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Adsorption of nucleic acid bases on clays: an investigation using *Langmuir* and *Freundlich* isotherms and FT-IR spectroscopy

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Abstract In the present paper, the adsorption of nucleic acid bases (*A*, adenine; *C*, cytosine; *U*, uracil; and *T*, thymine) on clays (bentonite, kaolinite, and montmorillonite) was studied at different *pH* (2.00 and 7.20). It should be pointed out there is no reported study of adsorption of nucleic acid bases on clays using seawater (with the major elements), and a wide range of *pH*. The main finding of this study was that the ratio of *A* and *T* adsorbed on clays ranged from 4.68 to 25.1, much higher than the ratio of their occurrence in organisms ranging from 0.95 to 1.05. The weaker adsorption of *U* and *T* on clays raises the question of the possibility of a genetic code based on purines only. The FT-IR spectra at *pH* 2.00 showed that the interaction of *A*, *C*, *T*, and *U* with the clays occurs through positively charged, protonated groups.

Keywords Adsorption; Clays; Nucleic acid bases.

Introduction

Bernal was the one that first suggested that minerals could overcome the problem of infinite dilution of key monomers in the sea of the primitive earth, by adsorbing them on. He also suggested that the minerals could be used as catalysts for the formation of polymers from the monomers [1].

Numerous studies on the adsorption of nucleic acid bases on several materials have been published, such as adenine and uracil on chelating resin [2, 3], adenine and purine on pyrite [4], nucleotides on clays and minerals [5–8], several nucleic acid bases on clays and minerals [9–15], and various nucleic acid bases on graphite [16–18]. In general, many of these papers have shown that guanine and adenine are adsorbed more than other nucleic acid bases. This means that the purine/pyrimidine ratio has often been found to be greater than one in adsorption studies with clays, whereas the ratio of purines to pyrimidines in organisms is always about 1.00 [19]. Thus, the weaker adsorption of pyrimidines on these materials raises the question of the possible early existence of a genetic code with one type of nucleic acid base. The present paper describes the adsorption of nucleic acid bases (*A*, adenine; *C*, cytosine; *U*, uracil; and *T*, thymine) on clays (montmorillonite, bentonite, and kaolinite) at different *pH* (2.00 and 7.20). The nucleic acid bases were dissolved in artificial seawater (with the major elements). It should be pointed out that as far as we know there are no reported studies describing the adsorption of nucleic acid bases under such conditions mimicking a naturally occurring environment. The present paper also examines the interaction of nucleic acid bases and clays using FT-IR.

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Results and discussion

Tables 1 and 2 show *Langmuir*'s parameters/correlation coefficient of linear plots and *Freundlich*'s parameters/correlation coefficient of linear plots for nucleic acid bases adsorbed on clays, respectively. *Langmuir*'s and *Freundlich*'s parameters were obtained as shown in Experimental. Table 3 shows the amount of nucleic acid bases adsorbed on clays. The nucleic acid bases (*A*, adenine; *C*, cytosine; *U*, uracil; and *T*, thymine) were dissolved in seawater and adsorbed on clays (bentonite, montmorillonite, and kaolinite). It should be pointed out that while there are studies in the literature about the adsorption of nucleic acid bases on various materials, there is no reported study, as far as we know, using conditions approaching the naturally occurring condition used in this study. The seawater used in these experiments contains all the major elements, and we also used a wide range of *pH* (2.00–7.20). As reviewed by *Holm* and *Andersson* [20] and *Holm et al.* [21] this range of *pH* could be even wider in hydrothermal environments.

The results showed an equilibrium constant (k_{eq}) less than 1, indicating that the process of adsorption of nucleic acid bases on clays is not thermodynamically favorable (Table 1). Probably due to the observed low adsorption of nucleic acid bases on clays, the results fit well in both isotherm models (*Langmuir* and *Freundlich*) (Tables 1 and 2). The review by *Lahav* and *Chang* [9] showed that the adsorption coefficient for several compounds (purines, pyrimidines, and related compounds) adsorbed on various minerals (apatite, illite, and montmorillonite) was mostly less than 1. *Ayar* and *Yildiz* [2] and *Perezgasga et al.* [15] studied the adsorption of *A* and *U* on sporopollenin and montmorillonite. They observed that k_{eq} was greater than 1 for *A*, but less than 1 for *U*. However, *Sowerby et al.* [17] found an adsorption coefficient greater than 1 for nucleic acid bases (*G* guanine, *A* adenine, *H* hypoxanthine, *T* thymine, *C* cytosine, and *U* uracil) adsorbed on

