When RNA meets montmorillonite: Influence of the pH and divalent cations

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Abstract

A key question for the investigation of the origins of life is to understand the interaction between complex organic molecules and minerals. In this general frame, the present study investigated the nature of the interactions at the molecular level between a ribonucleic acid and ion-exchanged montmorillonites. We observed that the formation of RNA/clay mineral complexes was strongly pH-dependent. In a sufficiently acidic medium, the RNA molecules were intercalated in the interlayer space, in a flat-lying conformation, with a large contribution of electrostatic interactions that may be complemented by hydrogen bonds. The secondary structure of the RNA strands was strongly affected. The presence of different cations such as Na^+, Ca^{2+}, Mg^{2+}, and Sr^{2+} influenced the adsorption of organic molecules. Apparently, metal cations directly took part in the formation of bridges between the negative charges on the mineral surface and the phosphate groups of the biomolecule.

Keywords: Ribonucleic acid, Clay minerals, Montmorillonite, Prebiotic chemistry.

Introduction

Interactions between biomolecules and clay minerals are key for different fields like separations processes and hybrid nanomaterials. Among clay minerals, montmorillonites belong to the group of smectites with a 2:1 layer structure, negative surface charges (due to isomorphic substitutions), counterbalanced by cations present in the interlayer space, high internal surface area, cation exchange capacity and reactive groups on their surfaces, as well as the ability to confine molecules in the interlayer space (Jaber and Miehé-Brendlé, 2008; Wu et al., 2015; Escamilla-Roa et al., 2017). These intrinsic properties make them possible "hosts" for biomolecules such as RNA (James Cleaves et al., 2012; Ruiz-Mirazo et al., 2014).

The mechanisms of nitrogen bases, nucleotides and RNA/montmorillonite interactions have been reviewed and both electrostatic interactions and hydrogen bonding were found to play a role (Yu et al., 2013). Some studies suggested the participation of the interlayer cations as a “bridge” for interaction between the negatively charged clay surface and the phosphate of the RNA, which also bears a negative charge (Franchi et al., 2003; Pedreira-Segade et al., 2018; Hao et al., 2019). Alkaline earth metal cations have been observed to strongly enhance the adsorption of single-strand (but not double-strand) RNA to montmorillonite (Gujjari et al., 2018). Some studies suggest the likely presence of these metallic cations in rocks and in the primitive ocean (Eder and Rode, 1994; Izawa et al., 2010). They are important for the folding, structure and function of RNA, especially the divalent ion Mg^{2+} (Pyle, 2002; Leonarski et al., 2017), and their involvement has been suggested in the stabilization and/or activation of biological macromolecules (Black et al., 1994). Villafañe-Barajas et al. have also underlined the importance of added cations for the adsorption of nucleotides, the monomers of RNA; and

molecular modelling studies (Carrascoza et al., 2019) suggest that they could play a significant role in the reactivity of adsorbed organic molecules.

pH is a key parameter that controls the adsorption of biomolecules, since acido-basic speciation determines the electrostatic interaction between RNA and the surface (Theng et al., 1982; Lawless et al., 1985). Some studies in recent years have addressed the interaction of nitrogen bases (Benetoli et al., 2008; Hashizume et al., 2010; Carneiro et al., 2011), nucleotides (Feuillie et al., 2013; Pedreira-Segade et al., 2016; Hao et al., 2019) and less often nucleosides (Fornaro et al., 2018) with montmorillonite, varying the pH and the nature of metal cations, and trying to elucidate the types of interactions between these molecules and montmorillonite. However, the interactions of polymeric RNA with the surface of clay minerals remain understudied, especially at the molecular level. In this perspective, the aim of this study was to evaluate the adsorption of RNA (ribonucleic acid diethylaminoethanol salt) on montmorillonite in order to contribute to the understanding of a bigger puzzle, the origin of life. The organic-inorganic interactions were investigated at the molecular level and the influence of different parameters such as nature of the interlayer cation (Na^+, Ca^{2+}, Mg^{2+}, and Sr^{2+}) and the pH was evaluated.

2. Materials and Methods

2.1. Chemicals

Ribonucleic acid diethylaminoethanol salt (diethylethanolammonium; ribonucleic acid derived from Torula yeast, CAS 63231-63-0, Sigma-Aldrich), hydrofluoric acid (HF, 40% w/w, Fluka), sodium acetate (CH_3COONa, 99%, Sigma-Aldrich), magnesium acetate ($(CH_3COO)_2Mg.\ 4H_2O$, 99%, Sigma-Aldrich), Al_2O_3 (98%, Pural-Sasol), and SiO_2 (99.8%,

Aerosil 130, Evonik Industries). The others chemicals were purchased from Sigma-Aldrich (analytical grade). All chemicals were used without any previous purification.

2.2. Synthesis of montmorillonite

The sodium montmorillonite (Na-Mt) used in this work was synthesized according to the procedure described previously (Jaber and Miehé-Brendlé, 2005; Jaber and Miehé-Brendlé, 2008; Trigueiro et al., 2018b, 2018a) and had the ideal formula per half unit cell $Na_{0.2}[Si_4Al_{1.8}Mg_{0.2}O_{10}(OH,F)_2]$. The reagents were mixed in the following order: 196.860 g of deionized water, 2.410 g of hydrofluoric acid, 0.990 g of sodium acetate, 2.587 g of magnesium acetate, 3.325 g of alumina and 7.249 g of silica. The obtained hydrogel was aged under stirring at room temperature for 2 h and then was autoclaved at 220°C for 72 h. The autoclave was cooled to room temperature and the product was thoroughly washed with distilled water and centrifuged. Finally, the synthesized Na-Mt was dried at 50°C for 72 h. The synthesis conditions had previously been optimized for the crystallization of pure montmorillonite. The Al/Mg ratio influenced the preferential location of Al^{3+} in the tetrahedral sheets. The initial pH of the hydrogel, around 5, determined the di- or tri-octahedral character of the obtained phyllosilicate and the Si/F ratio as well as the synthesis time influenced the crystallinity of pure montmorillonite (Reinholdt et al., 2001, 2005).

2.3. Ion exchange reaction

The ion exchange of Na-Mt was performed according to previously published procedures (Lepoitevin et al., 2014). Na-Mt was suspended in 1 mol L^{-1} of $CaCl_2.2H_2O$, $MgCl_2.6H_2O$ or $Sr(NO_3)_2$ solutions for 24 h under magnetic stirring at 25°C. Then, the obtained materials were centrifuged, washed in deionized water and dried at 50°C. The same process was repeated twice. Finally, the solids were washed and dried in the same conditions. The exchanged montmorillonites will be designated as Ca-Mt, Mg-Mt, and Sr-Mt.

2.4. Adsorption of RNA salt on montmorillonite

The adsorption of RNA on the exchanged montmorillonites was performed according to a procedure published previously (Greaves and Wilson, 1969) with some modifications. Initially, the clay mineral samples (Na-Mt, Ca-Mt, Mg-Mt and Sr-Mt) were dispersed in water at a 1.25 mg mL^{-1} concentration over a period of 4 h under magnetic stirring at 25°C. In all cases, the natural pH of all clay mineral dispersions was around 5.0. Then, 1 mg mL^{-1} of RNA solution (pH 6.8) was slowly added. The RNA/Mt ratio was 2:1 w/w for all systems. After this step, the pH of all systems was between 6.2-6.5 without pH adjustment. The system was kept under magnetic stirring for 16 h at 25°C. The same procedure was also performed for all systems at pH 2.9-3.1, adjusted with 0.5 mol L^{-1} of the HCl solution. The resulting samples were centrifuged, washed with deionized water and dried at 50°C overnight. As a control, Na-Mt was maintained at pH 3.0 without RNA in the same conditions and analyzed after the reaction.

2.5. Characterizations

X-ray diffraction (XRD): powder X-ray diffraction was carried out on a Shimadzu XRD-6000 model diffractometer equipped with a monochromatic CuKα (λ = 0.15406 nm) source operating at 2 kVA, 40 kV, and 30 mA. The diffraction patterns were recorded from 3 to 80° with a scan rate of 0.5° min^{-1} in the same conditions as for previous works (França et al., 2020; da Silva et al., 2021).

