



J. Serb. Chem. Soc. 75 (10) 1381–1389 (2010)
JSCS–4060

UV Radiation and the reaction between ammonium and thiocyanate under prebiotic chemistry conditions

HENRIQUE de SANTANA¹, LEIDIMARA PELISSON¹, DIOGO R. JANIASKI¹,
CÁSSIA THAÍS B. V. ZAIA² and DIMAS A. M. ZAIA^{1*}

¹Departamento de Química – CCE, Universidade Estadual de Londrina, 86051-990,
Londrina-PR and ²Departamento de Ciências Fisiológicas–CCB, Universidade
Estadual de Londrina, 86051–990, Londrina–PR, Brazil

(Received 19 February, revised 26 May 2010)

Abstract: The reaction between ammonium and thiocyanate under prebiotic chemistry conditions was studied using FT-IR spectroscopy. Ammonium thiocyanate (1.0×10^{-3} mol L⁻¹) was dissolved in sodium chloride solution (28.57 g L⁻¹) at two different pH values (5.30 and 7.20). FT-IR results showed that it was possible that some compound that resembles dithiooxamides was synthesized when samples of ammonium thiocyanate were exposed to UV radiation under a regular atmosphere, as UV radiation in the presence of oxygen leads to the formation of perchlorate ions (ClO₄⁻) due to the presence of Cl⁻ as well. After acid hydrolysis of the samples of ammonium thiocyanate irradiated under a nitrogen atmosphere, yellow and white compounds were obtained, which could not be identified. These results were different from those reported in the literature, where other authors found methionine. However, they used higher concentrations of ammonium thiocyanate and a different type of UV lamp. On the other hand, in the present study, a lower concentration of ammonium thiocyanate was used, which probably resembled more the concentration of ammonium thiocyanate of primitive earth.

Keywords: ammonium thiocyanate; prebiotic chemistry; UV radiation; origin of life.

INTRODUCTION

Amino acids play an important role in the biochemistry of present living organisms, such as: inhibitory and excitatory neurotransmitters, glucose, hormone and neurotransmitters precursors and peptides and proteins subunits. It should be emphasized that proteins participate 18 % w/w to the composition of mammalian cells.¹ Thus, the study of their synthesis is relevant to an understanding of the origin of life on Earth. As pointed out by Zaia *et al.*,² when planning prebiotic

*Corresponding author. E-mail: damzaia@uel.br
doi: 10.2298/JSC100219112S

chemistry experiments, it is important to know what were the sources for amino acid synthesis on the primitive Earth and which amino acids were synthesized from these sources.

Ammonium and thiocyanate were synthesized in experiments under prebiotic chemistry conditions. They were also found in places that resemble these environments. Dowler and Ingmanson³ determined the presence of thiocyanate in Atlantis II Deep Brine, located at the bottom of the sea in the Rift Valley. Thiocyanate was synthesized due to the reaction between HCN and H₂S. HCN, H₂S and nitrogen compounds (CNS⁻, amines, amino acids – Arg, Ser, Thr, Gly, ABA, Phe) are found in sub-marine volcanoes.^{4,5} Comets⁶ could be a source of NH₃, HCN and H₂S, as well as of other sulfur and nitrogen compounds (NH₂CHO, HNC, HNCO, CH₃CN, HC₃N, CS₂, CS, SO₂, SO, OCS, H₂CS, NS). Summers⁷ showed that nitrite could be reduced to ammonia by iron(II). As reviewed by Raulin and Toupance,⁸ ammonium thiocyanate can be synthesized when a mixture of gases (CH₄–NH₃–H₂O_(vap)–H₂–N₂–CO₂–H₂S), resembling the primitive atmosphere of Earth, is submitted to electric discharges. Thus, ammonium and thiocyanate are substances that probably were easily found on the primitive Earth.

