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ABSTRACT

The polycondensation of amino acids to oligopeptides is an important step in the origins of life, and known to be effective on several mineral surfaces. The data available on clay mineral surfaces are heterogeneous and sometimes contradictory, however. The objective of the present work is to investigate the adsorption of a selected amino acid, alanine, in expanding and non-expanding clays and then study the possible peptide condensation by thermal activation. A multi-technique approach was used, including macroscopic measurements of the adsorption process (adsorption isotherms and pH-metry), and in situ molecular-level characterization of the solids obtained (X-ray diffraction, thermogravimetric analysis and infrared spectroscopy). The results indicate that only weak interaction is established between alanine molecule and kaolinite surfaces, most of the deposited molecules being only physically retained. Thermal activation of alanine/kaolinite only led to desorption. In contrast, higher-energy adsorption mechanisms were at play in hectorite, including cation exchange and coordinative adsorption to the interlayer ions, and alanine species adsorbed in this way were observed to form cyclic dimers upon activation between 160 and 270 °C.

Keywords: Kaolinitte, hectorite, alanine, Prebiotic Chemistry, peptide bond.

1 INTRODUCTION

How did life emerge? This question keeps intriguing researchers in different fields as well as non-scientists. One of its aspects is prebiotic chemistry, the study of the primordial formation of biomolecules before the appearance of micro-organisms, and research scientists start to realize that prebiotic pathways may have originated on the surface of ancient minerals (Fontecilla Camps, 2019). Bernal (Bernal, 1949) was the first researcher to associate clay minerals with the origin of life. According to his hypothesis, clays could have played an important role in amino acid concentration, which was necessary to induce their polymerization to peptides, as they are good adsorbents (Ramos and Huertas, 2013; Villafane-Barajas et al., 2018; Zaia, 2012).

Now, clays have been invoked in several other roles connected with Origins of Life studies, including confinement - allowing to concentrate, confine and protect molecules, creating a "safe" environment for proto-biochemical reactions (Yang et al., 2013). Here, however, we will concentrate on their potential effect on amino acids adsorption and polymerization. Bernal's hypothesis has gained strength over the years. First, clay minerals are widely distributed, have been historically prevalent along the whole timeline of geological and biological events on Earth, and have affinity for organic molecules (Aguilar-Ovando and Negrón-Mendoza, 2015; Carneiro et al., 2011; Georgelin et al., 2015; Kalra et al., 2000; Pedreira-Segade et al., 2016; Ponnamperuma et al., 1982). Second, amino acids polymerization has been observed experimentally in the presence of clay minerals starting in the 1970's, (Bujdák et al., 1994; Jaber et al., 2014; Lahav et al., 1978; Paecht-Horowitz et al., 1970). A summary of work related to amino acids surface polymerization on various supports may be found in (Lambert, 2008). The implications of the formation of oligopeptides to "kick-start" the evolution of Life are discussed e.g. in Brack, 2008.

However, these studies mostly have an exploratory character. Little justification is offered for the choice of the mineral phases to assess peptide formation reactivity; the experimental procedures applied may be quite different, and the experimental results have little in common beyond the bare occurrence of peptide formation (see Tables 1 and 2). Clays are not systematically compared with other minerals, much less between themselves. Two important exceptions may be found in the work of Bujdák and Rode ((Bujdák and Rode, 1999b) and (Bujdák and Rode, 1999a), respectively). Recent work on amino acids polymerization more often uses silica or titania as a support, in part for reasons regarding the ease of characterization (Guo and Holland, 2015; Martra et al., 2014; Sakhno et al., 2019) or, if clays are used, montmorillonite is often chosen as a generic smectite (Kollár et al., 2003; Bu et al., 2019). In the present paper, we report on the comparative polymerization ability of two different clay minerals, kaolinite and hectorite, that differ in layer structure as well as in layer charge.

Kaolinite [$Al_2Si_2O_5(OH)_4$] belongs to the kaolin subgroup of minerals, and is one of the most common of the aluminosilicate clay minerals. Kaolinite has a 1:1 layer structure (T-O), exhibits two distinctly different types of surface, base planes (001) and edge planes (110) and (010). The basal planes are anisotropic: one face exposes tetrahedral siloxane surface (–Si–O–Si–), and the opposite one consists of an octahedral gibbsite-like ($Al(OH)_3$) sheet. The basal planes are electrically neutral, but the reactivity of kaolinite surfaces is often driven by hydroxyl groups located at the edge planes, whose net charge is pH depend (Spence and Kelleher, 2012). Studies reporting adsorption of organic molecules in kaolinites have appeared extensively in the literature (Duarte-Silva et al., 2014).

Smectites make up another important group of clay minerals that likely occurred widely in the environments from weathering of volcanic rocks on the early Earth. They are characterized by a 2:1 layer structure (T-O-T), which consists of an octahedral sheet sandwiched between two tetrahedral sheets (BU et al., 2019b). Due to the negative layer charge originating from substitutions, they generally exhibit cation exchange capacities in the range of 50 to 100 meq.g^{-1}. Hectorite is a well-known representative mineral of the smectite family. The ideal chemical formula of hectorite is $Na_{0.3}Mg_{2.7}Li_{0.3}Si_4O_{10}(OH)_2$, in which the layer structure is composed of an Mg and Li-containing octahedral sheet sandwiched between two tetrahedral Si-containing sheets. It is a trioctahedral smectite, and the origin of the layer charge is in the octahedral sheet, so that the negative layer charge is more spread out (less localized) than in other smectites such as saponites where the electric charge originates from tetrahedral sheet.

The objective of the present work is to characterize the interaction between a simple amino acid, alanine (Ala) and these two clay minerals, by means of the different characterizations (X-Ray Diffraction, Thermogravimetric Analysis and Infrared spectrometry), and especially to check whether or not these clays give rise to peptide formation. As can be seen in table 1, studies of amino acid (AA) polymerization on kaolinite supports are rather heterogeneous and the systems were activated in very different conditions, with correspondingly different results. Studies on hectorite have been carried out in more homogeneous conditions, mostly involving wetting-and-drying cycles, with a body of data obtained by Bujdák, Rode and coworkers. It is generally found to induce significant polymerization of at least small amino acids (such as Ala and Gly), although some contradictions are found, e.g. as regards its relative efficiency as compared to montmorillonite. The activation conditions applied, however, involve quite low drying temperatures that only lead to low or moderate peptide yields.

In the present study, we chose a simple activation by heating in dry air at the target temperature because wetting-and-drying procedures involve many steps of hydrolysis, condensation and bond rearrangement and the underlying chemistry is difficult to understand.

Table 1: Studies that reported amino acids polymerization on kaolin or kaolinite clays. AA = amino acid, Gly = glycine, Ala = Alanine, Val = valine, Asp = aspartic acid, Ser = serine, Phe = phenylalanine; Gly_2 = linear Glycylglycine, DKP = diketopiperazine (cyclic dimer). TF = temperature fluctuation, WD = wetting and drying.

Ref	Amino acid(s) adsorbed	Activation procedure	Activation temperatures	Results
(Jackson, 1971)	Asp, Ser, Phe	Heating in sealed tubes in presence of solution	80 °C, up to 10 days	Unspecified peptides, up to 25%. Enantioselectivity claimed
(Lahav et al., 1978)	Gly	TF cycles, compared with WDTF cycles	dehydration 60 °C, then heating at 94 °C	Up to Gly_5. WDTF much more efficient than TF
(White et al., 1984)	Gly	Probably heating in vacuo	70 to 145 °C	Low amounts of unspecified polymers
(Ito et al., 1990)	Gly, Ala, Val, Asp together – introduced as the amides!	7 WD cycles with constant adding of AA	Drying at 100 °C, open beaker	Very long linear polymers
(Zamaraev et al., 1997)	Gly	Hydrothermal reactions (no drying)	100 to 150 °C, autogenic pressure	Slow formation of Gly_2.
(Bujdák and Rode, 1999a)	Gly, Gly_2, Gly+Ala, Gly_2+Ala	5 WDTF cycles	Drying at 80 °C	Small amounts (≤2%) of Gly and DKP. Ala less reactive than Gly
(Meng et al., 2007)	Gly	Direct heating in air gas flow	In steps, followed by in situ IR, from 110 to 510 °C	Amide bands, assigned to DKP, present from 210 to 310 °C
(Dalai et al., 2017)	Gly	Dry heating under N_2	200 or 250 °C	Mostly desorbs, 5% transformed to Gly_2 and DKP

Table 2: Studies that reported amino acids polymerization on hectorite clays. Mt, montmorillonite; Hec, hectorite; Kao, kaolinite. Val = valine, Pro = proline, Lys = lysine, Tyr = tyrosine. SIPF = salt-induced peptide formation.