Scanning electron microscopy (SEM): Scanning electron microscopy was performed using a FEI/Thermo Fischer, model Quanta FEG 250 with cannon per field and an acceleration voltage of 1 to 30 kV. Samples were fixed on carbon tape and coated with gold in a Quorum model Q150R sputter-coater for 35 s at 20 mA by a plasma generated under an argon atmosphere.

Fourier transform infrared spectroscopy: the FTIR spectra of samples - dispersed in KBr pellets containing approximately 1% (w/w) of the sample - were recorded using a Shimadzu PESTIGE – 21 model spectrometer over the 4000 – 400 cm^{-1} range at a resolution of 4 cm^{-1} and with 30 scans for each run.

Thermal analyses (TG-DTG): Thermogravimetric analyses and derivate thermogravimetry were performed using equipment from TA Instruments SDT Q600 model. The samples were heated from 20 to 1000°C in an alumina crucible with a heating rate of 10°C min^{-1} under nitrogen flow.

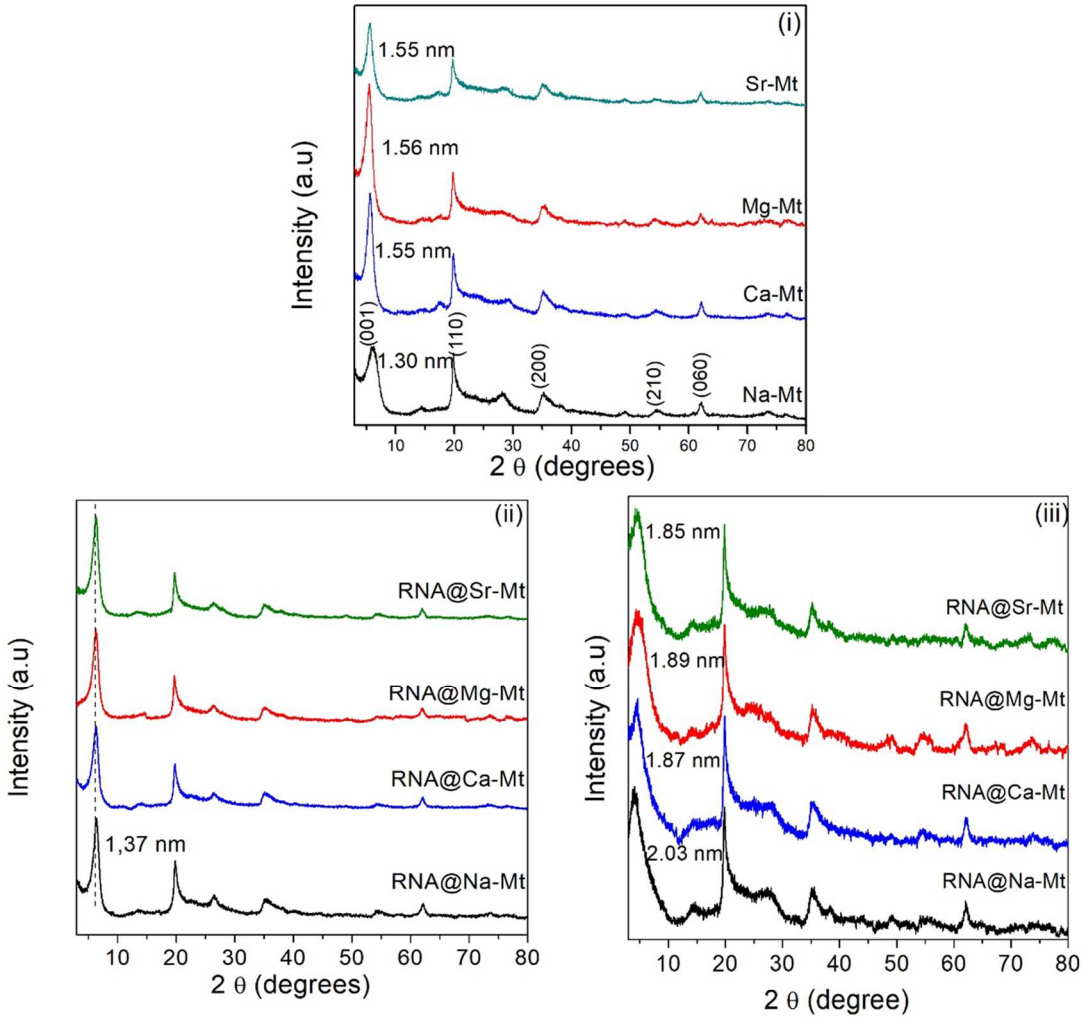
Solid state nuclear magnetic resonance: ^{31}P, ^{13}C, and ^{27}Al MAS NMR spectra were obtained on a Bruker Avance III spectrometer equipped with a 4 mm H-X probe, operating at Larmor frequencies of 500.17 MHz (^{1}H), 202.47 MHz (^{31}P), 125.77 MHz (^{13}C) and 130.33 MHz (^{27}Al) based on previous studies (Guillermin et al., 2019; Rodrigues et al., 2019; Cavalcanti et al., 2021). Chemical shifts were calibrated using triphenylphosphine (19.64 ppm) for ^{31}P, the carboxyl signal of adamantane (38.52 ppm) for ^{13}C, and $AlNO_3$ (0 ppm) for ^{27}Al as external standards. All spectra were recorded with a spinning rate of 14 kHz. The ^{13}C Cross-Polarization spectra were acquired with a ramp-CP contact time of 2 ms and a 1.5 s recycle delay and with a ^{1}H decoupling spinal 64. The acquisition time was 40 ms. The number of scans to obtain the spectra varied depending on the S/N obtained for each sample. Spectra were processed with a zero-filling factor of 2 and with an exponential decay corresponding to a 50 Hz line broadening in the transformed spectra. Only spectra with the same line broadening are directly compared. ^{27}Al single-pulse spectra using a 30° pulse were recorded with 100 kHz spectral width and with a recycle delay of 500. ^{31}P experiments using a 90° pulse were acquired with a recycle delay of 5 s and with a ^{1}H decoupling spinal 64.

3. Results

3.1. XRD diffraction

XRD patterns of the sodium and exchanged montmorillonites and resulting RNA-Mt hybrids are presented in Figure 1. A reflection at 6.77° (2θ), corresponding to a d_{001}-value of 1.30 nm, was observed for Na-Mt. Other reflections were observed at 2θ = 19.91°, 35.31°, 54.71° and 62.13° and were indexed to (110) (0.445 nm), (200) (0.253 nm), (210) (0.167 nm) and (060) (0.149 nm) reticular planes, respectively (Jaber et al., 2014; Trigueiro et al., 2018a). The formation of the Na-Mt was monitored by SEM (Figure SM-1). Well-formed particles in typical plates morphology were observed without the presence of other phases.

For all ion-exchanged samples, the (001) reflections shifted up to 1.56 nm.



161

162 **Figure 1**:XRD patterns of (i) Na-Mt, Ca-Mt, Mg-Mt, and Sr-Mt montmorillonites and of the
163 RNA@cations-Mt hybrids prepared at (ii) pH 6.2-6.5 and (iii) pH 2.9-3.1.

164

165 After adsorption, the observed pH of the solutions varied between 6.2-6.5 and 2.9-3.1,
166 for systems without and with pH adjustment. Figure 1(ii) depicts the XRD patterns of
167 RNA@cations-Mt at pH 6.2-6.5 and 1(iii) pH 2.9-3.1. At pH 6.2-6.5, the d_{001}-value is 1.37
168 nm for all samples. At pH 2.9-3.1, in Figure 1(iii), the (001) reflection shifted to higher basal
169 distances: d_{001}-value= 2.03 nm, 1.87 nm, 1.89 nm, and 1.85 nm in RNA@Na-Mt, RNA@Ca-
170 Mt, RNA@Mg-Mt and RNA@Sr-Mt, respectively, corresponding to interlayer spacings in
171 the 0.9 nm range. In contrast, XRD (Figure SM-2) of the Na-Mt maintained in an acidic

medium (pH 3) without the presence of RNA molecules presented the same reflections as the original sample. In particular, the d_{001} value stayed at 1.30 nm and did not show any broadening, suggesting that no alteration in long-range order or formation of exfoliated phase occurred in the conditions of the synthesis.

