Amino Acids (2010) 38:1089–1099
DOI 10.1007/s00726-009-0318-8

ORIGINAL ARTICLE

Cysteine, thiourea and thiocyanate interactions with clays: FT-IR, Mössbauer and EPR spectroscopy and X-ray diffractometry studies

Henrique de Santana · Andrea Paesano Jr. · Antonio C. S. da Costa · Eduardo di Mauro · Ivan G. de Souza · Flávio F. Ivashita · Cláudio M. D. de Souza · Cássia T. B. V. Zaia · Dimas A. M. Zaia

Received: 10 March 2009 / Accepted: 19 June 2009 / Published online: 5 July 2009

Abstract The present study examined the adsorption of cysteine, thiourea and thiocyanate on bentonite and montmorillonite at two different pHs (3.00, 8.00). The conditions used here are closer to those of prebiotic earth. As shown by FT-IR, Mössbauer and EPR spectroscopy and X-ray diffractometry, the most important finding of this work is that cysteine and thiourea penetrate into the interlayer of the clays and reduce Fe^{3+} to Fe^{2+}, and as consequence, cystine and c,c′-dithiodiformamidinium ion are formed. This mechanism resembles that which occurs with aconitase. This is a very important result for prebiotic chemistry; we should think about clays not just sink of molecules, but as primitive vessels of production of biomolecules. At pH 8.00, an increasing expansion was observed in the following order for both minerals: thiourea > thiocyanate > cysteine. At pH 3.00, the same order was not observed and thiourea had an opposite behavior, being the compound producing the lowest expansion. Mössbauer spectroscopy showed that at pH 8.00, the proportion of Fe^{2+} ions in bentonite increased, doubling for thiourea, or more than doubling for cysteine, in both clays. However, at pH 3.00, cysteine and thiourea did not change significantly the relative amount of Fe^{2+} and Fe^{3+} ions, when compared to clays without adsorption. For thiocyanate, the amount of Fe^{2+} produced was independent of the pH or clay used, probably because the interlayers of clays are very acidic and HSCN formed does not reduce Fe^{3+} to Fe^{2+}. For the interaction of thiocyanate with the clays, it was not possible to identify any potential compound formed. For the samples of bentonite and montmorillonite at pH 8.00 with cysteine, EPR spectroscopy showed that intensity of the lines due to Fe^{3+} decreased because the reaction of Fe^{3+}/cysteine. Intensity of EPR lines did not change when the samples of bentonite at pH 3.00 with and without cysteine were compared. These results are in accordance with those obtained using Mössbauer and FT-IR spectroscopy.

Keywords Adsorption · FT-IR spectroscopy · Mössbauer spectroscopy · X-ray diffractometry · EPR spectroscopy · Clays

Introduction

Bernal (1951) was the first to propose that minerals could have played an important role in the origin of life on

H. de Santana · C. M. D. de Souza · D. A. M. Zaia (✉)
Departamento de Química-CCE,
Universidade Estadual de Londrina, Londrina,
PR 86051-990, Brazil
e-mail: damzaia@uel.br

A. Paesano Jr. · F. F. Ivashita
Departamento de Física-CCE,
Universidade Estadual de Maringá, Maringá,
PR 87020-900, Brazil

A. C. S. da Costa · I. G. de Souza
Departamento de Agronomia-CCA,
Universidade Estadual de Maringá, Maringá,
PR 87020-900, Brazil

E. di Mauro
Laboratório de Fluorescência e Ressonância Paramagnética
Eletrônica (LAFLURPE)-CCE, Universidade Estadual de
Londrina, Londrina, PR 86051-990, Brazil

C. T. B. V. Zaia
Departamento de Ciências Fisiológicas-CCB,
Universidade Estadual de Londrina, Londrina,
PR 86051-990, Brazil

earth. According to him, minerals could have taken part in processes such as: the selection and concentration of biomolecules as well as catalyzing their reactions. In the living beings of today, amino acids are very important since they are involved in most of life's reactions (Darnell et al. 1990; Bittner et al. 2000; Alia and Matysik 2001; Wen et al. 2007; Fan et al. 2009), and thus, the study of their production and reactions are very important issues in prebiotic chemistry (Zaia et al. 2008; Lambert 2008; Zaia 2004).

As reviewed by Zaia et al. (2008), cysteine could be synthesized under simulated conditions of prebiotic atmospheres and hydrothermal vents. There are several experiments describing the synthesis of thiourea under prebiotic chemistry conditions, and only a few are cited here, such as heated/irradiated ammonium thiocyanate in the solid state using UV radiation (Talreja et al. 1967), heated ammonium thiocyanate plus sand plus transition metals in solid state (Zaia et al. 2004), and mixture of gases resembling the atmosphere of primitive earth (Raulin and Toupance 1977). Thiocyanate has been found in Atlantis II Deep Brine located at the bottom of the sea in the Rift Valley (Dowler and Ingmanson 1979) as well as produced in experiments simulating the atmosphere of prebiotic earth (Raulin and Toupance 1977). Thus, all the substances (cysteine, thiourea, thiocyanate) studied here could be easily found on prebiotic earth.

The adsorption of cysteine, thiourea or thiocyanate have been studied on several materials such as pyrite (Bebié and Schoonen 2000), clays (Benetoli et al. 2007; Brigatti et al. 1999), silica (Basiuk 2002) and metals (Garcia et al. 2004; Bron and Holze 1999). There are several reports about the adsorption of amino acids on minerals/clays/sediments, as well as some good reviews about this subject (Lambert 2008; Hazen 2006; Zaia 2004; Lahav and Chang 1976).

The adsorption of cysteine on kaolinite and bentonite was recently studied by Benetoli et al. (2007), and several questions were raised from this study as follows: Is the interaction of cysteine/clays pH dependent? Which are the products of the interaction of cysteine/clays? Could other thiol compounds show the same interactions with clays? The main goal of the present work was to address these questions. Thus, we studied the interaction of cysteine, thiourea, and thiocyanate with bentonite and montmorillonite at two different pH levels (3.00 and 8.00), using FT-IR, Mössbauer and EPR spectroscopy, and X-ray diffractometry. It also should be pointed out that as far as we know there are no prior published studies on the interaction of these substances with clays under conditions simulating those of prebiotic earth as used in the present study.

Materials and methods

Materials

All reagents were of analytical grade.

Clays

Bentonite and montmorillonite were purchased from Acros Organics-NJ, USA. All clays were used without further preparation.

Bentonite: *chemical composition (%)*: $SiO_2 = 73.0$, $Al_2O_3 = 14.0$, $Fe_2O_3 = 2.7$, $CaO = 0.2$, $MgO = 1.1$, $Na_2O = 0.6$, $K_2O = 1.9$; *surface area* = 240 m^2/g. Montmorillonite: *chemical composition (%):* $SiO_2 = 54.0$, $Al_2O_3 = 17.0$, $Fe_2O_3 = 5.2$, $CaO = 1.5$, $MgO = 2.5$, $Na_2O = 0.4$, $K_2O = 1.5$; *surface area* = 100 m^2/g.

Cysteine, thiourea, and thiocyanate

L-Cysteine, thiourea, and potassium thiocyanate were purchased from Synth (Brazil) and were used as received.