Table 1 *Langmuir* parameters of nucleic acid bases adsorbed on clays

Base	Clay	*Range of *pH*	**b (μg/mg)	***K_{eq} (cm^3/μg)	$^{\nabla}r^2$
A	*b*	2.21–2.41	$29.5 \pm 1.9(5)$ $[5]^{C,+}$	$0.0238 \pm 0.0043(5)$ $[5]^{B,+}$	$0.9924 \pm 0.0020(5)$ $[5]^{+}$
		6.80–7.80	$11.0 \pm 3.3(8)$ $[10]^{B,D,F,I,+}$	$0.0110 \pm 0.0033(5)$ $[10]^{B,+}$	$0.8790 \pm 0.0384(8)$ $[10]^{+}$
	m	2.17–2.58	$33.9 \pm 0.4(4)$ $[5]^{A,+}$	$0.0490 \pm 0.0027(4)$ $[5]^{A,+}$	$0.9869 \pm 0.0011(4)$ $[5]^{+}$
		6.13–7.90	(0) $[13]^{+}$	(0) $[13]^{+}$	(0) [13]
	k	1.93–2.90	$0.175 \pm 0.086(4)$ $[13]^{B,D,F,H,J,+}$	(0) $[13]^{+}$	$0.5965 \pm 0.1286(4)$ $[13]^{+}$
		7.17–8.00	NA	NA	NA
C	*b*	2.20–2.60	$16.1 \pm 0.9(4)$ $[6]^{B,D,F,G,+}$	$0.0267 \pm 0.0071(3)$ $[6]^{B,+}$	$0.9955 \pm 0.0010(4)$ $[6]^{+}$
		6.60–7.55	$8.45 \pm 4.30(4)$ $[10]^{B,D,F,+}$	$0.0118 \pm 0.0061(4)$ $[10]^{B,+}$	$0.4698 \pm 0.0851(4)$ $[10]^{+}$
	m	2.14–2.36	$27.8 \pm 1.8(5)$ $[5]^{E,+}$	$0.0184 \pm 0.0036(5)$ $[5]^{B,+}$	$0.9903 \pm 0.0044(5)$ $[5]^{+}$
		6.75–7.90	$8.89 \pm 3.46(6)$ $[10]^{B,D,F,+}$	$0.0084 \pm 0.0026(5)$ $[10]^{B,+}$	$0.5516 \pm 0.0622(6)$ $[10]^{+}$
U	*b*	1.99–2.28	$0.047 \pm 0.015(5)$ $[5]^{B,D,F,H,J,+}$	(0) $[5]^{+}$	$0.9280 \pm 0.0240(5)$ $[5]^{+}$
		6.90–7.30	$0.176 \pm 0.059(4)$ $[6]^{B,D,F,H,J,+}$	0.0181(1) $[6]^{+}$	$0.8843 \pm 0.0374(4)$ $[6]^{+}$
	m	1.92–2.06	(0) $[5]^{\#}$	(0) $[5]^{\#}$	(0) $[5]^{\#}$
		6.98–7.54	(0) $[5]^{+}$	(0) $[5]^{+}$	(0) $[5]^{+}$
T	*b*	1.85–2.35	(0) $[6]^{+}$	(0) $[6]^{+}$	(0) $[6]^{+}$
		6.55–7.37	(0) $[5]^{+}$	(0) $[5]^{+}$	(0) $[5]^{+}$
	m	1.94–2.31	(0) $[5]^{\#}$	(0) $[5]^{\#}$	(0) $[5]^{\#}$
		6.84–7.54	(0) $[5]^{+}$	(0) $[5]^{+}$	(0) $[5]^{+}$

A adenine, *C* cytosine, *U* uracil, *T* thymine, *m* montmorillonite, *k* kaolinite, and *b* bentonite; $^{+}$Method 1 and $^{\#}$Method 2 Results are presented as mean ± standard error of mean. ( ) Number of duplicate experiments with *Langmuir*'s parameters and/or correlation coefficient of straight lines were not negative. [ ] Number of experiments with two sets each. *NA* Not adsorption; *Ranges of *pH*s after the samples were tumbled: 3 h for bentonite and montmorillonite and 16 h for kaolinite, the *pH*s were adjusted at time $t = 0$–2.00 or 7.20. $^{**}b$ theoretical limit of adsorbed solute; $^{***}K_{eq}$ equilibrium constant (adsorbate-adsorbent); $^{\nabla}$Correlation coefficient of straight lines For *b* ANOVA test ($F = 21.35$, $p = 0.000$), SNK test values statistically different ($p < 0.05$) from each other: A/B, C/D, E/F, G/H, and I/J; For *k* ANOVA test ($F = 10.84$, $p = 0.000$), SNK test values statistically different ($p < 0.05$) from each other: A/B