3.2. Infrared spectroscopy

FTIR spectra of the solids before and after RNA adsorption are presented in Figure 2. Irrespective of the compensating cation, the spectra were similar with absorptions at 3638 cm^{-1} and 3400 cm^{-1} (broad) assigned to OH stretching of structural OH and water, respectively (Bizovská et al., 2018). Bands at 1640 and 922 cm^{-1} were attributed to water deformation, and to the OH bending bands in Al_2OH, respectively (Madejová et al., 2017).

Absorptions at 1120 cm^{-1} and 1046 cm^{-1} were assigned to Si-O-Si asymmetric and symmetric stretchings, respectively, in the silicate matrix (Bizovská et al., 2018). Two bands at 790 cm^{-1} and 622 cm^{-1} were associated with Al-O-Si bending and to Al-O out-of-plane and Si-O vibrations (Madejová et al., 2017). Furthermore, Al-O-Si and Si-O-Si bending modes were observed at 528 and 471 cm^{-1}, respectively (Pentrák et al., 2018). If amorphous silica was present, it would be detected by absorption bands at 800 - 810 cm^{-1} (Costa et al., 1997; Pentrák et al., 2012; Santos et al., 2015); they were not observed in present case.

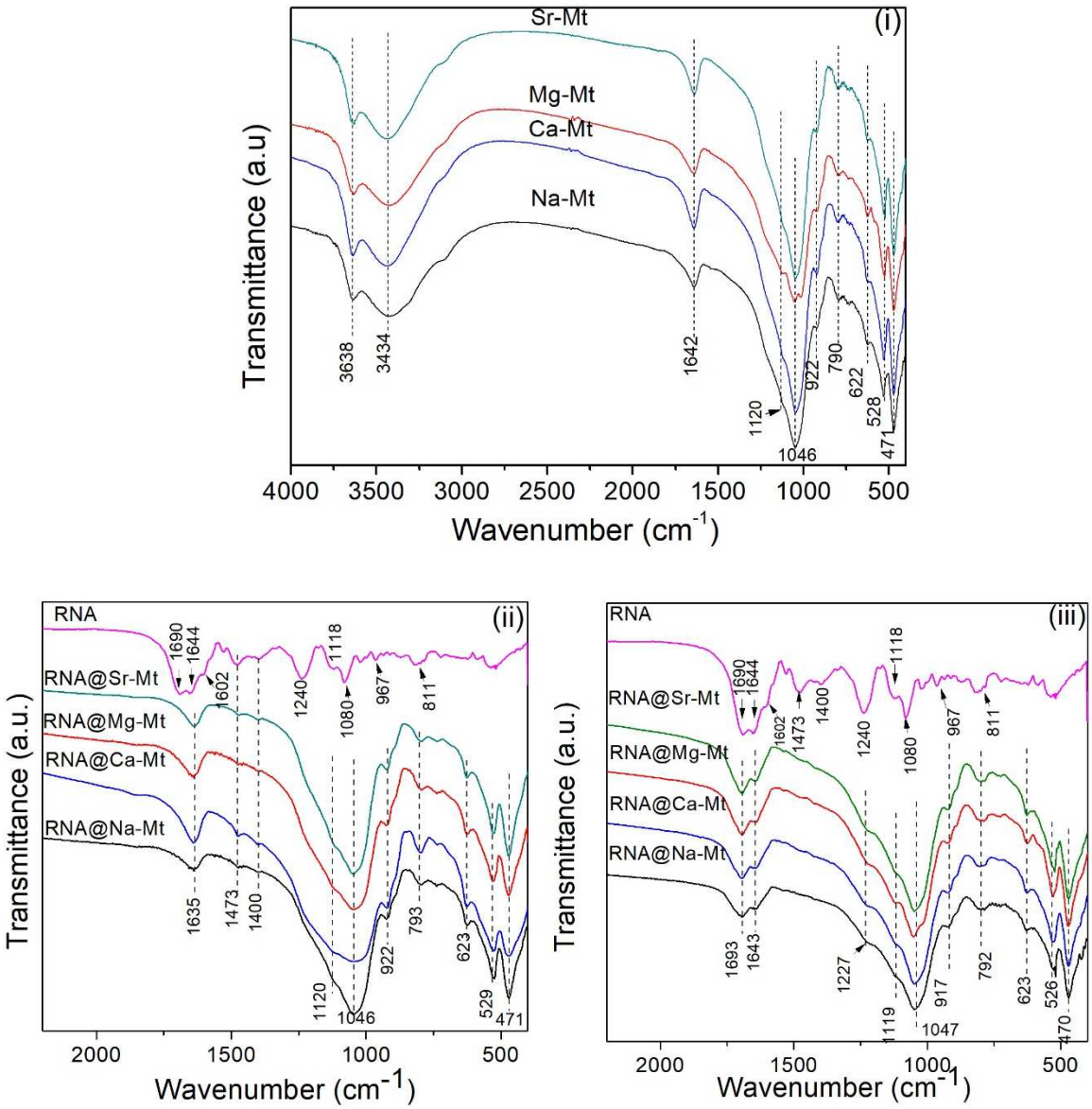


Figure 2. FTIR spectra for (i) Na-Mt, Ca-Mt, Mg-Mt and Sr-Mt montmorillonites, and spectra in range 2200 – 400 cm^{-1} for RNA and RNA@cations-Mt hybrids obtained at (ii) pH 6.2-6.5 and (iii) pH 2.9-3.1.

Figure 2ii and iii shows FTIR spectra in the 400-2000 cm^{-1} range of RNA and solids after RNA adsorption. FTIR spectra in the complete 400-4000 cm^{-1} range are presented in Figures SM-2i and ii. The spectrum of RNA presents a band at 3387 cm^{-1} (Figure SM-2i and ii) associated with the N-H stretch and one at 2954 cm^{-1} due to the C-H stretch (Tsuboi, 1970). The bands at 1690, 1644 and 1602 cm^{-1} were associated with the C=O vibration on

200 purine and pyrimidines (Tsuboi, 1970; Banyay et al., 2003). The absorption band at 1473 cm^{-1}
201 has been attributed to ring vibrations of the uracil base (Kumar and Singh, 2013) and the one
202 at 1400 cm^{-1} was attributed to in-plane C-O-H deformation mode, at 2' in the ribose ring
203 (Tsuboi, 1970; Banyay et al., 2003). The bands at 1240 and 1080 cm^{-1} were attributed to
204 asymmetrical and symmetrical PO_2^- stretch, respectively (Tsuboi, 1970; Benedetti et al., 1997)
205 and the band at 1118 cm^{-1} to the C-O stretch in ribose (Benedetti et al., 1997). Finally, the
206 band at 967 cm^{-1} is an unidentified ribose-phosphate main chain vibrations, and that at 811
207 cm^{-1} is a sugar marker of complex origin (Tsuboi, 1970; Banyay et al., 2003).

208 The spectra of the solids after RNA adsorption in the 400-2000 cm^{-1} range are
209 depicted in Figures 2ii and iii. At pH 6.2-6.5 (Figure 2ii), similar new bands were observed in
210 the FTIR spectra of the solids, both at the same position as in the spectra of RNA: 1473 cm^{-1}
211 (ring vibrations) and 1400 cm^{-1} (in-plane C-O-H deformation of ribose). Other diagnostic
212 bands of RNA may be present as shoulders, but are hidden by the strong absorption of the
213 clay mineral lattice in the 900-1200 cm^{-1} region.