Seawater

The following substances were weighed and dissolved in 1.0 l of distilled water: 28.57 g of sodium chloride, 3.88 g of magnesium chloride, 1.787 g of magnesium sulfate, 1.308 g of calcium sulfate, 0.832 g of potassium sulfate, 0.124 g of calcium carbonate, 0.103 g of potassium bromide, and 0.0282 g of boric acid. The pH of the seawater was adjusted to 3.00 or 8.00 with NaOH or HCl. This solution contains the major components of the seawater; thus the conditions used here are closer to those of the prebiotic earth.

Sample preparation

L-Cysteine was dissolved in seawater at a concentration of 1.00 mol l^{-1}, and thiourea and potassium thiocyanate were dissolved in seawater as a saturated solution. Each clay (bentonite and montmorillonite) was processed as follows: 500 mg of clay (bentonite or montmorillonite) were added to two separate sets of three tubes (15 ml) to which (a) 5.00 ml of seawater and (b) 5.00 ml of seawater containing 1.00 mol l^{-1} L-cysteine or saturated with thiourea or potassium thiocyanate. The pH was adjusted to 3.00 or 8.00 with NaOH or HCl. The tubes were mixed for 24 h, and afterward spun for 15 min at 2,000 rpm. The aqueous phase was lyophilized and used for FT-IR spectroscopy and the solids were dried in an oven at 40°C for 24 h. A portion of the dried solids was used for FT-IR, Mössbauer and EPR spectroscopy, and X-ray diffractometry. Another portion of the dried solids were transferred to 15-ml tubes, and 5.00 ml of 0.20 mol l^{-1} $CaCl_2$ were added. The tubes were mixed for 24 h, and afterward spun for 15 min at 2,000 rpm. The aqueous phase was again lyophilized, and the solids were dried in an oven at 40°C for 24 h; both samples were used for FT-IR spectroscopy.

Methods

Mössbauer spectroscopy (MS)

Mössbauer spectroscopy (MS) characterizations were performed in transmission geometry, using a conventional Mössbauer spectrometer, operated in a constant acceleration mode. The γ-rays were provided by a $^{57}Co(Rh)$ source. The Mössbauer spectra were analyzed by a nonlinear least-squares routine, with Lorentzian line shapes. All isomer shift (IS) data given are relative to α-Fe throughout this paper.

Electron paramagnetic resonance (EPR) spectroscopy

The samples were submitted to EPR experiment at X- band (ca. 9 GHz) with 20 G modulation amplitude and a magnetic field modulation of 100 kHz using a JEOL (JES-PE-3X) spectrometer at room temperature. Mn^{2+}:MgO ($g = 1.981$) was used as g marker and standard of line intensity, using its fourth spectrum line.

X-ray diffractometry

Clay materials before and after the adsorption experiments were analyzed by X-ray diffraction in a Shimadzu D-6000 apparatus with a Cu source and Ni filter. Oriented glass slides of the materials were prepared by the smear method and analyzed at room temperature conditions. Each material was analyzed in step scan mode from 2 to 30° 2θ, 0.02 spacing for 0.6 s each step. All peak positions were analyzed using Grams software®.

Infrared spectroscopy

The IR spectra were recorded with an FT-IR 8300 Shimadzu spectrophometer using pressed KBr disks and a spectral resolution of 4 cm^{-1}, and each final spectrum was obtained after acquiring 85 spectra. FT-IR analysis was carried out with clay samples with and without cysteine, thiourea and thiocyanate adsorption, clay samples after desorption with 0.20 mol l^{-1} $CaCl_2$, lyophilized aqueous phase, and lyophilized aqueous phase after desorption with $CaCl_2$. About 10 mg of each sample plus 200 mg of KBr was weighed and ground in an agate mortar with pestle until a homogeneous mixture was obtained. Disc pellets were prepared and spectra were recorded from 400 to 4,000 cm^{-1}. FT-IR spectra were analyzed using the Origin program (5.0, 2001).

Results and discussion

The adsorption of cysteine, thiourea, and thiocyanate on bentonite and montmorillonite at two different pH levels (3.00, 8.00) was studied using FT-IR, EPR and Mössbauer spectroscopy and X-ray diffractometry. X-ray diffractometry was used to determine not only whether these compounds are adsorbed on the surface of clays but also if they penetrate the interlayer of the clays. Mössbauer and EPR spectroscopy were used to acquire information about the reaction of these compounds with iron present in the clays. The high affinity of thiol compounds for metals is well known. FT-IR spectroscopy was used to obtain information about how these compounds are adsorbed or absorbed by clays, as well as to determine the products formed from reactions with metals.

X-ray diffraction shows interesting aspects of the process of adsorption onto materials. Bentonite and montmorillonite have mostly a permanent negative charge and some pH-dependent charges (Sposito 1984) that can be compensated by surface functional groups of compounds (SFGC) such as amino ($\sim NH_2$), carboxylic ($\sim COOH$) and thiol ($\sim SH$) groups (Brigatti et al. 2003). The expansion of clay minerals are dependent on several chemical attributes of the solution, mostly associated with the presence of cations (Na^+, Ca^{2+}, Mg^{2+}) or metals (Cu^{2+}, Zn^{2+}). Among the cations, basal d001 spacing increases in the following order for montmorillonite: $Na^+ < Ca^{2+} < Cu^{2+}$ (Siffert and Kessaissia 1978; Benincasa et al. 2000). Besides the saturating cation, the layer charge also has a strong influence on the expansion of 2:1 clay minerals. Bentonite, with lower layer charge, shows smaller expansion than clay minerals with higher layer charges (montmorillonite and beidellite) (Benincasa et al. 2000).

At pH 3.00, most of the permanent negative charge is compensated by protons from the solution and a very small net charge will be available for the adsorption of SFGC. At pH 8.00, most of the charge, pH-dependent and permanent, will be negative, and strong binding of positive SFGC will dominate the complexation reactions.

At pH 8.00, expansion of the clay minerals was more pronounced for bentonite than montmorillonite. At this pH, the net mineral charge is greater than at pH 3.00 and more negative charge associated with the permanent and pH-dependent groups protrude (Fig. 1).