Ref	Amino acid(s) adsorbed	Activation procedure	Activation temperatures	Results
(Paecht-Horowitz, 1978)	Ala, Lys – introduced as the adenylates!	?	?	Gives longer polymers than Mt
(Bujdák and Rode, 1997; Jackson, 1971)	Ala, Gly+Ala, Gly_2+Ala, DKP	1 to 14 WDTF cycles, with variable amounts of water.	Drying at 80 °C	Better yields than Mt - More efficient for chain elongation than for initial peptide formation.
(Le Son et al., 1998)	Gly, Ala	WDTF cycles, with constant adding of AA; also carried out in the presence of $CuCl_2$ and NaCl (SIPF)	Drying at 80 °C	Hec less efficient than Mt.
(Porter et al., 1998)	Gly	10 WDTF cycles	Drying at 90 °C	Large yields of DKP and Gly_2, smaller yields of peptides up to Gly_6
(Bujdák and Rode, 1999a) (cf. Table 1, line 6)	Gly, Gly_2, Gly+Ala, Gly_2+Ala	5 WDTF cycles	Drying at 80 °C	Hec gives polymer yields up to 10 times higher than Kao
(Bujdák and Rode, 1999b)	Gly, Ala, Pro, Val, Leu, and mixtures	7 WDTF cycles	Drying at 85 °C	Gly gives linear and cyclic dimers, Ala less reactive; other AAs unreactive.
(Porter et al., 2001)	Gly, Tyr	10 WDTF cycles	Drying at 85 °C	Cu^{2+}-hectorite was tested – somewhat less efficient than Mt

2 EXPERIMENTAL PART

2.1. Chemicals

Kaolinite purchased from sigma Aldrich (CAS Number: 1318-74-7) with the following theoretical formula per half unit cell $Al_2(Si_2O_5)(OH)_4$), deionized water, sodium acetate (sigma aldrich, 99% wt), magnesium acetate tetrahydrate (sigma Aldrich, 99% wt), lithium acetate (sigma aldrich, 99.5% wt), sodium silicate solution (sigma aldrich, CAS Number: 127-09-3), L-alanine (sigma Aldrich, 98% wt), hydrochloric acid (sigma aldrich, 37% wt), sodium hydroxide (sigma aldrich, 99% wt), acetic acid (sigma Aldrich, 99% wt) ammonia (sigma Aldrich, 28% wt). All chemicals applied in this work were purchased from Aldrich or Sigma-Aldrich with an analytical grade and used without any previous purification.

2.2 Synthesis of the hectorite (HecS)

For the synthesis of sodium hectorite, a first solution of sodium acetate (0.4 g) in water 15g) and sodium silicate (14.8 g) was prepared. A second solution of deionized water (35g), lithium acetate (0.2g), magnesium acetate (9.1g), and acetic acid was added to the first one under stirring. The composition corresponded to the following theoretical formula: $Na_{0.3}[(Si_4)(Mg_{2,7}Li_{0.3})]O_{10}(OH)_2$ (formula of Hectorite per half unit cell for a substitution ratio of Magnesium by lithium cations of 0.3) The hydrogels were aged under stirring at room temperature for 6 h and then were autoclaved for reaction at 300 °C and 90 bar for 6 h. The autoclave was cooled to room temperature and the solid product was washed thoroughly with distilled water and separated by centrifugation. The solids were then dried at 50 °C for 24 h.

2.3 Samples prepared by experimental isotherms

Alanine solutions were used in the 0.05-1.25 mol.L^{-1} concentration range (Table 3). Thus, 150.0 mg of the adsorbent material were contacted with the amino acid solution (5.0 ml for Kao and 15.0 ml for HecS) and subjected to mechanical stirring for 2 h. They were then centrifuged and analyzed.

Table 3: Specific alanine concentrations used for deposition on kaolinite and hectorite.

Concentration	**Sample #**
0.05 mol.L^{-1}	1
0.10 mol.L^{-1}	2
0.15 mol.L^{-1}	3
0.20 mol.L^{-1}	4
0.35 mol.L^{-1}	5
0.50 mol.L^{-1}	6
0.65 mol.L^{-1}	7
0.85 mol.L^{-1}	8
1.0 mol.L^{-1}	9
1.25 mol.L^{-1}	10

The experimental adsorption isotherm was made in duplicate.

2.5 Heat treatment of the samples

Samples obtained by adsorption were subjected to heat treatment (20.0 mg) in a temperature-programmed oven in a U-shaped cell, with a heating ramp of 5 °C.min^{-1} and different final temperatures: 280 °C for Kao and Ala/Kao 5 (0.35 mol.L^{-1}) samples, 160 °C and 270 °C for Ala/HecS 1 (0.05 mol.L^{-1}), Ala/HecS 2 (0.1 mol.L^{-1}) and Ala/Kao 5 (0.35 mol.L^{-1})), under a dry oxygen flow with a flow rate of 100 mL.min^{-1}.

2.6 Characterization

2.6.1 Fourier transform infrared (FT-IR)

FT-IR spectra were recorded in the transmission mode in KBr pellets (7.5 mg of the substances in 142.5 mg KBr), using a Magna-IR 500 series II spectrometer, with 128 scans, between 4000 and 400 cm^{-1}. Data were analyzed with the software OMNIC S.P. 52.

2.6.2 X-ray diffraction (XRD)

Powder X-ray diffractograms were recorded using a D8 Advance Bruker-AXS Powder X-ray diffractometer and working with the Cu Kα radiation (λ = 1.5404 Å). The diffractograms were recorded between 5-70° (2θ), with a scan rate of 0.5 °.min^{-1}.

2.6.3 Thermal analysis (TG/DTG)

Thermogravimetric analyses were carried out using a TA Instrument SDT Q600 analyzer. The heating rate was 5 °C.min^{-1} from 25 °C to 1000 °C, under a dry air flow of 10 mL.min^{-1}, and using alumina pans.

3. RESULTS

3.1 Alanine on kaolinite

The adsorption process was followed by measuring the pH of the alanine solutions before contact, immediately after contact, and after 2 hours equilibrium prior to separation of the solids. As shown in Figure 1, the pH does not evolve much, except in the most dilute solutions where a small acidic drift in observed. At higher concentrations, it has the constant value of 6.2, dictated by the alanine buffer.

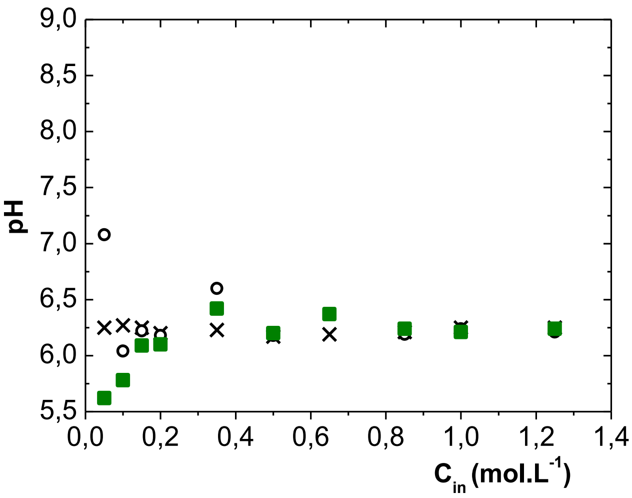

Figure 1: pH evolution upon contact of Ala solutions with kaolinite, as a function of the initial [Ala] concentration. Crosses, before contact; empty circles, immediately after contact; full squares, after equilibrium.