214 Infrared spectra of the hybrids obtained in an acidic medium (Figure 2iii) showed
215 different additional bands at 1693 cm^{-1} (C=O stretching in nucleobases). The band of PO_2^-,
216 observed at 1240 cm^{-1} in RNA spectra (Figure 3), shifted to 1227 cm^{-1} in the RNA@cations-
217 Mt.

218 Bathochromic shifts of the bands attributed to the structural OH of the clay mineral
219 lattice (cations-Mt: 3638 cm^{-1}, RNA@cations-Mt at pH 6.2-6.5: 3632 cm^{-1} and
220 RNA@cations-Mt at pH 2.9-3.1: 3630 cm^{-1}), were observed (see Supplementary materials
221 Fig. SM-3i and ii).

3.3. ^{27}Al, ^{13}C and ^{31}P solid state NMR

^{27}Al NMR spectra for samples before and after adsorption of the biomolecule at pH 2.9-3.1 are shown in Figure 3i and ii. All precursor samples presented characteristic chemical shifts at 3.9 and 69.1 ppm. Another chemical shift was observed in both spectra at 55 ppm. The same chemical shifts were observed in the RNA@cations-Mt samples.

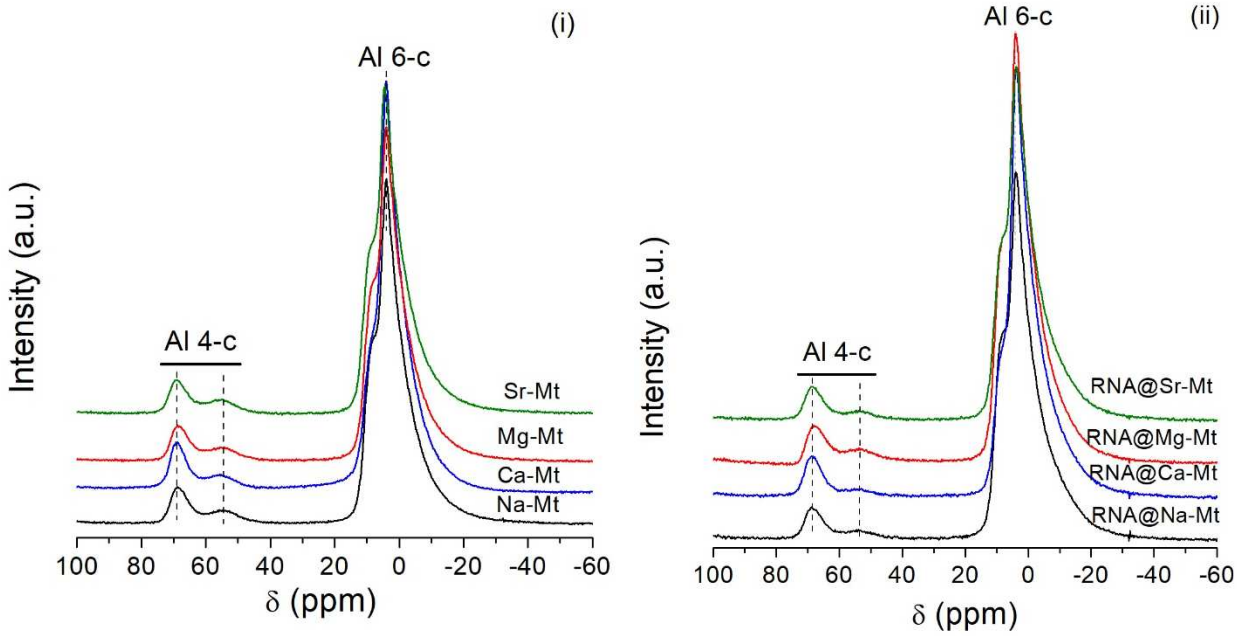
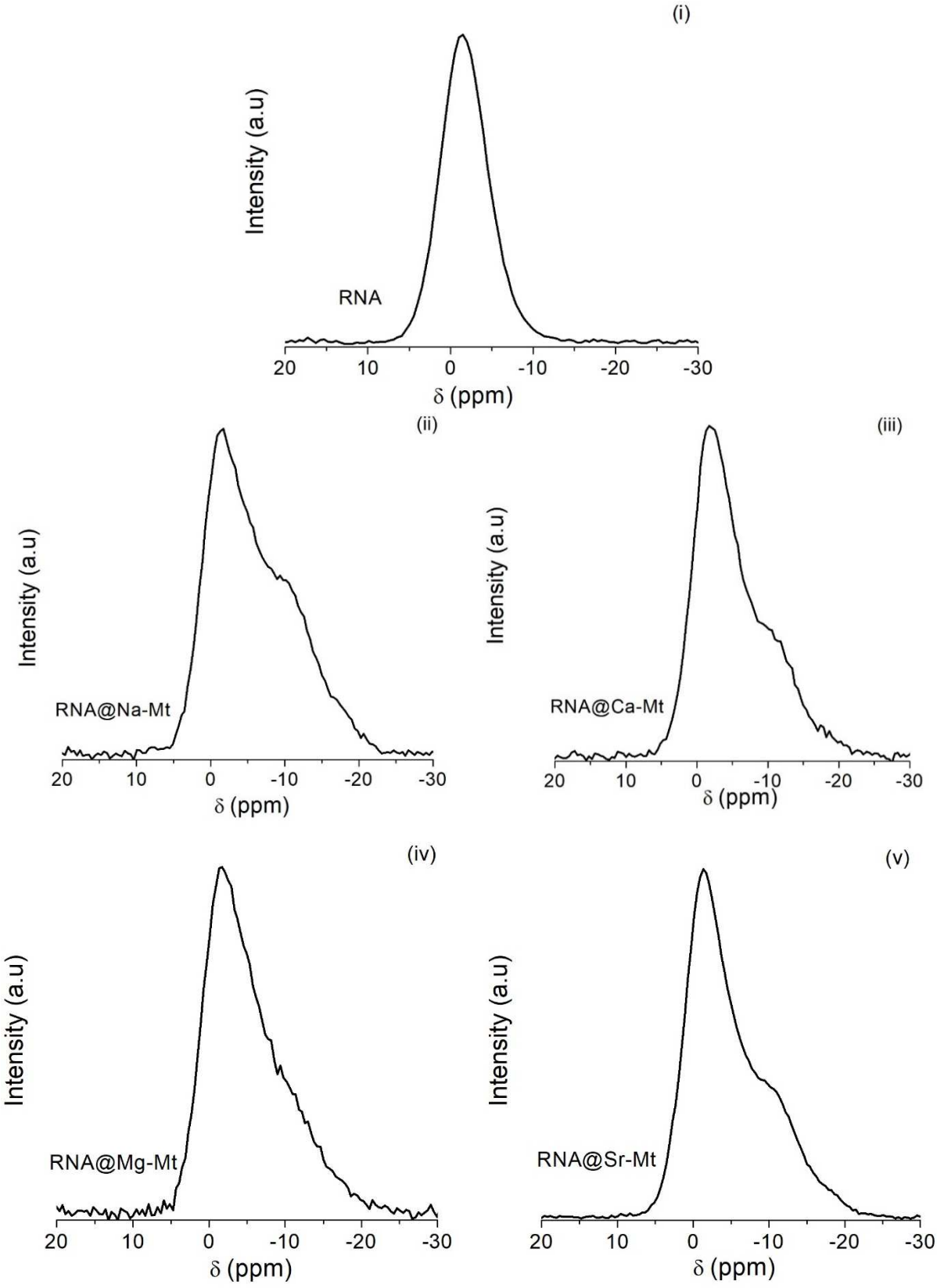


Figure 3. ^{27}Al NMR spectra of the (i) exchanged montmorillonites and (ii) RNA@Mt hybrids obtained at pH 2.9-3.1.

The ^{31}P spectrum of RNA is shown in Figure 4i. A broad, asymmetric signal is observed with a maximum at +1.4 ppm. For the RNA@cations-Mt samples, a strong new component appeared at -9.3 to -10.6 ppm, i.e., 8 to 9 ppm upfield from the signal in pristine RNA.