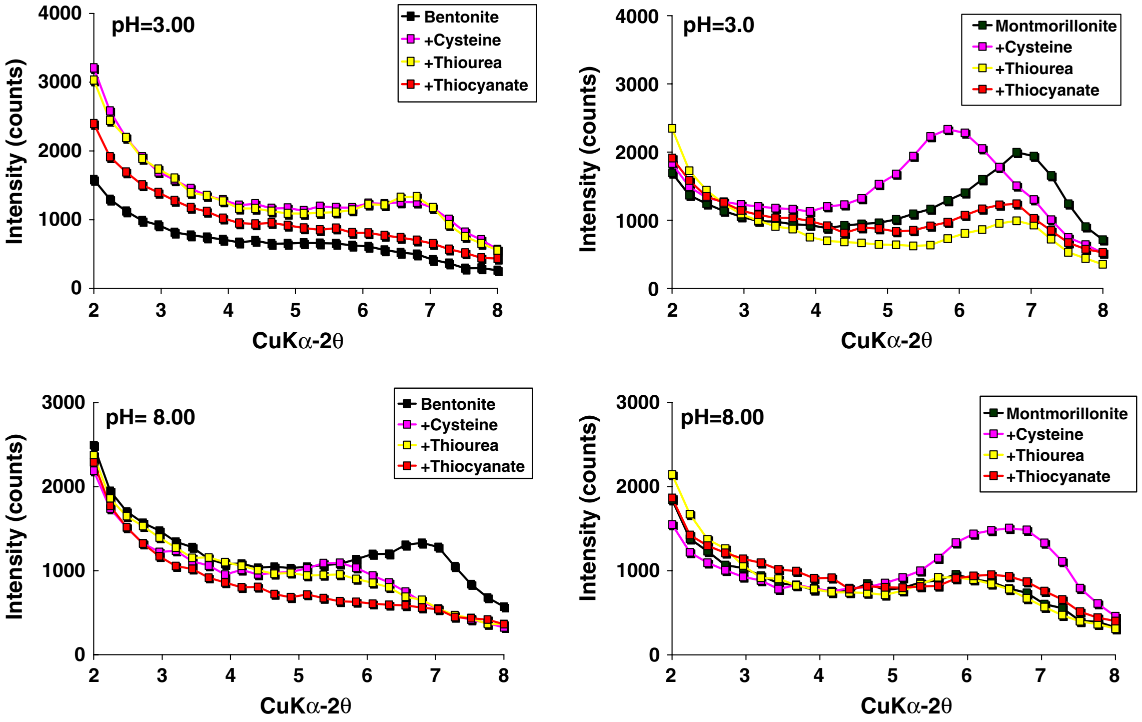


Fig. 1 X-ray diffraction patterns for bentonite and montmorillonite under different pH and adsorption compounds. Clay samples were tumbled for 24 h with seawater, cysteine was dissolved in seawater at concentration of 1.00 mol l^{-1} and thiourea and thiocyanate were dissolved in seawater as a saturated solution. All samples were spun for 15 min at 2,000 rpm, and the solids were dried in an oven at 40°C for 24 h

According to the d_{001} dimension of natural oriented clays, expansion, and therefore adsorption occurred with all the tested compounds. The extent of the expansion was compared to the original clay mineral without the presence of the test compound (Fig. 1).

In the present work, the original expansion for the 001 diffraction plane observed for pure bentonite was higher (12.86 Å) than for pure montmorillonite (12.73 Å), and the composition of the seawater used in the batch adsorption experiments explains this difference (Fig. 1).

Overall, the average expansion was not significantly different between the clay minerals at pH 3.00. Bentonite, independent of the compound intercalated, expanded to an average of 13.40 Å and montmorillonite to 13.54 Å (Greenland et al. 1965). Higher expansions are observed for the clay minerals when metals, such as Cu^{2+}, are intercalated in the presence of amino acids or other organic compounds (Brigatti et al. 1999, 2003).

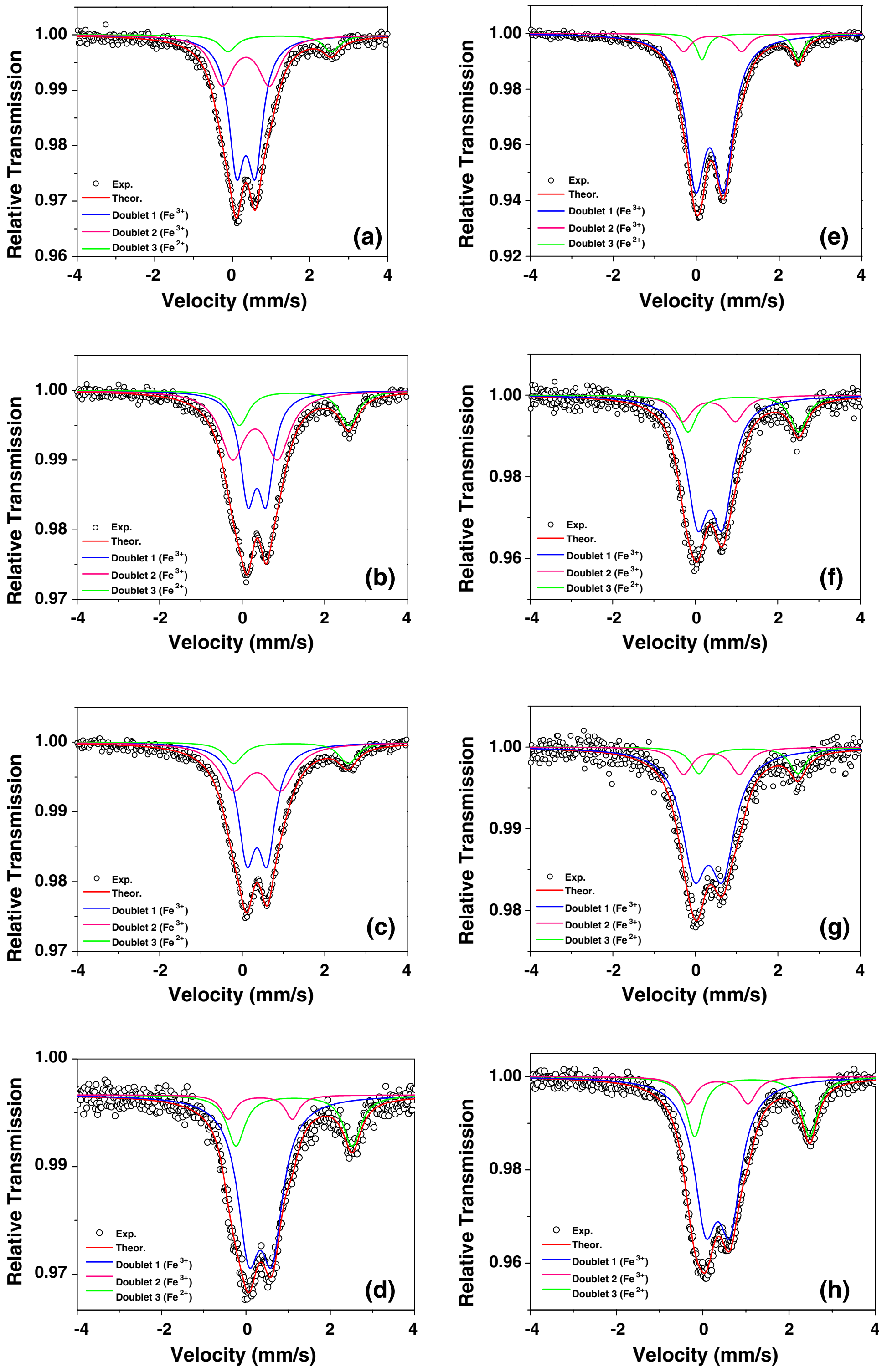
At pH 3.00, thiocyanate adsorbed onto bentonite showed the highest expansion ($d_{001} = 13.96$ Å), while the adsorption of cysteine showed highest expansion for montmorillonite. At this pH condition, the adsorption of cysteine onto montmorillonite showed the highest expansion among all treatments with a d_{001} spacing of 14.63 Å. At this pH, the SFGC have maximal protonation ($\sim NH_2^+$ and $\sim SH_2^+$) and compete with the protons for the negative charge adsorption sites.

Thiourea displays a peculiar behavior at pH 8.00. This compound showed the highest expansion value of the d_{001} spacing for both clay minerals at this pH. This suggests that protonation increases the two negative charges on the SFGC ($\sim N^-$) as the pH increases from 3.00 to 8.00. At pH 8.00, expansion in both minerals increased in the following order: thiourea > thiocyanate > cysteine (Fig. 1). At pH 3.00, the same order was not observed and thiourea had an opposite behavior, where it caused the lowest expansion (Fig. 1).

The Mössbauer spectra for bentonite and montmorillonite with and without adsorbed cysteine, thiourea or thiocyanate, prepared at pH 3.00 and 8.00 were fitted with three doublets, two of them corresponding to Fe^{3+} and the third to Fe^{2+}. Two representative spectra are shown in Fig. 2. The hyperfine parameters, averaged over all samples and pH ranges, are presented in Table 1.

