Mechanism of acridine orange removal from water by low-charge swelling clays

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A B S T R A C T
Swelling clays have been extensively studied to remove contaminants from aqueous solution. This research aimed at elucidating the mechanism of adsorption of acridine orange (AO), a cationic dye, on low charge montmorillonite in order to better understand the principles behind AO removal using swelling clays. Proportional desorption of exchangeable cations from the clays accompanying AO adsorption confirmed cation exchange as the most dominant mechanism for AO removal. FTIR analyses further confirmed the presence of cationic dye AOH⁺ on the surface and in the interlayer of the swelling clays. XRD and TG-DTG analyses revealed interlayer adsorption, thus, intercalation of AO molecules with different conformations at low and higher AO adsorption levels. Molecular simulation showed dimer and horizontal bilayer formation of intercalated AO at the low and high adsorption levels. At the intermediate AO adsorption levels, the dimer intercalated and bilayer intercalated swelling clays formed mixtures at the single crystal scale, resulting in a significant broadening of (0 0 1) peak. The results showed that the uptake of AO could far exceed the cation exchange capacity, an advantage of using swelling clays to treat wastewater containing cationic dyes.

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1. Introduction

Extensive use of colorants resulted in their frequent detection in wastewater streams. However, current methods relying on activated sludge systems for the removal of color arising from the presence of the water-soluble reactive dyes are inadequate, both at on-site installations and even after dilution with domestic wastewater at sewage works [1].

Dyes are classified as acid and basic dyes depending whether their water-soluble form is an anion or a cation. Acid dyes show anionic properties when dissolved in water and are thought to fix to fibers by hydrogen bonding, van der Waals forces, and ionic bonding. A typical example of acid dye is azo dyes. On the contrary, the basic dyes show cationic properties when dissolved in water, react on the basic side of the isoelectric points, and are salts, usually chlorides. A typical example of basic dyes is methylene blue (MB) used in biology to stain cells. Acridine orange (AO) is a nucleic acid selective fluorescent cationic dye. Together with MB, it is often used to probe DNA structure in drug-DNA and protein-DNA interactions [2].

AO absorbs on a variety of solvents. Earlier studies of AO adsorption on montmorillonite were mainly focused on metachromasy [3–5].

In addition to studying adsorption of AO or MB onto montmorillonite separately, interactions of a pair of different cationic dyes (e.g. MB and AO) in the presence of dilute aqueous suspensions of SWy-1 and laponite were also investigated using spectrophotometric techniques [6]. Although cation exchange was attributed to the major mechanism of AO adsorption, no cation exchange capacity (CEC) data for the minerals studied were given [7]. In addition, the charge-transfer reaction hypothesis was also attributed to the metachromasy [3,5], which was not widely accepted. It was contradictory to the experimental observations of dye molecular aggregations on the surface, as well as low metachromasy observed for some saponites that are tetrahedrally charged and should have the highest metachromasy based on the charge-transfer reaction hypothesis [7].

For the purpose of dye removal from water, sorbents tested include granular kohlrali peel [8], yeast-based biosorbent [9], magnetic charcoal [10], magnetic nanoparticles [11], carbon nanotubes inserted into cavities of diatomite [12], and superparamagnetic mesoporous silica [13]. In the presence of AO or quinoline, the adsorption of pyrene increased due to the formation of regions of reduced polarity, but significant reduction in AO adsorption was seen when the solution pH was above 7 at which AO was still in its cationic form [14].

The purpose of this study was to understand the mechanism of AO adsorption on swelling clays used for colorant removal using batch studies in conjunction with X-ray diffraction (XRD), Fourier
transform infrared (FTIR), and thermal gravity (TG) analyses. The state of presence of adsorbed AO molecules on the surface and in the interlayer of low-charged montmorillonite was discussed.

2. Materials and methods

The swelling clay used was SWy-2, a low-charge Na-montmorillonite, and SHCa-1, a low-charge hectorite (a Li-bearing triocahedral smectite). Both were obtained from the Clay Mineral Repositories in Purdue University (West Lafayette, IN) and were used without further purification. Their CEC was $5.8 \pm 3$ and $66 \pm 4$ mmol/100 g [15], layer charge was 0.35 and 0.23 eq/mol per half unit cell [16], and external surface area was 23 and 36 $\text{m}^2/\text{g}$, respectively [17].