237 **Figure 4**. ^{31}P NMR spectra of RNA (i) and hybrids RNA@Na-Mt (ii), RNA@Ca-Mt (iii),
238 RNA@Mg-Mt (iv) and RNA@Sr-Mt (v) in pH 2.9-3.1.

239

240 ^{13}C NMR spectra of the RNA and the hybrids obtained at pH 2.9-3.1 are depicted in
241 Figure 5 and values of chemical shifts and respective attributions are summarized in Table 1.
242 The observed NMR signals for the RNA@Mt hybrids were similar.

243 The signals at 60 – 90 ppm were assigned to carbons in the ribose moiety (Chang et
244 al., 1982; Rodrigues et al., 2019). Signals in the 100 - 170 ppm range were attributed to the
245 nucleotides bases (Chang et al., 1982; Fujiwara et al., 1995). In particular, the signals of
246 carbon positions G_4 and A_4 were located at 150.3 ppm, and were assigned to C=C groups in
247 both nucleotides bases. This signal was also present in the RNA@Mt hybrids, but shifted to
248 151.5 ppm. The signal at 165.4 ppm associated with the carbon at position 4 in cytosine (-
249 (NH_2) C=N) group, shifted to 166.4, 166.6, 166.4, and 166.5 ppm, in the RNA@Na-Mt,
250 RNA@Ca-Mt, RNA@Mg-Mt, and RNA@Sr-Mt, respectively. The other well-individualized
251 signals did not shift as much.

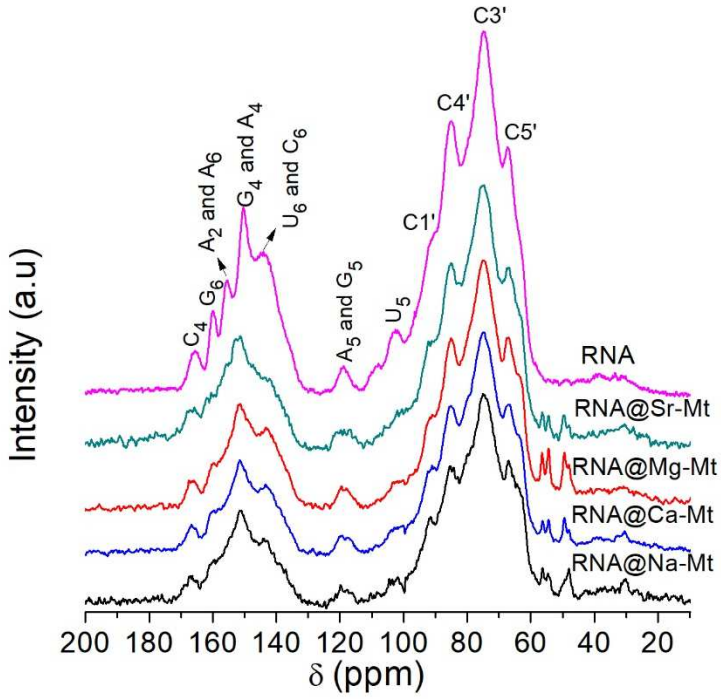


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Figure 5. ^{13}C NMR spectra of the RNA and RNA@Mt hybrids obtained at pH 2.9-3.1 (The symbol C' represents the carbons present in the ribose group and U_5, for example, represents the carbon 5 in the uracil nitrogen base).

Table 1. ^{13}C chemical shifts of RNA and RNA@clay hybrids in pH 2.9-3.1*.

RNA	RNA@Na-Mt	RNA@Ca-Mt	RNA@Mg-Mt	RNA@Sr-Mt	Attributions
67.2	67.0	67.1	67.1	67.1	C5'[a]
74.7	74.7	74.8	74.8	74.9	C3'[b]
85.0	85.0	85.0	85.0	85.0	C4'[a]
91.2	91.4	91.2	91.4	91.5	C1'[b]
102.5	102.7	102.6	102.6	102.4	U5[b]
119.0	119.0	119.0	119.0	119.0	A5 and G5[b]
144.1	143.9	144.0	143.9	143.8	C6 and U6[b,d]
150.3	151.5	151.5	151.5	151,5	G4 and A4[b]
155.4	-	-	-	-	A2 and A6[b,c]
159.8	159.7	159.5	159.5	160.0	G6[d]
165.4	166.4	166.6	166.4	166.5	C4[b]

**Chemical Shifts in ppm.* [a](Rodrigues et al., 2019); [b](Chang et al., 1982); [c](Fujiwara et al., 1995); [d](Lapper et al., 1973). *C' refers the carbons of the ribose group; A4, C4, U4 and G4 for example, refer to carbon 4 in the adenine, cytosine, uracil, and guanine nitrogen base, respectively.*

3.4. Thermogravimetry

TG and DTG curves of the solids before and after RNA adsorption at pH 2.9-3.1 are presented in Figure SM-3, and summarized in Table SM1. It was hoped that the amount of intercalated RNA could be estimated by integrating the weight losses after 200°C and correcting for the weight loss of the raw montmorillonite clay. Some thermal events attributable to the degradation of RNA are indeed apparent between 200 and 460°C, but organic matter elimination is not complete even at 1000°C, so that the amounts tabulated are to be interpreted as lower bounds. They appear to be only little sensitive to the nature of the compensation cation, or even to its charge.

4. Discussion

XRD diffraction

The XRD patterns of the sodium montmorillonite confirm the expected dioctahedral character of the sample according to the 2θ = 62.13° reflection, indexed (060) plane; in other words, they indicate the predominance of trivalent cations in the octahedral sheet of Na-Mt (Moore and Reynolds, 1997; Reinholdt et al., 2001, 2005). For the exchanged samples, the observation of the (001) reflections at 1.56 nm for all samples are compatible with a two-layer hydrate in the interlayer, as compared to a one-layer hydrate in the starting material (Cases et al., 1997; Cavalcante et al., 2016; Chang et al., 2018).

After RNA adsorption, there is no intercalation of bulky species in the interlayer space in the pH 6.2-6.5 systems, as the d_{001} still corresponds to a two-water layer hydrate. The possibility of intercalation strongly depends on the electrostatic interactions between the clay mineral layers (negatively charged) and the molecule to be intercalated. Individual nucleotides exhibit two or three acid-basic couples due to the protonation/deprotonation of nucleobase nitrogens and of the bridging phosphate P-OH (Martin, 1985; Feuillie et al., 2013). Deprotonation of the heterocycle NH group is possible for GMP and UMP nucleotides (pKa = 9.4 to 9.5) but this equilibrium is not active in our pH conditions. The nucleobases may be protonated in the cases of AMP (N1, pK_a = 3.84), CMP (N3, pK_a = 4.33) and GMP (N7, pK_a = 2.48) (Feuillie et al., 2015). This part of the nucleotides would then be mostly neutral at pH 6.2 to 6.5, while at pH 2.9 to 3.1, the majority of AMP and CMP groups, and a minority of the GMP (20 to 30%) would be protonated. Finally, the phosphate group has a pKa of the order of 0.5 and is always deprotonated in our conditions.

Thus, in a neutral medium, each nucleotide residue would bear a net negative charge of -1 and the intercalation of the biopolymer would be electrostatically disfavored because of

296 repulsive interactions with the negative clay mineral layers, since in all pH conditions,
297 montmorillonite has a negative charge (Saka and Güler, 2006; Yukselen and Erzin, 2008; Liu
298 et al., 2017; Şans et al., 2017; Guillermin et al., 2019). It is therefore likely that RNA
299 molecules at pH 6.2-6.5 are adsorbed on the external surface and edges of the clay mineral
300 samples (Mignon et al., 2009; Mignon and Sodupe, 2012). In addition, the cations (Na^{+}, Ca^{2+},
301 Mg^{2+} and Sr^{2+}) may be contributing to adsorption, by creating "ion bridges" between the
302 negative surface of montmorillonite and the phosphate groups present in RNA. A similar
303 mechanism was invoked for the adsorption of DNA in montmorillonite in the presence of
304 different cations (Sheng et al., 2019).