The occurrence of two Fe^{3+} sites, in contrast to the single one observed for bentonite samples with and without adsorbed cysteine, prepared in the same way (Benetoli et al. 2007), reveals that the final clays are very sensitive to eventual small variations in the chemical route of adsorption. However, the hyperfine parameters and subspectral

Fig. 2 Mössbauer spectra of bentonite and montmorillonite samples. (**a**) Bentonite without thiocompounds adsorbed on; (**b**) bentonite with thiourea adsorbed on; (**c**) bentonite with thiocyanate adsorbed on; (**d**) bentonite with cysteine adsorbed on; (**e**) montmorillonite without thiocompounds adsorbed on; (**f**) montmorillonite with thiourea adsorbed on; (**g**) montmorillonite with thiocyanate adsorbed on and (**h**) montmorillonite with cysteine adsorbed on. Clay samples were mixed for 24 h with seawater (pH 8.00), thiourea and thiocyanate were dissolved in seawater at concentration of saturated solutions (pH 8.00). The tubes were spun for 15 min at 2,000 rpm and the solid was dried in an oven at 40°C for 24 h



areas representing the Fe^{2+} site and one of the Fe^{3+} sites are consistent with earlier reported results. The third site (Fe^{3+}) observed in the present samples may be attributed to iron atoms at the very surface of the clay aggregates, where regular coordination is broken and the site symmetry is far from cubic.

Through the subspectral areas (see Fig. 3), cysteine and thiourea, at pH 3.00, did not appear to change significantly the relative amount of Fe^{2+} and Fe^{3+} ions, in comparison with the clays without adsorption (i.e., blank samples). However, at pH 8.00, the proportion of Fe^{2+} ions in bentonite increased, doubling with thiourea, or more than

Table 1 Mössbauer hyperfine parameters for bentonite and montmorillonite samples

Clay	Subspectrum	IS[a] (mm/s) (±0.02)	QS[b] (mm/s) (±0.02)
Bentonite	Doublet 1 (Fe^{3+})	0.35	0.50
	Doublet 2 (Fe^{3+})	0.35	1.25
	Doublet 3 (Fe^{2+})	1.21	2.72
Montmorillonite	Doublet 1 (Fe^{3+})	0.34	0.61
	Doublet 2 (Fe^{3+})	0.38	1.32
	Doublet 3 (Fe^{2+})	1.23	2.48

[a] Isomer shift/relative to α-Fe foil at room temperature

[b] Quadrupole splitting

doubling with cysteine, in both clays. It is also observed that the concentration of Fe^{2+} increased with increasing concentration of cysteine. For thiocyanate, in all cases, about 12% of the resultant iron ions were divalent, revealing that the final ratio of the two cation species, Fe^{2+}/Fe^{3+}, was not significantly affected by pH or the clay used. Ng and Henry (1975) studied the oxidation of thiocyanate by tris(1,10-phenanthroline)Fe(III), and they observed that k_{obs} decreased with increasing concentration of H^+, probably because of the formation of an inactive species of HSCN. Our results of constant ratio of Fe^{2+}/Fe^{3+} independent of the clay used or pH could be explained considering that the interlayer of clays is very acidic and the ratio $SCN^-/HSCN$ is also constant. Thus, HSCN formed in the interlayer of clays does not appear to reduce Fe^{3+} ions to Fe^{2+} ions.

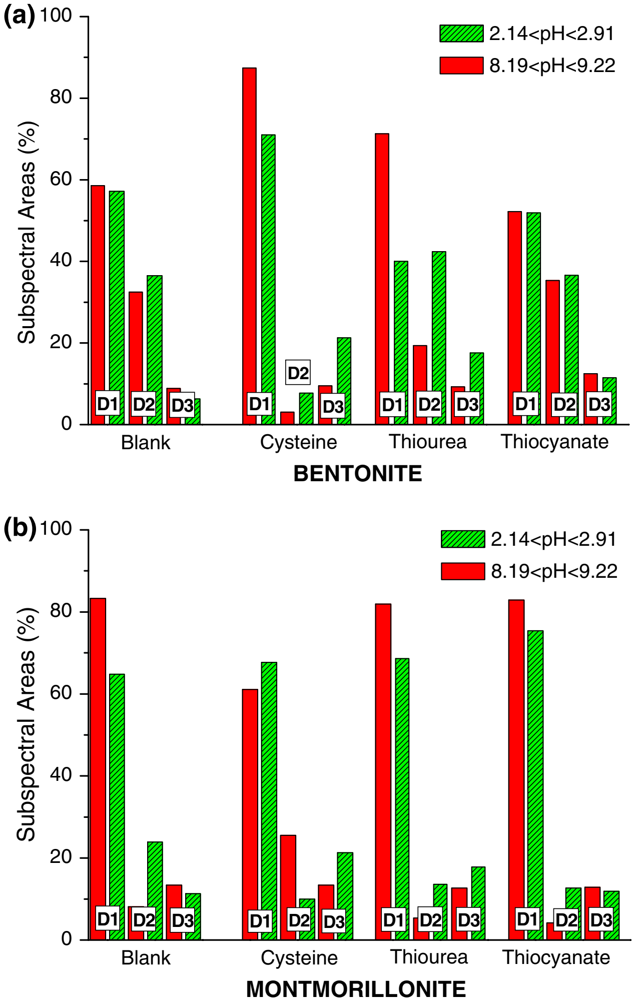


Fig. 3 Mössbauer subspectral areas for blank and adsorbed samples: Bentonite (**a**) and Montmorillonite (**b**); D1, D2 and D3 refer to doublet 1, doublet 2 and doublet 3, respectively

These results show that cysteine, thiourea, and thiocyanate partially reduced the iron present in the clays (bentonite, montmorillonite). These findings are similar to the situation with aconitase where ferric ions are reduced to Fe 2.5, meaning that one electron is shared by two ferric ions (Cowan, 1993).

The EPR spectra of the clay samples showed three resonance lines (Fig. 4) at $g = 8.1$, $g = 4.3$ and $g = 2.0$. These lines are ascribed to Fe^{3+} complexes (McBride 1978; Senesi 1990; Martin-Neto et al. 1991). The intensities of these three lines were determined with regard to the signal of Mn^{2+}:MgO and the values are at Table 2. The line intensities in the sample of bentonite without cysteine, prepared at pH 8.00 are higher than the lines in the sample with cysteine prepared at pH 8.00 (Table 2); thus the quantity of Fe^{3+} complexes is greater in the sample of bentonite without cysteine. These results are in accordance with those obtained with Mössbauer (Figs. 2, 3) and FT-IR (Fig. 5) Spectroscopy. As it was showed by Mössbauer spectroscopy (Figs. 2, 3) cysteine decreased the quantity of Fe^{3+}, thus the intensity lines of EPR were less intensity than in the sample without cysteine. FT-IR spectroscopy (Fig. 5) showed cystine was synthesized in large amounts using Fe^{3+}. The intensities of the first and second lines in the sample of bentonite with cysteine prepared at pH 3.00 are a little higher than the same lines in the sample of bentonite without cysteine prepared at pH 3.00, but the third line in the sample of bentonite with cysteine is a little less intense than the same line in the sample of bentonite without cysteine (Table 2), so the quantity of Fe^{3+} complexes is about equivalent in these samples. These results are in accordance with those obtained by Mössbauer (Figs. 2, 3) and FT-IR (Fig. 5) spectroscopy. Mössbauer spectroscopy showed that in this pH (3.00) the quantity of Fe^{3+} did not change in the sample with cysteine when compared to the sample without cysteine. The FT-IR spectra showed that they were not well resolved probably because of the small amount of cystine formed. The line intensities in the sample of montmorillonite without cysteine prepared at pH 3.00 are higher than the sample lines in the sample of montmorillonite with cysteine prepared at