The AO (CAS # 65-61-2) was used in an HCl form with a molecular weight of 301.8 g/mol purchased from Sigma–Aldrich (St. Louis, MO). It has an isosbestic point of $1-5 \times 10^{-5}\text{M}$ [18]. The $pK_a$ of AO was 10.4 [19] below which the molecule is protonated to form $\text{AOH}^+$ (Scheme 1). At any concentration, there will be up to three types of species present in solution, which may be defined as monomers, dimers, and oligomers that are aggregates of three or more AO units [18].

The input concentrations for AO adsorption varied from 2 to 12 mmol/L with a 2 mmol/L increment. To each 50-mL centrifuge tube 20 mL of AO solution and 0.2 g of swelling clays were combined. The mixtures were shaken at 150 rpm for 24 h and then were centrifuged at 10,000 rpm for 20 min, followed by filtering the supernatant through a 0.45-µm syringe filter.

The equilibrium AO concentrations were measured using a UV–vis spectrophotometer (SmartSpec 3000, Bio-Rad Corp.) at wavelengths of 490 and 470 nm, corresponding to the maximal absorbance of monomer and dimer of AO.

The exchangeable cations desorbed were determined by an ion chromatography (Dionex 100) with an IonPac CS12A column (4 × 250 mm) and a mobile phase of 1.922 mL of 20 mM methane-sulfonic acid in 1 L of water. At a flow rate of 1.0 mL/min, the retention time for $\text{Na}^+$, $K^+$, $\text{Mg}^{2+}$, and $\text{Ca}^{2+}$ was 2.3, 3.1, 4.6, and 5.8 min, respectively.

Powder XRD analyses were performed on a Rigaku D/max-IIia diffractometer with a Ni-filtered $\text{Cu}\kappa_\alpha$ radiation at 30 kV and 20 mA. Orientated samples were scanned from 2° to 10° 20 at 1°/min with a scanning step of 0.01°/step. A 1° divergent slit and scatter slit and 0.3 mm receiving slit were used.

FTIR spectra were acquired on a Jasco FT/IR-4100 Spectrometer equipped with a ZnSe Attenuated Total Reflection (ATR) accessory. The spectra were obtained from 650 to 4000 cm$^{-1}$ by accumulating 256 scans at a resolution 4 cm$^{-1}$.

The TG-DTG analyses were performed on a Pryis Diamond TG/DTA (Perkin Elmer). The heating rate was 10°C/min under air. The initial sample weight was between 6 and 9 mg.

3. Results and discussion

3.1. AO adsorption and cation desorption

A complete removal of AO from water by both clays was observed at an input concentration of 12 mmol/L, resulting in an AO adsorption of 1.2 mmol/g, even though the CEC of SWy-2 and SHCa-1 was 0.85 and 0.66 mmol/g, respectively [15]. AO adsorption on low charge swelling clays was much higher than 200 mg/g or 0.67 mmol/g on magnetic charcoal [10]. Previous results showed that AO adsorption could reach as high as 1.4 mmol/g on SHCa-1 with an equilibrium solution concentration of 0.12 mmol/L [20]. An AO adsorption capacity of 2 mmol/g was observed on a saponite, much larger than the CEC of 0.78 mmol/kg for the clay [4]. Similarly, an AO adsorption capacity as high as 3 mmol/g was found on Na–beidellite although its CEC was only 1.09 mmol/kg [5]. Thus, in general, the results in this study agreed well with the published data. On the contrary, the desorption of AO on SWy-1 was only 0.055 mmol/g [14] much less than its CEC of 0.85 mmol/g [15], in contrast to 1.2 mmol/g in this study. The AO adsorption above the CEC was attributed to hydrophobic interactions [4,5].

The uptake of AO by clays was attributed to cation exchange [3]. The amount of exchangeable cations desorbed was linearly proportional to that of AO adsorbed for the swelling clays (Fig. 1). The desorption of different cations was in very good agreement with other studies focusing on the alkaline and earth-alkaline cations when the adsorption energies for cations were compared [21]. However, the slope was 0.46 and 2.0 for AO adsorption on SWy-2 and SHCa-1, respectively, suggesting significant differences in AO uptake by these clays. For SWy-2, even though the slope was much lower than 1, when the amount of AO adsorbed was at the CEC value of 0.85 mmol/g, the amount of exchangeable cations desorbed was 0.8 meq/g, showing about 1:1 relationship between AO adsorbed and cations desorbed. For SHCa-1, although the major
exchangeable cation was Ca\(^2+\), the amount of Ca\(^2+\) desorbed far exceeded that of AO adsorbed, suggesting other contributions to the Ca\(^2+\) solution concentration. As the mineral had appreciable amount of calcite [22] while the AO used was in HCl form, the excess of Ca\(^2+\) may come from calcite dissolution.