305 For the system at pH 2.9-3.1, higher basal reflections were observed for all samples,
306 indicating the intercalation of a rather large biomolecule in the interlayer space of the clay
307 mineral. The occurrence of intercalation is associated with the partial protonation of the
308 nitrogen bases in the RNA nucleotides at pH values lower than their pKa. With the pKa
309 values listed above, and supposing that the four possible nucleotide monomers have similar
310 abundances in the RNA chain, it is easy to calculate that at pH 2.9-3.1, the chain bears a
311 charge that is reduced by over one half with respect to pH 6.2-6.5 – about 0.48 negative
312 charges per monomer. In these conditions, electrostatic repulsion between the RNA chain and
313 the negatively charged surface is reduced, permitting intercalation. These interpretations are
314 compatible with other studies in RNA adsorption (Greaves and Wilson, 1969; Jelavić et al.,
315 2017) and also studies about the adsorption of the nitrogen bases (Carneiro et al., 2011) and
316 nucleotides (Pedreira-Segade et al., 2016) on montmorillonite. Similar results were observed
317 in the RNA intercalation in montmorillonite in an acidic medium (pH 5 to 3.5) (Greaves and
318 Wilson, 1969). Other studies observed no displacement of the basal plane in torula yeast
319 RNA/Mg-smectite (Viennet et al., 2020) and DNA/ion-exchanged Mt (Li^{+}, Na^{+}, Mg^{2+}, Ca^{2+},
320 Fe^{3+} and Al^{3+}) Mt (Xie et al., 2019)., but they were not conducted at low pH.

It is obvious that what is intercalated is not the intact torula yeast RNA, which has an intricate ternary structure and exists in solution as a Y-shaped molecule (Tripathi et al., 2017) with a 30 nm gyration radius (Manzano and Zydney, 2017). Indeed, a double strand of nucleic acid has a diameter of about 26 Å, and consequently, even a single strand lying in the interlayer with its nucleobases perpendicular to the clay layers would give an interlayer spacing of about 13 Å, significantly higher than the values we observe. This is why in the structural sketch of intercalated RNA we propose in Figure 6, the RNA strand is represented with the bases lying flat in the interlayer. This would involve a strong degree of RNA deformation; therefore, it is likely that the whole RNA molecule does not enter the interlayer. It is proposed that only fragments of the RNA chain are intercalated, while the rest of the molecule may be adsorbed on the surface or edges of the Mt. A similar adsorption mechanism has been documented for other biomacromolecules in Mt, such as enzymes (Sanjay and Sugunan, 2006; Gopinath and Sugunan, 2007).

Infrared spectroscopy

At both pHs, new bands were observed in the FTIR spectra of samples after the absorption of RNA. For example, at pH 6.2-6.5, bands assigned to ring vibrations and in-plane C-O-H deformation of ribose were observed. At pH 2.9-3.1, new bands assigned to C=O and PO_2^- stretching were apparent. Furthermore, changes in the position of the PO_2^- band were observed. This shift is probably associated with distortions in the RNA molecule, possibly caused by the interactions of cations with phosphate groups (Duguid et al., 1993; Andrushchenko and Bouř, 2009; Pedreira-Segade et al., 2018). It is likely that the clay/RNA interaction simultaneously includes several contributions from different local interactions such as H-bonds. It may be relevant from this point of view that the stretching vibration of the clay layer structural O-H shifts by a few cm^{-1}, which may be indicative of their implication in

H-bonding (Schnetzer et al., 2017), but a more precise study would be needed to identify H-bonds with confidence.

^{27}Al, ^{13}C and ^{31}P solid state NMR

^{27}Al NMR spectra in all precursor samples presented characteristic chemical shifts at 3.9 and 69.1 ppm related to hexacoordinated (Al 6-c) and tetracoordinated (Al 4-c) aluminum located in octahedral and tetrahedral sheets of the Mt, respectively (Cadars et al., 2012; Marsh et al., 2018). Another chemical shift corresponding to the Al 4-c environment was observed in both spectra at 55 ppm and can be attributed to small impurities of amorphous aluminosilicate, also observed in the literature for synthetic (Cadars et al., 2012) and natural (Garg and Skibsted, 2014) montmorillonites. In the RNA@cations-Mt samples (Figure 3ii), no change was observed with respect to cations-Mt, indicating the absence of interaction between the biomolecule and Al sites in the Mt.

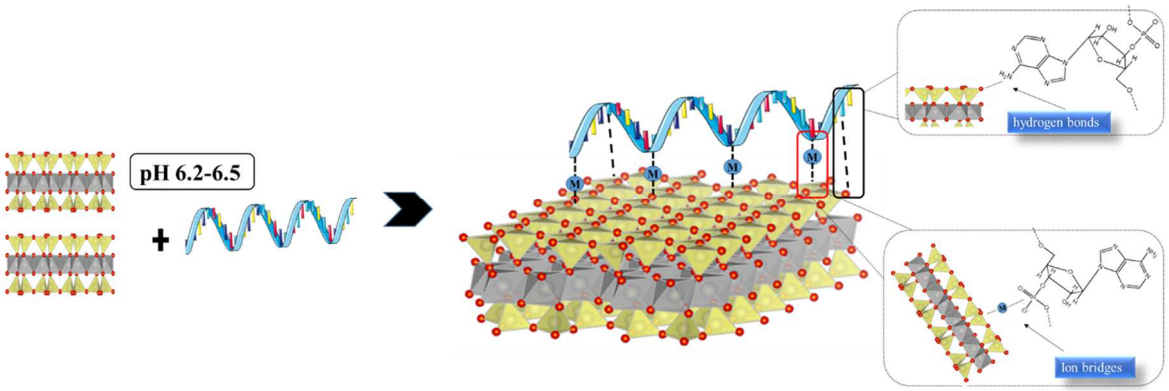
As regards the ^{31}P spectrum of RNA, Figure 4i, there are surprisingly few literature results available for comparison. Solution studies of short RNA sequences (Legault and Pardi, 1994; Maderia et al., 2000) revealed a series of narrow peaks in the (+2.0, -2.0) ppm range corresponding to all different phosphodiester bridges in the RNA chain. In the solid state, the loss of resolution may produce a signal such as the one we are observing. For the RNA@cations-Mt samples, a strong new component appeared. This finding is quite unexpected since the shift is comparable to the chemical shift difference separating the Q^0 signal in monophosphate from the Q^1 in diphosphate. The formation of diphosphate seems unlikely however, as it would require a breakdown and rearrangement of the RNA structure. Another possibility is that the new signal could correspond to the effect of the adsorption interaction on the bridging phosphate groups. Among likely interactions, only coordinative bonds to metal cations would be expected to result in such shifts. Intuitively, however, they

370 would also be expected to induce a decrease in electron density in the phosphate moiety, and
371 therefore a downfield shift (increase in chemical shift values) – the opposite of the effect
372 observed here. The already quoted study by Maderia et al (2000) indeed reports shifts of
373 several ppm when modified hairpin RNA is contacted with Cd^{2+} or Mg^{2+} cations. For
374 different RNA structures, they can be either upfield or downfield. The authors could not
375 provide any clear explanation for the upfield shifts; they supposed that the affected phosphate
376 groups did not directly coordinate to the cations, but were indirectly affected through changes
377 in RNA conformations. We will not speculate any further on the reasons for the new signal
378 observed here, except to say that it reveals a strong RNA/clay mineral interaction. One can
379 also note that in at least three of the four samples, there are indications of a third, even more
380 upfield shifted component in the ^{31}P spectrum; attempts at signal deconvolution necessitated
381 the introduction of a component at -14 to -17 ppm. The hypothesis of strong deformations of
382 the RNA chain is in line with the structural picture deduced from XRD.