In TG, raw kaolinite (Figure 2) does not lose any significant weight prior to 300 °C. In particular, it does not hold physisorbed water. At higher temperatures, it presents a single, slightly endothermic weight loss, peaking at 497 °C in our conditions, and corresponding to the transformation to metakaolin (Lambert et al., 1989), with a 14.5 to 14.9% weight loss. Finally, at 985 °C, the transformation to mullite is betrayed by a sharp exothermic DTA signal without weight loss.

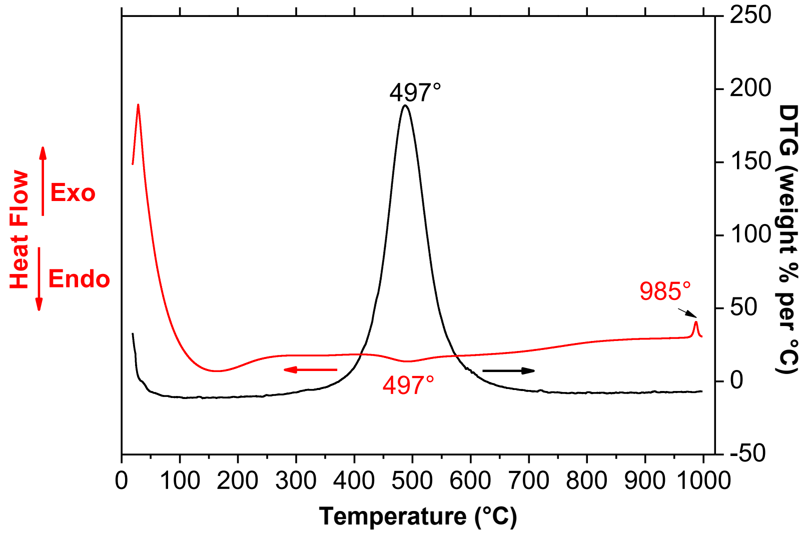

Figure 2: Thermal analysis of raw kaolinite.

The kaolinite to metakaolinite transition event is unchanged in Ala/Kao samples. The latter, however, also present an additional, endothermic weight loss in the 175-265 °C region, whose intensity increases monotonously with the amount of alanine that was contacted to the kaolinite (Figure 3). The lack of any IR bands except those of the kaolinite support (vide infra) after this thermal event indicates that all the organic matter is lost before 265 °C, and thus the weight loss of the endothermic event can be used to determine the amount of alanine (or alanine-derived compounds) contained in the initial dry solid.

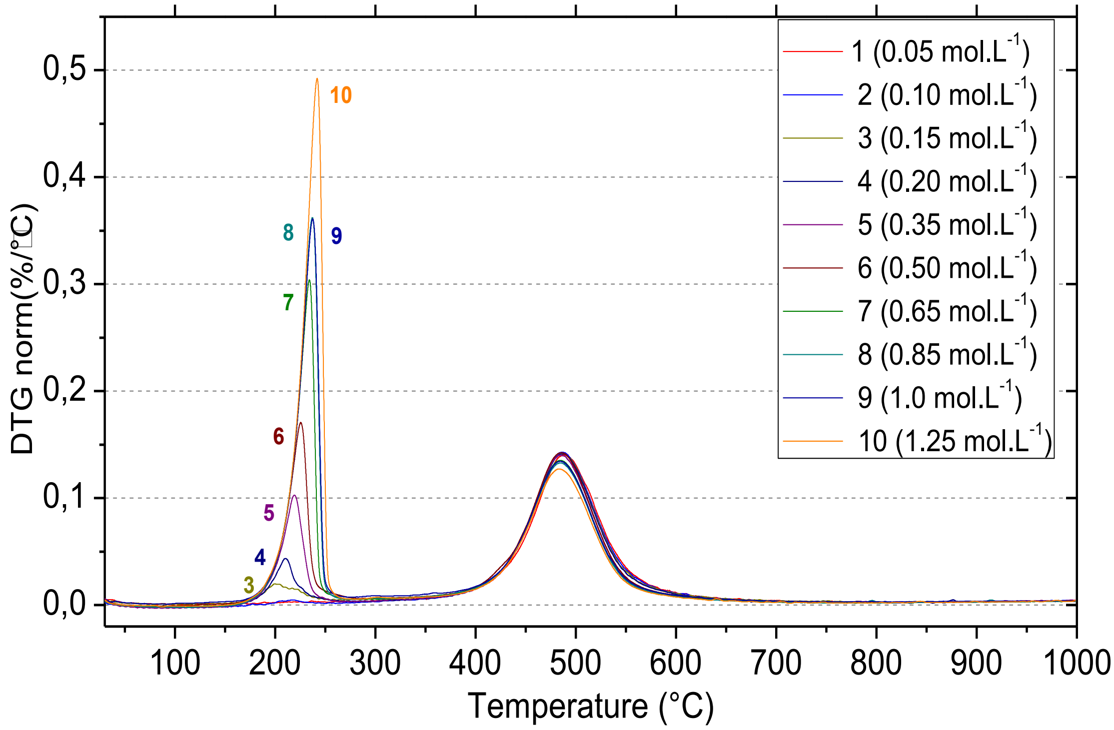

Figure 3: DTG traces of raw kaolinite, and Ala/Kao samples containing increasing amounts of alanine (1 to 10).

The amount of alanine retained is plotted as a function of the solution concentration in Figure 4.

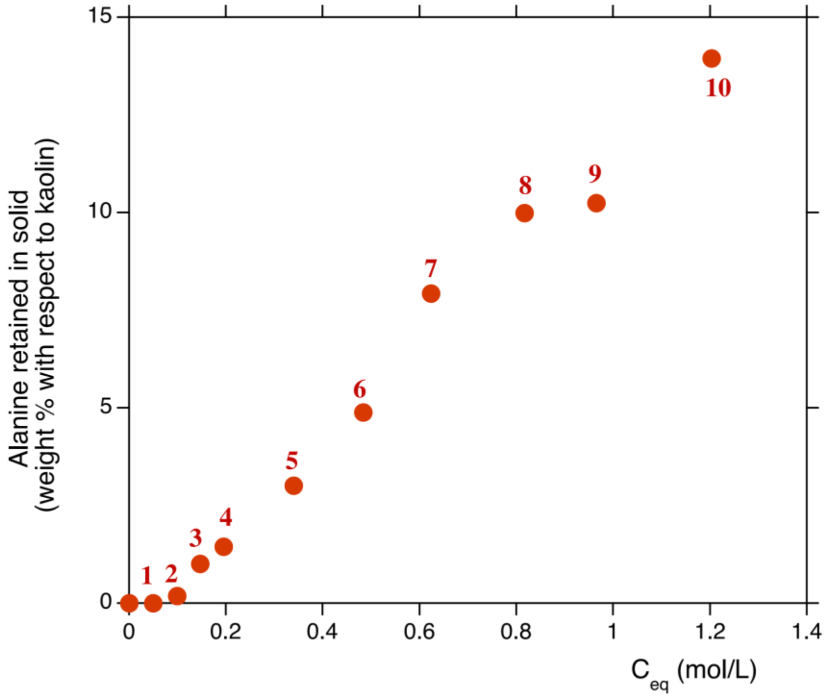

Figure 4: Amounts of alanine (from TG quantification) retained in solid Ala/Kao samples as a function of the final concentration in solution (increasing concentrations from 1 to 10)

In principle, it could be interpreted as an adsorption isotherm. In this case however, and since no washing step was applied, it is likely that a small amount of the alanine-containing solution was physically retained by the solid prior to drying – the amount retained is indeed almost linearly proportional to the initial solution concentration, corresponding to about 4% of the total amount of alanine in solution; in other words, kaolinite would physically retain about 1.3 mL solution per gram. The only other published "adsorption isotherm" for an amino acid (Gly) on kaolinite has the same general aspect (Meng et al., 2007).

