Heteroatom Chemistry
Volume 21, Number 3, 2010

Resolving the Enigma of Prebiotic C—O—P Bond Formation: Prebiotic Hydrothermal Synthesis of Important Biological Phosphate Esters

Gull Maheen,[1] Ge Tian,[1] Yingwu Wang,[2] Chao He,[1] Zhan Shi,[1] Hongming Yuan,[1] and Shouhua Feng[1]

[1]*State Key Laboratory of Inorganic Synthesis and Preparative Chemistry, College of Chemistry, Jilin University, Changchun 130012, People's Republic of China*

[2]*College of Life Science, Jilin University, Changchun 130012, People's Republic of China*

Received 23 November 2009; revised 25 February 2010

ABSTRACT: *Important biological phosphate esters such as* sn*-glycerol-3-phosphate, glycerol-2-phosphate, and phosphoethanolamine were synthesized under hydrothermal conditions. Phosphorus was incorporated into the biomolecules, leading to the formation of C—O—P type compounds hydrothermally. Only perlite-catalyzed reaction at 180°C could result in the formation of sn-glycerol-3-phosphate, whereas glycerol-2-phosphate could be easily synthesized at 100°C with or without minerals and phosphoethanolamine was obtained within a temperature range of 100 to 120°C.* © 2010 Wiley Periodicals, Inc. Heteroatom Chem 21:161–167, 2010; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.20591

INTRODUCTION

Hydrothermal systems are one of the most potential sites for the origin of life. Many significant evidences convince that life originated hydrothermally [1–6]. It was suggested that life emerged in marine hot springs, which were equipped with all necessary conditions that could facilitate the origin of life [7]. Acidic hydrothermal vents are known to support the early life, in which acidophiles (one of the most primitive extremophiles) reside [8]. Volcanic activities are known to be the most plausible sources of acidic hydrothermal vents in which acidity is mainly generated by the biological oxidation of elemental sulfur [8].

$$S^{\circ} + \frac{3}{2}O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+$$

Many evidences suggest that iron-rich acidic hydrothermal sites are the relics of ancient (Archaean) iron world [8] where life thrived very well. Not only this, but also such sites are the potential sources of important prebiotic minerals. Copious amounts of minerals such as kaolinite, hematite, magnetite, perlite, and zeolites are present in the acidic hydrothermal systems, along with many others [9,10]. Kaolinite mineral is abundant in acidic hydrothermal systems [11]. It is also abundantly located in the western Australian acid lake systems [12]. Deposits of kaolinite along with quartz are also found in the slightly acidic hydrothermal systems [13]. As mentioned previously, acidic hydrothermal systems also have abundance of zeolites. It is believed that

Correspondence to: Shouhua Feng; e-mail: shfeng@mail.jlu.edu.cn.

Contract grant sponsor: National Natural Science Foundation of China.
Contract grant numbers: 20631010, 90922034, and 20901026.

© 2010 Wiley Periodicals, Inc.

