все другие исследуемые монтмориллониты. В случае пуринов и пиримидинов, сильная абсорбция происходит от pH 3 до pH 7–8; а у нуклеозидов она бывает очень разной, в зависимости от соединения уменьшаясь в порядке аденозин > цитидин » гуанозин » инозин.