3.2. FTIR analyses

The FTIR spectra of swelling clays adsorbed with different amounts of AO are plotted in Figs. 2. Overall, the vibrations of clay minerals matched well with those listed for the standard clays [23]. Because of the presence of noises in absorption coefficients above 1700 cm\(^{-1}\) due to localization near the band edges, and above 3400 cm\(^{-1}\) due to coulomb repulsion among free charge carriers in the conduction band [24], only the vibrations in the fingerprint region of 1100–1700 cm\(^{-1}\) were plotted. The broader band at 1410 cm\(^{-1}\) of SHCa-1 was attributed to the CO\(_2\) stretching of calcite while the band at 1630 cm\(^{-1}\) of SHCa-1 and SWy-2 was due to OH deformation of water [23]. The band at 1116 cm\(^{-1}\) of SWy-2 was originated from Si–O stretching (longitudinal mode) that occurred only with ATR method. The rest of the bands were from AO.

Most of the band positions shifted a few cm\(^{-1}\) except the bands at 1637 and 1350 cm\(^{-1}\), which remained the same regardless of the amount of AO adsorbed (Fig. 2). A ring vibration at 1600 cm\(^{-1}\) was reliable for the purpose of following the changes in the character of the interactions in which the cationic dye took part. The former was thought at 1613 cm\(^{-1}\) assigned to the mode from coupling of \(v_{1b}\) of pyridine with \(v_{3b}\) of benzene for neutral AO molecule and blue-shifted to 1637 cm\(^{-1}\) after protonation [25]. In this study it was located at 1633–1634 cm\(^{-1}\), confirming that the adsorbed AO was in a cationic form of AOH\(^+\). The band at 1590 cm\(^{-1}\) in the spectrum of AO microcrystals shifted to 1602 cm\(^{-1}\) when adsorbed on clays and shifted to even lower wavenumbers with increasing amount of AO to the clay [3]. However, in this study, the band was located at 1589 cm\(^{-1}\) for crystalline AO and blue-shifted to 1601 cm\(^{-1}\) rather than to a lower wavenumber (Table 1).

The intense band at 1508 cm\(^{-1}\) was attributed to an aliphatic \(\sigma_{CN}\) stretch coupled to aromatic \(\sigma_{CC}\) stretch motion. The \(\sigma_{CN}\) contribution was mainly responsible for the large protonation-induced blue shift of 32 cm\(^{-1}\) from 1474 cm\(^{-1}\) [25]. In this study the band is at 1505 cm\(^{-1}\) again confirming the presence of AOH\(^+\).

The band at 1352 cm\(^{-1}\) was due to the protonated form of AOH\(^+\), while the 1337 cm\(^{-1}\) band was due to the organic base AO, and the main difference between AOH\(^+\) and AO is the positions of the two peaks. When AO was positively charged as in AOH\(^+\), the IR peak shifted from 1337 to 1352 cm\(^{-1}\) [26]. In this study, the peak was at 1351 cm\(^{-1}\) for crystalline AO and 1352 to 1353 cm\(^{-1}\) for AO adsorbed on the clays. A well resolved peak at 1352 cm\(^{-1}\) in this study is another clear indication that the adsorbed AO was in cationic form adsorbed on the mineral surfaces.

The structural O–H groups are oriented perpendicular to the 001 plane for a trioctahedral phyllosilicate, such as SHCa-1, but are oriented nearly in the 001 plane in the case of a dioctahedral 2:1 phyllosilicate, such as SWy-2 [27]. The orientation of the OH groups in the nearly parallel position to 001 plane may prevent extensive hydrogen bonding between OH and N if the AO molecules are taking a horizontal orientation. Since the difference in band shifting between AO adsorption on SWy-2 and SHCa-1 was not significant, it is suggested that hydrogen bonding may not be an important process for AO adsorption in the low charged montmorillonite.

