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Synthesis of Guanidine from Ammonium Thiocyanate in Solid State

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Guanidina é uma substância importante para os seres vivos atuais assim como para a química prebiótica. No presente trabalho foi estudada a reação entre as espécies amônio e tiocianato em estado sólido com diferentes substâncias (areia de rio, caulin, metais de transição e sais da água do mar) e temperaturas (80, 120 e 150 °C). As recuperações de tiocianato ou amônio nas amostras com areia de rio ou caulin a 150 °C foram estatisticamente diferentes (p<0,05) das recuperações de tiocianato e amônio nas amostras sem aquelas substâncias. A decomposição do tiocianato de amônio nas amostras com areia de rio ou caulin a 150 °C foi de 40%. Caulin mostrou ser mais efetivo na síntese de guanidina do que areia de rio, visto que a concentração de guanidina sempre foi maior nas amostras com caulin do que nas amostras com areia de rio. A decomposição do tiocianato de amônio nas amostras com metais de transição mais areia de rio a 120 °C foi de 30%. Sais da água do mar ou metais de transição mais tiocianato de amônio mais areia de rio (120 °C) aumentaram a concentração de tiouréia (água do mar: 538%, metais de transição: 357%) ou guanidina (água do mar: 393%, metais de transição: 806%) quando os resultados foram comparados com amostras sem estas substâncias. Comparando os resultados obtidos com as amostras de tiocianato de amônio (120 °C) mais sais da água do mar ou metais de transição com a amostra de tiocianato de amônio observamos que houve um aumento na produção de tiouréia (168%) e guanidina (268%) respectivamente. Os rendimentos obtidos para a síntese de guanidina mostrados neste trabalho são melhores do que os mostrados por outros autores. Experimentos mostraram que Fe (III) está envolvido na formação do composto amarelo obtido da reação entre amônio e tiocianato e espectros Raman mostraram que este composto pode ser o enxofre.

Guanidine is an important substance for modern living beings as well as for the prebiotic chemistry. We studied the reaction between ammonium and thiocyanate species in solid state with different substances (river sand, kaolin, transition metals, salts of seawater) and temperatures (80, 120 and 150 °C). The recoveries of thiocyanate and ammonium in the samples with river sand or kaolin at 150 °C were statistically different (p<0.05) from the recoveries of thiocyanate and ammonium in the sample without them. The decomposition of ammonium thiocyanate in the samples river sand or kaolin at 150 °C was about 40%. Kaolin showed to have more effect on the synthesis guanidine than river sand, because, the concentrations of guanidine in the samples with kaolin were always bigger than in the samples with river sand. The decomposition of ammonium thiocyanate in the samples with transition metals plus river sand at 120 °C was about 30%. The salts of seawater or transition metals plus ammonium thiocyanate plus river sand (120 °C) showed to increase the concentration of thiourea (seawater: 538%; transition metals: 357%) or guanidine (seawater: 393%, transition metals: 806%) when they were compared to the samples without them. When the results obtained with sample of ammonium thiocyanate were compared to the samples of ammonium thiocyanate (at 120 °C) plus salts of seawater or transition metals also an increased on the production of thiourea (168%) and guanidine (268%) was obtained, respectively. The yield for the synthesis of guanidine showed in this paper is better than that obtained by other authors. Experiments showed that iron (III) is involving in the formation of the yellow compound and Raman spectra showed this compound could be sulphur.

Keywords: guanidine, ammonium thiocyanate, clay, prebiotic chemistry, transition metals

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Introduction

The so-called mineral theories of origin of life have become widely considered, since Bernal (1951)[1] first suggested that minerals could concentrate biomolecules as well as catalyse their reactions for the formation of biopolymers. The adsorption of biomolecules on minerals was studied by several authors.[2-6] Ferris[2] showed that montmorillonite catalysis the elongation of oligo-nucleotides, so probably this clay was important in the origin of the RNA world. However, Zaia *et al.*[3] have raised some doubts about the importance of sand as concentration mechanism for amino acids.

There are several environments that the synthesis of biomolecules and biopolymers under plausible prebiotic conditions could occur and role of minerals should be considered. The synthesis of biomolecules and peptides, using wetting and drying cycles, that mimicks small lagoons or tidal pools or puddles under the influence of sun, rain and tidal changes has been studied by several authors.[7-12] Using clays and transitions metals in the wetting/drying cycles the yields for the synthesis of biomolecules or peptides were higher than using other methods, so these cycles probably played an important role in the origin of life on earth.[7-12] The discovery and suggestion by Corliss *et al.*[13,14] that submarine hydrothermal vents could be played an important role in the origin of life on earth has stimulated numerous studies on this subject. Several advantages can be cited to use simulated submarine hydrothermal vent conditions as prebiotic environment: the biomolecules and biopolymers were protected from UV rays of the sun, the gradient of temperature provides energy and different sites for different reactions, at high temperature and high pressure the water behavior like an organic solvent and salts of seawater, transition metals and clays of hydrothermal vents catalyze the syntheses of biomolecules and biopolymers.[14,15] Under hydrothermal conditions, the syntheses of biomolecules,[15-17] bio-polymers[15,18-22] and microspheres,[15,22] was obtained. The prebiotic environments that could be corresponding of laboratory experiments of reaction of substances in solid state are: cooling lava from volcanoes on the ground and impact of bolides (meteor, comet) on the earth.[23] Heating substances with minerals (alumina, silica, sand, clays, etc) in solid state was possible to obtain several biomolecules and biopolymers: amino acids,[24] diketopiperazines,[25] phosphite,[26] and peptides.[27, 28]