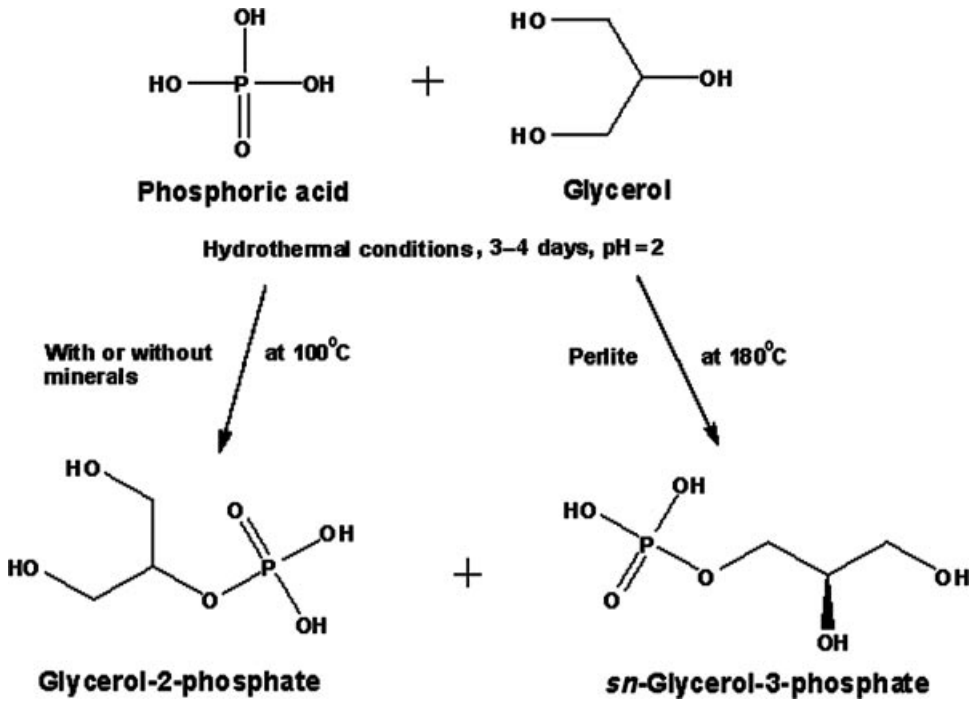
the formation of zeolites and clays is the most specific link between impact cratering and the origin of life [9]. Recently discovered world's oldest rocks, which resemble volcanic rocks, are rich in minerals such as garnet, quartz, and many other silicates [14]. Similarly, hematite is ubiquitous both in highly acidic hydrothermal vents [8] and in highly acidic rivers, along with quartz [15,16]. Perlite, a hydrated volcanic glass, is formed in acidic volcanic systems. The spatial mineralogical zonation of Alternation Halos beneath Australian volcanic hosted massive sulfide deposits is rich in perlite, quartz, hematite, and many other silicates [17]. Hence, it can be assumed that acidic hydrothermal environments would have played a significant role, not only in the origin of life but also in the accumulation of the necessary minerals and clays that had catalytic properties to synthesize biomolecules. As discussed previously, there is a strong possibility of the origin of life under hydrothermal conditions with the aid of various prebiotic minerals as catalysts. However, there still remains a problem that needs to be resolved: the biological C—O—P bond formation under hydrothermal conditions. It is necessary to find out the possible sources of phosphorus (P) for the prebiotic synthesis under hydrothermal conditions, the ultimate incorporation of P into organic biomolecules, leading to the formation of C—O—P type biological compounds under hydrothermal conditions. The only known example of phosphorylation reaction under simulated hydrothermal conditions does not demonstrate the very incorporation of P into the organic systems [18]. Biomolecules need C—O—P and C—P linkages, which are formed from inorganic P by utilizing enzymes [19]. To the best of our knowledge, hydrothermally induced C—O—P bond formation has not been demonstrated yet. Orthophosphate is the most common source of P-type compounds and it exists in the form of minerals [19,20]. These orthophosphate minerals are considered to be the major carriers of P on the earth [19]. There are many known sources of PO_4 (phosphate) compounds [21]; however, it is believed that the most reliable sources are the volcanic activities, which can produce water-soluble polyphosphates via partial hydrolysis of P_4O_{10} [22,23]. Although, orthophosphate shows quite sluggish reactivity toward biomolecules but still it could be considered as one of the most acceptable source for phosphorylation for the early developing life in the oceans, owing to its easy availability [24].

Biological phosphate esters such as glycerol phosphates and phosphoethanolamine enjoy a special place in the biochemistry of living organisms. Prebiotic synthesis of glycerol phosphates has been demonstrated earlier, which, so far, is the only remarkable prebiotic synthesis of glycerol phosphates [25]. In addition, there are no efficient methods described to synthesize the phosphate esters under prebiotic conditions [26]. Under ordinary organic conditions, *sn*-glycerol-3-phosphate has been synthesized [27–29], but these kinds of reactions are very intricate. Here, for the first time, we report the prebiotic hydrothermal synthesis of phosphate esters including glycerol phosphates (Scheme 1) and phosphoethanolamine (Scheme 2). Our work is one of the most initial works to demonstrate the incorporation of P into the biological world hydrothermally and it also suggests how biological phosphates would have synthesized for the hyperthermophiles such as acidophiles. Moreover, it also throws some light on the synthesis of biological phosphate esters in the acidic hydrothermal systems.