The reaction between ammonium and thiocyanate has been studied under different conditions, some of them resembling the environments of the primitive earth. For example, Holmes⁹ irradiated a concentrated aqueous solution of ammonium thiocyanate (4.0 mol L⁻¹) and observed the formation of colloidal sulfur. Talreja *et al.*¹⁰ heated/irradiated with UV radiation ammonium thiocyanate in the solid state and observed the formation of thiourea. Zaia *et al.*¹¹ also studied the reaction of ammonium thiocyanate in the solid state. They heated samples of ammonium thiocyanate, sand and transition metals and detected the formation of guanidine. Spheres were detected in samples of ammonium thiocyanate and formaldehyde with or without UV irradiation and ammonium thiocyanate with UV irradiation.^{12,13} Steinman *et al.*¹⁴ detected the formation of methionine when an aqueous solution of ammonium thiocyanate was exposed to UV radiation.

The climate of the primitive Earth is uncertain, there are models of hot and cold primitive Earth and both are plausible.^{15,16} The atmosphere of the primitive Earth was probably neutral-redox (CO, CO₂, N₂, H₂) and the O₂ concentration was very low, it could have been about 0.10 % of the present atmospheric level.¹⁷ The main extraterrestrial source of carbon compounds was interplanetary dust particles, IDPs.^{18–20} The main proposed sources of energy on the primitive Earth are: UV radiation, electric discharges, volcanic heating, radioactivity, cosmic rays and meteor impacts. According to Kobayashi *et al.*,²¹ UV radiation was the greatest source of energy for the synthesis of biomolecules on the primitive Earth. Thus in the present research, UV radiation was used as the source of energy for the synthesis of biomolecules from the reaction between ammonium and thiocyanate under prebiotic chemistry conditions. To simulate the action of UV

rays, a UV lamp was used. Ammonium thiocyanate ($1.0 \times 10^{-3} \text{ mol L}^{-1}$) was dissolved in a sodium chloride solution (28.57 g L^{-1}), which is the same concentration found in seawater, at two different pH values (5.30 and 7.20). It should be emphasized that, to the best of our knowledge, this reaction has not been studied before under conditions plausible to those of the primitive Earth.

EXPERIMENTAL

All employed reagents were of analytical grade. For all the experiments, a wooden box with the following dimensions (40 cm×40 cm×40 cm) was used. The box contained an Hg lamp with a 120 W bulb. The pH of the samples was adjusted to 5.30 and 7.20, because the former is found in hydrothermal environments and the latter is the average pH of seawater.²²

Experiment 1: UV irradiation of ammonium thiocyanate solution

50 mL of a solution containing ammonium thiocyanate ($1.0 \times 10^{-3} \text{ mol L}^{-1}$) and sodium chloride (28.57 g L^{-1}) were added to four beakers. The pH of the samples was adjusted to 5.30 or 7.20 with HCl (0.10 mol L^{-1}) or NaOH (0.10 mol L^{-1}). The samples were irradiated for 0.5, 1.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0 and 15.0 h. During all the irradiation period (0–15 h), the beakers were kept on an ice bath. After irradiation, aliquots were withdrawn for ammonium and thiocyanate analysis, the samples were lyophilized, and the FT-IR spectra were recorded using the pressed KBr disc technique.

Experiment 2: UV irradiation of ammonium thiocyanate solution under a nitrogen atmosphere

A volume of 50 mL of ammonium thiocyanate ($1.0 \times 10^{-3} \text{ mol L}^{-1}$) dissolved in a sodium chloride solution (28.57 g L^{-1}) was added to four beakers. The pH of the samples was adjusted to 5.30 or 7.20 with HCl (0.10 mol L^{-1}) or NaOH (0.10 mol L^{-1}), respectively. The samples were irradiated for 3.0 h. During the irradiation period, the beakers were kept on an ice bath and under a nitrogen atmosphere. After irradiation, the samples were lyophilized and then were submitted to acid hydrolysis with an HCl solution (6.0 mol L^{-1}) at 120 °C for 24 h. The FT-IR spectra were recorded using the pressed KBr disc technique.

