The Interactions of Nucleosides on Clay Mineral Surfaces

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Abstract:

Up to 75% of the carbon on land is contained in soil organic matter (SOM). SOM can interact with mineral surfaces to prevent or slow SOM decay. Clay minerals are layered aluminosilicates that are less than 2 μm in diameter. Their high surface areas and permanent negative charge on their layers allow them to interact with SOM via several mechanisms including cation exchange. It has been hypothesized that clay minerals had a role in the development of primitive forms of RNA by concentrating and protecting nucleic acids. In this study, the interactions of adenosine, cytidine, guanosine and uridine with montmorillonite surfaces were quantified. The amount of nucleoside adsorbed on the clay surface was quantified using UV spectroscopy. To determine the interactions of the nucleoside with clay mineral surface, infrared spectroscopy (IR) and X-ray diffraction (XRD) were used. Nucleosides at various concentrations were mixed with clay mineral solutions and adsorbed at pH 3. After 48 hours, the dried samples were analyzed using IR and XRD. IR bands representing the NH$_2$ deformation in adenosine and guanosine shifted when interacting with the clay. Additional IR bands representing the C=O stretch in guanosine also shifted. Both bands suggest adenosine and guanosine are physically interacting with the clay. XRD of adenosine, guanosine, cytidine and uridine show d-values indicative of some portion of the nucleoside in the interlayers of montmorillonite. Future experiments will investigate the orientation of the nucleosides when interacting with the clay as well as the effects of pH on the interactions of nucleosides with clay mineral surfaces.
Introduction:

The purpose of this paper was to evaluate the adsorption of nucleosides at variable initial concentrations onto montmorillonite under acidic conditions to describe the interactions and location of the nucleosides onto the surface of montmorillonite. These interactions are important because they explore the possibilities that clay minerals could have been involved in the initial development of living forms (Bernal, 1954). The adsorption and concentration of organic matter in the interlayers of montmorillonite gives a better understanding of how compounds, such as nucleosides, could have come together and potentially synthesize into larger organic compounds.

Soil organic matter (SOM), through the process of carbon sequestering, stores roughly 75-80% of the available carbon on land (Ontl, 2012). SOM can interact with various mineral surfaces found in the soil that act to concentrate the organic matter. The concentration of organic matter helps slow and prevent SOM decay over time. Increased concentrations of SOM are associated with high amounts of clay in the soil. Clay minerals are small, layered structures less than 2 µm in diameter with a high surface area due to the layers (Hashizume, 2012). Montmorillonite is a layered aluminosilicate clay that contains octahedral layers of AlO$_6$ and tetrahedral layers of SiO$_4$ (Fig. 1). During formation, Al$^{3+}$ may be substituted in the octahedral layer for a lower valent element such as Mg$^{2+}$ (Ferris et al., 1989). The negative charge that the substitution of Mg$^{2+}$ generates allows montmorillonite to interact with SOM through several mechanisms including the presence of cations to balance out the charged surface (Ghadiri, 2015). Montmorillonite has different surface sites including planar surfaces, edges and interlayer spaces that can interact with organic molecules.
The space between the layers of clay is known as the interlayer space and possesses a net negative charge in montmorillonite (Ferris et al., 1989). The planar surfaces of montmorillonite provide interaction sites for water, ions, and organic molecules. Water molecules are found in the interlayers of montmorillonite and aid in expanding the layers. In addition to the planar exchange sites, the broken edges of clay minerals are also reactive but are pH-dependent (Ghadiri, 2015). The edges only accounts for a small amount of cation exchange space relative to the clay surface area because the edges of the clay may or may not possess a negative charge.

The interlayer spaces of the clay are sites for cation exchange and a space for water molecules to reside. The fluctuating amount of water in the interlayer space of montmorillonite gives it the unique property of being a swelling clay (Castellni et al., 2017. The substitution of Al\(^{3+}\) for Mg\(^{2+}\) generates a low net negative charge so the layers of the clay mineral loosely hold on to the next layer causing the montmorillonite to swell (Ferris et al., 1989). In wet conditions, the interlayer spacing is large whereas the interlayer spacing decreases under dry conditions. In the same manner, the clay interlayers expand when organic compounds interact with them (Ferris, 2005). The aluminosilicate layers of montmorillonite have a consistent distance that is unchanged by the surrounding environment but the distance between the layers fluctuates based on several factors including hydration. Together, the layered structure of montmorillonite and its swelling
characteristics influence the interactions that take place between the clay mineral surface and organic compounds.