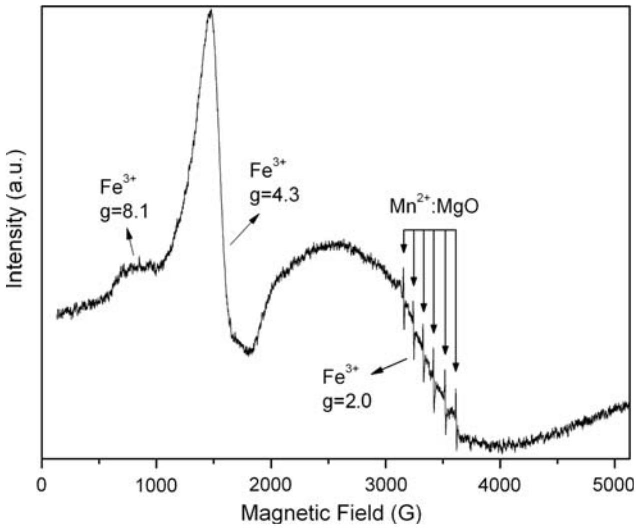


Fig. 4 EPR spectrum of clay sample 18 at room temperature. Mn^{2+}:MgO was used as *g* marker and standard of line intensity. Clay samples were tumbled for 24 h with seawater, and cysteine was dissolved in seawater at concentration of 1.00 mol l^{-1}. All samples were spun for 15 min at 2,000 rpm, and the solids were dried in an oven at 40°C for 24 h

pH 3.00 (Table 2), so the quantity of Fe^{3+} complexes is greater in the sample of montmorillonite without cysteine. There is no explanation why the lines of EPR for the sample without cysteine were bigger than in the sample with cysteine, since Mössbauer spectroscopy showed that quantity of Fe^{3+} in those samples did not change much. The line intensities in the sample of montmorillonite without cysteine prepared at pH 8.00 are higher than the sample lines in the sample of montmorillonite with cysteine prepared at pH 8.00 18 (Table 2), so the quantity of Fe^{3+} complexes is greater in the sample of montmorillonite without cysteine. These results also are in accordance with the results of Mössbauer (Figs. 2, 3) and FT-IR (Fig. 5) spectroscopy. Mössbauer spectroscopy (Figs. 2, 3) showed that cysteine decreased the quantity of Fe^{3+} in the sample with it; thus the intensity lines of EPR were of less intensity than in the sample without cysteine. FT-IR spectroscopy (Fig. 5) showed cystine was synthesized in large amounts using Fe^{3+}. It should be pointed out that free radical was not detected in this reaction (Fe^{3+}/cysteine); however, the formation of free radical was observed in other reactions involving cysteine as well as other amino acids (Augusto and Vaz 2007).

Figure 5 shows the FT-IR spectra of bentonite, montmorillonite, cysteine adsorbed on bentonite (pH 8.00) and on montmorillonite (pH 8.00), lyophilized solution after adsorption of cysteine on clays (pH 8.00), clays after desorption by $CaCl_2$, lyophilized solution after desorption of cysteine on clays by $CaCl_2$, and solid cysteine and cystine. The bands observed in the samples cysteine adsorbed on bentonite and on montmorillonite and lyophilized solution after cysteine adsorption on clays, demonstrate that these spectra are characteristic of cystine, as observed in the spectra of Fig. 5a–g and 5-B–g. The main bands at 1,622, 1,589, 1,490, 1,408, 1,342, 1,295, 1,193, 1,123, 964, 875, 849, 779, 677, 615, 540 cm^{-1} (Fig. 5-A-b, B-b, A-d, B-d), are shown in the spectrum of cystine (Fig. 5-A-g, 5-B-g). The bands at 1,743, 1,518, 1,430, 1,202, 1,140, 1,107, 1,058, 866, 772 and 520 cm^{-1} relative to the cysteine spectrum (Fig. 5-A-f, B-f), were not observed in the cysteine adsorbed on bentonite (Fig. 5-A-b) and on montmorillonite (Fig. 5-B-b) and lyophilized solution after adsorption of cysteine on clays (Fig. 5-A-d, B-d). This means that after the reaction between cysteine and Fe^{3+} of clays, excess cystine that is formed could diffuse into the aqueous phase. The formation of cystine was also observed when cysteine was intercalated into layered double hydroxides and its oxidation occurred through H_2O_2 (Wei et al. 2006). The FT-IR spectra of cysteine samples at pH 3.00 were not much different from those at pH 8.00, but the spectra were not well resolved probably because of the small amount of cystine formed. This result is in accordance with the Mössbauer spectroscopy results, where at pH 3.00 the amount of Fe^{2+} was low (Figs. 2 and 3). We also can say this result is in accordance with the EPR spectroscopy results, where at pH 8.00 lines due to Fe^{3+} in the samples with cysteine decreased when

Table 2 Resonance line intensities of Fe^{3+} complexes

Clay	pH	Sample	Line 1 Fe^{3+} ($g = 8.1$)	Line 2 Fe^{3+} ($g = 4.3$)	Line 3 Fe^{3+} ($g = 2.0$)
Bentonite	3.00	Without Cys	0.32	1.24	1.87
		With Cys	0.36	1.68	1.71
	8.00	Without Cys	0.37	1.51	2.44
		With Cys	0.30	1.34	1.22
Montmorillonite	3.00	Without Cys	0.51	2.78	2.68
		With Cys	0.30	2.01	1.39
	8.00	Without Cys	0.50	3.11	2.68
		With Cys	0.40	2.97	1.74

Clay samples were tumbled for 24 h with seawater; cysteine was dissolved in seawater at concentration of 1.00 mol l^{-1}. All samples were spun for 15 min at 2,000 rpm, and the solids were dried in an oven at 40°C for 24 h