Table 2 *Freundlich* parameters of nucleic acid bases adsorbed on clays

Base	Clay	*Range of *pH*	**n	***K_f	$^{\nabla}r^2$
A	*b*	2.21–2.41	3.76 ± 0.38(5) [5]$^{+}$	5.67 ± 0.59(5) [5]$^{B,E,+}$	0.9830 ± 0.0039(5) [5]$^{+}$
		6.80–7.80	1.71 ± 0.56(8) [10]$^{+}$	0.586 ± 0.291(8) [10]$^{B,D,F,H,+}$	0.8747 ± 0.0437(8) [10]$^{+}$
	m	2.17–2.58	5.68 ± 0.63(5) [5]$^{+}$	11.4 ± 0.2(5) [5]$^{A,+}$	0.9194 ± 0.0230(5) [5]$^{+}$
		6.13–7.90	0.499 ± 0.045(9) [13]$^{+}$	0.0018 ± 0.0012(9) [13]$^{B,D,F,H,+}$	0.8504 ± 0.0524(9) [13]$^{+}$
	k	1.93–2.90	0.089 ± 0.012(7) [13]$^{+}$	$<10^{-10}$(7) [13]$^{+}$	0.8407 ± 0.0780(7) [13]$^{+}$
		7.17–8.00	NA	NA	NA
C	*b*	2.20–2.60	12.4 ± 5.8(6) [6]$^{+}$	5.91 ± 1.59(6) [6]$^{B,C,+}$	0.9104 ± 0.0508(6) [6]$^{+}$
		6.60–7.55	0.737 ± 0.155(7) [10]$^{+}$	0.0140 ± 0.0092(7) [10]$^{B,D,F,H,+}$	0.7908 ± 0.0544(7) [10]$^{+}$
	m	2.14–2.36	2.96 ± 0.32(5) [5]$^{+}$	3.52 ± 0.54(5) [5]$^{B,D,F,G,+}$	0.9864 ± 0.0073(5) [5]$^{+}$
		6.75–7.90	1.15 ± 0.20(10) [10]$^{+}$	0.108 ± 0.049(10) [10]$^{B,D,F,H,+}$	0.7515 ± 0.0540(10) [10]$^{+}$
U	*b*	1.99–2.28	0.423 ± 0.173(3) [5]$^{+}$	0.0013 ± 0.0012(3) [5]$^{B,D,F,H,+}$	0.8542 ± 0.0958(3) [5]$^{+}$
		6.90–7.30	1.99 ± 1.63(3) [6]$^{+}$	0.0319 ± 0.0319(3) [6]$^{B,D,F,H,+}$	0.7524 ± 0.2074(3) [6]$^{+}$
	m	1.92–2.06	0.257 ± 0.024(5) [5]$^{\#}$	$<10^{-8}$(5) [5]$^{\#}$	0.9791 ± 0.0024(5) [5]$^{\#}$
		6.98–7.54	0.445 ± 0.053(5) [5]$^{+}$	$<10^{-4}$(5) [5]$^{+}$	0.9793 ± 0.0076(5) [5]$^{+}$
T	*b*	1.85–2.35	0.339 ± 0.043(5) [6]$^{+}$	$<10^{-6}$(5) [6]$^{+}$	0.9495 ± 0.0161(5) [6]$^{+}$
		6.55–7.37	0.299 ± 0.047(5) [5]$^{+}$	$<10^{-6}$(5) [5]$^{+}$	0.9778 ± 0.0069(5) [5]$^{+}$
	m	1.94–2.31	0.524 ± 0.007(5) [5]$^{\#}$	$<10^{-5}$(5) [5]$^{\#}$	0.9859 ± 0.0078(5) [5]$^{\#}$
		6.84–7.54	0.456 ± 0.062(5) [5]$^{+}$	$<10^{-4}$(5) [5]$^{+}$	0.9469 ± 0.0297(5) [5]$^{+}$

A adenine, *C* cytosine, *U* uracil, *T* thymine, *m* montmorillonite, *k* kaolinite, and *b* bentonite; $^{+}$Method 1 and $^{\#}$Method 2 Results are presented as mean ± standard error of mean. ( ) Number of duplicate experiments with *Freundlich*'s parameters and/or correlation coefficient of straight lines were not negative. [ ] Number of experiments with two sets each. *NA* Not adsorption; *Ranges of *pH*s after the samples were tumbled: 3 h for bentonite and montmorillonite and 16 h for kaolinite, the *pH*s were adjusted at time $t = 0$–2.00 or 7.20. **n = adsorption intensity; ***K_f = adsorption capacity; $^{\nabla}$Correlation coefficient of straight lines, For k_f ANOVA test ($F = 44.61$, $p = 0.000$), SNK test values statistically different ($p < 0.05$) from each other: A/B, C/D, E/F, and G/H

Table 3 Amount of nucleic acid bases adsorbed on clays

Base	Clay	*Range of *pH*	**K (μg/mg)
A	*b*	2.21–2.41	19.5 ± 0.3 [5]$^{B,C,+}$
		6.80–7.80	6.06 ± 0.26 [10]$^{B,D,F,H,J,K,+}$
	m	2.17–2.58	21.7 ± 0.8 [5]$^{A,+}$
		6.13–7.90	7.44 ± 0.25 [13]$^{B,D,F,H,I,+}$
	k	1.93–2.90	0.407 ± 0.039 [13]$^{B,D,F,H,J,L,N,P,S,U,+}$
		7.17–8.00	NA
C	*b*	2.20–2.60	13.1 ± 0.3 [6]$^{B,D,F,G,+}$
		6.60–7.55	2.15 ± 0.16 [10]$^{B,D,F,H,J,L,N,P,R,+}$
	m	2.14–2.36	17.7 ± 0.1 [5]$^{B,D,E,+}$
		6.75–7.90	3.44 ± 0.20 [10]$^{B,D,F,H,J,L,N,O,+}$
U	*b*	1.99–2.28	0.212 ± 0.022 [5]$^{B,D,F,H,J,L,N,P,S,U,+}$
		6.90–7.30	0.287 ± 0.018 [6]$^{B,D,F,H,J,L,N,P,S,U,+}$
	m	1.92–2.06	1.24 ± 0.14 [5]$^{B,D,F,H,J,L,N,P,S,\#}$
		6.98–7.54	1.35 ± 0.06 [5]$^{B,D,F,H,J,L,N,P,+}$
T	*b*	1.85–2.35	0.776 ± 0.049 [6]$^{B,D,F,H,J,L,N,P,S,+}$
		6.55–7.37	0.700 ± 0.018 [5]$^{B,D,F,H,J,L,N,P,S,+}$
	m	1.94–2.31	4.23 ± 0.08 [5]$^{B,D,F,H,J,L,M,\#}$
		6.84–7.54	1.59 ± 0.04 [5]$^{B,D,F,H,J,L,N,P,T,+}$