Experiment 3: UV irradiation of ammonium thiocyanate solution under a nitrogen atmosphere without sodium chloride¹⁴

The samples for Experiment 3 were prepared as described by Steinman *et al.*¹⁴ A volume of 50 mL of ammonium thiocyanate (0.10 mol L^{-1}) was added to four beakers. The samples were irradiated for 3.0 h. During irradiation, the beakers were kept on an ice bath and under a nitrogen-saturated atmosphere. After irradiation, the samples were lyophilized and then were submitted to acid hydrolysis in 6.0 mol L^{-1} HCl at 120 °C for 24 h. The FT-IR spectra were recorded using the pressed KBr disc technique.

Acid hydrolysis of the samples from Experiments 2 and 3

An aliquot of 500 μL of each sample and 500 μL of concentrated HCl were added to tubes used for acid digestion of amino acids. The tubes were heated at 110 °C for 24 h. After cooling, the solids (white and yellow) were withdrawn and washed several times with distilled water. The solids, white and yellow aggregates, separated from each other and thus were separated manually.

Infrared spectrophotometric method

The IR spectra were recorded on a Shimadzu FT-IR 8300 instrument, using the pressed KBr disk technique. The spectral resolution was 4 cm^{-1} and each spectrum was determined

after acquiring 120 spectra. About 10 mg of samples plus 200 mg of KBr were weighed and then ground in an agate mortar until a homogeneous mixture was obtained. The disc pellets were prepared and the spectra were recorded from 400 to 4000 cm^{-1} . The FT-IR spectra were analyzed with the program Origin (version 5.0).

RESULTS AND DISCUSSION

Prebiotic chemistry studies require that the experiments must be performed under conditions plausible to those that prevailed on the primitive Earth.²³ The concentration of thiocyanate in present seawater is $1.0 \times 10^{-5} \text{ mol L}^{-1}$,² and the concentration of total nitrogen compounds around submarine volcanoes is $2.2 \times 10^{-2} \text{ mol L}^{-1}$.⁴ Thus, the concentration used in the experiments ($1.0 \times 10^{-3} \text{ mol L}^{-1}$) was close enough to real conditions and a reasonable value to perform prebiotic chemistry experiments. Seawater as described by Benetoli *et al.*²⁴ could not be used because sulfates and carbonates would interfere with the FT-IR experiments. Thus, ammonium thiocyanate was dissolved in distilled water with sodium chloride at the same concentration present in seawater (28.57 g L^{-1}). Experiments 1 and 2 were performed at pH 5.30 and 7.20 because the first is found in hydrothermal environments and the second is the average of seawater today.²² Thus experiments 1 and 2 were realized under conditions of an ammonium thiocyanate concentration of $1.0 \times 10^{-3} \text{ mol L}^{-1}$, control of pH (5.30 or 7.20) and NaCl (at the same concentration as in seawater), *i.e.*, under conditions more close to those of prebiotic Earth than any of the mentioned studies. It should also be emphasized that Experiment 1 simulated a primitive atmosphere with oxygen and Experiment 2 without it.

Experiments 1 and 2 showed a change in the solution and slight white suspension appeared in the solution after 2 h of irradiation. Smith *et al.*¹² irradiated aqueous solutions of ammonium thiocyanate ($0.01\text{--}2.0 \text{ mol L}^{-1}$) and perceived the formation of an intense white suspension. After 10 min, the irradiation was stopped and formation of microspheres that aggregated to form large spheres and chains was observed. According to them, the formation of spheres depended on the time of irradiation and the concentration of ammonium thiocyanate (NH_4SCN). In the present experiments a white suspension appeared but the formation of microspheres was not observed. On the other hand, Holmes⁹ studied the irradiation of a concentrated solution of NH_4SCN (4.0 mol L^{-1}) and observed the formation of solids believed to be colloidal sulfur. Experiments 1 and 2 did not show the formation of microspheres or colloidal sulfur and after a few hours, the irradiation was stopped and the white suspension disappeared. These results could be explained by the fact that a low concentration of ammonium thiocyanate ($1.0 \times 10^{-3} \text{ mol L}^{-1}$) compared to those employed in the experiments of Holmes⁹ (4.0 mol L^{-1}) and Smith *et al.*¹² ($0.01\text{--}2.0 \text{ mol L}^{-1}$) was used. In addition, it should be mentioned that the experimental conditions used by Holmes⁹ and Smith *et al.*¹² were unrealistic from a prebiotic chemistry view point,