Organic compounds are the building blocks of life on earth. One type of organic compound is a nucleobase, such as adenine, which can be found in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) and is classified as a small bio-molecule (Hashizume, 2012). Nucleosides, such as adenosine, each contain a nucleobase and a sugar and are used in the formation of RNA chains with the addition of a phosphate group. These are the three essential ‘ingredients’ for forming RNA and DNA chains. This work focused on the interactions of nucleosides, such as adenosine, guanosine, cytidine and uridine, with montmorillonite. Nucleosides are a ribose ring bound to a nucleobase at C1, with an alcohol group on C5, where the phosphate groups would normally bind (Table 1).

Nucleosides are positively charged when the surrounding environment has a pH less than the first pK\textsubscript{a} of the nucleoside, pK\textsubscript{a1}. This encourages the nucleosides to cation exchange into the negatively-charged clay interlayers, which provides a protective environment for nucleosides to reside. This is an important characteristic of the clay mineral-nucleoside interaction because previous research has shown that this type of interaction could facilitate the creation of the first ribonucleic acid (RNA) strand; this could be where life started (Yu, et al., 2013). In order to understand how life could have started in clay interlayers, the interactions of RNA components with the clay need to be understood. Clay mineral-nucleobase interactions have been studied previously (Benetoli, 2008, Lailach et al., 1968, 1969, Ferris 2005) but nucleosides interactions with clay minerals are understudied in terms of how and where the interactions take place (Lailach et al., 1968, Winter and Zubay, 1993).
Nucleobases and clay mineral surfaces interact via hydrogen bonding, van der Waals forces, and ionic bonds (Benetoli, 2008). The layers of montmorillonite are held together by van der Waals forces, but the bond is relatively weak to accommodate the changing environment due to water and organic material present in the interlayers (Ferris et al., 2005). Water also contributes to the interactions by mediating hydrogen bonds between organic matter and montmorillonite (Pucci, 2010). The interaction between cations and anions, including negatively charged montmorillonite, have the potential to form ionic bonds. Montmorillonite has been shown to facilitate cationic exchange between protonated nucleobases and clay interlayers (Pucci, 2010).

Previous research has investigated the interactions of nucleosides with montmorillonite in relation to the synthesis of living forms (Lailach et al., 1967, Ferris et al., 1989, Winter & Zubay, 1995). Adenosine, guanosine, and cytidine adsorb to montmorillonite at a range of pH values with the greatest amount of adsorption occurring under acidic conditions (Ferris et al., 1989, Lailach et al., 1967). The dependency on pH as well as the aromatic characteristics of nucleosides influence the amount of adsorption that can occur on the surface, edges, or interlayers of the clay (Lailach et al., 1967). Adenosine was found in the interlayers of montmorillonite, but the exact mechanism resulting in this interaction is unknown. Similarly, it is unknown how other nucleosides interact with montmorillonite (Ferris, et al., 1989).

It has been hypothesized that clay minerals had a role in the development of primitive forms of RNA by concentrating and protecting RNA components (Bernal, 1954). In this study, the adsorption of nucleosides was quantified on montmorillonite surfaces. The surface loadings of various nucleosides onto montmorillonite were identified by varying the concentration in
solution with montmorillonite. Then, the resulting solids and solutions were characterized.

Infrared spectroscopy (IR) was used to identify the interactions between the clay and each nucleoside and x-ray diffraction (XRD) was used to determine the location of the interactions between the nucleoside and the clay mineral surface.

**Methods:**

**Supplies**

A calcium-rich montmorillonite clay from Texas (STx-1b), purchased from the Source Clays Repository, was used for all experiments. The chemical formula for STx-1b is \[^{[4]}(Si_{7.753}Al_{0.247})^{[6]}(Al_{3.281}Mg_{0.558}Fe_{0.136}Ti_{0.024}Mn_{0.002})^{[12]}(Ca_{0.341}Na_{0.039}K_{0.061})O_{20}(OH)_4\] (Castellini, 2017). All experiments were performed using the material “as received”. Adenosine, guanosine, cytidine, and uridine were purchased from Sigma Aldrich. All chemical compounds used in the experimentation were of high-quality grade.