The elimination of all the organic matter in a single, endothermic event suggests a simple desorption is happening. Bulk alanine sublimates under the same heating conditions, with a maximum weight loss rate at 289 °C (T_{max}). A close-up of the alanine elimination event in our samples indicates that the T_{max} increases with the amount of alanine in our samples (Figures 5 and 6).

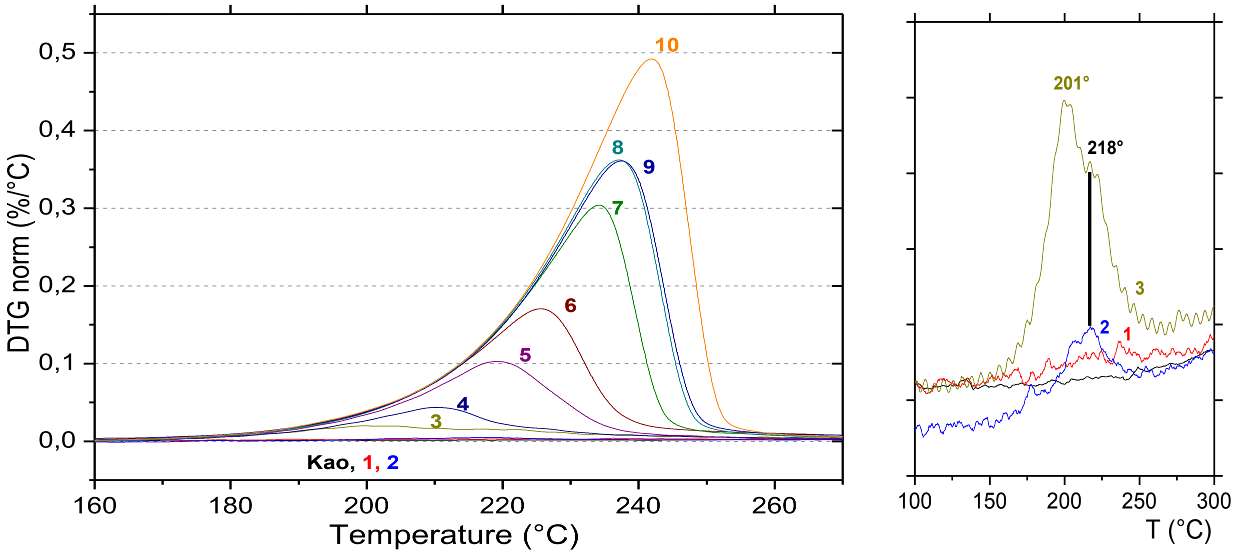

Figure 5: same as Figure 3, close-up of the alanine elimination region

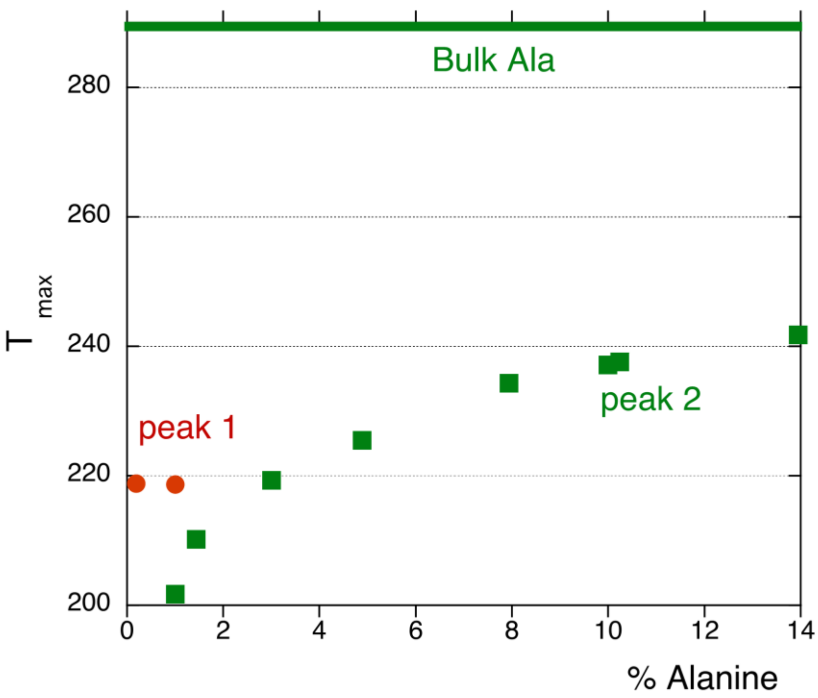

Figure 6: T_{max} of the alanine elimination events, plotted as a function of the amount of alanine retained by the Ala/Kao samples. "Peak 1" refers to the minority event shown in the right-hand part of Fig.5, "peak 2"to the main (sublimation) event.

This, and the general shape of the DTG events with their slow onsets and sharp endings, suggests a zero-order desorption mechanism, probably from small precipitated alanine particles. However, for the samples with the lowest Ala loadings (sample 2, and shoulder in sample 3, see inset of Figure 5), an additional small peak is apparent at a T_{max} of 218 °C. If present in higher-loading samples, it would be obscured by the much more intense sublimation event. We think that it could correspond to the desorption of a minor amount of alanine molecules (at most 0.3% by weight) interacting with specific kaolinite surface sites (such as silanols or aluminols on the edges).

XRD confirms the fact that most of the alanine in the solid samples is not interacting with kaolinite. When the alanine loading is increased, the peaks characteristic of bulk alanine start to appear in superposition to those of kaolinite that remain present. Figure 7 shows this trend for the two most intense peaks of L-Ala (indexed according to (Shkir et al., 2017).

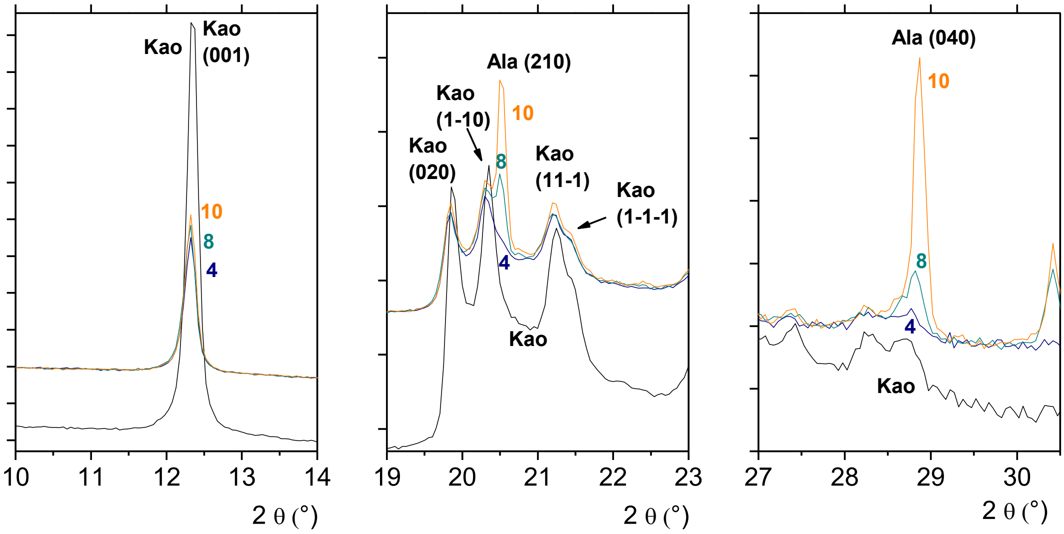

Figure 7: XRD powder diffractograms of raw kaolinite, and of three Ala/Kao samples with increasing Ala contents.