The strong detection of bands related to \(-\overset{\circ}{C}=C-\) and \(-\overset{\circ}{C}-N-\) stretching in the polyheterocycles around 1600 cm\(^{-1}\) and \(-\overset{\circ}{C}-N-\) stretching at around 1330 cm\(^{-1}\) suggested polymeric orientation of the polyheterocyclic dyes with respect to stainless steel surface [28]. In this study, although the band at 1600 cm\(^{-1}\) was present, the absence of the band at 1330 cm\(^{-1}\) after AO adsorption suggests that the adsorbed AO molecule might not take a nearly perpendicular orientation to the mineral surface.

3.3. XRD analyses

The XRD patterns of SWy-2 and SHCa-1 adsorbed with different amounts of AO were plotted in Figs. 3 and 4. At the MB adsorption maxima, the d-spacing expanded to 1.87 and 1.84 nm for SWy-2 and SHCa-1, respectively [29]. Compared to MB adsorption, AO adsorption resulted in a slightly less d-spacing expansion. For SHCa-1, as the amount of AO adsorbed increased, a progressive increase in d-spacing was observed. At the AO adsorption of 1.2 mmol/g, the d\(_{001}\) spacing was 1.7 nm in contrast to 1.35 nm at the AO adsorption of 0.2 mmol/g. For SWy-2, the 0 0 1 peak became asymmetric and could be considered as a composite peak as the amount of AO adsorption increased. The d\(_{001}\) spacing increased to 1.63 nm at AO adsorption of 1.2 mmol/g in agreement with a previous observation for AO adsorption on a Wyoming bentonite [30].

The peaks were fitted to either Gaussian or Pearson VII functions (Figs. 3 and 4). For SWy-2, two major peaks occurred at 1.31 and 1.43 nm at lower AO loading levels and three peaks occurred at 1.32, 1.44, and 1.62 nm at higher AO loading levels (Table 2). For SHCa-1, the two peaks occurred at 1.36–1.43 and 1.68–1.71 nm (Table 3). The monomeric adsorption at a d-spacing of 1.32 nm was attributed to the aromatic ring assumed to be parallel to the alumino-silicate layer [3]. In contrast, the d-spacing of 1.59–1.78 nm was due to bilayer formation in the interlayer [30]. The d-spacing in this study may suggest bilayer and monolayer conformation of AO intercalated in the interlayer of the swelling clays. However,
Table 1
Band positions and suggested assignments for the infrared absorption frequency of AO adsorbed on SWy-2 at the 1100–1700 cm⁻¹ region.

<table>
<thead>
<tr>
<th>Assignment*</th>
<th>Band positions (cm⁻¹)</th>
<th>AO adsorption (mmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AOH⁺</td>
<td>AOH⁻</td>
</tr>
<tr>
<td>Ring stretching</td>
<td>1603</td>
<td>1635</td>
</tr>
<tr>
<td>Ring stretching</td>
<td>1502</td>
<td>1505</td>
</tr>
<tr>
<td>Ring stretching</td>
<td>1409</td>
<td>1404</td>
</tr>
<tr>
<td>Ring stretching</td>
<td>1383</td>
<td>1380</td>
</tr>
<tr>
<td>CH + CH₃ deformation</td>
<td>1359</td>
<td>1352</td>
</tr>
<tr>
<td>Ring deformation, stretching</td>
<td>1295</td>
<td>1284</td>
</tr>
<tr>
<td>Ring deformation, stretching CC,CN</td>
<td>1250</td>
<td>1252</td>
</tr>
<tr>
<td>CH₃ deformation</td>
<td>1231</td>
<td>1219</td>
</tr>
<tr>
<td>CH deformation</td>
<td>1137</td>
<td>1140</td>
</tr>
</tbody>
</table>

* Ref. [39].
* Ref. [2].

Table 2
Fitting of XRD data to Pearson VII function for AO adsorption on SWy-2.