**Adsorption**

Adsorption solutions were prepared with a solid to solution ratio of 1.6 g montmorillonite/L, and a final nucleoside concentration ranging from 60-3500 μM. Solutions also contained 0.001 M CaCl\(_2\) to maintain a constant ionic strength. Solutions were adjusted to a pH of 3.0 ± 0.1 using 0.5 M and 1.0 M NaOH and HCl and a pH probe. The solutions were equilibrated for 48-hours on an orbital shaker at 110-120 revolutions per minute, wrapped in aluminum foil to avoid light contamination. After 48-hours, solutions were centrifuged at 5000 rpm for 10 minutes to separate the solution and the solid.
Table 1: Nucleoside characteristics

<table>
<thead>
<tr>
<th>Nucleoside</th>
<th>Adenosine</th>
<th>Cytidine</th>
<th>Guanosine</th>
<th>Uridine</th>
</tr>
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<tr>
<td>Structure</td>
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<td><img src="structure.png" alt="Structure" /></td>
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<td>pK&lt;sub&gt;a&lt;/sub&gt;</td>
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<td>12.5</td>
<td>4.22</td>
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</tr>
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<td>Solubility in water (mg/mL)</td>
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<td>100.0</td>
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<td>50.0</td>
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<td>260</td>
<td>268</td>
<td>258</td>
<td>262</td>
</tr>
</tbody>
</table>

**Ultraviolet Spectroscopy**

After 48-hours, solution analysis was performed by finding the absorbance at λ<sub>max</sub> for each nucleoside remaining in solution, in triplicate. Corning UV 96-well half area plates with UV transparent bottoms and a Biotek microplate spectrophotometer were used. The λ<sub>max</sub> for adenosine, cytidine, guanosine, and uridine were 260 nm, 268 nm, 258 nm, and 262 nm, respectively. Samples were diluted using deionized water when necessary. Standard curves were used to determine the concentration of nucleoside adsorbed to the clay. The 0.001 M CaCl<sub>2</sub> was used as the blank.

**Infrared Spectroscopy**

Sample analysis was performed after the clay samples were dried at 65 °C overnight. The clay was ground into a fine powder using an agate mortar and pestle. Using a Perkin Elmer Spectrum 100 FT-IR spectrometer, the samples were analyzed from 700 to 4000 cm<sup>-1</sup>. A consistent pressure of 150 N was maintained while the clay was scanned for an accumulation of
50 scans at a resolution of 2 cm\(^{-1}\). Attenuated total reflectance (ATR) correction was performed on all spectra.

**X-Ray Diffraction**

To evaluate the spacing between layers of the clay, ground samples were dried again at 115 °C for one hour. Samples were immediately packed into 1 mm Special Glass capillaries and sealed. Data was collected from 1-50° 2θ and a step size of 0.033° using the Empyrean X-ray Diffractometer with molybdenum X-ray source.

**Results:**

**Surface Loading**

The maximum amount of nucleoside adsorbed to the clay was determined. At pH 3, the solutions were below the pK\(_{a1}\) values for adenosine and cytidine, so the dominant species of each nucleoside is protonated. The surface loading of each nucleoside increased with increased initial nucleoside concentration in solution (Fig. 2). Increased concentrations of adenosine and guanosine adsorbed to montmorillonite while cytidine and uridine had lower concentrations adsorb to the clay under the same conditions. Adenosine adsorbed to montmorillonite at a maximum surface loading of 129 mg/g. Guanosine showed a 16% increase in adsorbed organic matter, compared to the adsorption of adenosine, and the highest concentration of adsorbed nucleoside at 134 mg/g adsorbed (Fig. 2). Cytidine also adsorbed to montmorillonite at a concentration of 44.4 mg/g of clay (Fig. 2) Uridine was not previously reported to adsorb to montmorillonite but as the concentration of nucleoside increased, 24.1 mg/g of uridine was adsorbed (Fig. 2) (Lailach et al., 1968).
In infrared (IR) spectroscopy, the functional groups and bonds between atoms that occur within a molecule or compound are evaluated. IR was used to characterize the interactions between the four nucleosides and montmorillonite. Montmorillonite has a characteristic IR peak at 1629 cm\(^{-1}\) (Fig. 3A). When nucleosides physically interact with montmorillonite, the characteristic peaks of the nucleoside of interest can shift while montmorillonite peaks remain fixed. Adenosine has a characteristic peak at 1662 cm\(^{-1}\) that represents \(\text{NH}_2\) deformation (Benetoli, 2008). When adenosine is adsorbed to montmorillonite at pH 3, the IR peak belonging to adenosine at 1662 cm\(^{-1}\) shifts to 1698 cm\(^{-1}\) (Fig. 3A). The \(\text{NH}_2\) deformation peak shifts for all initial concentrations of