383 The ^{13}C NMR spectra show chemical shifts values that are practically the same for
384 the different montmorillonites. This suggests that the nucleotide bases, mainly adenine and
385 cytosine, can be protonated in an acidic medium (pH 2.9-3.1). These results are in keeping
386 with calculations based on the pKa values. They corroborate the FTIR and XRD results,
387 indicating that the protonation of these moieties induces hydrogen bonds and electrostatic
388 interactions with the montmorillonite surface.

389 Figure 6 sketches a model of the RNA-clay composites that is compatible with the
390 information from XRD, IR and ^{13}C and ^{31}P NMR.

391



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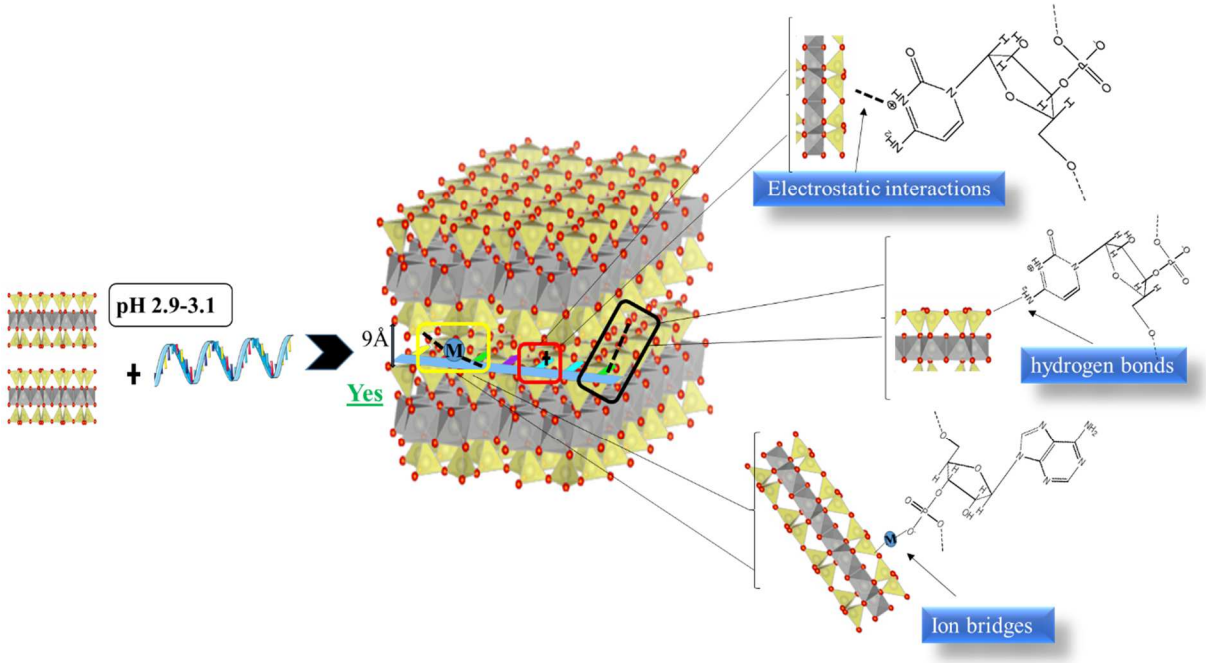
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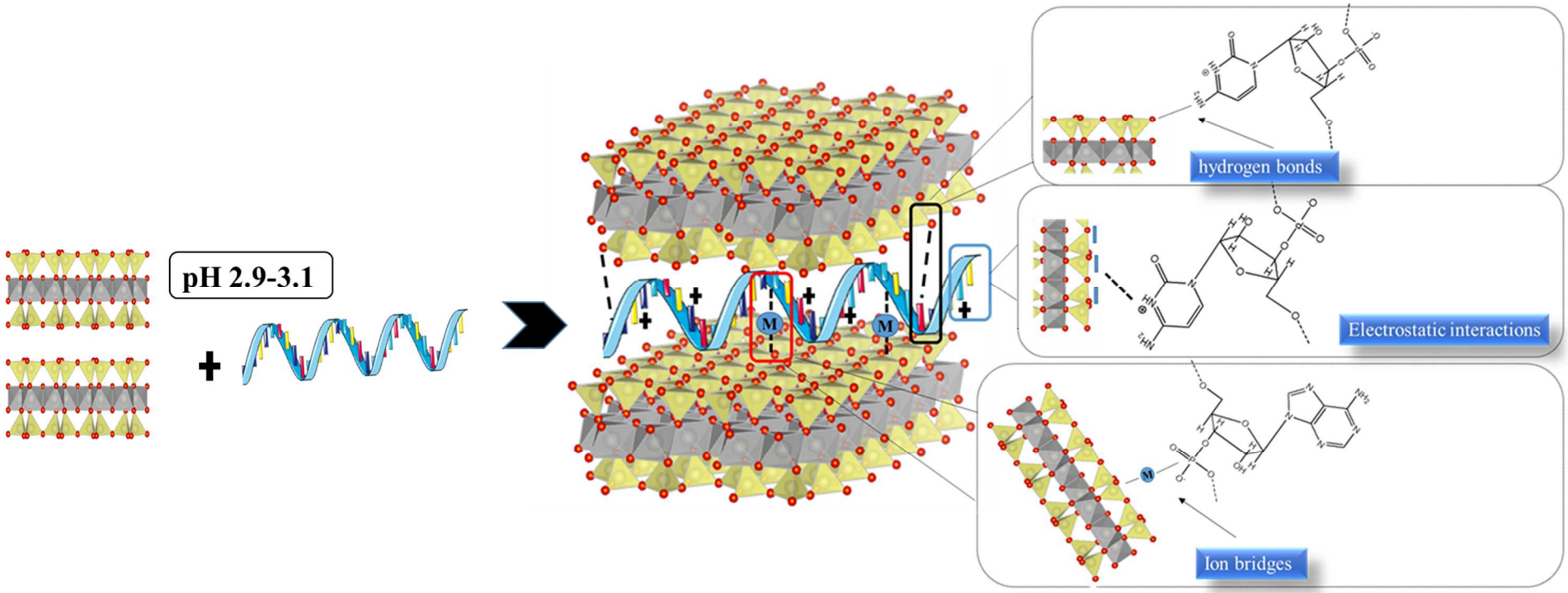
9Å
M
Yes
Electrostatic interactions
hydrogen bonds
Ion bridges

393



394

395 **Figure 6**. Illustrative scheme for proposed interactions between fragment of RNA and montmorillonite in neutral and acidic medium.

5. Conclusions

We have investigated the interaction of an RNA-derived molecule with synthetic montmorillonites having different cations in their interlayer space. The systems were prepared in both neutral and acidic media.

The interaction of the biomolecule with montmorillonites was pH-dependent: the intercalation of RNA in the interlayer space of montmorillonite was largely controlled by electrostatic interactions. At close to neutral pH, the RNA molecules have a significant negative charge and are prevented from entering the interlayer space by electrostatic repulsion from the clay mineral layers. It is likely that RNA is adsorbed on edge sites, maybe with compensating cations acting as ions bridges.

At lower pH values, extensive intercalation occurs, as evidenced by the value of basal spacing observed in XRD. Indeed, the nucleobases are then expected to be partly protonated, resulting in significantly lower negative charges on the RNA molecules. The driving force for intercalation could include contributions of localized electrostatic interactions (between the positively charged aromatic nuclei and the negatively charged clay mineral layers), probably accompanied by H-bonding, and also of coordinative bonding with the interlayer cations. Some authors have suggested that at pH close to 7, van der Waals interactions between the montmorillonite layers and the purine and pyrimidine bases of the nucleotides may also be acting (Kawamura and Ferris, 1999; Ferris, 2006).

Indeed, both FTIR and ^{13}C NMR indicate small but significant modifications of the ring carbon atoms local environment. FTIR also shows a rather important shift of the phosphate band. Taken together, these results suggest that both groups are involved in the interaction mechanism. ^{31}P NMR shows a surprisingly strong effect of adsorption on the

phosphates, but the direction of the shift does not seem compatible with a coordination to the electropositive compensating cations. Rather, it might be indicative of important configuration changes induced by the intercalation of the RNA backbone into a constrained space.