RESULTS

The glycerol phosphates were detected and identified by comparing the retention time and MS (mass spectrometric) fragments with those of standard. The isomer of *sn*-glycerol-3-phosphate (glycerol-2-phosphate) was identified by its same molecular weight and retention time that showed a slight difference with that of the standard *sn*-glycerol-3-phosphate, whereas phosphoethanolamine was characterized by peaks at 140, 79, and 97 (negative-ion mode of MS). Its best yield was 0.83%, and it could be obtained within a temperature range of 100 to 120°C. We obtained glycerol-2-phosphate at 100°C in both ways (with and without minerals). However, the presence of minerals did increase the yield (Table 2). Only perlite-catalyzed reaction at 180°C could catalyze the formation of *sn*-glycerol-3-phosphate. The yield of this reaction was 0.99%. We then repeated the experiment without the mineral, but we were unsuccessful; rather, we obtained glycerol-2-phosphate. This means that perlite was essential for the synthesis of *sn*-glycerol-3-phosphate. In our synthesis, the two isomers could not be obtained simultaneously in the same reaction.

In our experiments, we also verified the effects of temperature and pressure on the yields of our products. We selected glycerol phosphates as models to find out the effects of aforementioned factors. It was found that temperature greatly affects the yields of glycerol phosphates. The effect of temperature on the yields of glycerol phosphates is shown in Fig. 1. The maximum yields were obtained at 160°C. We also studied the effects of pressure on our synthesis. We conducted a series of experiments with a wide range of pressure from 1 to 1.8 MPa and found that

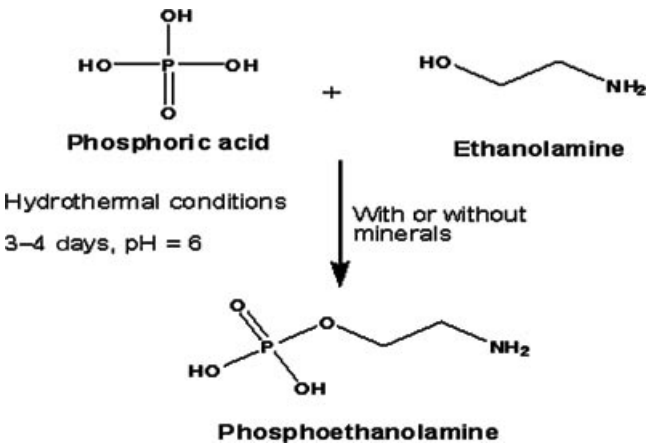


SCHEME 1 Synthesis of glycerol phosphates.

the pressure had a little effect on the reaction. However, the yields of the glycerol phosphates were very slightly improved with maximum possible pressure (1.8 MPa). The best yields were obtained at pressure and temperature of 1.8 MPa and 160°C, respectively.

DISCUSSION

It can be seen that minerals play a significant role by increasing the percentage yield of the reaction (Table 2). We successfully carried out the phosphorylation of organic biomolecules. Our work clearly reveals that it is possible to synthesize the biological phosphate esters under simple acidic hydrothermal conditions. An interesting observation was the synthesis and existence of the glycerol phosphate even at 200°C. Furthermore, as mentioned previously,



SCHEME 2 Synthesis of phosphoethanolamine.