Figure 2: Surface loadings of nucleosides to montmorillonite at pH 3. Adenosine and guanosine had the highest surface loading. Cytidine and uridine also adsorbed to the clay in lower quantities at pH 3. All samples were collected after a 48-hour equilibration period and solutions were analyzed using UV-vis spectroscopy in triplicate.
adenosine adsorbed to the clay. Adsorption of adenosine does not shift the clay peak. However, the intensity of the clay peak decreases as the concentration of nucleoside increases.

Similar trends in IR peak shifts are observed when guanosine is adsorbed to montmorillonite. Guanosine has peaks corresponding to NH$_2$ deformation at 1621 cm$^{-1}$ and C=O stretching at 1725 cm$^{-1}$ as well as a clay peak that remains steady at 1629 cm$^{-1}$ (Fig. 3B). When adsorbed, the guanosine NH$_2$ deformation shifts to 1640 cm$^{-1}$ and the C=O stretch shifts to 1696 cm$^{-1}$ (Fig. 3B). When guanosine is adsorbed, the clay peak often appears as a shoulder off the NH$_2$ deformation peak of guanosine.

Figure 3: IR analysis of nucleosides adsorbed to montmorillonite. A) Adenosine B) Guanosine C) Cytidine D) Uridine. Solid nucleoside and montmorillonite were evaluated for each. Representative nucleoside concentrations were analyzed for each. All samples were collected after a 48-hour equilibration period at pH 3 and the solids were dried in an oven at 65 °C for 24 hr.
The IR spectrum of cytidine had similar trends compared to the IR spectra of adenosine. Cytidine alone has characteristic peaks at 1600 cm\(^{-1}\), corresponding to NH\(_2\) deformation and at 1642 cm\(^{-1}\) corresponding to the C=O stretch (Fig. 3C) (Benetoli, 2008). At pH 3, cytidine is adsorbed to the clay, though the clay peak remains around 1629 cm\(^{-1}\), decreasing in absorbance as the concentration of cytidine increases. The peaks representing the NH\(_2\) deformation shifts from 1600 cm\(^{-1}\) to 1681 cm\(^{-1}\), when cytidine is adsorbed to the clay. The peak representing the C=O stretch at 1646 cm\(^{-1}\) also shifts to an increased wavenumber of 1713 cm\(^{-1}\) when cytidine is adsorbed, at all concentrations.

The characteristic peaks of uridine occur at 1663 cm\(^{-1}\) and a small shoulder located at 1699 cm\(^{-1}\) (Fig. 3D). In the region of the IR spectra from 1450-188 cm\(^{-1}\), there are no obvious signatures of uridine present with montmorillonite. The clay peak remains present in all samples at 1629 cm\(^{-1}\) (Fig. 3D).

**XRD**

With the use of XRD, the d-value of montmorillonite was analyzed, which is a measure of the clay layers and the space between them, including any water or organic molecules in the interlayer. Dehydrated montmorillonite has a d-value of 9.8 Å (Fig. 4). When the nucleosides are adsorbed to the clay, the d-values of the samples increase. Adenosine has the largest d-value of 14.7 Å when 129 mg/g of adenosine is adsorbed to montmorillonite at pH 3. Cytidine adsorbed to montmorillonite increased the d-value to 13.9 Å when 44 mg/g of cytidine was adsorbed, reaching a maximum d-value of 15.6 Å (Fig. 3, 4). Guanosine has a d-value of 13.1 Å
when 134 mg/g of nucleoside was adsorbed to montmorillonite (Fig. 4). The XRD of
montmorillonite with uridine shows a d-value of 12.7 Å when 24.0 mg/g was adsorbed (Fig. 4).