<table>
<thead>
<tr>
<th>AO loading (mmol/g)</th>
<th>Peak one</th>
<th>Peak two</th>
<th>Peak three</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d-spacing (nm)</td>
<td>Peak area (%)</td>
<td>d-spacing (nm)</td>
</tr>
<tr>
<td>0.2</td>
<td>1.44</td>
<td>96</td>
<td>1.30</td>
</tr>
<tr>
<td>0.4</td>
<td>1.43</td>
<td>80</td>
<td>1.32</td>
</tr>
<tr>
<td>0.6</td>
<td>1.62</td>
<td>7</td>
<td>1.44</td>
</tr>
<tr>
<td>0.8</td>
<td>1.63</td>
<td>10</td>
<td>1.44</td>
</tr>
<tr>
<td>1.0</td>
<td>1.61</td>
<td>31</td>
<td>1.44</td>
</tr>
<tr>
<td>1.2</td>
<td>1.63</td>
<td>64</td>
<td>1.37</td>
</tr>
</tbody>
</table>

The separation of peaks based on line fitting rather than physical separation on the XRD patterns indicated that the two conformations were mixed at the molecular levels, suggesting closer to mixed layers rather than a physical mixture. For SHCa-1, as the amount of AO adsorbed increased, the percentage of the peak at 1.68–1.71 nm increased proportionally, reflecting a transition from a monolayer to bilayer AO interlayer conformation (Table 3).

The SWy-2 samples were also tried to fit into two peaks rather than three. Removal of the third peak (at 1.44 nm) would not affect the peak positions at 1.32 and 1.62 nm significantly. But undeniable extra intensities would result in an increase in the fitting error. It seemed impossible to have reasonable fitting with two peaks in the case of 0.6, 0.8, and 1.0 mmol/g AO adsorption levels, especially the 0.6 and 0.8 patterns.

One of the possible explanations for the intermediate peak could be a third material with an intermediate degree of expansion, but that would require a third interlayer configuration. Assuming that the mixed layers do exist, the possible cause for its occurrence could be a mixed layer material (at 1.44 nm) plus the two end-members (at 1.32 and 1.62 nm) (Table 2).

Peak width is a reflection of the number of stacking layers along the c direction and becomes broader as the number of stacking layers decreases. The full width at half maximum (FWHM) can be used to estimate the vertical thickness of the layered silicates using the Scherrer equation [31]:

$$ t = \frac{0.9\lambda}{B \cos \theta_B} $$

where B is the FWHM in radian, θB is the angle of the peak in degree, λ is the wavelength of X-ray radiation (1.54178 Å for CuKα). With the fitted FWHM, the calculated thickness would be 4.7–8.7 nm for both peaks, corresponding to three to five fundamental particle stacking along c direction.

MB had a dimension of 1.7 nm x 0.76 nm x 0.33 nm [32]. AO would have a similar dimension due to their similar structure. Using only the external surface area of 23 and 36 m²/g [17] for AO adsorption, the area occupied per AO molecule at the highest amount of AO adsorbed would be 0.03 and 0.05 nm², far less than the surface area of 1.3 nm² when the AO molecule lies in a flat orientation, an indication of intercalation of AO into the swelling clays.

Table 3
Fitting of XRD data to Gaussian function for AO adsorption on SHCa-1.

<table>
<thead>
<tr>
<th>AO loading (mmol/g)</th>
<th>Peak one</th>
<th>Peak two</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d-spacing (nm)</td>
<td>Peak area (%)</td>
</tr>
<tr>
<td>0.2</td>
<td>1.50</td>
<td>32</td>
</tr>
<tr>
<td>0.4</td>
<td>1.49</td>
<td>100</td>
</tr>
<tr>
<td>0.6</td>
<td>1.68</td>
<td>46</td>
</tr>
<tr>
<td>0.8</td>
<td>1.68</td>
<td>72</td>
</tr>
<tr>
<td>1.0</td>
<td>1.69</td>
<td>90</td>
</tr>
<tr>
<td>1.2</td>
<td>1.71</td>
<td>100</td>
</tr>
</tbody>
</table>
Intercalation of AO into DNA was noticed between the polynucleotides G–C and A–T base pairs with minor external binding [2].

3.4. TG-DTG analyses

The TG-DTG analyses of AO adsorbed swelling clays and crystalline AO are plotted in Fig. 5. A peak at 50–60 °C on the DTG graph indicated the removal of adsorbed water while that at 115 °C was due to removal of interlayer water [33]. The height of the second peak increased as the amount of AO adsorption increased, suggesting the association of interlayer water with AO adsorption. A similar trend of DTG peak increase as the amount of AO adsorption increased was found in a previous study [34]. This trend is completely different from MB adsorption onto the same montmorillonite, in which as the MB adsorption increased the DTG peak for dehydration of interlayer water was missing, indicating the absence of interlayer water associated with MB adsorption [29].