A adenine, *C* cytosine, *U* uracil, *T* thymine, *m* montmorillonite, *k* kaolinite, and *b* bentonite; $^{+}$Method 1 and $^{\#}$Method 2 Results are presented as mean ± standard error of mean. [ ] Number of experiments with two sets each. *NA* not adsorption; *Ranges of *pH*s after the samples were tumbled: 3 h for bentonite and montmorillonite and 16 h for kaolinite, the *pH*s were adjusted at time $t = 0$–2.00 or 7.20. **For each duplicate curve, K was obtained: K = [Total amount of base adsorbed on clay/Total amount of clay], For K ANOVA test ($F = 751.63$, $p = 0.000$), SNK test values statistically different ($p < 0.05$) from each other: A/B, C/D, E/F, G/H, I/J, K/L, M/N, O/P, R/S, and T/U graphite. Thus, the adsorption coefficient depends on the material on which the nucleic acid bases are adsorbed.

For both of the isotherm models (*Langmuir* and *Freundlich*) used, the correlation coefficient of linear plots obtained at *pH* 2.00 was better than that was obtained at *pH* 7.20 (Tables 1 and 2). This effect could not be attributed to decreased adsorption with increase in *pH*, because even those nucleic acid bases whose adsorption was lower showed a very good linear fit of the data at *pH* 2.00 (Tables 1 and 2).

Kaolinite only adsorbed *A* at *pH* 2.00, and this adsorption was much lower than for other nucleic acid bases on clays as shown by b (Table 1), k_f (Table 2) and k (Table 3). This low adsorption of *A* as well as no adsorption of other nucleic acid bases could be attributed to the lower concentration of Fe_2O_3 in kaolinite when compared to the other clays (see chemical composition of clays). *Sawhney* and *Singh* [22] showed that Al-saturated smectite adsorbs much greater amounts of atrazine than does Ca-saturated smectite. According to these authors, atrazine is adsorbed as a neutral molecule, and the stronger H-bonding with the more polarized water molecules associated with the trivalent Al ion compared to the divalent Ca ion, was responsible for the greater adsorption by Al-smectite. *Bebié* and *Schoonen* [4] have observed that an increase in the concentration of metals (Cu^{2+} and Fe^{2+}) in solution enhanced the adsorption of *A* and purine on pyrite. However, *Lahav* and *Chang* [9] and *Cohn et al.* [14] have shown that the adsorption of nucleic acid bases on minerals are not related to surface area of minerals or the concentration of iron. Thus, there is no consensus in the literature on which parameters are important for the adsorption of nucleic acid bases on minerals.

The highest values for theoretical limit of adsorbed solute (b) were obtained for *A* adsorbed on bentonite and montmorillonite at *pH* 2.00 and *C* adsorbed on montmorillonite at *pH* 2.00 (Table 1). These values did not differ statistically ($p > 0.05$, SNK test), but they were statistically different ($p < 0.05$, SNK test) from other values shown in Table 1. The adsorption capacity (k_f) was highest for *A* adsorbed on bentonite at *pH* 2.00 and was statistically different ($p < 0.05$, SNK test) from other values shown in Table 2. High k_f values were also seen for *A* and *C* on bentonite at *pH* 2.00 and *C* on montmorillonite at *pH* 2.00. *A* adsorbed on montmorillonite at *pH* 2.00 showed the highest k value (Table 3) which was statistically different ($p < 0.05$, SNK test) from other values shown. High k values were also seen for *A* and *C* on bentonite at *pH* 2.00 and *C* on montmorillonite at *pH* 2.00. In general, there appears to a good agreement among the values for b, k_f, and k (Tables 1–3). As shown by these latter parameters, the adsorption of *A* on montmorillonite at *pH* 7.20, *U* on bentonite and montmorillonite at *pH* 2.00 and 7.20, and *T* on bentonite and montmorillonite at *pH* 2.00 and 7.20, was comparatively much lower (Tables 1–3).

The results also show that, in general, b, k_f, and k were significantly ($p < 0.05$, SNK test) lower at the higher *pH* (7.20) (Tables 1–3). The decrease in adsorption of nucleic acid bases on minerals at basic *pH* has been reported by other authors [4, 9, 10, 15].

A review published by *Lahav* and *Chang* [9] shows the results of adsorption coefficients for *A* and *C* adsorbed on illite and montmorillonite. In general, Illite adsorbs twofold more *A* than *C*, and montmorillonite adsorbs almost equal amounts of these nucleic acid bases. *Ayar* and *Yildiz* [2] and *Ayar* and *Mercimek* [3] obtained a greater adsorption for *A* than *U* when these bases were adsorbed on Co(II)-carboxylated diaminoethyl sporopollenin. *Winter* and *Zubay* [10] and *Perezgasga et al.* [15] have studied the adsorption of *A* and *U* on Na^+-montmorillonite and HDTMA-montmorillonite, and found that the adsorption of *A* was greater than that of *U* on either clay. The results obtained by *Lahav* and *Chang* [9], *Ayar* and *Yildiz* [2], *Perezgasga et al.* [15] and *Ayar* and *Mercimek* [3] are in good agreement with the results presented here.