![Figure 4: XRD analysis of nucleosides adsorbed to montmorillonite. Adenosine, guanosine, cytidine and uridine adsorbed to montmorillonite were evaluated. Representative initial nucleoside concentrations of 3.5 mM were analyzed for each. All samples were collected after a 48-hour equilibration period at pH 3 and the solids were dried in an oven at 115 °C for 1 hr.](image)

Nucleosides adsorbed to montmorillonite have d-values representative of the orientation of nucleoside predicted in the interlayer (Fig. 5). Adenosine-montmorillonite complexes had d-values ranging from 11.8 Å to 14.7 Å (Fig. 5). Cytidine-montmorillonite complexes created a larger interlayer spacing around 15.4 Å even at low surface loadings (Fig. 5). Similarly, uridine-montmorillonite complexes had interlayer spacings clustered around 11.6 Å for all amounts of nucleoside adsorbed. Guanosine-montmorillonite complexes had a wider range in d-values when compared to the amount of nucleoside adsorbed to montmorillonite. A d-value around 13.0 Å
was achieved at 19.0 mg guanosine per gram of montmorillonite adsorbed and maintained through a surface loading of 134 mg/g.

Discussion:

Surface Loading of Nucleosides

The interactions between nucleosides and clay mineral surfaces were evaluated using surface loading experiments, IR spectroscopy, and XRD analysis. The adsorption of nucleosides to montmorillonite showed high levels of adenosine and guanosine adsorbed at pH 3. At 1.5 mM, the relative amount of adenosine adsorbed to montmorillonite was the greatest, followed by guanosine, cytidine, and uridine. A similar trend in the relative amount of nucleoside adsorbed
to Ca-montmorillonite after 24-hours was previously reported with adenosine adsorbing the largest quantities followed by guanosine and cytidine (Lailach et al., 1968). Qualitative discrepancies are most likely the result of procedural differences, specifically the previous study used a much larger solid to solution ratio of 27 g/L (Lailach et al., 1968).

Nucleosides can be positively charged, negatively charged, or have a neutral charge depending on the pH. At pH 3, adenosine and cytidine are positively charged based on their pK_{a1} values (Table 1). The positively charged molecules are able to interact with the net negative charge of the clay mineral and specifically in the interlayers of the montmorillonite clay. Adenosine generally adsorbed the most to the montmorillonite with additional adsorption at higher initial solution concentrations (Fig. 1). Guanosine has a pK_{a1} value of 1.79 so at pH 3, most of the guanosine in solution has a neutral charge with a small fraction that is positively charged. Guanosine also adsorbed to montmorillonite at pH 3 and adsorption increased as concentration increased (Fig. 1). Uridine has a high pK_{a1} so it is has a neutral charge at pH 3.

Cytidine adsorption increased by 21% compared to lower concentrations when the concentration of cytidine increased to 3.5 mM. Overall quantities of cytidine adsorbed to montmorillonite were significantly lower compared to adenosine and guanosine (Fig. 1). The pK_{a1} value of 4.22 for cytidine indicates that 94% of the nucleoside in solution will maintain a positive charge when the solution is at a pH of 3. For cytidine, the low amount of protonated nucleoside in solution should correlate to a relatively high amount of nucleoside adsorbed to montmorillonite (Fig. 1). However, the relative quantity of cytidine adsorbed to montmorillonite was comparable to the literature values in relation to adenosine and guanosine (Lailach et al., 1968). Although relatively low amounts of cytidine adsorbed, it was likely due to multiple
mechanisms required for the adsorption of the nucleoside to montmorillonite, including van der Waals forces and ionic exchange.

Low quantities of uridine adsorbed to the clay at pH 3 (Fig. 1). Previous research reported that uridine did not adsorb to the clay at any pH value ranging from 1.7 to 11 with concentration of 1.3 mM uridine and a solid to solution ratio of montmorillonite at 27 g/L (Lailach et al., 1968). In this experiment, uridine adsorbed to montmorillonite at initial concentrations of 1.5 mM and 3.5 mM with a solid to solution ratio of 1.6 g/L.

The positive and negative charges and the resulting interactions between them, contribute to the van der Waals forces and ionic bonds of nucleosides to montmorillonite (Benetoli, 2008). The adsorption values of adenosine, guanosine, and cytidine can be correlated to the interactions taking place between the nucleoside and the clay. The adsorption values achieved by the surfacing loading experiments include the adsorption on the surfaces of the clay, but surface loadings do not indicate if nucleosides are adsorbed in the layers between the clay. The movement of the nucleosides into the interlayers is possible in part because of the swelling nature of the clay (Kraehenbuehl et al., 1987). The layers of the clay are able to expand in the presence of water or other organic compounds therefore allowing the compounds to move into the interlayers (Ferris, 2005). IR and XRD were used to characterize the interactions and location of the interactions determined by surface loading experiments.