Ferris *et al.*[29] studied the condensation of cyano-acetaldhyde with guanidine in dilute aqueous solution to form 2,4-diaminopyrimidine, which was hydrolyzed to cytosine and uracil. Robertson *et al.*[30] studied the same reaction of Ferris *et al.*,[29] however instead using dilute aqueous solution they used the concentrated conditions of a drying/wetting cycles model obtaining high yields (40-85%) of 2,4-diaminopyrimidine. Ferris and Hagan Jr.,[31] in a review paper, pointed out the importance of cyanoguanidine as a condensing agent in phosphorylation and peptide formation. Guanidine has also important roles to play in modern living beings.[32]

In spite of the importance of guanidine in prebiotic chemistry as well as the modern living being, as far as we know there are two papers about prebiotic synthesis of guanidine. Halmann[33] reported that cyanamide solution converts to cyanoguanidine and Lohrmann[34] studied the formation of urea and guanidine by irradiation of ammonium cyanide.

Ammonium and thiocyanate have been synthesized in experiments under prebiotic chemistry conditions or have been found in places that resembling these environments. Dowler and Ingmanson[35] found thiocyanate in Atlantis II Deep Brine, located at the bottom of the sea in the Rift Valley Summers[36] showed that nitrite could be reduced to ammonia by iron (II) in the oceans. A review paper, published by Raulin and Toupance,[37] shows that ammonium thiocyanate was formed when a primitive atmosphere (CH_4, NH_3, H_2O, H_2, N_2, CO_2, H_2S) and electric discharge as energy source were used. So, ammonium and thiocyanate are substances that easily would be present on the primitive Earth.

In this paper we describe the synthesis of guanidine from the reaction of ammonium thiocyanate in solid state with different temperatures and several substances (river sand, kaolin, transition metals, salts of seawater).

Materials and Methods

Materials

Visible and infrared spectrophotometries were carried out on Shimadzu spectrophotometers UV-Vis 1203 and FTIR-8300, respectively. The Raman spectra were performed using a Renishaw Raman Imaging Microscope System 3000 Spectrometer, acoppled to a Olympus Microscopy BH-2 and a CCD detector (Wright, 600x400 pixels) cooled by a Peltier system. A He-Ne laser (Spectra Physics model 127) was used to obtain the 632.8 nm excitant radiation with 0.7 mW on the sample and spectral resolution of 7.0 cm^{-1}. The mineralogical composition of river sand was carried out on a petrographic microscope (KM Plival, Carl Zeiss/Aus Jena Company) and magni-fying glass (Carl Zeiss/Aus Jena Company).

All reagents were of analytical grade.

Kaolin. The median size of the kaolin particle was 2.13 μm (diameter at 10.0% = 0.54 μm and diameter at 90.0% = 9.83 μm) and the kaolin chemical composition was 45.7% of SiO_2, 0.6% of K_2O, 0.1% of CaO, 0.3% of Fe_2O_3, 38.9% of Al_2O_3, 0.1% of MgO, 0.1% of Na_2O, 0.2% of TiO_2 and 14.0% of loss on the ignition.

River sand. The river sand was collected from a river near by Londrina-PR-Brazil, and prepared as described by Rohlfing and McAlhaney.[27] The mineralogical analysis of river sand used in the experiments showed more than 99% quartz in its composition. The river sand has the following percentage (m/m) and particle size: 11.0%, >0.50 mm; 20.1%, 0.50-0.42 mm; 35.3%, 0.420-0.297 mm; 28.5%, 0.297-0.149 mm, and 5.1%, <0.149 mm, respectively.

Salts of seawater. The following substances were weighted: 114.26 g of sodium chloride, 15.55 g of magnesium chloride, 7.15 g of magnesium sulfate, 5.23 g of calcium sulfate, 3.33 g of potassium sulfate, 0.4960 g of calcium carbonate, 0.4120 g of potassium bromide, and 0.1128 g of boric acid; all the salts were ground with an agate mortar and pestle until a homogenized mixture was obtained.

Transition metals. The following substances were weighted: 2.700 g of sodium molybdate (VI), 2.750 g of iron (III) chloride, 2.950 g of zinc (II) sulfate, 2.080 g of manganese (II) nitrate, 2.650 g of copper (II) sulfate and 2.940 g of cobalt (II) nitrate; all these substances were ground with an agate mortar and pestle until a homogenized mixture was obtained.