The activation energies \( (E_a) \) of dehydration from the surface and interlayer could be calculated from [35]:

\[
E_a = RT_{\text{max}}^2 \frac{d((\Delta M/dT)_{T=T_{\text{max}}})}{M_t - \Delta M_{T_{\text{max}}}}
\]

where \( R \) is the gas constant, \( T \) is the absolute temperature, \( T_{\text{max}} \) is the temperature at which DTG is at maximum, \( M_t \) is the total mass of water initially contained in the sample, obtained from mass loss curve at the end of the dehydration, \( \Delta M_{T_{\text{max}}} \) is the mass loss at temperature \( T_{\text{max}} \), \( d((\Delta M/dT)_{T=T_{\text{max}}}) \) is the DTG at \( T_{\text{max}} \). The calculated values are listed in Table 4. At very low AO adsorption level (<0.2 mmol/g on SHCa-1, and <0.4 mmol/g on SWy-2), the \( E_a \) values were about 20 kJ/mol. At higher AO
Observed data
Fitting to observed data after background correction
Fitting to 1.36 – 1.43 nm
Fitting to 1.68 – 1.71 nm

Fig. 4. Fitting of observed data to Gaussian function. The number is the amount of AO adsorbed in mmol/g on SHCa-1.

adsorption levels, the $E_a$ values dropped significantly (Table 4). The change in $E_a$ matched with the XRD results (Figs. 3 and 4) well, suggesting a change in AO interlayer configuration above these levels.

In addition to these peaks, a new DTG peak occurred at 230–250 °C when the AO adsorption was greater than 0.4 mmol/g. This peak was absent for raw clays and crystalline AO. Thus, the appearance of this peak may indicate a breakage of bond that was formed after AO adsorption. The mass loss at this temperature range was attributed to combination of the hydrogen atoms from the AO molecules with the oxygen atoms of the air [34]. As the amount of AO adsorption increased, the mass loss at 600 °C increased proportionally. The progressive increase in mass loss confirmed the presence of AO on the surface or in the interlayer of the clays.

3.5. Configuration of AO molecules in the interlayer

The state of presence of adsorption organic dyes such as MB and AO as well as their conformation could be investigated by determining the spectral shift in the visible light range. Experiments with AO in the presence of clays showed absorptions at 500, 495, and 490 nm, which were assigned to dye monomers on the clay particles, internal monomers, and internal diprotonated monomers, respectively [6]. On the contrary, studies on AO adsorption on Na–saponite showed that monomeric AO adsorbed in the interlayer while AO dimers and polymers adsorbed at the solid–liquid interface [4]. In contrast to MB aggregation, AO also forms aggregates on the clay surfaces, although the changes in the spectra with time were not as significant as those observed in the MB/SWy-1 systems [6].
For molecular orientation of MB adsorbed on swelling clays, the plane of the dye heteroaromatic system was not arranged in a parallel fashion as assumed in earlier studies [34], but rather in a tilted or perpendicular fashion [36]. As the protonation at the nitrogen atom of the central ring was predicted to be the most stable protonation site based on infrared multiple photon dissociation spectroscopy observation and low level quantum chemical calculations [25], the longest axis of an AO cation may also be parallel to the clay surface plane. However, the orientation of the aromatic rings of the AO molecules in the interlayer of swelling clays was assumed to be parallel to the aluminosilicate layer with a monolayer or bilayer formation resulting in a d-spacing of 1.32 and 1.59–1.78 nm [3,30].

Using the total SSA of 725 m²/g determined by polyvinylpyrrolidone sorption [37] and the AO adsorption of 1.2 mmol/g, the calculated surface area occupied per single AO molecule would be 1 nm² on SWy-2 and SHCa-1, respectively. If the dimension of MB was used for AO, the contact area would be 0.55 nm² if it was made of 1.7 nm × 0.33 nm between AO and the mineral surfaces, or 1.3 nm² if it was made of 1.7 nm × 0.76 nm between AO and the mineral surfaces. Apparently, at the AO adsorption of 1.2 mmol/g, the flat-lying bilayer orientation would use about 65% of the total available surface area. On the other hand, a vertical dimer, i.e. a side-way overlap with the N in opposite direction would result in a total surface area of 1.1 nm². At higher AO adsorption, the AO molecules interact with each other on the silica surfaces and could be attributed to bilayer formation [38]. As the measured increase in d-spacing would not support a vertical dimer, bilayers of AO with a flat-lying orientation would be more likely the surface configuration of AO at a higher loading level.