**Interactions between nucleosides and montmorillonite**

The interactions that take place between organic matter, such as nucleosides, and montmorillonite include hydrogen bonding, van der Waals forces, ion-dipole attraction and ionic bonds (Benetoli, 2008, Ruiz-Hitzky et al., 2005). The clay sheets are held together by van der
Waals forces; therefore, the interaction between organic molecules and clay minerals must be stronger to separate the layers and accommodate any organics inside (Ferris, 2005). The adsorption of water in the interlayers, on the surface, or the broken edges shows a characteristic peak at 1635 cm\(^{-1}\) (Fig. 3). This peak belongs to the H\(_2\)O bending at locations where water is adsorbed to montmorillonite (Che, 2011). When organic matter is adsorbed to hydrated montmorillonite, the characteristic montmorillonite H\(_2\)O peak does not shift.

Within the interlayers of montmorillonite, the interactions that occur with nucleosides can be investigated with IR. The IR spectrum of solid adenosine had a characteristic peak at 1662 cm\(^{-1}\), which is attributed to an NH\(_2\) deformation (Fig. 3A) (Colthup, 1964). The deformation is the result of stress distribution and orientation distribution intramolecularly between the NH\(_2\) and C=O components in adenosine (Wool, 1980). The NH\(_2\) is positively charged at this pH promoting it to interact with the negatively charged montmorillonite. When adenosine is adsorbed to montmorillonite, the physical interaction taking place between adenosine and the clay through the NH\(_2\) deformation results in a weaker C=N bond shifting the C=N peak on the IR spectra to a higher wavenumber around 1631 cm\(^{-1}\) compared to 1600 cm\(^{-1}\) in solid adenosine (Benetoli, 2008). When adenosine is adsorbed to montmorillonite, the NH\(_2\) characteristic peak shifts from 1662 cm\(^{-1}\) to 1700 cm\(^{-1}\) (Fig. 3A) indicating there are physical interactions occurring between the nucleosides and montmorillonite. It has been proposed that cationic binding is occurring in the interlayers and responsible for the shift in the corresponding nucleoside characteristic peaks (Ferris, 1989).

Solid guanosine has a characteristic peak around 1622 cm\(^{-1}\) from NH\(_2\) and 1726 cm\(^{-1}\) from the C=O bond (Fig. 3B) (Mathlouthi et al., 1986). When guanosine is adsorbed to montmorillonite,
the NH$_2$ and C=O peaks shift to 1645 and 1700 cm$^{-1}$, respectively (Fig. 3B). The peak shifts suggest that there is an interaction between guanosine and montmorillonite and the strength of the interaction is larger than the van der Waals forces holding the clay layers together (Ferris, 2005). As with adenosine, it is possible that ionic interactions are occurring between guanosine and montmorillonite at pH 3. At pH 3, adenosine maintains a positive charge while guanosine possesses little positive charge in relation to its pK$_{a1}$.

Cytidine has characteristic IR peaks at 1640 and 1599 cm$^{-1}$ (Fig. 3C). Cytidine contains a C=O stretch at 1640 cm$^{-1}$ and an NH$_2$ deformation at 1599 cm$^{-1}$ (Fig. 3C). When cytidine is adsorbed to montmorillonite, the montmorillonite peak remained at 1635 cm$^{-1}$ (Fig. 3C). After adsorption, there were again shifts in both the NH$_2$ and C=O bonds in cytidine to 1760 and 1685 cm$^{-1}$, respectively (Fig. 3B). The peak at 1685 cm$^{-1}$ may also be attributed to the hydrogen bonding of the cytosine ring to montmorillonite, which is facilitated by water molecules (Pucci, 2010).