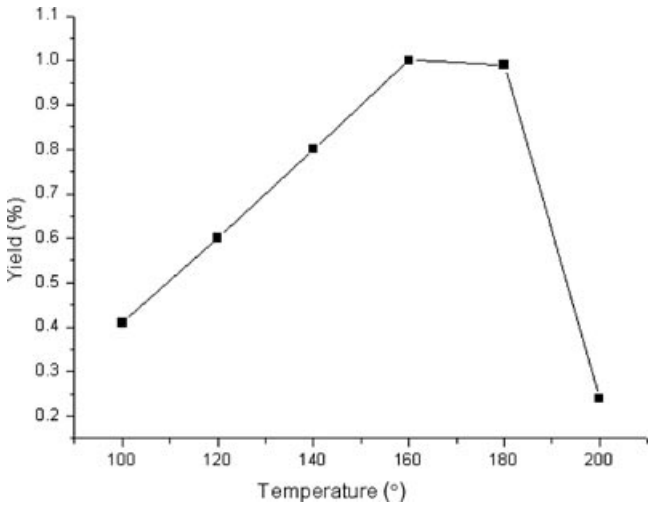


FIGURE 1 Effect of temperature on the synthesis of glycerol phosphates (percentage yield). The curve shows the effect of temperature on the rate of reaction. As seen in the curve, the reaction starts at 100°C, maximizes at 160°C, remains almost constant until 180°C, and then starts declining.

perlite was essential for the synthesis of *sn*-glycerol-3-phosphate because it catalyzed the attachment of PO_4 at the terminal position of glycerol. We can also conclude that orthophosphate is one of the ultimate sources of P for the biological world. Also, for the synthesis of glycerol phosphates, condensing agents such as cyanamide or urea are not essential and hydrothermal conditions also favor the formation of C—O—P type biological compounds unlike the previous works, which show that the presence of Mg^{2+} is somehow vital for the phosphorylation reactions [30]. We successfully synthesized the PO_4 compounds even without the presence of the former one. It can also be concluded that the hydrothermal systems could be the most likely havens for the high-energy PO_4 esters that could easily be transferred to the developing life. It is hard to get the ultimate answer about the most common ancestors of bacteria and archaea. Some believe that these were most likely to be mesophiles [31,32], whereas some consider that probably these could be hyperthermophiles [33,34]. But it is undeniable that the hyperthermophilic organisms do occupy a place somewhere near the basal position of the phylogenetic tree [33], and it is also worth mentioning that the structuring of the genetic code took place at acidic pH [35]. Hence, if it is believed that hyperthermophiles lie somewhere near the basal position of the phylogenetic tree and were present in hydrothermal vents and volcanic springs, then we can trace out all the possible routes by which biomolecules would have synthesized under such extreme conditions. Moreover, our experimental conditions, that is, high temperature and lower pH, fortify our belief that perhaps the earliest life could be acidophile-type bacteria, which lived under acidic conditions and could thrive very well at pH 2.

EXPERIMENTAL

All of the minerals (Fig. 2) were selected because of their natural occurrences in the acidic hydrothermal systems, as discussed previously. All of the prebiotic minerals were pure with known compositions (percentage of various elements or their oxides) and were obtained from Inner Mongolia Mineral Resource Co Ltd. (Chifeng, China). No prior grinding techniques were employed and the minerals were used without any further proceedings. The details about the minerals are given in the Table 1, which reveals the chemical compositions, average sizes of particles, physical appearances, and specific types or names of the minerals, respectively. Reagents such as glycerol, phosphoric acid, and ethanolamine were purchased