Molecular simulation was performed by using Materials Studio 5.0 software under the module Forcite to investigate the AO conformation in the interlayer of SWy-2. The supercell of the model was made of 8 unit cells at 4a × 2b × 1c. The established model was optimized geometrically. The temperature was set at 298K and simulation time was 1 ns with a time step of 1 fs. Universal force field was used during simulation. For the component of d-spacing = 1.35 nm, the interlayer was assumed to be made of two AO cations plus three Na⁺, due to a lower rate of cation exchange. The interlayer was assumed to be made of five AO cations plus two neutral AO molecules based on the CEC values at the high cation exchange level. The results of molecular simulation showed

### Table 4

<table>
<thead>
<tr>
<th>Sample</th>
<th>T max₁ (°C)</th>
<th>E₀₁ (kJ/mol)</th>
<th>T max₂ (°C)</th>
<th>E₀₂ (kJ/mol)</th>
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**Fig. 5.** TG and DTG analyses of SHCa-1 (a, b) and SWy-2 (c, d), adsorbed with different amounts of AO. The numbers are amount of AO adsorbed in mmol/g. Crystalline AO is with the right Y-axis in (a) and (c).
a monolayer AO formation made of AO dimers at the low AO adsorption level and a horizontal bilayer formation at the high AO adsorption level (Fig. 6), confirmed the observation of different d-spacings at different AO adsorption levels.

3.6. Mechanisms of AO adsorption on low charge swelling clays

Adsorption of AO on SHCa-1 was attributed to surface protonation of AO [14,20]. Monitoring of solution pH change, thus, protonation of MB after dissolution in water and after 24 h mixing with water showed that protonation occurred right after MB dissolution and before addition of clays [29]. Thus, protonation of AO should be considered as a reaction with water only, not with clays. However, as the pKₐ of AO was 10.4, below which the protonated AOH⁺ form dimers [19], the AO should have been protonated as a cation before being adsorbed, similar to the observation of MB adsorption on these clays.

The cation exchange reaction alone was not sufficient enough to account for AO surface concentrations in excess of the CEC of the clays. Thus, “cooperative” sorption with preexisting AOH⁺ was attributed to additional AO sorption beyond the CEC of the mineral, resulting in a clustering of sorbates [20]. Dimer sorption could also be responsible for additional AO adsorption as observed for MB adsorption on the same clays [29]. The slope of exchangeable cations desorbed against AO adsorbed was 0.5 for AO sorption on SWy-2 (Fig. 2). Thus, dimer adsorption may contribute significantly to the total AO adsorption on SWy-2. However, when the amount of AO adsorbed was at the CEC value of 0.85 mmol/g, the amount of exchangeable cations desorbed was 0.8 meq/g, showing about 1:1 relationship between AO adsorbed and cations desorbed. Thus, this research confirmed previous findings that AO adsorption on montmorillonite and laponite was dominant by monolayer at low loading levels, while bilayers were formed as the loading level increased [4,5,30,34].

As for the much larger slope for AO adsorption on SHCa-1, the major cation in solution was Ca²⁺ while the exchangeable cation was Na⁺. The elevated Ca²⁺ concentration would be attributed to dissolution of calcite after addition of AO solution under acidic condition as the mineral had appreciable amount of calcite [39].

4. Conclusions

Through this study the following conclusions can be reached:

1. The amount of AO adsorbed far exceeded the CEC of the low charge swelling clays. A distinct advantage of using swelling clays to removal cationic dyes from water.
2. The uptake of AO was attributed to cation exchange when the amount of AO adsorbed was less than the CEC. The intercalated AO molecules may adopt a dimer and bilayer conformation confirmed by molecular simulation.
3. Peak fitting of the XRD patterns revealed that these intercalated clay minerals with dimer and horizontal bilayer formation were formed at the singular unit cell scales rather than physical mixture, resulting in a composite (0 0 1) peak.

References