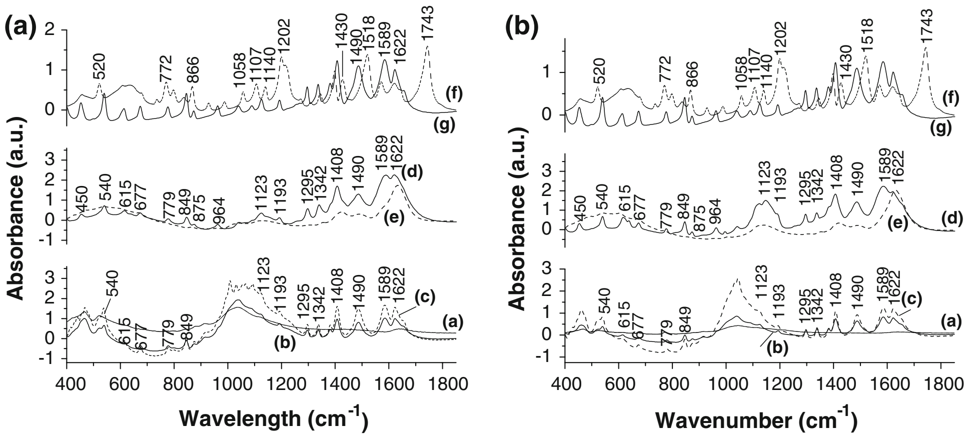


Fig. 5 FT-IR spectra: bentonite, 2-**A**–*a*; montmorilonite 2-**B**–*a*; cysteine adsorbed on bentonite, 2-**A**-*b* and on montmorilonite, 2-**B**–*b*; clays after desorption by $CaCl_2$ (0.20 mol l^{-1}), 2-**A**-*c* and 2-**B**–*c*; cysteine solution after adsorption (*lyophilized solid*), 2-**A**-*d* and 2-**B**–*d*; solution after desorption by $CaCl_2$ (0.20 mol L^{-1}), 2-**A**-*e* and 2-**B**-*e*; solid cysteine, 2-**A**-*f* and 2-**B**–*f*; solid cystine, 2-**A**-*g* and 2-**B**–*g*. Clays samples were mixed for 24 h with seawater (pH 8.00), cysteine was dissolved in seawater at concentration of 1.00 mol l^{-1} (pH 8.00). The tubes were spun for 15 min at 2,000 rpm; the aqueous phase was lyophilized and the solid was dried in an oven at 40°C for 24. A portion of the dried solid was transferred to a tube (15 ml) and 5.00 ml of $CaCl_2$ 0.20 mol l^{-1} was added. The tube was mixed for 24 h, after it was spun for 15 min at 2,000 rpm; the aqueous phase was again lyophilized and the solid was dried in an oven at 40°C for 24

compared to the samples without cysteine (Fig. 4; Table 2). For all experiments, the adsorption of cysteine (1,200 µg/5.0 ml) on bentonite (500 mg) and kaolinite (500 mg) at three different pH values (3.00, 6.00, 8.00) was almost complete (Benetoli et al. 2007). This could mean that the reaction of cysteine with Fe^{3+} was not the only process occurring, even though the amount of Fe^{3+} in the clays (kaolinite 0.3% of Fe_2O_3, bentonite 2.7% of Fe_2O_3) was much larger than the amount of cysteine (1,200 µg/5.0 ml). We can also conclude that pH 3.00 did not favor the formation of cystine. The adsorption of cysteine on Na, Ca, Cu homoionic smectites, on pyrite, and on silica was studied by Brigatti et al. (1999), Bebié and Schoonen (2000) and Basiuk (2002), respectively. Brigatti et al. (1999) observed the formation of a stable chelate complex between cysteine and Cu^{2+}-rich-smectites. Bebié and Schoonen (2000) reported a strong interaction between pyrite and cysteine through its thiol group. Basiuk (2002) showed that the thiol group of cysteine decreases $\Delta G°$, and as consequence adsorption is increased when compared to other amino acids.

An experiment using HPLC was carried out to determinate if any cysteine could be recovered from the clays. The following amino acids were adsorbed on bentonite: cysteine, histidine and alanine. Alanine, histidine, and cysteine showed the following percentages of adsorption, 28.3, 40.0, and 100%, respectively. These results are in accordance with those obtained by Benetoli et al. (2007). Clays with amino acids adsorbed on were dried for 24 h at 40°C and after a solution of KCl 1.0 mol l^{-1} was added. The solutions were tumbled for 24 h and the amino acids were analyzed by HPLC. Amino acids were recovered from the clays in the following percentages: 110% for alanine, 73% for histidine, and 0% for cysteine. The chromatograms of HPLC did not show the characteristic peak of cysteine. This experiment is showing that amino acids histidine and alanine are being adsorbed by clays, but cysteine is reacting with clays.

Figure 6 shows the FT-IR spectra of bentonite, montmorillonite, thiourea adsorbed on bentonite (pH 8.00) and on montmorillonite (pH 8.00), lyophilized solution after adsorption of thiourea on clays (pH 8.00), clays after desorption by $CaCl_2$, lyophilized solution after desorption of thiourea on clays by $CaCl_2$, and solid thiourea. The bands observed in the samples thiourea adsorbed on bentonite (Fig. 6-A-b) and on montmorillonite (Fig. 6-B-b) and thiourea solution (Fig. 6-A-d, B-d) after adsorption, demonstrates that these spectra are not characteristic of thiourea (Fig. 6-A-f, B-f). The main bands at 1,622, 1,414, 1,085 and 732 cm^{-1} of the thiourea spectrum (Fig. 6-A-f, 6-B-f) were seen at 1,636, 1,433–1,388, 1,143–1,094 and 729 cm^{-1} in the spectra of lyophilized solution after thiourea adsorption (Fig. 6-A-d, 6-B-d) and thiourea adsorbed on bentonite (Fig. 6-A-b), and on montmorillonite (Fig. 6-B-b). The bands in the spectrum of thiourea at 1,469, 1,414, and 1,085 cm^{-1} can be attributed to stretching (N–C–N); rocking (NH_2), stretching (C=S) stretching (N–C–N); and stretching (C=S), respectively (Yamaguchi et al. 1958). The frequency at 1,469 cm^{-1} was the same as that observed in the spectra of thiourea adsorbed on clays and thiourea solution after adsorption, which could be an indication that adsorption occurred through the C=S bond.

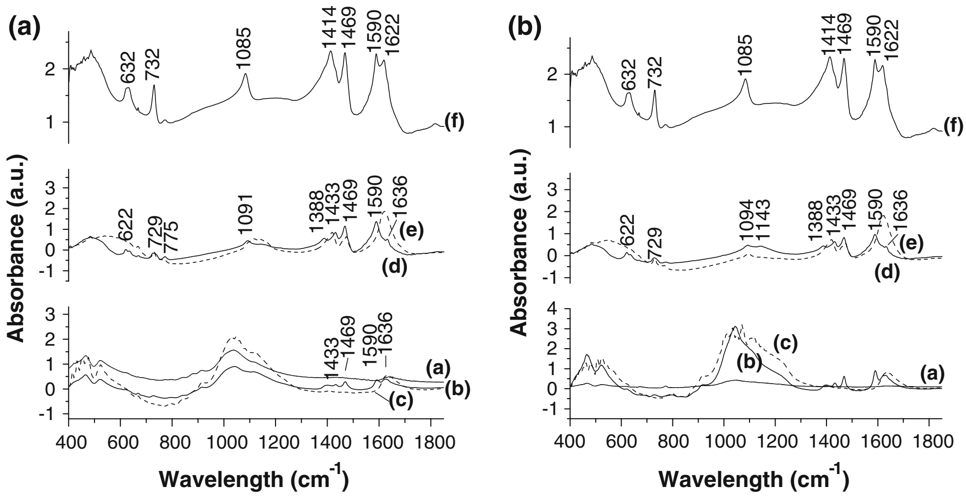


Fig. 6 FT-IR spectra: bentonite, 3-**A**–*a*; montmorilonite 3-**B**–*a*; thiourea adsorbed on bentonite, 3-**A**-*b* and on montmorilonite, 3-**B**–*b*; clays after desorption by $CaCl_2$ (0.20 mol l^{-1}), 3-**A**-*c* and 3-**B**–*c*; thiourea solution after adsorption (*lyophilized solid*), 3-**A**-*d* and 3-**B**–*d*; solution after desorption by ($CaCl_2$ 0.20 mol l^{-1}), 3-**A**-*e* and 3-**B**–*e*; solid thiourea, 3-**A**-*f* and 3-**B**–*f*. Clay samples were mixed for 24 h with seawater (pH 8.00) and thiourea was dissolved in seawater as a saturated solution (pH 8.00). The tube was spun for 15 min at 2,000 rpm; the aqueous phase was lyophilized and the solid was dried in an oven at 40°C for 24. A portion of the dried solid was transferred to a tube (15 ml) and 5.00 ml of $CaCl_2$ 0.20 mol l^{-1} was added. The tube was mixed for 24 h after it was spun for 15 min at 2,000 rpm; the aqueous phase was again lyophilized and the solid was dried in an oven at 40°C for 24