Peaks indicative of bulk alanine crystallites are discernible when the sample contains at least 2% Ala. At lower loadings, alanine may be adsorbed on the kaolinite surface, and/or form crystallites too small and/or in too small quantities to be evidenced by XRD.

Another, negative result must be underlined: the d_{001} of kaolinite is not shifted from its position in raw kaolinite (7.2 Å), ruling out the possibility of significant alanine intercalation between the kaolinite layers.

The FTIR spectra of the samples are shown in Figure 8. Kaolinite exhibits well-known signatures in the 550-1200 cm^{-1} (lattice vibrations) and the 3600-3750 cm^{-1} (OH stretching) regions (Castellano et al., 2010). Figure 8A compares the spectrum of an Ala/Kao sample with that of raw kaolinite. The former presents a set of sharp, but low-intensity additional bands in the "fingerprint" region that can all be assigned to the vibrations of crystalline α-L-Alanine (Rosado et al., 1997) (see inset in right part of the figure). Weak C-H stretching bands are also apparent in the 2850-3000 cm^{-1} region (not shown). Band positions are listed in Table 4 and compared with those for bulk alanine. Almost all the expected absorptions are present, with little or no shift from the bulk positions. This confirms that alanine molecules are intact after deposition, and only weakly interacting with the surface (no covalent bonds).

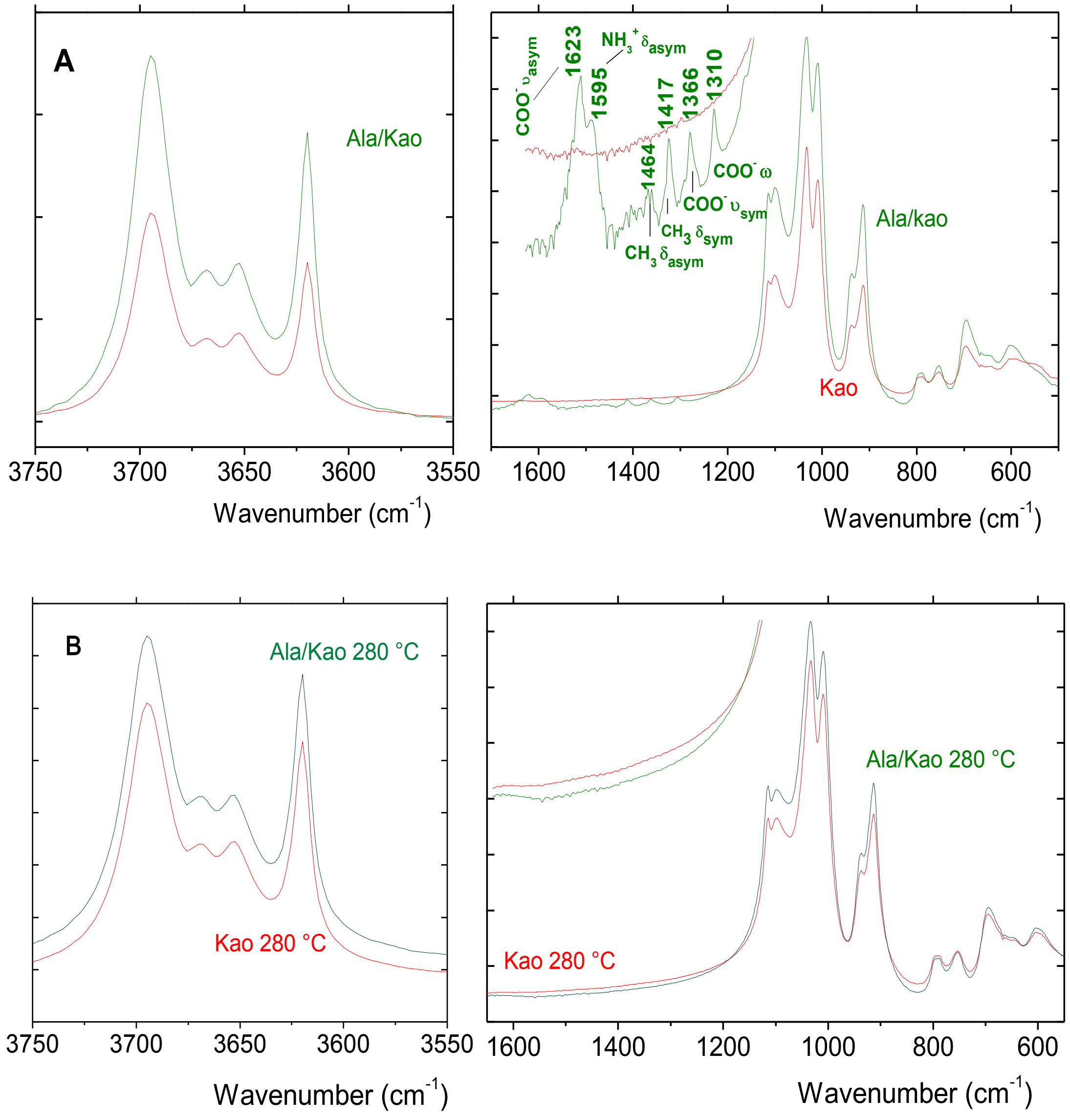

Figure 8: middle IR spectrum of Ala/Kao sample compared with that of raw Kaolinite. (A) before and (B) after heat treatment at 280 ° C.

Table 4: Band positions (cm^{-1}) for bulk alanine and Ala/Kao; n.o. = not observed.

Assignment	Position in Ala	Ala/Kao
CH δ	1235	n.o.
NH_3^+ δ_{sym}	1307	1310
COO^- ν_{sym}	1366	1366
CH_3 δ_{sym}	1408	1417
CH_3 δ_{asym}	1458	1464
NH_3^+ δ_{asym}	1523	n.o.
NH_3^+ δ_{asym}	1592	1595
COO^- ν_{asym}	1623	1623

Figure 8B shows the raw kaolinite and Ala/Kao after heat treatment. The characteristic bands assigned to the vibrations of crystalline α-L-Alanine disappeared after heating up to 280 °C (Completion of thermal event 1). In fact, no bands attributable to adsorbed organic molecules are observable (see magnified fingerprint region in right part of Figure 8 B) and the spectrum is superposable to that of raw kaolinite, confirming the conclusion from TG that all organic matter is desorbed from the solid in the low-temperature event.

3.2 Alanine on hectorite

Upon contact with hectorite, alanine solutions are significantly modified. The pH immediately increases upon contact and a further slow increase is observed upon equilibrium (Figure 9), to reach values between 7.3 and 8.3 from the initial 6.2. The strongest increases are observed for the lowest alanine concentrations.

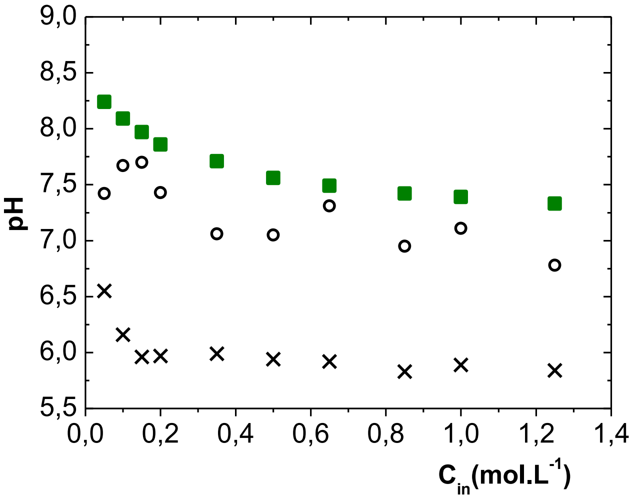

Figure 9: pH evolution upon contact of Ala solutions with hectorite, as a function of the initial [Ala] concentration. Crosses, before contact; empty circles, immediately after contact; full squares, after equilibrium.