Uridine has a characteristic peak around 1660 cm$^{-1}$ (Fig. 3D) and a small shoulder at 1695 cm$^{-1}$ (Fig. 3C). The large peak likely corresponds to a C=O stretch found in uridine. Even though the uridine surface loading experiments had a maximum of 24 mg uridine per gram of clay, the IR region under investigation for uridine-montmorillonite complexes only exhibited the characteristic peak for montmorillonite at 1629 cm$^{-1}$ (Fig. 3D). The interactions are influenced by the pK$_{a1}$ of uridine, which at pH 3 means uridine is neutral. There was less uridine adsorbed to montmorillonite, which decreases the amount of potential interactions between the nucleoside and montmorillonite as well as the intensity of any uridine peaks that might be observed in the IR spectra. Therefore, possible interactions of uridine with montmorillonite are not observed in the IR spectra.
Effect of nucleoside on d-value of montmorillonite

XRD analysis allows for the quantification of the d-values of montmorillonite and organic matter adsorbed in the montmorillonite interlayers. The interlayers of montmorillonite contain water molecules because of the clay’s swelling properties. To evaluate if water or organic matter is in the clay interlayer, the clay must be dried to remove water molecules. When water is removed from the interlayer by heating the samples at 115 °C, approximately ½ H₂O remains in the interlayer per ion present to give a d-value of 9.8 Å (Kraehenbuehl et al., 1987, Bala et al., 2000). The adsorption and interaction of organic matter with montmorillonite has the potential to increase the d-value, depending on its location and orientation. A d-value around 12.5 Å indicates that nucleobases, such as adenine or cytosine, are in the interlayer of montmorillonite (Lailach and Brindley, 1969). Specifically, a spacing of 12.5 Å corresponds to the adsorption of nucleobases parallel to the clay layer (Lailach et al., 1968). Nucleosides have additional steric constraints compared to their respective nucleobases that affect their ability to enter the interlayer. Based on the 3-dimensional structure of the nucleosides, one possible orientation is for just the nucleobase to enter the interlayer while the ribose ring is wedge to the outside edge, unable to enter due to the steric characteristics and give a d-value around 12.5 Å (Lailach et al., 1968). A d-value greater than 12.5 Å is required to fit more of the nucleoside structure into the interlayer. The strained interaction of the ribose ring with the clay layers could be forcing the layers open, thus increasing the d-value of the clay. A d-value of 16.6 Å indicates the entire cytidine nucleoside is in the interlayer of montmorillonite. Lailach et al. found that it is likely for the cyclic ring structure to interact with one face of the silicate interlayer while ribose loosely interacts with the opposite inner surface of montmorillonite (1968). Additionally, increased d-
values also suggest that the nucleoside is in the interlayer because a large amount of nucleoside is required to overcome the van der Waals forces between the layers and separate them for molecules to move in (Lailach et al., 1968). Based on the literature, less that 10% of nucleosides are predicted to flatten out in the interlayer because there is a large amount of strain on the glycosidic bond between the nucleobase and the ribose ring (Lailach et al., 1968).

In these experiments, d-values varied based on the adsorption location of the nucleoside with montmorillonite. When adenosine was adsorbed to montmorillonite, the d-value ranged from 11.8 Å to 14.7 Å from lower to higher initial nucleoside concentration in solution. The d-values around 12.5 Å indicate that the nucleobase is in the interlayer of the clay, but it is possible that the entire nucleoside is in the interlayer at larger d-values. Guanosine adsorbed to montmorillonite had d-values ranging from 11.7 Å to 13.4 Å. The increased d-values above 12.5 Å indicate that some portion of the nucleoside was adsorbed to the clay is in the interlayer, therefore expanding the space between the layers to accommodate the nucleobase, the ribose ring, or parts of both. When cytidine is adsorbed to montmorillonite, the d-values ranged from 13.4 Å to 15.6 Å. The d-values are all above 12.5 Å indicating that at the minimum, the nucleobase is in the interlayer, but it also suggests that the entire cytidine structure may be in the interlayer. The monocyclic structure of cytidine may play a role in its interactions with the interlayer because of its smaller structure. While size may influence the movement of cytidine, the interactions between cytidine and montmorillonite also contribute to the expansion of the interlayer spacing.

The maximum amount of uridine adsorbed to montmorillonite increased the d-value to 12.7 Å. The d-value of 12.7 Å indicates that it is most likely the nucleobase portion of uridine that is in the interlayer. The entire uridine molecule was not able to enter the interlayer because low
quantities were adsorbed and unable to overcome the van der Waals forces to enter the interlayer. Conversely, the minimum d-value of uridine adsorbed to montmorillonite was 11.0 Å. With this d-value, it is greater than the d-value of dehydrated montmorillonite but smaller than 12.5 Å corresponding to montmorillonite containing the nucleobase in the interlayer. It is possible that there may be uridine in some of the interlayers while others remain empty. This also reflects the low quantities of uridine adsorbed during surface loading.