Ferris and *Kamaluddin* [5] observed that the binding of 2′-d-5′-GMP to montmorillonite 22 A was comparable to that of 2′-d-5′-AMP, while 2′-d-5′-CMP was comparable to 5′-TMP. *Ferris et al.* [7] also found that 2′-d-5′-GMP bound two times more strongly to Na^+-montmorillonite than did 2′-d-5′-CMP. According to *Ertem* and *Ferris* [8] the interaction of adenine derivatives and 5′-UMP or uridine with montmorillonite differs, where the adsorption of adenine derivatives involves ionic bonds and *Van der Waals* forces. On the other hand, *Holm et al.* [23] studied the adsorption of adenosine, 5′-AMP, 3′-AMP, 5′-UMP, and 5′-CMP on goethite and akaganéite and observed little difference among the nucleotides in their adsorption, suggesting that the binding was between the negative charges of the phosphate group and the positive charges on the mineral surface. These results show that the manner in which molecules are adsorbed depends greatly on the material on which they are adsorbed.

As determined by *De Santana et al.* [24] the point of zero charge (pH_{pzc}) for bentonite, kaolinite, and montmorillonite is 1.88, 4.52, and 1.47. Thus, at *pH* 2.00, the surface of kaolinite is positively charged and bentonite and montmorillonite are negatively charged. At *pH* 2.00, *A* and *C* are positively charged and *T* and *U* are neutral [15, 25]. This difference in charge between the bases (*A*, *C*) and clays (bentonite and montmorillonite) could explain why these nucleic acid bases were adsorbed much more at *pH* 2.00 than *pH* 7.20, or even why kaolinite did not adsorb any of the nucleic acid bases (Tables 1–3). At *pH* 7.20, the surface of all clays is negatively charged and the bases are neutral, and thus, a lower adsorption of nucleic acid bases on the clays should be expected at this *pH* (Tables 1–3). However, a difference in charge does not explain why *A* and *C* adsorbed much more than *U* and *T* on the clays at *pH* 7.20 (Table 3). Thus, other interactions should be taken into account, such as *Van der Waals* forces and hydrogen bonding [4, 14, 15, 17, 22].

Based on b, k_f, and k values determined (Tables 1–3), we propose the following order of adsorption of nucleic acid bases (*A*, *C*, *U*, and *T*) on clays (bentonite, montmorillonite, and kaolinite): *A* on bentonite and montmorillonite at *pH* 2.00 > *C* on bentonite and montmorillonite at *pH* 2.00 > *A* on bentonite at *pH* 7.20 > *C* on bentonite and montmorillonite at *pH* 7.20 > *A* on kaolinite at *pH* 2.00 ≈ *A* on montmorillonite at *pH* 7.20 ≈ *U* on bentonite and montmorillonite at *pH* 2.00 and 7.20 ≈ *T*, on bentonite and montmorillonite at *pH* 2.00 and 7.20. However, *Sowerby et al.* [17] obtained the following order of adsorption of nucleic acid bases on graphite: *G*, guanine > *A*, adenine > *H*, hypoxanthine > *T*, thymine > *C*, cytosine > *U*, uracil. This order of adsorption is different from the one obtained in the present paper. It should be pointed out that graphite and clays (kaolinite, montmorillonite, and bentonite) are very different from each other.

Table 4 shows the ratio of *A–T* adsorbed on bentonite and montmorillonite at *pH* 2.00 and 7.20, along with *A/T* ratios in various several organisms [19]. The ratio of *A–T* adsorbed on clays ranged from 4.68 to 25.1, while the *A/T* ratio found in the organisms listed ranged from 0.95 to 1.05. The weaker adsorption of *T* and *U* on clays raises the question: is it possible to have a genetic code with only one type of nucleic acid base? *Sowerby et al.* [17] also observed that graphite adsorbed much more *G* and *A* than other nucleic acid bases. Using the results of the adsorption of *A* and *T* and *G* and *C* on graphite obtained by *Sowerby et al.* [17] the *A/T* and *G/C* ratios are about 3.1 and 10.3, respectively. *Sowerby et al.* [17] also raised some questions about the difficulties of a genetic code with one nucleic acid base. Other authors have also ascertained that purines are adsorbed more than pyrimidines [2, 3, 9, 10, 15] and that nucleic acid bases could even influence the adsorption of nucleotides [5]. All these results show that perhaps a genetic code was a later invention in the origin of life. It is also possible that adsorption of nucleic acid bases on minerals did not play an important role in giving rise to a primitive genetic code using nucleic acid bases as seen in for today's organisms. The importance of minerals for the adsorption of biomolecules (amino acids) has been questioned before [26–29].

Table 4 Ratios of *A/T*

**A/T*-Clay-range of *pH*	***A/T*-organism
25.1-bentonite-2.00	1.05-homo sapiens
8.66-bentonite-7.20	1.03-sheep
5.13-montmorillonite-2.00	1.02-chicken
4.68-montmorillonite-7.20	1.05-turtle
	1.02-salmon
	1.02-sea urchin
	1.00-grasshopper
	0.95-yeast
	1.04-*E. coli*
	1.05-*Staphylococcus aureus*

A adenine, *T* thymine; *Ratios of *A* and *T* adsorbed on clays, for the calculation of these ratios were used the values of Table 3 were used. **Ratios of *A* and *T* of several organisms [19]

A study using FT-IR spectroscopy was undertaken to better understand the interaction between clays and nucleic acid bases. For all bases (*A*, *C*, *U*, and *T*) adsorbed on clays (bentonite and montmorillonite), FT-IR spectra were better at *pH* 2.00.