Sample preparations

Samples of ammonium thiocyanate and ammonium thiocyanate plus river sand or kaolin. Two sets with two samples each were always run. First set: each sample with about 3.0 g of ammonium thiocyanate plus 5.0 g of river sand or kaolin was weighted and mixed until a homogenized was obtained. Second set: each sample with about 3.0 g of ammonium thiocyanate was weighted. Aliquots were withdrawn for determination of concentration of ammonium and thiocyanate as a zero time. The samples were added to a sublimation apparatus and heated for 3 days at different temperatures (80, 120, 150 °C). After heating, the crude sublimate and sample at the bottom were removed from sublimation apparatus and mixed. Aliquots were withdrawn and analysis of ammonium, thiocyanate, thiourea, and guanidine thiocyanate were carried out as described in methodology.

Samples of ammonium thiocyanate plus salts of seawater and ammonium thiocyanate plus salts of seawater plus river sand. Two sets with two samples each were always run. First

set: each sample with about 3.0 g of ammonium thiocyanate plus 5.0 g of river sand plus 5.0 g of salts of seawater was weighted and mixed until a homogenized was obtained. Second set: each sample with about 3.0 g of ammonium thiocyanate plus 5.0 g of salts of seawater was weighted and mixed until a homogenized was obtained. Aliquots were withdrawn for determination of concentration of thiocyanate as a zero time. The samples were added to a sublimation apparatus and heated for 3 days at 120 °C. After heating, crude sublimate and sample at the bottom were removed from the sublimation apparatus and mixed. Aliquots were withdrawn and analysis of thiocyanate, thiourea, and guanidine thiocyanate were carried out as described in methodology.

Samples of ammonium thiocyanate plus transition metals and ammonium thiocyanate plus transition metals plus river sand. Two sets with two samples each were always run. First set: each sample with about 3.0 g of ammonium thiocyanate plus 5.0 g of river sand plus 200 mg of transition metals was weighted and mixed until a homogenized was obtained. Second set: each sample with about 3.0 g of ammonium thiocyanate plus 200 mg of transition metals was weighted and mixed until a homogenized was obtained. Aliquots were withdrawn for determination of concentration of thiocyanate as a zero time. The samples were added to sublimation apparatus and heated for 3 days at 120 °C. After heating, crude sublimate and sample at the bottom were removed from sublimation apparatus and mixed. Aliquots were withdrawn and analysis of thiocyanate, thiourea, and guanidine thiocyanate were carried out as described in methodology.

General method

Before each run, the sublimation apparatus was shaked with hot concentrated sulfuric acid to oxidize any organic substance. After, the sublimation apparatus was washed out with plenty of distilled water to remove the sulfuric acid. Then, It was dried at 200 °C for 2 h, cooled to room temperature and immediately used.

Visible spectrophotometric method

Determination of ammonium and thiocyanate. Ammonium and thiocyanate were extracted from the samples with distilled water. The ammonium was determined using the ninhydrin method as described by Fisher *et al.*[38] The thiocyanate was determined using the method described by Kolthoff *et al.*[39] As a standard in all analysis, a solution of ammonium thiocyanate was prepared and standardized as described by Kolthoff *et al.*[39]

Determination of thiourea. The thiourea was extracted from the samples with 0.012 mol L^{-1} of NaOH and it was determined using an adaptation of the p-benzoquinone method described by Zaia *et al.*[40] For all samples, the absorbance was read at 420 nm and acetate buffer (1.0 mol L^{-1}, pH=3.0) was used.

Infrared spectrophotometric method

Determination of guanidine thiocyanate. About 3.0 mg of guanidine thiocyanate (Sigma-PA) per mg of KBr (with appropriate internal standard) was used as standard in all determinations. The absorbance of NH deformation at 1656 cm^{-1} was used as characteristic band of guanidine thiocyanate. Different internal standards were used for different samples. For the samples of ammonium thiocyanate and ammonium thiocyanate plus river sand, sulfate (5.2 μg of K$_2$SO$_4$ per mg of KBr) was used as internal standard and it was measured the absorbance of the asymmetric stretch of sulfate (1111 cm^{-1}). For the samples of ammonium thiocyanate plus salts of seawater, ammonium thiocyanate plus salts of seawater plus river sand, ammonium thiocyanate plus transition metals and ammonium thiocyanate plus transition metals plus river sand, iodate (27.9 μg of KIO$_3$ per mg of KBr) was used as an internal standard and it was measured the absorbance of the stretch of IO (756 cm^{-1}). For the samples of ammonium thiocyanate plus kaolin, boric acid (20.1 μg of H$_3$BO$_3$ per mg of KBr) was used as an internal standard and it was measured the absorbance of the BH$_2$ deformation vibration (1194 cm^{-1}). Depending on the concentration of guanidine thiocyanate in the samples, a different mass of the samples and the KBr (with appropriate internal standard) were weighted: from 20 to 100 mg for the samples and from 200 to 300 mg for the KBr. They were ground with an agate mortar and pestle until a homogenized mixture was obtained, after a disc pellet was prepared and the absorbances at wavelengths described above were measured.