This strong variation corresponds to an increase in the base/acid ratio of the alanine/alaninate couple. These two species can be denoted as $HAla^{\pm}$ and Ala^{-}, the first symbol underlining the fact that alanine is present as a zwitterion. According to the well-known Henderson-Hasselbach equation (Eq. 1), one has:

$$pH = pKa + \log\left(\frac{[Ala^{-}]}{[HAla^{\pm}]}\right)$$, with pKa being the pKa_2 of alanine (9.7). (1)

Hectorite, in contrast to kaolinite, is a cation exchanger. The original compensating cations may be substituted by cations present in solution such as the alaninium ions, H_2Ala^{+}. While they are a very minority species in the solutions we used, the alanine zwitterion disproportionation reaction, according to equation (2):

$$HAla^{\pm}_{aq} = Ala^{-}_{aq} + H_2Ala^{+}_{aq} \quad (2)$$

would be displaced to the right if the clay (noted as Hec^-) had a strong affinity for alaninium cations, because it would be coupled to the cation exchange reaction, Eq. (3):

$$H_2Ala^+_{aq} + Na^+Hec^-_{solid} = Na^+_{aq} + (H_2Ala^+)Hec^-_{solid} \quad (3)$$

so that the global reaction would be the Eq. (4):

$$HAla^{\pm}_{aq} + Na^+Hec^-_{solid} = Na^+_{aq} + Ala^-_{aq} + (H_2Ala^+)Hec^-_{solid}. \quad (4)$$

Thus, in principle, the amount of Ala^- in solution would be equal to the amount of H_2Ala^+ ion-exchanged. The H_2Ala^+ values estimated from this calculation are plotted as a function of the equilibrium alanine concentration in Figure 10. The shape of the curve is indeed reminiscent of those observed for ion exchange in binary systems, but the maximum amounts remain a factor of three inferior to the CEC.

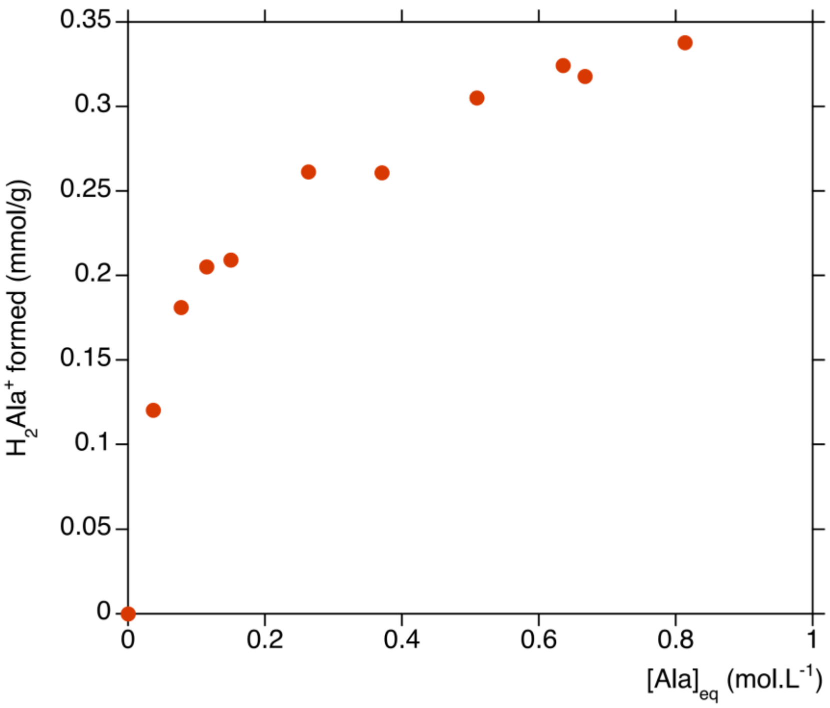

Figure 10: Amount of alaninium cations formed (from Henderson-Hasselbach equation) as a function of equilibrium alanine concentration in solution

In TG (Figure 11), raw hectorite mostly shows the endothermic elimination of physisorbed water, including water in the interlayers, peaking at 65 °C, and possibly the beginning of a degradation of the hectorite structure, not yet complete at 1000 °C.

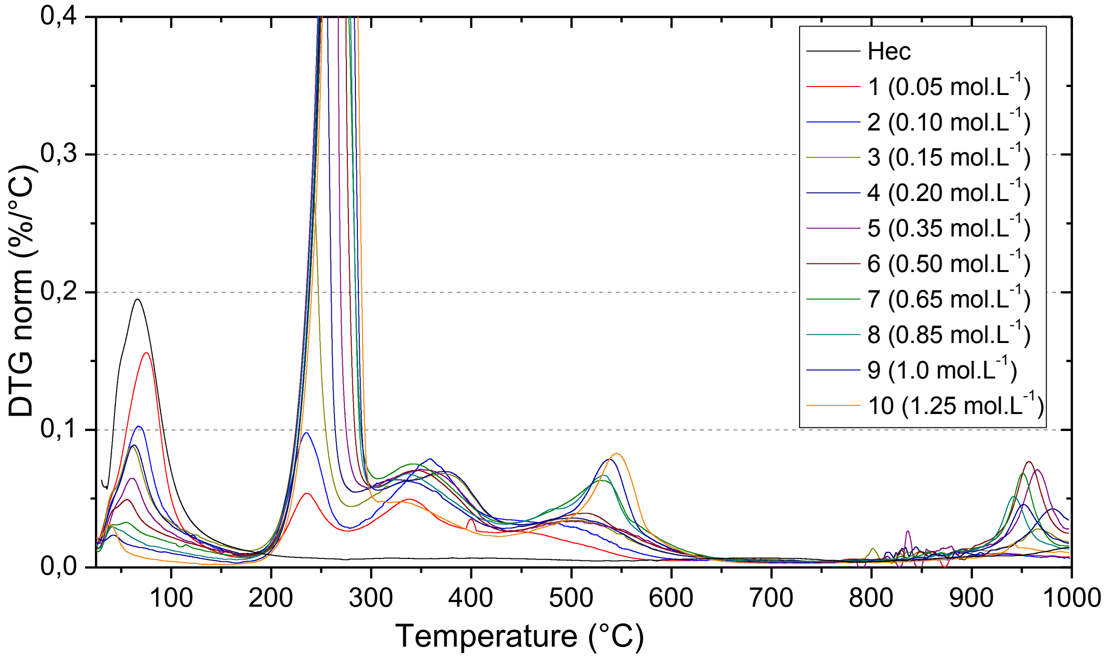

Figure 11: DTG traces of raw Hectorite, and Ala/Hec samples containing increasing amounts of alanine (1 to 10).

Ala/Hec samples show intense peaks in the region of 200-700 °C, indicative of the elimination of a large amount of organic molecules. More specifically, three regions can be identified. The first endothermic event between 200 and 300 °C is present for all samples but strongly increases with the [Ala] concentration in solution. The second event between 300 and 600 °C has a complex shape, is exothermic and partly superimposed on the third event. The latter peaks at 530-545 °C, is strongly exothermic and only appears for the highest loading samples. A quantification of these events is shown in Figure 12.