since they used a high concentration of ammonium thiocyanate that could never have existed on the primitive Earth.^{3,4,25}

The FT-IR spectra of solid ammonium thiocyanate (lyophilized samples of ammonium thiocyanate dissolved in sodium chloride solution after ultraviolet irradiation for 2, 7 and 15 h at pH 7.20) are shown in Fig. 1. The experiment (Experiment 1) was performed under a regular atmosphere. The intensity of the bands was determined using the internal standard KIO_3 (784 cm^{-1}). The bands at 1400 and 3125 cm^{-1} are characteristic of the ammonium ion, attributed to NH_4 deformation and stretch, respectively, and the feature at 2065 cm^{-1} is due to the SCN^- group, assigned to the CN bond stretching. After ultraviolet irradiation, the bands at 2065 and 3125 cm^{-1} vanished and two new bands (at 1152 and 1633 cm^{-1}) were observed (Fig. 1). The intensity of the bands at 1152 and 1633 cm^{-1} increased with irradiation time. The same results were observed for the samples irradiated at pH 5.30. Besides the decomposition of SCN^- and NH_4^+ , only the vanishing of the band at 2065 cm^{-1} occurred. The band at 1400 cm^{-1} did not disappear, thus suggesting a coupling between thiocyanates $[\text{S}=\text{C}=\text{N}]^-$. This coupling could explain the retention of the band at 1400 cm^{-1} and the new band at 1633 cm^{-1} . The product of the coupling of thiocyanates $[\text{S}=\text{C}=\text{N}]^-$ resembles dithiooxamides $[\text{NH}_2\text{C}(=\text{S})\text{C}(=\text{S})\text{NH}_2]$ which have characteristic bands at 1592 and 1434 cm^{-1} due to the groups NH_2 and H_2NCS .²⁶ The band at 1152 cm^{-1} could not be explained by the synthesis of compounds that resemble dithioox-

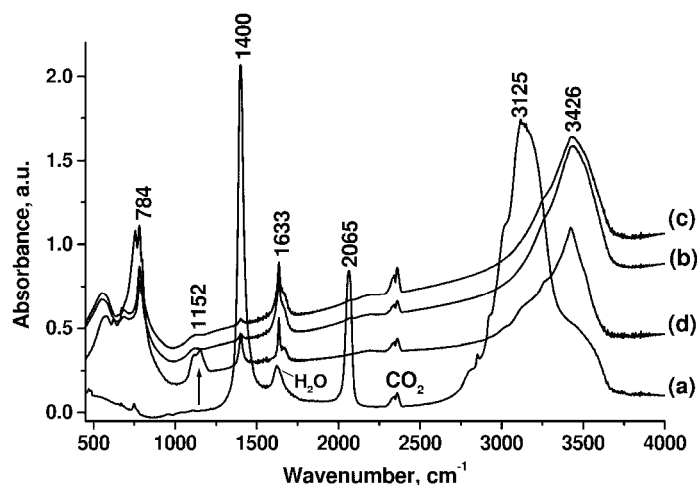


Fig. 1 FT-IR spectra: solid ammonium thiocyanate (a); lyophilized samples of ammonium thiocyanate ($1.0 \times 10^{-3}\text{ mol L}^{-1}$) dissolved in sodium chloride solution (28.57 g L^{-1}) after irradiation for 2 (b), 7 (c) and 15 h (d) at pH 7.20. The FT-IR spectra of the samples (b), (c) and (d) were recorded with an internal standard of KIO_3 (784 cm^{-1}). The FT-IR spectra of the samples were recorded in pressed KBr discs. The experiments were performed under a regular atmosphere, as described in the experimental section (Experiment 1).