These results indicate that RNA oligomers and polymers may have interacted effectively with clay minerals, such as montmorillonite, in an acidic medium on the primitive Earth. Such an RNA/montmorillonite interaction may have stabilized and protected RNA molecules from an environment of likely hostile conditions on the primitive earth, such as high UV radiation.

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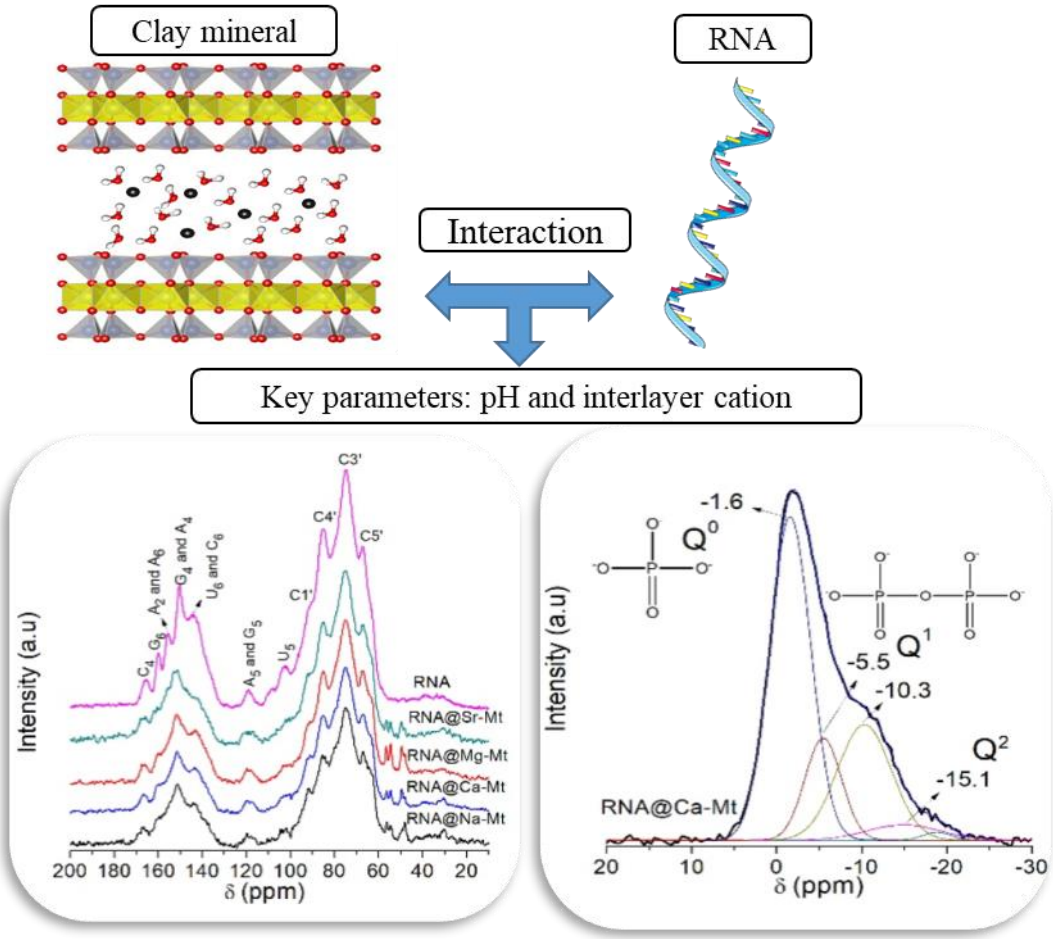

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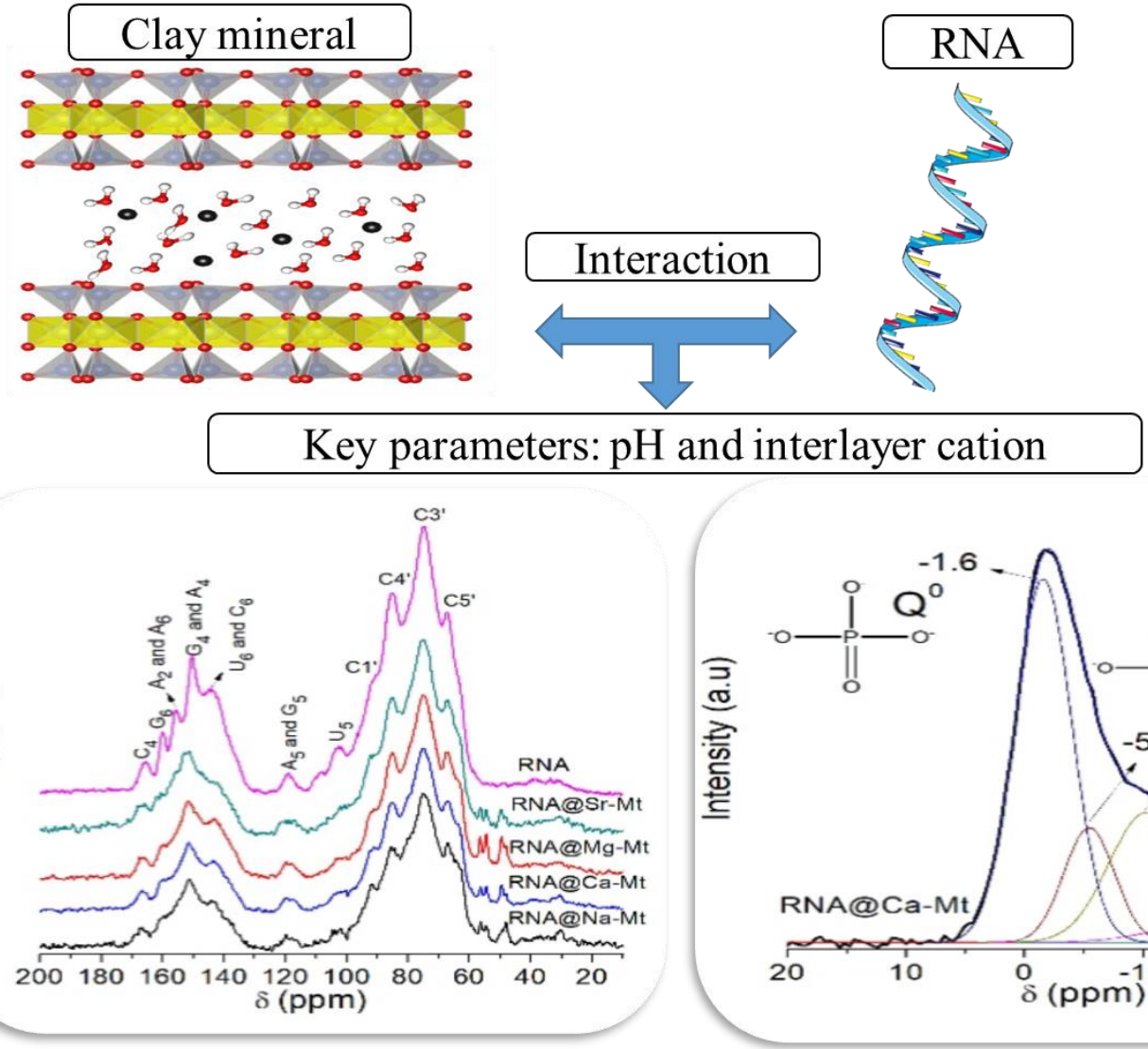
Clay mineral
RNA
Interaction
Key parameters: pH and interlayer cation
Intensity (a.u)
δ (ppm)
C3'
C4'
C5'
C1'
C4 G8 A2 and A6
G4 and A4
U6 and C6
A5 and G5
U5
RNA
RNA@Sr-Mt
RNA@Mg-Mt
RNA@Ca-Mt
RNA@Na-Mt
200 180 160 140 120 100 80 60 40 20
-1.6
Q0
Q1
Q2
-5.5
-10.3
-15.1
RNA@Ca-Mt
20 10 0 -10 -20 -30