Figures 1 and 2 show the FT-IR spectral results of bentonite (Fig. 1-A-a) and montmorillonite (Fig. 2-A-a), solid bases and bases adsorbed on bentonite and montmorillonite. The FT-IR spectrum of solid *A* (Figs. 1-A-b and 2-A-b) shows bands at 1603 and 1672 cm^{-1} which could be attributed to C=N stretching and NH_2 deformation [30]. The spectrum

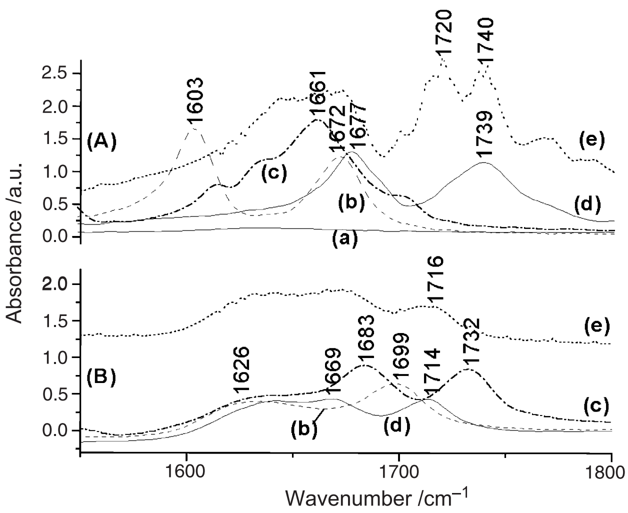

Fig. 1 FT-IR spectra of (A): bentonite (a), solid adenine (b), solid cytosine (c), solid thymine (d), and solid uracil (e); (B): adenine (b), cytosine (c), thymine (d), and uracil (e) adsorbed on bentonite. Clay samples were tumbled for 3 h with seawater and saturated solution (adenine, cytosine, thymine, or uracil) dissolved in seawater in the range of *pH* 2.00. All samples were filtered, and the solids were dried in an oven at 40°C for 24 h

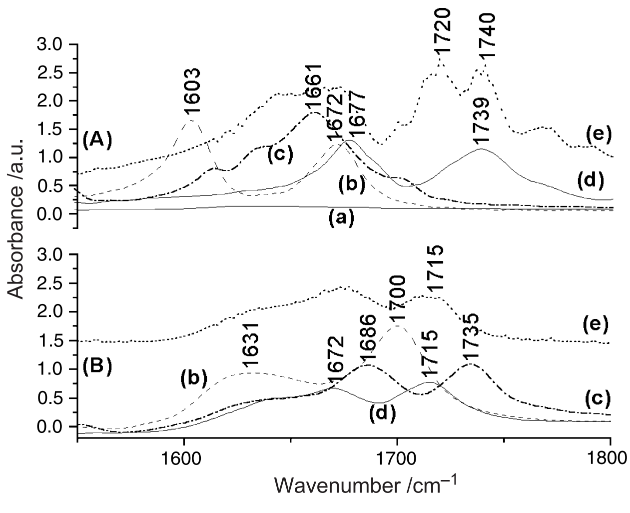

Fig. 2 FT-IR spectra of (A): montmorillonite (a), solid adenine (b), solid cytosine (c), solid thymine (d), and solid uracil (e); (B): adenine (b), cytosine (c), thymine (d), and uracil (e) adsorbed on montmorillonite. Clay samples were tumbled for 3 h with seawater and saturated solution (adenine, cytosine, thymine or uracil) dissolved in seawater in the range of *pH* 2.00. All samples were filtered, and the solids were dried in an oven at 40°C for 24 h

of *A* adsorbed on bentonite (Fig. 1-B-b) and montmorillonite (Fig. 2-B-b) shows a shift in the frequencies to 1626 and 1699 cm^{-1} and 1631 and 1700 cm^{-1}. Since the clays are negatively charged at this *pH*, the interaction of *A* with clay occurred through the protonated NH_2^+, resulting in the weakness of the C=N bond on clays.

The FT-IR spectrum of solid *C* (Fig. 1-A-c and 2-A-c) shows a band at 1661 cm^{-1} which could be attributed to C=O stretching [31]. The spectrum of *C* adsorbed on bentonite (Fig. 1-B-c) and montmorillonite (Fig. 2-B-c) shows a splitting in the frequency to 1683 and 1732 cm^{-1} and 1686 and 1735 cm^{-1}, respectively. It is possible that the interaction of *C* with the clays was also through the protonated NH_2^+, showing the new band at 1732–1735 cm^{-1} and a shift in the ν(C=O) frequencies to 1683–1686 cm^{-1}.

The FT-IR spectra of solid *T* (Figs. 1-A-d and 2-A-d) and solid *U* (Figs. 1-A-e and 2-A-e) show bands at 1677 and 1739 cm^{-1} and 1720 and 1740 cm^{-1}, respectively, which could be attributed to C=C and C=O stretching [32, 33]. The spectra of *T* adsorbed on bentonite (Fig. 1-B-d) and montmorillonite (Fig. 2-B-d) and *U* adsorbed on bentonite (Fig. 1-B-e) and montmorillonite (Fig. 2-B-e) show a shift in the frequencies to 1669 and 1714 cm^{-1} and 1672 and 1715 cm^{-1}, and 1720 cm^{-1} to 1716 cm^{-1} and 1713 cm^{-1}, and a vanishing of the band at 1740 cm^{-1}. It is possible that the interactions of *T* and *U* occurred through the protonated NH^+, resulting in the weakness of the C=C and C=O bonds on clays.