This notion was verified with the band at 1,085 shifted and duplicated at 1,143–1,094 cm^{-1} (Fig. 6-A-b, B-b, A-d, B-d). Another possibility is the formation of a C–S bond, due to a new band at 775 cm^{-1}, in the spectrum of thiourea solution after adsorption (Fig. 6-A-d, B-d), attributed to υ(C–S). The possible mechanism is the formation of c,c′-dithiodiformamidinium ion {$[NH_2(NH)CSSC(NH)NH_2]^{2+}$}. This reaction resembles that which occurred with adsorption of cysteine on clays, meaning the formation of cystine. Thus, the C=S bond present in thiourea had been changed due to an interaction with the clays and the excess of the compound $[NH_2(NH)CSSC(NH)NH_2]^{2+}$ that was formed could have diffused into the aqueous phase during the process. It also should be pointed out that the oxidation of thiourea with the formation of the compound $[NH_2(NH)CSSC(NH)NH_2]^{2+}$ is well known to occur on the surface of metal electrodes (Parker and Hope 2008; Yan et al. 1996). Again, the FT-IR spectra of these samples at pH 3.00 were not much different from those at pH 8.00, but the spectra were not well resolved probably because of the low concentration of $[NH_2(NH)CSSC(NH)NH_2]^{2+}$ formed. This result is in accordance with the Mössbauer spectroscopy results, where at pH 3.00 the amount of Fe^{2+} was low (Figs. 2, 3).

Figure 7 shows the FT-IR spectra of bentonite, montmorillonite, thiocyanate adsorbed on bentonite (pH 8.00) and on montmorillonite (pH 8.00), lyophilized solution after adsorption of thiocyanate on clays (pH 8.00), clays after desorption by $CaCl_2$, lyophilized solution after desorption of thiocyanate on clays by $CaCl_2$, and solid thiourea. The interaction of thiocyanate with bentonite resulted in several bands (Fig. 7-A-b) that were not observed in the interaction of thiocyanate with montmorillonite (Fig. 7-B-b). It should also be pointed out that the substance adsorbed on bentonite (Fig. 7-A-b) was totally removed by $CaCl_2$ as shown by the spectrum (Fig. 7-A-e) of this lyophilized solution. The band at 2,071 cm^{-1} is due to stretching (CN) (Fig. 7-A-c, A-d, A-f, B-b,c,d,e). Bron and Holze (1999) studied the adsorption of thiocyanate on gold electrodes, and they observed besides the band at 2,065 cm^{-1}, three other bands at 2,115, 2,145, and 2,168 cm^{-1}. They attributed the bands at 2,145 and 2,168 cm^{-1} to gold-thiocyanate complex and cyanate, respectively. Probably, the bands at 2,051, 2,058, 2,080 are due to the interaction of thiocyanate with metals (Fe^{3+}, Al^{3+}) in bentonite (Fig. 7-A-b) and the bands at 2,037, 2,083, and 2,106 cm^{-1} (Fig. 7-A-e) of lyophilized solution after desorption with $CaCl_2$ are due to interaction of thiocyanate and desorbed metals. However there is no explanation why the interaction of thiocyanate with bentonite was so different from that with montmorillonite. The FT-IR spectra of these samples at pH 3.00 were not different from those at pH 8.00, and this result is in accordance with the Mössbauer spectroscopy data (Figs. 2, 3).

In general, FT-IR spectra (Figs. 5, 6, 7) are showing that when cysteine, thiourea or thiocyanate is added to clays a reaction occurred. Adsorption will be occurring only when there is not Fe^{3+} left. The product of reaction between cysteine and thiourea and clays were cystine and c,c′-dithiodiformamidinium ion, respectively. The product of reaction between thiocyanate and clays could not be

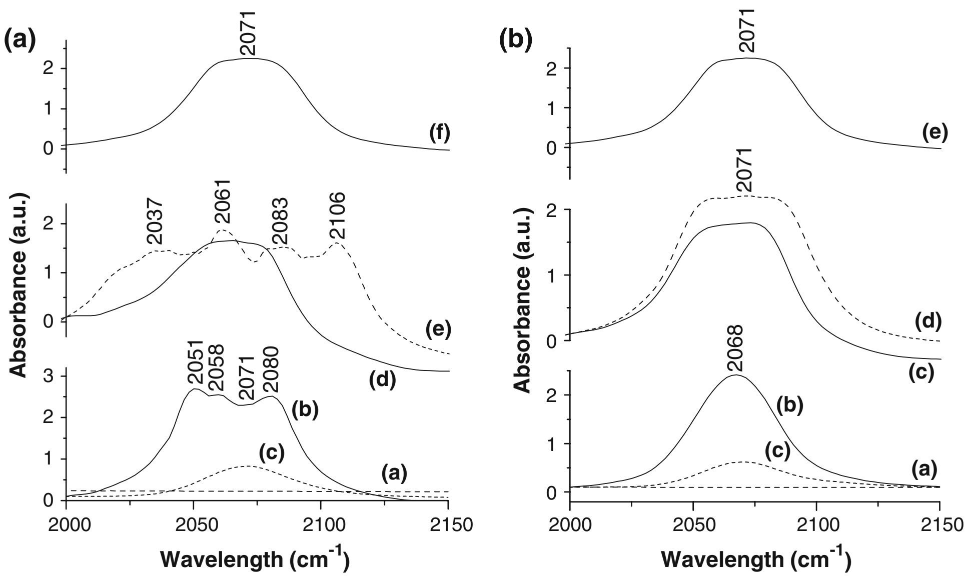


Fig. 7 FT-IR spectra: bentonite, 4-**A**–*a*; montmorilonite 4-**B**–*a*; thiocyanate adsorbed on bentonite, 4-**A**-*b* and on montmorilonite, 4-**B**–*b*; clays after desorption by CaCl$_2$ (0.20 mol L^{-1}), 4-**A**-*c* and 4-**B**–*c*; thiocyanate solution after adsorption (lyophilized solid), 4-**A**-*d* and 4-**B**–*d*; solution after desorption by CaCl$_2$ (0.20 mol l^{-1}), 4-**A**-*e* and 4-**B**–*e*; solid thiocyanate, 4-**A**-*f* and 4-**B**–*f*. Clay samples were mixed for 24 h with seawater (pH 8.00) and thiocyanate was dissolved in seawater as a saturated solution (pH 8.00). The tube was spun for 15 min at 2,000 rpm; the aqueous phase was lyophilized and the solid was dried in an oven at 40°C for 24. A portion of the dried solid was transferred to the tubes (15 ml) and 5.00 ml of CaCl$_2$ 0.20 mol l^{-1} was added. The tube was mixed for 24 h after it was spun for 15 min at 2,000 rpm; the aqueous phase was again lyophilized and the solid was dried in an oven at 40°C for 24

identified. The experiment using HPLC also showed that cysteine could not be desorbed from bentonite. The characteristic peak of cysteine was not showed in the chromatograms.

Conclusion

X-ray diffraction showed that at pH 8.00 there was an increase in expansion for both minerals with thiol compounds in the following order: thiourea > thiocyanate > cysteine. At pH 3.00, the same order was not observed and thiourea had an opposite behavior, where it produced the lowest expansion.