Raman spectroscopy. Raman spectra were obtained from solid samples. Because the samples are not homogeneous, several spectra were performed from different points of each sample used in the experiments.

Statistical analysis. Comparisons between means were assessed by using: Student's *t*-test, ANOVA test and Student-Newman-Keuls test (S.N.K. test) at a significance level of p<0.05.

Results and Discussion

Table 1 shows the recoveries of thiocyanate and ammonium in the samples of ammonium thiocyanate (A), ammonium thiocyanate plus river sand (B) and ammonium thiocyanate plus kaolin (C), heated for 3 days at different temperatures (80, 120 and 150 °C) in solid state. For all samples, it was obtained lower recoveries of thiocyanate and ammonium with an increasing of the temperature. In spite of, the recoveries of thiocyanate (ANOVA test, F=4.36 and p=0.038) and ammonium in the samples B and C at 120 °C were lower than those obtained for the sample A, the Student-Newman-Keuls test (S-N-K test) showed that, only the recovery of thiocyanate in the sample C was statistically different (p<0.05) from the recovery of thiocyanate in the sample A (Table 1). In the sample A (150 °C) the decomposition of ammonium thiocyanate was about 10%, but this result was not statistically different from 100%. The S-N-K test showed that the recoveries of thiocyanate (ANOVA test, F=6.53 and p=0.011) and ammonium (ANOVA test, F=7.55 and p=0.007) in the samples B and C at 150 °C were statistically different

Table 1. Recoveries of thiocyanate[39] and ammonium[38] in samples: ammonium thiocyanate (A), ammonium thiocyanate plus river sand (B) and ammonium thiocyanate plus kaolin (C). The samples were heated at different temperatures for 3 days in solid state

Temperature (°C)	Recovery (%)					
	A		B		C	
	SCN$^-$	NH$_4^+$	SCN$^-$	NH$_4^+$	SCN$^-$	NH$_4^+$
80	99.5 ± 0.7 (4)	101.5 ± 6.4 (4)	97.9 ± 5.6 (4)	100.4 ± 3.3 (4)	-	-
120	98.6 ± 2.5^a (7)	97.1 ± 4.4 (7)	92.4 ± 2.0 (4)	92.7 ± 7.4 (4)	86.8 ± 4.0 ^{b} (4)	80.4 ± 7.9 (4)
150	90.5 ± 4.5^a (7)	92.3 ± 5.8^d (7)	62.6 ± 7.1^b (5)	41.3 ± 13.3 ^{e} (5)	64.8 ± 9.1^c (4)	59.3 ± 12.7^f (4)

The results are presented as mean ± standard error of the mean. The number of sets is given in parentheses. Each set with two samples. For comparison among recoveries of thiocyanate in the samples A, B and C ANOVA test: line 2 F=4.36 and p=0.038; line 3 F=6.53 and p=0.011 S-N-K test that were statistically different (p<0.05) from each other: line 2 a/b; line 3: a/b and a/c For comparison among recoveries of ammonium in the samples A, B and C ANOVA test: line 3: F=7.55 and p=0.007. S-N-K test that were statistically different (p<0.05) from each other: line 3 d/e and d/f.

(p<0.05) from the recoveries of thiocyanate and ammonium in the sample A (Table 1). The decomposition of ammonium thiocyanate in the samples B and C at 150 °C was about 40%. If dry salt of ammonium thiocyanate is heated at 160-170 °C, 25% will partially isomerize to thiourea,[41,42] and on further heating (190-200 °C) guanidine thiocyanate is obtained and hydrogen sulfide, ammonia and carbon disulfide are evolved.[42] These experiments are showing that using a lower temperature a bigger decomposition of ammonium thiocyanate was obtained, so river sand or kaolin catalyzes the reaction between thiocyanate and ammonium in solid state.