amides. An explanation for this band is the synthesis of perchlorate (ClO_4^-); in fact, it is known to occur naturally through the formation of ozone (O_3) by UV radiation of oxygen and, subsequently, perchlorate is formed by the oxidation of chloride by ozone.²⁷ The FT-IR spectrum of sodium perchlorate showed a band at 1152 cm^{-1} due to Cl–O stretch (data not shown). However, it should be emphasized that this reaction probably did not play an important role in prebiotic chemistry, since the amount of oxygen was too low on the primitive Earth.¹⁵

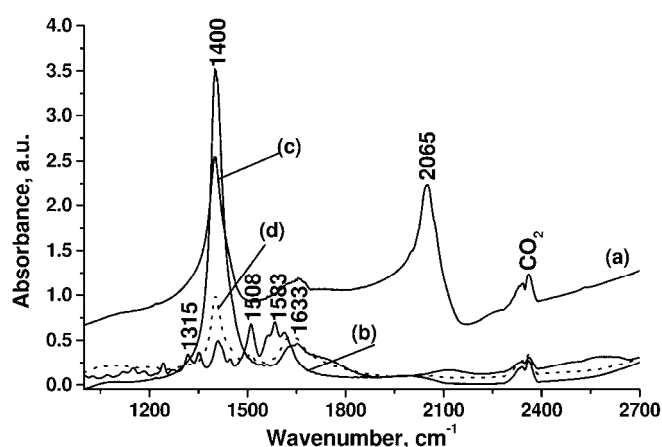


Fig. 2. FT-IR spectra of: solid ammonium thiocyanate (a); solid methionine (b); white solid (c) and yellow solid (d). The white and yellow solids were obtained in Experiment 3. The samples in Experiment 3 (50 mL of 0.10 mol L^{-1} ammonium thiocyanate) were irradiated for 3.0 h. During irradiation, the samples were kept on an ice bath and under a nitrogen saturated atmosphere. After irradiation, the samples were lyophilized, and then submitted to acid hydrolysis in 6.0 mol L^{-1} HCl at $120\text{ }^\circ\text{C}$ for 24 h. The FT-IR spectra of the samples were recorded in pressed KBr discs.

Samples prepared as described in experiment 2 and 3 were irradiated for just 3 h under a nitrogen atmosphere. Experiment 3 was performed under the same conditions as described by Steinman *et al.*¹⁴ In both experiments, two solids (white and yellow) were obtained after acid hydrolysis. The acid hydrolysis was performed to verify if the precursors of amino acids were synthesized.²⁸ The FT-IR spectra of solid ammonium thiocyanate, solid methionine and the observed white solid (Experiment 3) and yellow solid (Experiment 3) are shown in Fig. 2. The band at 2065 cm^{-1} was absent in spectrum d (yellow solid) and bands at 1400 and 1633 cm^{-1} were again observed. However, these bands are not characteristic of methionine, which shows bands at 1315 , 1508 and 1583 cm^{-1} (Fig. 2). The solids (white and yellow) obtained in experiment 2 showed the same FT-IR spectra as in Experiment 3. As shown by Lapinski *et al.*,²⁹ the synthesis of the solids (white and yellow) could be attributed to phototautomeric compounds of dithioamides. However, this could be true only for the yellow solid because the S–H

stretch was observed in the FT-IR spectrum of this sample. As expected, the products in Experiments 2 and 3 did not show a band at 1152 cm^{-1} , because both experiments were carried out under a nitrogen atmosphere and in Experiment 3 there was also no sodium chloride present.