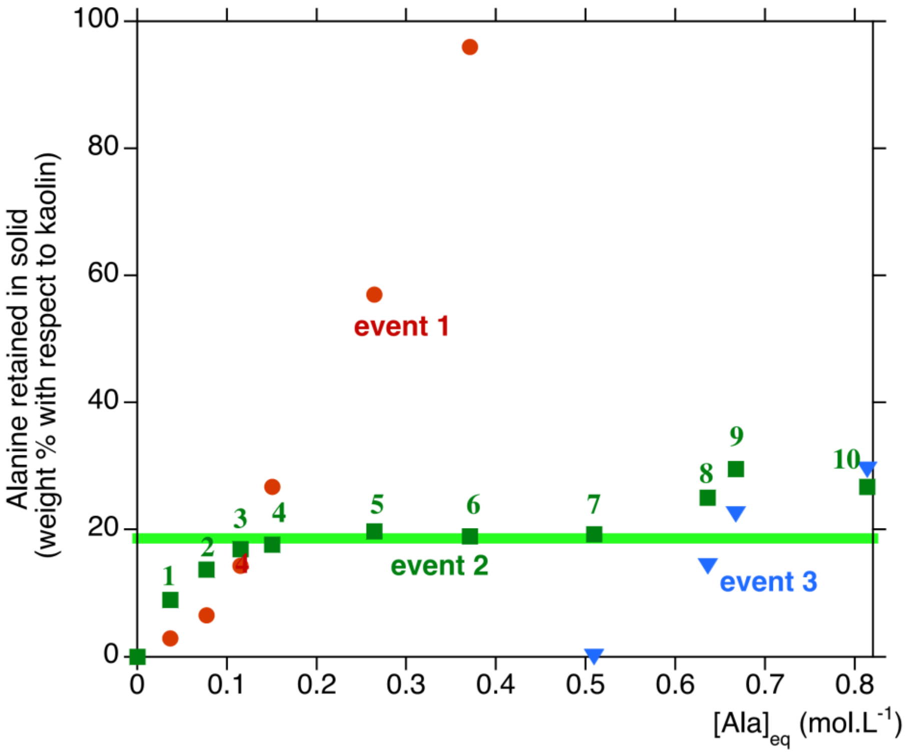

Figure 12: Quantification of the 3 TG events for solid Ala/Hec samples, as a function of the final concentration in solution (increasing concentrations from 1 to 10; event 1 is off scale for samples 7 to 10)

For initial Ala concentrations in excess of 0.35 M, the amount of alanine retained in solid samples is more than 100% of the initial hectorite weight. It seems reasonable to exclude these samples (7 to 10) from further discussion, at least in the context of prebiotic chemistry where such situations would be highly unrealistic.

Event 1 is very similar to the one we attributed to bulk-like alanine sublimation in Ala/Kao systems, except that much larger amounts of alanine are retained. The dependency of the amount corresponding to event 1 on alanine concentration is not linear however: the retention of bulk-like alanine must involve a phenomenon more complicated than simple physical retention.

The amount corresponding to event 2 follows a different trend, with a possibly Langmuirian shape, reaching a plateau for an amount of alanine of 19% with respect to hectorite, or 2.1 mmol Ala per gram hectorite – to be compared with the CEC, which is *1* mmol.g^{-1}. The event is exothermic, meaning that the corresponding alanine molecules do not desorb upon thermal activation – rather, they are eliminated by (possibly catalytic) combustion by oxygen from the air flow.

XRD (Figure 13) confirms that alanine crystallites are present starting at least from sample 2, that would contain 6.5% of "bulk-like" alanine according to TG. They are not discernible in sample 1 (2.9% bulk-like alanine). The intensity of alanine peaks increases with the same trend as that of event 1 in TG.

The peaks/bands of hectorite remain visible in Ala/Hec although scaled down due to the large amounts of alanine in the samples. This includes the d_{001}, which is observed at 13.0 Å in raw hectorite, and at 13.8 to 14.05 Å in the alanine-containing samples. It is hard to tell if this indicates intercalation of the organic molecules, since shifts of this order could well simply be due to different hydration states.

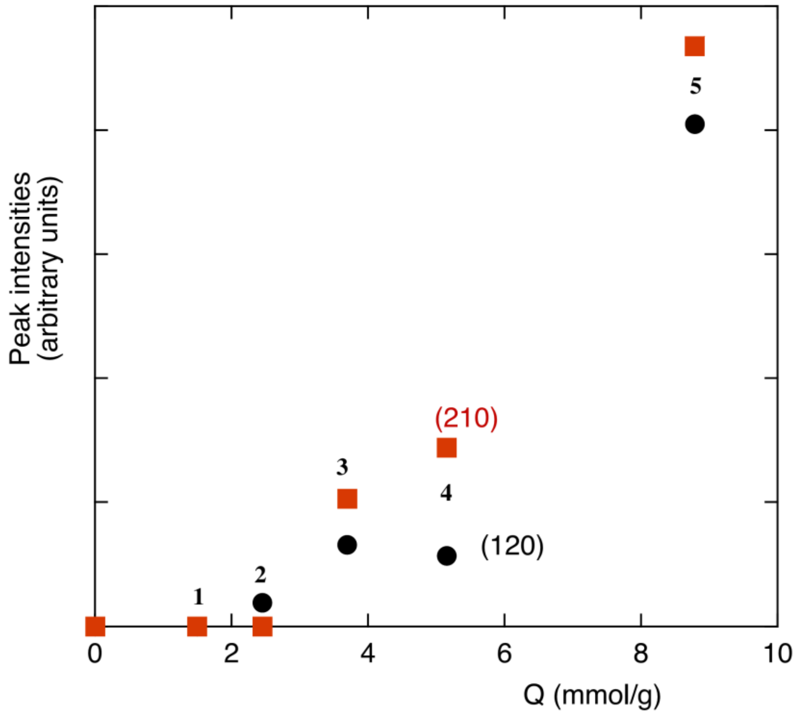

Figure 13: XRD peak intensities of Ala/Hec samples containing increasing alanine amounts.

For the IR investigations, we concentrated on samples with low alanine loadings, so that the information on adsorbed alanine species would not be drowned out by the bulk-like one. Figure 14 presents the IR spectra of the two samples with the lowest alanine contents. In the fingerprint region, hectorite only has a small band at 1639 cm^{-1} mainly due to the δ_{HOH} of residual H_2O.

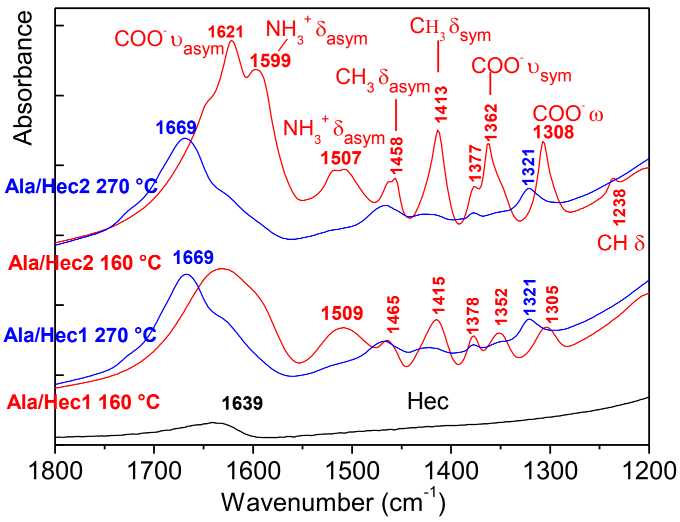

Figure 14: middle IR spectrum of Ala/Hec samples 1 and 2 with band assignments

The samples contacted with alanine and heated to 160 °C (a temperature where dehydration is complete but the alanine-associated events have not started yet) show a series of new bands that can all be assigned to alanine, more especially its zwitterionic form, as was already the case for Ala/Kao. Most of them (Table 5) are shifted by at most a few cm^{-1} as compared to bulk alanine. However, the NH_3^+ δ_{asym} is red-shifted by 12-15 cm^{-1} (main band in sample 1, coexists with unshifted in sample 2), and the COO^- ν_{sym} region appears to show two components: one close to the bulk alanine position, and a doublet that might result from a splitting of this vibration because of an interaction of the carboxylate. Thus, at low loadings, a modified form of the zwitterion seems to be present. The modification would concern both the COO^- and the NH_3^+ moieties; the accompanying band shifts are larger than those observed for H-bonds, but smaller than for covalent bonding.