The FT-IR spectra show that the interaction of *A* and *C* with the clays occurs according to the model proposed by *Ferris et al.* [6]. Although *T* and *U* are neutral in solution at *pH* 2.00, the FT-IR spectra showed that they were protonated and interacted with the clays through positively charged groups. Protonation may be taking place when *T* and *U* enter the interlayer regions of the clays, which are known to be acidic [8].

In conclusion this paper showed that the results for the equilibrium constant (k_{eq}) were less than 1, indicating that the process of adsorption of nucleic acid bases on clays is not thermodynamically favorable.

In most cases of the isotherm models (*Langmuir* and *Freundlich*), the correlation coefficients for linear plots obtained at *pH* 2.00 were better than those obtained at *pH* 7.20.

Kaolinite only adsorbed *A* at *pH* 2.00, and this adsorption was much lower than that of other nucleic acid bases on clays.

In general, there appears to be a good agreement among the values for *b* (theoretical limit of adsorbed nucleic acid base), k_f (the adsorption capacity) and k (total amount of base adsorbed on clay/total amount of clay).

At the higher *pH*, b, k_f, and k values were lower, and in most cases, these values were statistically different ($p < 0.05$, SNK test) compared with those at the lower *pH*.

Based on the values for b, k_f, and k the following conclusions could be withdrawn: bentonite and montmorillonite adsorbed nucleic acid bases much more than kaolinite; the nucleic acid bases were more adsorbed at *pH* 2.00 than at *pH* 7.20 and the nucleic acid bases *A* and *C* were much more adsorbed on clays than the nucleic acid bases *T* and *U*.

The ratio of *A*–*T* adsorbed on clays ranged from 4.68 to 25.1, and the ratio of their occurrence in organisms ranges from 0.95 to 1.05. The weaker adsorption of *U* and *T* on clays raises the question of the possibility of a genetic code with one nucleic acid base.

The FT-IR spectra at *pH* 2.00 showed that the interaction of *A*, *C*, *T*, and *U* with the clays occurs between positively charged, protonated bases and negatively charged clays.

Although *T* and *U* are uncharged in solution at *pH* 2.00, the FT-IR spectra showed that they were protonated. Protonation may be taking place when *T* and *U* enter the interlayer regions of the clays, which are known to be acidic.

Experimental

All reagents were of analytical grade. Bentonite and montmorillonite were purchased from Acros Organics-NJ, USA. Kaolinite was a gift from Dr. *Carlos H. Sampaio* of LAPROM/CT-UFRGS-RS, Brazil. All clays were used without further preparation. All nucleic acid bases were purchased from Acros Organics (USA) or Sigma (Canada) and were used as received. Base abbreviations are as follows: *A* adenine, *C* cytosine, *U* uracil, and *T* thymine.

Seawater

The following substances were weighed and dissolved in 1.0 dm^3 of distilled water: 28.57 g sodium chloride, 3.88 g magnesium chloride, 1.787 g magnesium sulfate, 1.308 g calcium sulfate, 0.832 g potassium sulfate, 0.124 g calcium carbonate, 0.103 g potassium bromide, and 0.0282 g boric acid.

Sample preparation for isotherms

A and *C* were dissolved in seawater at a concentration of 240 and 720 $\mu g\,cm^{-3}$ and as a saturated solution. *U* and *T* were dissolved in seawater at a concentration of 240, 360, 480, 600, and 720 $\mu g\,cm^{-3}$ and as a saturated solution. The isotherms were obtained using two methods.

Method 1: The concentration of the nucleic acid base was kept constant and the amount of clay was varied. For *A* (bentonite/*pH* 2.00 and 7.20; montmorillonite/*pH* 2.00 and 7.20) and *C* (bentonite/*pH* 2.00; montmorillonite *pH* 2.00), 5.00 cm^3 of seawater with 240 $\mu g\,cm^{-3}$ of the nucleic acid base were added to each tube of two different sets of five tubes (15 cm^3), each containing 10, 20, 30, 40, and 50 mg of clay (bentonite or montmorillonite). For *A* (kaolinite/*pH* 2.00), 5.00 cm^3 of seawater with 720 $\mu g\,cm^{-3}$ of the nucleic acid base were added to each tube of two different sets of five tubes (15 cm^3), each containing 100, 300, 500, 700, and 1000 mg of clay (kaolinite). For *C* (bentonite/*pH* 7.20; montmorillonite/*pH* 7.20) and *U* (montmorillonite/*pH* 7.20), 5.00 cm^3 of seawater with 240 $\mu g\,cm^{-3}$ of the nucleic acid base were added to each tube of two different sets of five tubes (15 cm^3), each containing 50, 100, 150, 200, and 250 mg of clay (bentonite or montmorillonite). For *U* (bentonite/*pH* 2.00 and 7.20) and *T* (bentonite/*pH* 2.00 and 7.20; montmorillonite *pH* 7.20), 5.00 cm^3 of seawater with 240 $\mu g\,cm^{-3}$ of the nucleic acid base were added to each tube of two different sets of five tubes (15 cm^3), each containing 100, 200, 300, 400, and 500 mg of clay (bentonite or montmorillonite). The *pH* was adjusted to 2.00 or 7.20 with HCl or NaOH. The tubes were tumbled for 3 h for bentonite and montmorillonite or for 16 h for kaolinite, after which they were spun for 15 min at 2,000 rpm; the aqueous phase was used for base analysis.