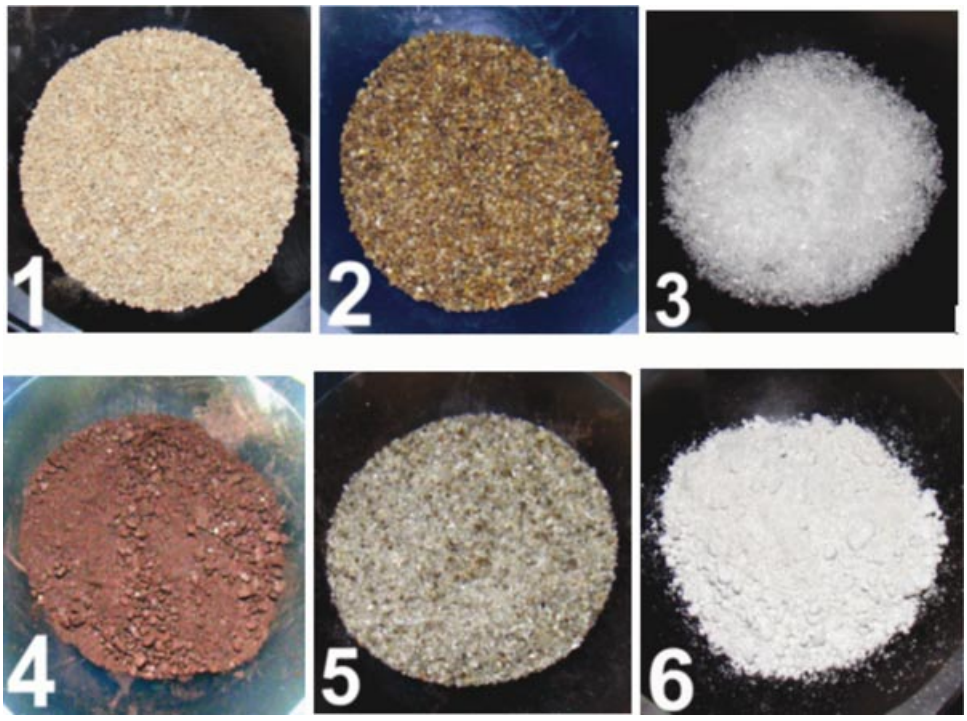


FIGURE 2 Different minerals used in the synthesis of phosphate esters. Different minerals used in our experiment and their names are as follows: 1, offretite; 2, garnet; 3, quartz; 4, hematite; 5, perlite; and 6, kaolinite. In our experiment, perlite was proven to be as an excellent catalyst for the incorporation of PO_4 at the terminal carbon of glycerol. Only perlite-catalyzed reaction at 180°C could give *sn*-glycerol-3-phosphate.

TABLE 1 Characterization of the Minerals Used

Entry	*General Name*	*Special Name*	*% Composition*	*Particle Size (Diameter)*	*Physical Appearance*
1	Zeolite	Offretite	63.54% SiO_2 13.22% MgO 3.56% Al_2O_3 0.29% K_2O 2.8% FeO 2.81% CaO 1.48% Fe_2O_3 1.91% Na_2O	0.6–1 mm	Brownish orange grains
2	Garnet (andradite)	Topazolite	23.7% Ca 21.97% Fe 16.57% Si 37.76% O	0.4–1 mm	Brown grains
3	Quartz	White quartz	46.7% Si 53.3% O	0.3–0.5 mm	White crystal
4	Hematite		70.0% Fe 30.0% O	0.1–0.8 mm	Brick red grains
5	Perlite		74% SiO_2 15% Al_2O_3 3% Na_2O 4% K_2O 1% Fe_2O_3 0.5% MgO 1.5% CaO 1% H_2O	0.3–0.9 mm	Gray grains
6	Kaolinite		39.5% Al_2O_3 46.5% SiO_2 14.0% H_2O	8–9 μm	White amorphous

from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and were of AR grade, whereas standards, *sn*-glycerol-3-phosphate and phosphoethanolamine, were purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan) and ACROS (Geel, Belgium), respectively.

Synthesis of Phosphate Esters

Glycerol phosphates were synthesized as follows: 2 mmoles of phosphoric acid, 4 mmoles of glycerol, and 500 mg of the selected mineral were added into steel alloy (Fe-Cr-Ni, alloy GB1220-92) autoclaves with a filling capacity of 90%. Finally, water was added to the reaction mixture up to the filling capacity of the autoclave. The same procedure was repeated for another set of autoclaves but with a difference that minerals were not included. The pH of this experiment was 2. The autoclaves were then sealed tightly and kept at a temperature range of 100 to 200°C for 3 to 4 days. After the completion of reaction, the products were taken out and analyzed. Many prebiotic minerals including quartz, offretite, garnet, perlite, hematite, and kaolinite were used as catalysts at different temperatures. In the other experiment, ethanolamine phosphate was synthesized (Scheme 2) by adding ethanolamine and phosphoric acid (2 mmoles ethanolamine and 3 mmoles phosphoric acid, respectively) into water. The solution was added into the autoclaves. The autoclaves were sealed and placed in the ovens at a temperature range of 80 to 120°C for 3 days. This reaction was completed successfully without the aid of minerals. After the completion of reaction, the products were taken out and analyzed by liquid chromatography-mass spectrometry (LC-MS) (Figs. 3a and 3b). The characterization was performed on the HPLC system (Agilent 1100), which consisted of a binary pump, an autosampler, an oven, and a Welchrom-C18 column (5 μm, 250 × 4.6 mm i.d.; Welch Materials, Shanghai, China). Chromatography was performed at 25°C with an 8-min linear gradient elution from 3% to 50% methanol in water containing 1% formic acid at a flow rate of 0.5 mL/min. The products were detected by liquid chromatography-tandem mass spectrometry (LC-MS-MS) with an Applied Biosystems Sciex API 2000 (Foster, USA) Q-trap mass spectrometer set at unit resolution in the multiple-reaction monitoring mode. The transition of the deprotonated molecular ions of glycerol phosphates was set at m/z 130 → 60, with collision energy of −30 eV. Electrospray ionization was used for ion production in negative mode. Optimum values for gas 1, gas 2, and curtain gas (N_2)