Table 5: Wavenumbers (cm^{-1}) of vibrational bands of bulk alanine and Ala/Hec. n.o. not observed.

Assignment	Position in Ala (Rosado et al., 1997; Caroline, et al., 2009; Mohsin, et al. 2018)	Ala/HecS
CH δ	1235	1238
NH_3^+ δ_{sym}	1307	1305-1308
COO^- ν_{sym}	1366	1377-1378 and 1352 1362 (sample 2 only)
CH_3 δ_{sym}	1408	1413-1415
CH_3 δ_{asym}	1458	1458-1465
NH_3^+ δ_{asym}	1523	1507-1509 1518 (sample 2 only)
NH_3^+ δ_{asym}	1592	1599
COO^- ν_{asym}	1623	1621

The spectra are also interesting for what they do not show: COO^- and NH_3^+ are present, but not the protonated form COOH, which would be evidenced by a C=O stretching at 1730-1750 cm^{-1}. This means that the cationic form (alaninium ion) is either absent or present in small amounts.

After heating up to 270 °C (completion of thermal event 1), strong changes are observed. The bands attributable to COO^- and NH_3^+ decrease strongly (although they may not completely disappear), while the CH_3 bands are not affected, and new bands appear at 1321 and 1669 cm^{-1}. By comparison with similar systems on silica (Sakhno et al., 2019), the latter is probably an amide I band, the amide groups being formed by condensation of the ammonium and carboxylates of the original amino acids. Note that the amide II band expected in the 1500-1600 cm^{-1} region appears to be absent, an occurrence that has been correlated with the formation of the cyclic dimer (diketopiperazine).

4. Discussion

For the Alanine/kaolinite system, alanine in aqueous solution hardly shows any specific interaction with kaolinite. The solid samples separated from such solutions do contain significant amounts of alanine, but this appears to be due only to physical retention of limited amounts of solution by the solid; when the systems are dried, most of the alanine precipitates in crystals separated from the kaolinite phase, which are apparent upon XRD and IR characterization. Furthermore, the solutions are not significantly modified by contact with kaolinite, except for a small pH drift in the most dilute ones. Careful examination of the DTGs provides evidence for a very small amount of specific adsorption (at most 0.3% by weight). Identification and characterization of species present in such small quantities would require specific spectroscopic techniques, such as solid-state NMR of isotopically marked molecules.

Alanine did not form intercalates with kaolinite since the d_{001} was completely unaffected. Such compounds have been reported before (Sato, 1999; Wang et al., 2017), but they used the "guest-exchange" method, i.e. kaolinites pre-swollen with water or ethylene glycol. In a colloidal chemistry study of aspartic acid adsorption on kaolinite, Ikhsan et al (Ikhsan et al., 2004) only found weakly adsorbed molecules, probably by H-bonding, which is compatible with what we observed for alanine.

After heat treatment, kaolinite did not retain the alanine adsorbed: it desorbed in a zero-order thermal event before 250 °C. With the techniques we used, there was no evidence of a peptide formation reactivity. Of course, one cannot completely rule out that some peptides were formed and immediately desorbed, but in the present state of investigations this would be a completely ad hoc hypothesis. This lack of peptide bond formation on kaolinite does not stand in contradiction with previously published data.

As shown in Table 1, peptide formation on kaolinite was either very minor and/or observed in very different conditions from ours. The most comparable study is probably that of (Dalai et al., 2017), and it showed almost total desorption at 250 °C, in conformity with our observations.

The study of the Alanine/hectorite system shows a quite distinct picture. First, the adsorption mechanism is obviously different. The shift to basic pH upon contact with hectorite is well explained if there is a specific intercalation of the cationic form: the selective entrapment of the cationic form from the solution shifts the zwitterion disproportionation balance to the right. X-Ray diffractograms are compatible with alanine intercalation. However, the intercalation may not be completely explained by ion exchange. The amount of ion-exchanged alanine deduced from pH measurements is at most 0.35 $mmol.g^{-1}$, or one third of the CEC, while the total amount of strongly retained alanine deduced from TG is 2 $mmol.g^{-1}$, i.e., 6 times higher. Furthermore, IR spectroscopy does not show a clear evidence of the alaninium ion, the species that would be intercalated in an ion-exchange mechanism. Instead, it shows that the predominant alanine species is a zwitterion, whose ammonium and carboxylate moieties are however significantly modified by adsorption. The observed modifications in the carboxylate region may be due to a coordination to remaining Na^+ ions in the interlayer; the ammonium groups, being positively charged, could not take part in the coordinative binding to Na^+, but they could interact with the negatively charged basal surfaces of hectorite through H-bonding. Thus, the adsorption of alanine on/in hectorite would involve two parallel mechanisms: the cation exchange of protonated alaninium cations (minority), and the coordinative binding of unprotonated alanine zwitterions to remaining compensating cations (majority).

After thermal treatment at 270 °C (but not at 160 °C), the Ala/hectorite samples present new FTIR signals indicating the formation of peptide bonds, i.e., amide I bands. The absence of amide II signals can be due to the majority formation of the cyclic dimer of alanine (cyclo-(Ala-Ala), aka dimethyl-DKP). Thus, the strongly adsorbed forms of alanine in hectorite do not desorb upon heating, as opposed to what happens in Alanine/kaolinite, but finally react together to form the cyclic dimers. This reactivity is similar to what has been observed on silica (Guo and Holland, 2015; Lambert et al., 2013) but it needs higher temperatures: on silica, DKP formation is essentially complete at 160 °C, while it has not yet started on hectorite. This may be an illustration of Sabatier's principle for catalysis efficiency: the most effective catalysts for a reaction are those where the reagents/catalyst interaction has just the "right" energy. If the interaction is too weak (as in alanine/kaolinite), the reaction is not catalyzed. If it is too strong (as in alanine/hectorite), the reagents are trapped within the catalyst and take higher activation to react; if the interaction is "just right"(Davies, 2007) (as in alanine/silica), the catalysis is optimal and the reaction may thus proceed at low temperatures.

5. Conclusion

Although peptide formation from amino acids adsorbed on mineral surfaces is a well-established phenomenon, examination of the literature shows that in the case of clay minerals, published results are hard to interpret due to the use of very heterogeneous deposition and activation conditions between the various studies. We have compared in the same conditions two clay minerals with different structures and chemical properties, kaolinite and hectorite, and observed divergent behaviors. On kaolinite, a T:O clay mineral with negligible exchange capacity, alanine was not specifically adsorbed from aqueous solutions, only physically retained. Upon drying, it established at most weak H-bonds with the surface, and upon heating it desorbed extensively with no indication of any prior catalytic reaction on the surface. On hectorite, a T:O:T clay mineral, there was evidence for two simultaneous adsorption mechanisms: a minority cation exchange of the protonated alaninium cation, and a quantitatively more important intercalation probably driven by complexation to the remaining interlayer Na^+ cations. Amino acids retained by these more energetic mechanisms did not desorb upon heating, but converted to peptides (probably the cyclic DKP) at temperatures comprised between 160 and 270 °C. Thus, in order to react, the amino acids must be adsorbed with enough strength to avoid thermal desorption; but the adsorption mechanisms available in smectites such as hectorite are probably too energetic, only allowing reaction at temperatures significantly higher than on silica. Reflexion on the catalytic mechanism for amino acids condensation is probably premature, in view of the lack of consensus for the equivalent reaction on silica supports, even though they have been much more studied. At any rate, our results show that one should be wary of over-generalizing results obtained on a particular support. Amino acids/mineral surfaces systems show a rich and diverse chemistry, and each system must be studied at the molecular level in its own right.

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