In our experiments, we did not observed the evolving of hydrogen sulfide, ammonia, and carbon disulfide or the formation of sodium sulfides because when hydrochloric acid (0.1 mol L^{-1} or concentrated or hot concentrated) was added to samples, sulfide was not released. In the samples with kaolin or river sand, after the heating, the formation of a yellow compound was observed; this yellow compound could be sulphur. Experiments were carried out to find out more about the reaction studied herein that was different from only heat dry salt of ammonium thiocyanate. When the kaolin and ammonium thiocyanate were added to each other a red color was obtained because the complex between thiocyanate and iron from the kaolin. Using equipment for measurement of melting point a mixture of kaolin and ammonium thiocyante was heated. We observed the first yellow crystal at 120 °C, the same was observed with the sample of river sand. We hypothesize that iron from the kaolin or the river sand (traces) could be involving in the formation of yellow compound. So, sand white quartz from Sigma was added to ammonium thiocyanate, no red compound was observed because there is not iron in the sand. The sample was heated but a yellow crystal was no obtained. After, ferric chloride was added to the sample of sand white quartz plus ammonium thiocyanate, heated and the same behavior as observed for the samples of kaolin and sand river was obtained. Aluminum chloride was also tested however it did not show to have any role in this reaction. The FT-IR spectra did not show any new absorption that could be identified the yellow compound, so experiments were undertaken to identify this compound using Raman spectroscopy. Figure 1 shows the Raman spectra of the following compounds: sulphur, river sand, thiourea, guanidine thiocyanate, ammonium thiocyanate, and the sample river sand plus transition metals plus ammonium thiocyanate. The spectra of the samples with kaolin were not obtained because using the 632.8 nm as excitant radiation these samples showed a strong fluorescence. For the sample river sand plus transition metals plus ammonium thiocyanate, the Raman spectrum showed the bands at 439
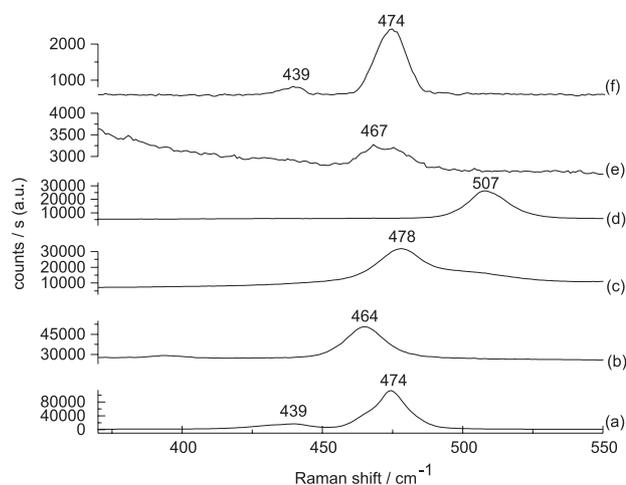

Figure 1. Raman spectra of sulfur (a), river sand (b), thiourea (c), guanidine thiocyanate (d), ammonium thiocyanate (e), and river sand plus ammonium thiocyanate plus transition metals (f).

and 474 cm^{-1}, which are the same bands observed in the spectrum of the sulphur sample (Figure 1). All other substances tested (river sand, thiourea, guanidine thiocyanate, ammonium thiocyanate) did not show the same bands at that region. The Raman spectra of the sample of river sand plus ammonium thiocyanate also showed the same bands of sulphur (spectrum not shown). So the results are shown that in this reaction sulphur has been formed.

Table 2 shows the analysis of thiourea (mg/100 g of NH_4SCN) and guanidine thiocyanate (g/100 g of NH_4SCN) in the samples of ammonium thiocyanate (A), ammonium thiocyanate plus river sand (B) and ammonium thiocyanate plus kaolin (C), heated for 3 days at different temperatures (80, 120 and 150 °C) in solid state. The concentration of thiourea for all samples studied increased with an increasing of the temperature (Table 2). The S-N-K test showed for all results in Table 2 with one exception at 80 °C (ANOVA test, F=27.97 and p=0.003) the concentration of thiourea in the samples B and C was not statistically different (p>0.05) from the concentration of thiourea in the sample A. It should be also pointed out that the concentrations of thiourea in the samples B (150 °C) and C (120 and 150 °C) are very small when compared to the decomposition of ammonium thiocyanate in those samples (Table 1). This could mean that guanidine thiocyanate has been formed immediately after isomerization of ammonium thiocyanate to thiourea. Guanidine thiocyanate was not identified in the samples A and B heated at 80 °C. Since, it is necessary to heat dry ammonium thiocyanate at least 190-200 °C to obtain guanidine thiocyanate,[42] thus even using river sand this temperature (80 °C) is too low to synthesize it (Table 2). The ANOVA test for the samples (guanidine thiocyanate) heated at 120 °C or 150 °C showed the following results F=41.62,

Table 2. Determination of thiourea[40] and guanidine thiocyanate in samples: ammonium thiocyanate (A), ammonium thiocyanate plus river sand (B) and ammonium thiocyanate plus kaolin (C). The samples were heated at different temperatures for 3 days in solid state

Temperature (ºC)	A		B		C	
	Thiourea (mg)[B]	GT^A (g) [B]	Thiourea (mg) [B]	GT^A (g) [B]	Thiourea (mg) [B]	GT^A (g) [B]
80	18.1 ± 1.6^a (4)	NI^{AB}	8.0 ± 0.3^b (3)	NI^{AB}	-	-
120	37.4 ± 3.8 (7)	0.91 ± 0.27^c (7)	30.6 ± 0.2 (4)	0.94 ± 0.30^d (4)	41.5 ± 2.8 (4)	7.47 ± 1.08^e (4)
150	119.4 ± 61.4 (5)	2.02 ± 0.46^f (7)	91.1 ± 17.4 (5)	9.36 ± 1.27^g (4)	195.2 ± 93.1 (4)	11.82 ± 1.72^h (3)