The experiments described in this paper (Experiments 1–3) did not show the presence of methionine, even when the experiment (Experiment 3) described by Steinman *et al.*¹⁴ was reproduced. This failure to synthesize methionine could be due to the difference in UV lamps used here and by Steinman *et al.*¹⁴ The radiation emitted by the UV lamp used in the present experiments has emission lines from 185 to 580 nm,³⁰ while the UV lamp used by Steinman *et al.*¹¹ had 90 % of intensity emission at 254 nm.³¹ It should also be emphasized that the only method used by Steinman *et al.*¹⁴ to identify methionine was paper chromatography, which could not have been conclusive. However, this experiment was realized in 1969 and that time paper chromatography was best analytical tool available.

CONCLUSIONS

In conclusion, this paper showed the following results: a) UV radiation is an important source for the synthesis of biomolecules; b) a compound that resembled dithiooxamides was probably synthesized; c) perchlorate (ClO_4^-) was synthesized when the samples were irradiated with UV radiation in the presence of oxygen and Cl^- ; d) two non-identified solids (white and yellow) were obtained after acid hydrolysis of the samples of ammonium thiocyanate irradiated under a nitrogen atmosphere; e) methionine was not obtained as reported other authors. However, they used higher concentrations of ammonium thiocyanate and a different type of UV lamp; f) the formation of microspheres was not observed, probably because of the low concentration of ammonium thiocyanate employed in the experiments; g) no biomolecules were found in this work.

Acknowledgments. LP and DRJ acknowledge the fellowships from PIBIC/CNPq/UEL. This research was supported by grants from CNPq (473076/2004) and Fundação Araucária (2421). Dr. A. Leyva helped with the English editing of the manuscript.

ИЗВОД

UV РАДИЈАЦИЈА И РЕАКЦИЈА ИЗМЕЂУ АМОНИЈАКА И ТИОЦИЈАНАТА ПРИ ПРЕБИОТИЧКИМ ХЕМИЈСКИМ УСЛОВИМА

HENRIQUE DE SANTANA¹, LEIDIMARA PELISSON¹, DIOGO R. JANIASKI¹,
CÁSSIA THAÍS B. V. ZAIA² и DIMAS A. M. ZAIA¹

¹Departamento de Química – CCE, Universidade Estadual de Londrina, 86051–990, Londrina–PR u

²Departamento de Ciências Fisiológicas – CCB, Universidade Estadual de Londrina, 86051–990, Londrina–PR, Brazil

Применом FT-IR спектроскопије изучавана је реакција између амонијака и тиоцијаната при пребиотичким хемијским условима. Амонијум-тиоцијанат ($1,0 \times 10^{-3}\text{ mol/dm}^3$) је растворен у раствору натријум-хлорида ($28,57\text{ g/dm}^3$) при две различите рН вредности (5,30 и 7,20).

На основу FT-IR спектроскопских резултата нађено је да у току UV радијације амонијум-тиоцијаната долази до грађења дитиооксимида, док у присуству Cl⁻ и кисеоника настаје перхлорат (ClO₄⁻). При радијацији у инертним условима (струја азота) и киселој хидролизи узорка амонијум-тиоцијаната долази до грађења производа жуте боје, чија структура није одређена. Приказани резултати се разликују од одговарајућих литературних података, где су аутори претпоставили да при наведеним условима долази до грађења метионина. Међутим, ови аутори су користили веће концентрације амонијум-тиоцијаната и различите UV лампе. Насупрот овоме, у овом раду коришћене су ниже концентрације амонијум-тиоцијаната, што више одговара његовом садржају у земљи.