Method 2: The concentration of the nucleic acid base was varied and the amount of clay was kept constant. For *U* (montmorillonite/*pH* 2.00) and *T* (montmorillonite/*pH* 2.00), 5.00 cm^3 of seawater with 240, 360, 480, 600, and 720 $\mu g\,cm^{-3}$ of the nucleic acid base were added to each tube of two different sets of five tubes (15 cm^3), each containing 100 mg of clay (bentonite or montmorillonite). The *pH* was adjusted to 2.00 or 7.20 with HCl or NaOH. The tubes were tumbled for 3 h, after which they were spun for 15 min at 2,000 rpm; the aqueous phase was used for base analysis.

Samples for FT-IR

Saturated solutions of the nucleic acid bases (*A*, *C*, *U*, and *T*) were prepared in seawater. Each clay (bentonite, montmorillonite, and kaolinite) was processed as follows: 500 mg of clay (bentonite, montmorillonite or kaolinite) in two different sets of four tubes (15 cm^3) were mixed with a) 5.00 cm^3 of seawater without bases and b) 5.00 cm^3 of seawater saturated with nucleic acid base (*A*, *C*, *U*, or *T*). The *pH* was adjusted to 2.00 or 7.20 with HCl or NaOH. The tubes were tumbled for 3 h (bentonite and montmorillonite) or 16 h (kaolinite), after which they were filtered. The aqueous phase was discarded, and the solids were dried in an oven at 40°C for 24 h and used for FT-IR.

Langmuir isotherm model

The *Langmuir* isotherm model, which is commonly represented by the equation $C/\theta = 1/k_{eq}b + C/b$, where C ($\mu g\,cm^{-3}$) is the concentration of nucleic acid base in the clay solution after equilibration, θ (μg mg^{-1}) is the concentration of nucleic acid base adsorbed on the clay (difference between initial nucleic acid base concentration, and the concentration after the equilibration was assumed to represent the amount adsorbed on the clay), b (μg mg^{-1}) is the theoretical limit of adsorbed nucleic acid base, and k_{eq} (cm^3 μg^{-1}) is equilibrium constant (adsorbate–adsorbent). The parameters b and k_{eq} can be obtained from the graph of the linear relationship between C/θ and C, known as the *Langmuir* plot [34, 35].

Freundlich isotherm model

The *Freundlich* isotherm model, which is commonly represented by the equation $\log\theta = \log k_{\mathrm{f}} + (1/n) \log \mathrm{C}$, where C ($\mu$g cm^{-3}) is the concentration of nucleic acid base in the clay solution after equilibration, θ (μg mg^{-1}) is the concentration of nucleic acid base adsorbed on clay (difference between initial nucleic acid base concentration and concentration after the equilibration was assumed to represent the amount adsorbed on the clay), k_{f} is the adsorption capacity, and n is adsorption intensity. The parameters k_{f} and n can be obtained from the graph of the linear relationship between $\log\theta$ and log C, known as the *Freundlich* plot [34, 35].

Obtaining k values

For each curve of the experiments used to obtain the *Langumuir* and *Freundlich* parameters a value of *k* was calculated by adding the amount of nucleic acid base adsorbed on the clay and this was divided by the amount of clay used. The following equation was used: $K =$ [Total amount of base adsorbed on clay/Total amount of clay].

UV spectrophotometric methods

Absorbance was read with a spectrophotometer, Spectronic Genesys. The nucleic acid bases (*A*, *C*, *U*, and *T*) were determined by reading the absorbance in the UV region (260–265 nm). The following equation was used for the calculation of the amount of base adsorbed on clay (bentonite, kaolinite, and montmorillonite). $\mathrm{C}_{\mathrm{adsorbed}}/\mu\mathrm{g} = (\mathrm{C}_{\mathrm{initial}} - \mathrm{C}_{\mathrm{solution}})$, where $\mathrm{C}_{\mathrm{solution}} = [(\mathrm{C}_{\mathrm{initial}})\ (\mathrm{Abs}_{\mathrm{sample}}/\mathrm{Abs}_{\mathrm{initial}})]$.

Infrared spectrophotometric method

All FT-IR spectra were recorded from 400 to 4,000 cm^{-1} with a spectral resolution of 4 cm^{-1} and 120 acquiring spectra. Disc pellets of all samples (clays with and without nucleic acid bases adsorbed on, solid nucleic acid bases) were prepared as follows: about 10 mg of the sample plus 200 mg of KBr were weighted and ground in an agate mortar with a pestle until a homogeneous mixture was obtained. FT-IR spectra of these pellets were recorded using a spectrophotometer Shimadzu FT-IR 8300. FT-IR spectra were analyzed by the Origin program (5.0, 2001).

Statistical analysis

The ANOVA and Student–Newman–Keuls test (SNK test) were used for the comparisons between means at a significance level of $p < 0.05$. When two or more means were statistically different from each other superscripts with capital letters were used.

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