GT^A (guanidine thiocyanate), [B]mg of thiourea or [B]g guanidine thiocyanate per 100 g of ammonium thiocyanate NI^{AB} (not identified) The results are presented as mean ± standard error of the mean. The number of sets is given in parentheses. Each set with two samples. For comparison among concentrations of thiourea in the samples A, B and C ANOVA test: line 1 F=27.97 and p=0.003. S-N-K test that was statistically different (p<0.05) from each other: line 1 a/b. For comparison among concentrations of guanidine thiocyanate in the samples A, B and C ANOVA test: line 2 F=41.62 and P=0.000; line 3 F=30.73 and p=0.000. S-N-K test that were statistically different (p<0.05) from each other: line 2 c/e and d/e; line 3 f/g and f/h.

p=0.000 and F=30.73, p0.000 respectively (Table 2). For the samples B (150 ºC) and C (120 and 150 ºC), the S-N-k test showed that the concentration of guanidine thiocyanate was statistically different (p<0.05) from the concentration of guanidine thiocyanate in the sample A (120 or 150 ºC) (Table 2). These are the same samples whose recoveries of ammonium and thiocyanate were statistically different (Table 1). About 12% (m/m) of guanidine thiocyanate was the best yield (sample C, 150 ºC) obtained (Table 2). Watt and Makosky[43] obtained a yield of 89% for the synthesis of guanidine thiocyanate, however they did not use a condition of reaction that exist on the earth (temperature 300 ºC, equimolar amounts of lead salts and ammonium thiocyanate). Halmann,[33] studying the condensation of glycine by cyanamide, reported that 12% of 0.05 mol L^{-1} cyanamide solution (pH 6.5) converted to cyanoguanidine after 5 days at 60 ºC. Lohrmann[34] studied the formation of urea and guanidine by irradiation of ammonium cyanide. The best yields for guanidine formation using daylight radiation (iron, pH 8.4, 10 weeks) and 254 nm radiation (iron, chloride, pH 8.5, 7 days) were 20% and 8%, respectively. The yields for synthesis of guanidine obtained by Halmann[33] and Lohrmann[34] are not better than that showed in this paper, actually using less time at least the same yield was obtained (Table 2).

Table 3 shows the recoveries of thiocyanate in the samples ammonium thiocyanate, ammonium thiocyanate plus river sand, ammonium thiocyanate plus salts of

Table 3. Recoveries of thiocyanate[39] in samples: ammonium thiocyanate, ammonium thiocyanate plus river sand, ammonium thiocyanate plus salts of seawater, ammonium thiocyanate plus salts of seawater plus river sand, ammonium thiocyanate plus transition metals and ammonium thiocyanate plus transition metals plus river sand. The samples were heated at 120 ºC for 3 days in solid state.

Samples	Recovery (%)	
	Without river sand SCN^-	With river sand SCN^-
ammonium thiocyanate	98.6±2.5 (7)	92.4 ± 2.0^a (4)
ammonium thiocyanate plus salts of seawater[B]	101.7±4.7 (5)	91.1 ± 3.0^b (5)
ammonium thiocyanate plus transition metals[BB]	91.3±1.8 (4)	69.7 ± 6.0^{Ac} (4)

The results are presented as mean ± standard error of the mean. The number of sets is given in parentheses. Each set with two samples. [A]For comparison between recoveries of thiocyanate in the samples without river sand and recoveries of thiocyanate in the samples with river sand (p<0.05) For comparison among recoveries of thiocyanate in the samples: with river sand plus ammonium thiocyanate, with river sand plus ammonium thiocyanate plus salts of seawater and river sand plus ammonium thiocyanate plus transition metals. ANOVA test: column 2 F=10.16 and p=0.004. S-N-K test that was statistically different (p<0.05) from each other: column 2 a/c b/c. [B]The following substances were weighted: 114.26 g of sodium chloride, 15.55 g of magnesium chloride, 7.15 g of magnesium sulfate, 5.23 g of calcium sulfate, 3.33 g of potassium sulfate, 0.4960 g of calcium carbonate, 0.4120 g of potassium bromide and 0.1128 g of boric acid, the salts were ground with an agate mortar and pestle until a homogenized mixture was obtained. [BB]The following substances were weighted: 2.700 g of sodium molybdate (VI), 2.750 g of iron (III) chloride, 2.950 g of zinc (II) sulfate, 2.080 g of manganese (II) nitrate, 2.650 g of copper (II) sulfate, and 2.940 g of cobalt (II) nitrate; they were ground with an agate mortar and pestle until a homogenized mixture was obtained.