(Примљено 19. фебруара, ревидирано 26. маја 2010)

REFERENCES

1. B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, J. D. Watson, *Molecular Biology of the Cell*, 3rd ed., Garland Publishing Inc., New York, 1994
2. D. A. M. Zaia, C. T. B. V. Zaia, H. de Santana, *Origins Life Evol. Biosphere* **38** (2008) 469
3. M. J. Dowler, D. E. Ingmanson, *Nature* **279** (1979) 51
4. G. Aloisi, M. Drews, K. Wallmann, G. Bohrmann, *Earth Planet. Sci. Lett.* **225** (2004) 347
5. L. M. Mukhin, *Origins Life* **7** (1976) 355
6. J. Lhorca, *Int. Microbiol.* **8** (2005) 5
7. D. P. Summers, *Origins Life Evol. Biosphere* **29** (1999) 33
8. F. Raulin, G. Toupance, *J. Mol. Evol.* **9** (1977) 329
9. M. Holmes, *J. Chem. Soc.* (1926) 1690
10. S. T. Talreja, P. M. Oza, P. S. Rao, *Bull. Chem. Soc. Jpn.* **40** (1967) 2427
11. D. A. M. Zaia, H. de Santana, R. Toppan, C. T. B. V. Zaia, *J. Braz. Chem. Soc.* **15** (2004) 190
12. A. E. Smith, G. Steinman, C. Galand, *Experientia* **25** (1969) 255
13. A. E. Smith, J. J. Silver, G. Steinman, *Experientia* **24** (1968) 36
14. G. Steinman, A. E. Smith, J. J. Silver, *Science* **159** (1968) 1108
15. J. F. Kasting, M. T. Howard, *Philos. Trans. R. Soc. B* **361** (2006) 1733
16. J. F. Kasting, S. Ono, *Philos. Trans. R. Soc. B* **361** (2006) 917
17. I. H. Campbell, C. M. Allen, *Nat. Geosci.* **1** (2008) 554
18. C. Chyba, C. Sagan, *Nature* **355** (1992) 125
19. M. Bernstein, *Philos. Trans. R. Soc. B* **361** (2006) 1689
20. A. Brack, *Chem. Biodiversity* **4** (2007) 665
21. K. Kobayashi, H. Masuda, K. I. Ushio, A. Ohashi, H. Yamanashi, T. Kaneko, J. I. Takahashi, T. Hosokawa, H. Hashimoto, T. Saito, *Adv. Space Res.* **27** (2001) 207
22. N. G. Holm, E. Andersson, *Astrobiology* **5** (2005) 444
23. D. A. M. Zaia, *Quim. Nova* **26** (2003) 260
24. L. O. B. Benetoli, C. M. D. de Souza, K. L. da Silva, I. G. de Souza Jr, H. de Santana, A. Paesano Jr., A. C. S. da Costa, C. T. B. V. Zaia, D. A. M. Zaia, *Origins Life Evol. Biosphere* **37** (2007) 479
25. M. Pasek, D. Lauretta, *Origins Life Evol. Biosphere* **38** (2008) 5
26. N. B. Colthup, L. H. Daly, S. E. Wierly, *Introduction to Infrared and Raman Spectroscopy*, Academic Press, New York, 1964
27. P. K. Dasgupta, P. K. Martinelango, W. A. Jackson, T. A. Anderson, K. Tian, R. W. Tock, S. Rajagopalan, *Environ. Sci. Technol.* **39** (2005) 1569

28. K. Kobayashi, T. Kaneko, A. Kouchi, H. Hashimoto, T. Saito, *Adv. Space Res.* **23** (1999) 401
29. L. Lapinski, H. Rostkowska, A. Khvorostov, M. Yaman, R. Fausto, M. J. Novak, *J. Phys. Chem. A* **108** (2004) 5551
30. A. Cavicchioli, G. R. Gutz, *Quim. Nova* **26** (2003) 913
31. Pen-Ray UV lamps (2008), <http://ridl.cis.rit.edu/products/manuals/Acton/old/MANUAL/MS-416.pdf> (accessed on December 8, 2008).