The surface loading differences between the adenosine and guanosine compared to cytidine may be related to the structural differences of the nucleosides. Adenosine and guanosine contain purines (adenine and guanine), meaning their structure consists of a double ring structures, while cytidine and uridine contain pyrimidines (cytosine and uracil) that only consist of a one ring structure. The size difference between the nucleobases when added to the ribose ring to form the nucleoside could potentially affect their abilities to fit into the interlayer. In addition, the nucleoside structure is not planar, but rather includes steric characteristics and the possibility of resonance structures between the nucleobase and ribose ring (Lailach et al., 1968).

There is free rotation around the C-N bond at C1 on ribose. This could potentially allow for the entire nucleoside to move into the interlayer of montmorillonite increasing the interlayer spacing. It is possible that the entire structure of the nucleoside does not fit into the interlayer so only portions are able to fit into the interlayer.
Conclusions and Future Directions:

The interactions of nucleosides with montmorillonite were explored to gain a better understanding of the types of interactions that occur and where the nucleoside is located on the clay. Previous experiments were limited to one nucleoside concentration and one solid to solution ratio (Lailach et al., 1968). This work varied the initial concentration of nucleoside in solution to identify the maximum surface loading of various nucleosides on Ca-montmorillonite. Adenosine and guanosine adsorbed to montmorillonite with maximum surface loadings of 129 mg/g and 134 mg/g, respectively. Cytidine also adsorbed to montmorillonite with a maximum surface loading of 44.4 mg/g. Uridine did not adsorb to montmorillonite until initial concentrations of it in solution were much greater than literature concentrations therefore resulting in a maximum surface loading of 24.1 mg/g. Once adsorbed, it was determined that there were physical interactions between the nucleosides and surfaces of montmorillonite through investigation with IR spectroscopy. Shifts in the NH$_2$ deformation peak of adenosine, guanosine, and cytidine indicate van der Waals forces and possibly ionic bonds are responsible for the interactions between the nucleoside and the clay. Uridine did not show large changes in its characteristic IR peaks indicating limited interactions between the uridine and montmorillonite.

XRD was used to identify the location of the nucleosides adsorbed to montmorillonite. Average d-values suggested that the nucleosides are most likely oriented between the layers of clay. The average d-value when adenosine was adsorbed to montmorillonite was 13.0 Å indicating the nucleoside is the adsorbed in the interlayer of montmorillonite. The average d-value of guanosine, 12.9 Å, and cytidine, 15.0 Å, adsorbed to montmorillonite also indicate some
portion of the nucleoside is located in the interlayer, which could be the nucleobase, ribose ring, or the whole nucleoside, flattened out. Uridine adsorbed to montmorillonite produces an average d-value of 11.6 Å indicating some interlayers were empty or only part of the nucleoside was in the interlayer of montmorillonite because the amount of uridine adsorbed was not able to overcome the van der Waals forces between the interlayers. The d-values obtained from the adsorption of nucleosides to montmorillonite range from 11.0 Å to 15.6 Å. The lower end of the d-value range indicates very little organic matter is in the interlayer while a d-value near than 12.5 Å would suggest that the nucleobase is in the interlayer and above 12.5 Å corresponds to the nucleosides in the clay interlayer in some formation. Overall, when nucleosides are adsorbed to montmorillonite at pH 3, there are interactions occurring on the surfaces and the interlayer of the clay. The interactions between nucleosides and montmorillonite may have been important for concentrating and synthesizing organic molecules into primitive life forms.

In future experiments, the strength of the interactions between the nucleosides that are adsorbed to the clay should be evaluated by changing environmental conditions to be more or less acidic. Desorption experiments allow for the alteration of pH after maximum adsorption of the nucleosides to determine the environmental effects, such as pH, on the changes in the amount of organic material in the interlayer. Additionally, a 3-dimensional visualization of the XRD spectra can be used to model the nucleoside in the clay interlayer. This will allow for a clearer understanding of how exactly the nucleosides are orientated in the clay mineral interlayers.
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