seawater, ammonium thiocyanate plus salts of seawater plus river sand, ammonium thiocyanate plus transition metals and ammonium thiocyanate plus transition metals plus river sand, heated at 120 ºC for 3 days in solid state. Recoveries of ammonium were not shown (Table 3) because salts of seawater and transition metals interfere in the ninhydrin method. The following metals Mo (VI), Fe (III), Zn (II), Mn (II), Cu (II) and Co (II) were chosen because as pointed out by Egami[44] they played an important role in the origin of life on earth since they were available in the sea. It was chosen to use the same molar amount because the ranges of concentration of these metals in different environments (seawater, hydrothermal vents, etc) are not narrowed.[44-46] In spite of the recovery of thiocyanate in the sample of ammonium thiocyanate plus salts of seawater plus river sand is low (91%), as showed by the Student *t*-test, it is not statistically different ($p > 0.05$) from the recovery obtained for the samples of ammonium thiocyanate plus salts of seawater or ammonium thiocyanate plus river sand (Table 3). On the other hand, the recovery of thiocyanate in the sample ammonium thiocyanate plus transition metals plus river sand is statistically (Student *t*-test) different ($p < 0.05$) from the recovery obtained for the samples of ammonium thiocyanate plus transition metals or ammonium thiocyanate plus river sand (Table 3). For the samples ammonium thiocyanate plus river sand, ammonium thiocyanate plus salts of seawater plus river sand and

ammonium thiocyanate plus transition metals plus river sand (column 3) the ANOVA test showed the following results $F = 10.16$ and $p = 0.004$ (Table 3). For the same samples the S-N-K test showed that the recovery of thiocyanate in the sample ammonium thiocyanate plus transition metals plus river sand is statistically different ($p < 0.05$) from the recoveries obtained for the samples thiocyanate plus river sand and ammonium thiocyanate plus salts of seawater plus river sand (Table 3). So, transition metals are catalysers for the reaction between ammonium and thiocyanate in solid state. However, these metals do not play the same role of lead salts as described by Watt and Makosky.[43] Because we did not observed evolving of hydrogen sulfide and the Raman spectrum (Figure 1) showed for this sample the same bands of the sulphur.

Table 4 shows the analysis of thiourea (mg/100 g of NH_4SCN) and guanidine thiocyanate (g/100 g of NH_4SCN) in the samples ammonium thiocyanate, ammonium thiocyanate plus river sand, ammonium thiocyanate plus salts of seawater, ammonium thiocyanate plus salts of seawater plus river sand, ammonium thiocyanate plus transition metals and ammonium thiocyanate plus transition metals plus river sand, heated at 120 ºC for 3 days in solid state. The concentrations of thiourea in the samples of ammonium thiocyanate plus salts of seawater plus river sand and ammonium thiocyanate plus transition metals plus river sand were statistically (Student *t*-test) different ($p < 0.05$) from the samples with the same

Table 4. Determination of thiourea[40] and guanidine thiocyanate in samples: ammonium thiocyanate, ammonium thiocyanate plus river sand, ammonium thiocyanate plus salts of seawater, ammonium thiocyanate plus salts of seawater plus river sand, ammonium thiocyanate plus transition metals and ammonium thiocyanate plus transition metals plus river sand. The samples were heated at 120 ºC for 3 days in solid state

Samples	Without river sand		With river sand	
	Thiourea (mg)[AA]	GT[A] (g)[AA]	Thiourea (mg)[AA]	GT[A] (g)[AA]
Ammonium thiocyanate	37.4 ± 3.8[a] (7)	0.91 ± 0.27[d] (7)	30.6 ± 0.2[g] (4)	0.94 ± 0.30[j] (4)
Ammonium thiocyanate plus salts of Seawater[B]	62.8 ± 7.1[b] (5)	0.51 ± 0.21[e] (5)	164.2 ± 21.0[Ch] (5)	3.69 ± 0.29[Dk] (5)
Ammonium thiocyanate plus transition Metals[BB]	38.2 ± 5.8[c] (4)	2.43 ± 0.23[f] (4)	109.1 ± 4.2[Ci] (4)	7.58 ± 0.97[Dl] (4)

GT[A] (guanidine thiocyanate) [AA]mg of thiourea or [AA]g of guanidine thiocyanate per 100 g of ammonium thiocyanate. The results are presented as mean ± standard error of the mean. The number of sets is given in parentheses. Each set with two samples. [C]For comparison between concentration of thiourea in the samples without river sand and with river sand ($p < 0.05$). [D]For comparison between concentration of guanidine thiocyanate in the samples without river sand and with river sand ($p < 0.05$). For comparison among concentrations of thiourea in the samples: line 1, line 2 and line 3. ANOVA test: column 1 $F = 6.98$ and $p = 0.009$, column 3 $F = 21.98$ and $p = 0.000$. S-N-K tests that were statistically different ($p < 0.05$) from each other: column 1 a/b and b/c; column 3 g/h, g/i and h/i. For comparison among concentrations of guanidine thiocyanate in the samples: line 1, line 2 and line 3. ANOVA test: column 2 $F = 12.78$ and $p = 0.000$, column 4 $F = 31.73$ and $p = 0.000$. S-N-K tests that were statistically different ($p < 0.05$) from each other: column 2 d/f and e/f column 4 j/k, j/l and k/l. [B]The following substances were weighted: 114.26 g of sodium chloride, 15.55 g of magnesium chloride, 7.15 g of magnesium sulfate, 5.23 g of calcium sulfate, 3.33 g of potassium sulfate, 0.4960 g of calcium carbonate, 0.4120 g of potassium bromide, and 0.1128 g of boric acid; the salts were ground with an agate mortar and pestle until a homogenized mixture was obtained. [BB]The following substances were weighted: 2.700 g of sodium molybdate (VI), 2.750 g of iron (III) chloride, 2.950 g of zinc (II) sulfate, 2.080 g of manganese (II) nitrate, 2.650 g of copper (II) sulfate, and 2.940 g of cobalt (II) nitrate; they were ground with an agate mortar and pestle until a homogenized mixture was obtained.