Mössbauer spectroscopy showed that at pH 3.00, cysteine and thiourea did not change significantly the relative amounts of Fe^{2+} and Fe^{3+} ions, in comparison with the clays without adsorption. However, at pH 8.00, the proportion of Fe^{2+} ions in bentonite increased, doubling with thiourea, or more than doubling with cysteine, in both clays. For thiocyanate, the amount of Fe^{2+} produced was independent of the pH and clay used, probably because the interlayers of clays are very acidic and the HSCN formed did not reduce Fe^{3+} to Fe^{2+}.

EPR spectroscopy showed that at pH 3.00 for the samples of bentonite with and without cysteine, the intensity of lines due to Fe^{3+} did not change. For the samples of montmorillonite and bentonite at pH 8.00 with cysteine, the intensity of the lines due to Fe^{3+} decreased when compared to the samples without cysteine. The same was observed for the samples of montmorillonite at pH 3.00.

FT-IR spectroscopy showed that the interaction of cysteine and thiourea with the clays produced cystine and c,c′-dithiodiformamidinium ion, respectively. In the interaction of thiocyanate with the clays, any possible compound formed could not be identified.

Acknowledgments CMDS acknowledges the fellowship from PIBIC/CNPq/UEL. This research was supported by grants from CNPq (473076/2004) and Fundação Araucária (2421). Dr. A. Leyva provided English editing of the manuscript.

References

Alia MP, Matysik J (2001) Effect of proline on the production of singlet oxygen. Amino Acids 21:195–200

Augusto O, Vaz SM (2007) EPR spin-trapping of protein radicals to investigate biological oxidative mechanisms. Amino Acids 32:535–542

Basiuk VA (2002) Adsorption of biomolecules on silica. In: Somassundaran P (ed) Encyclopedia of surface and colloid science. Marcell-Dekker, New York, p 277

Bebié J, Schoonen MAA (2000) Pyrite surface interaction with selected organic aqueous species under anoxic conditions. Geochem Trans 1:47–53

Benetoli LOB, de Souza CMD, da Silva KL, de Souza IG Jr, de Santana H, Paesano A Jr, da Costa ACS, Zaia CTBV, Zaia DAM

(2007) Amino acid interaction with and adsorption on clays: FT-IR and Mössbauer spectrometry and X-ray diffractometry investigations. Orig Life Evol Biosph 37:479–493

Benincasa E, Brigatti MF, Lugli C, Médici L, Poppi L (2000) Interaction between glycine and Na–, Ca–, and Cu-rich smectites. Clay Miner 35:635–641

Bernal JD (1951) The physical basis of life. Routledge and Kegan Paul, London

Bittner S, Gorohovsky S, Lozinsky E, Shames AI (2000) EPR study of radicals of various N-quinonyl amino acids. Amino Acids 19:439–449

Brigatti MF, Lugli C, Montorsi S, Poppi L (1999) Effects of exchange cations and layer-charge location on cysteine retention by smectites. Clays Clay Miner 47:664–671

Brigatti MF, Malferrari D, Médici L, Poppi L (2003) Effect of amino acids on the retention of copper by beidellite. Environ Eng Sci 20:601–606

Bron M, Holze R (1999) The adsorption of thiocyanate ions at gold electrodes from an alkaline electrolyte solution: a combined in situ infrared and Raman spectroscopic study. Electrochim Acta 45:1121–1126

Cowan JA (1993) Inorganic biochemistry an introduction. VCH, New York, pp 154–155

Darnell J, Lodish H, Baltimore D (1990) Molecular cell biology. Scientific American Books, New York, p 48

Dowler MJ, Ingmanson DE (1979) Thiocyanate in Red Sea brine and its implications. Nature 279:552

Fan JP, Kim HS, Han GD (2009) Induction of apoptosis by L-carnitine through regulation of two main pathways in Hepa1c1c7 cells. Amino Acids 36:365–372

Garcia G, Rodríguez JL, Lacconi GI, Pastor E (2004) Spectroscopic investigation of the adsorption and oxidation of thiourea on polycrystalline Au and Au(III) in acidic media. Languimuir 20:8773–8780

Greenland DJ, Laby RH, Quirk JP (1965) Adsorption of amino acids and peptides by montmorillonite and illite. Part 1: cation exchange and proton transfer. Trans Faraday Soc 61:2013–2023

Hazen RM (2006) Mineral surfaces and the prebiotic selection and organization of biomolecules. Am Miner 91:1715–1729

Lahav N, Chang S (1976) The possible role of solid surface area in condensation reactions during chemical evolution: reevalution. J Mol Evol 8:357–380

Lambert JF (2008) Adsorption and polymerization of amino acids on mineral surfaces: a review. Orig Life Evol Biosph 38:211–242

Martin-Neto L, Nascimento OR, Talamoni J, Poppi NR (1991) EPR of micronutrients-humic substances complexes extracted from Brazilian soil. Soil Sci 151:369–376

McBride M (1978) Transition metal bonding in humic acid: an ESR study. Soil Sci 126:200–209

Ng FTT, Henry PM (1975) Kinetics and mechanism of the oxidation of thiocyanate by tris(1, 10-phenathroline) iron (III) and its derivates. Can J Chem 53:3319–3326

Parker GK, Hope GA (2008) Spectroelectrochemical investigations of gold leaching in thiourea media. Miner Eng 21:489–500

Raulin F, Toupance G (1977) The role of sulphur in chemical evolution. J Mol Evol 9:329–338

Senesi N (1990) Molecular and quantitative aspects of the chemistry of fulvic acids and its interactions with metals ions and organic chemicals. Part I: the electron spin resonance approach. Anal Chim Acta 232:51–75

Siffert B, Kessaissia S (1978) Contribution au mecanisme d'adsorption des α-amino-acides par la montmorillonite. Clay Miner 13:255–270

Sposito G (1984) The surface chemistry of soils. Oxford University Press, New York

Talreja ST, Oza PM, Rao PS (1967) Radiation induced conversion of ammonium thiocyanate to thiourea. Bull Chem Soc Jpn 40:2427–2428

Wei M, Shi Z, Evans DG, Duan X (2006) Study on the intercalation and interlayer oxidation transformation of L-cysteine in a confined region of layered double hydroxides. J Mat Chem 16:2102–2109

Wen Z, Li M, Li Y, Guo Y, Wang K (2007) Delaunay triangulation with partial least squares projection to latent structures: a model for G-protein coupled receptors classification and fast structure recognition. Amino Acids 32:277–283

Yamaguchi A, Penland RB, Mizushima S, Lane TJ, Curran C, Quagliano JV (1958) Infrared absorption spectra of inorganic coordination complexes. XIV: infrared studies of some metal thiourea complexes. J Am Chem Soc 80:527–529

Yan M, Liu K, Jiang Z (1996) Electrochemical oxidation of thiourea studied by in situ FTIR spectroscopy. J Electroanal Chem 408:225–229

Zaia DAM (2004) A review of adsorption of amino acids on minerals: was it important for origin of life? Amino Acids 27:113–118

Zaia DAM, de Santana H, Toppan R, Zaia CTBV (2004) Synthesis of guanidine from ammonium thiocyanate in solid state. J Braz Chem Soc 15:190–198

Zaia DAM, Zaia CTBV, de Santana H (2008) Which amino acids should be used in prebiotic chemistry studies? Orig Life Evol Biosph 38:469–488