composition but without river sand (Table 4). However the concentration of thiourea is low for decomposition of ammonium thiocyanate (Table 3), this means that guanidine is formed after isomerization of ammonium thiocyanate to thiourea. Salts of seawater have not effect on the reaction between ammonium and thiocyanate without river sand (Table 4). However when river sand is added to the sample the amount of thiocyanate guanidine produced is statistically (Student *t*-test) different (p<0.05) from the sample without river sand (Table 4). The concentration of thiocyanate guanidine in the sample ammonium thiocyanate plus salts of seawater plus river sand was statistically (Student *t*-test) different (p<0.05) from the sample ammonium thiocyanate plus river sand (Table 4). Transition metals increased the amount of guanidine thiocyanate synthesized. The concentration of guanidine thiocyanate in the sample ammonium thiocyanate plus transition metals plus river sand was statistically (Student *t*-test) different (p<0.05) from the samples ammonium thiocyanate plus transition metal and ammonium thiocyanate plus river sand. The concentration of guanidine thiocyanate in the sample ammonium thiocyanate plus transition metals was statistically (Student *t*- test) different (p<0.05) from the sample ammonium thiocyanate. The ANOVA and S-N-K tests were also used to analyze the results of the columns 1-4. For the columns 1 and 3 (thiourea results) the ANOVA test showed the following results F=6.98 , p=0.009 and F=21.98 , p=0.000 respectively. The results at the column 1 are showing that only salts of seawater increased the amount of thiourea and it was statistically different (p<0.05) from the other two results, as showed by the S-N-K test (Table 4, column 1). For the column 3 all the results were statistically different (p<0.05) from each other as showed by the S-N-K test (Table 4). For the columns 2 and 4 (guanidine thiocyanate results) the ANOVA test showed the following results F=12.78 , p=0.000 and F=31.73 , p=0.000 respectively. The results at the column 2 are showing that only transition metals increased the amount of guanidine thiocyanate and it was statistically different (p<0.05) from the other two results, as showed by the S-N-K test (Table 4, column 2). For the column 4 all the results were statistically different (p<0.05) from each other as showed by the S-N-K test (Table 4).

Conclusion

Guanidine is an important substance for the modern living beings as well as for prebiotic chemistry. The results show that river sand, kaolin, transition metals and salts of seawater catalyse the reaction between ammonium and thiocyanate in solid state under compatible prebiotic chemistry conditions. For all samples, it was obtained lower recoveries of thiocyanate and ammonium with an increasing of the temperature. At 120 ºC, only the recovery of thiocyanate (86.8%) in the sample with kaolin was statistically different (p<0.05) from the recovery of thiocyanate in the sample without kaolin. The recoveries of thiocyanate and ammonium in the samples with river sand or kaolin at 150 ºC were statistically different (p<0.05) from the recoveries of thiocyanate and ammonium in the sample without them. The decomposition of ammonium thiocyanate in the samples river sand or kaolin at 150 ºC was about 40%. The concentrations of thiourea or guanidine thiocyanate for all samples studied increased with an increasing of the temperature. The concentrations of thiourea in the samples with river sand or kaolin at 120 or 150 ºC were not statistically different (p>0.05) from the concentration of thiourea in the sample without them. Kaolin showed to have more effect on the synthesis guanidine than river sand. Because, the concentrations of guanidine in the samples with kaolin (120 and 150 ºC) were always bigger than in the samples with river sand (120 and 150 ºC). Salts of seawater did not show to increase the decomposition of ammonium thiocyanate even when river sand was added to the samples. However, the decomposition of ammonium thiocyanate in the samples with transition metals plus river sand was about 30%. The salts of seawater or transition metals plus ammonium thiocyanate plus river sand showed to increase the concentration of thiourea (seawater: 538%; transition metals: 357%) or guanidine (seawater: 393%, transition metals: 806%) when they were compared to the samples of ammonium thiocyanate plus river sand. When the results obtained with sample of ammonium thiocyanate were compared to the samples of ammonium thiocyanate (at 120 ºC) plus salts of seawater or transition metals also an increased on the production of thiourea (168%) and guanidine (268%) was obtained, respectively. The yield for the synthesis of guanidine showed in this paper is better than that obtained by other authors. Experiments showed that iron (III) is involving in the formation of the yellow compound and Raman spectra showed this compound could be sulphur.

Acknowledgements

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