TABLE 2 Effect of Minerals on the Synthesis of Phosphate Esters

Entry	*Temperature (°C)*	*Catalyst*	*Yield (%) of A*	*Yield (%) of B*	*Yield (%) of C*
1	100	Quartz	0.41	No	0.41
2	100	None	0.22	No	0.22
3	120	Kaolinite	0.60	No	0.60
4	120	None	0.32	No	0.32
5	140	Offretite	0.80	No	0.80
6	140	None	0.38	No	0.38
7	160	Garnet	1	No	1
8	160	None	0.53	No	0.53
9	180	Perlite	No	0.99	0.99
10	180	None	0.50	No	0.50
11	200	Hematite	0.24	No	0.24
12	200	None	0.02	No	0.02

Yield (%) of glycerol phosphates (reaction 1, Scheme 1), carried out at pH 2. Reactants' molar ratios were kept constant throughout the experiment, that is, 2:1 for glycerol and H_3PO_4, respectively, where, A = glycerol-2-phosphate, B = *sn*-glycerol-3-phosphate, and C = total sum of glycerol phosphates (A + B), respectively.

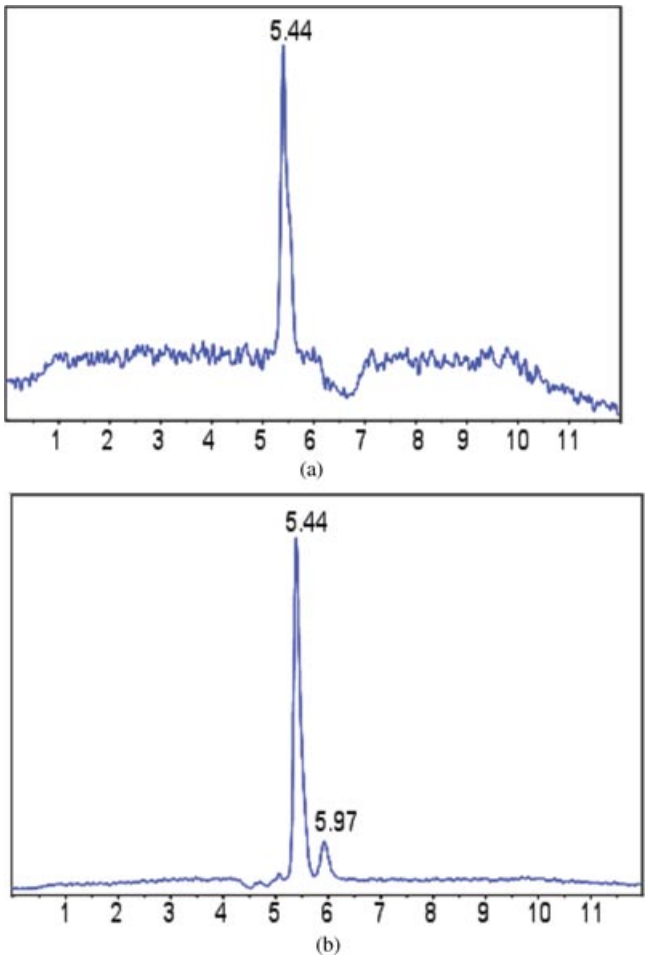


FIGURE 3 LC-MS spectrum of reaction 1. LC-MS-MS chromatogram of (a) the standard *sn*-glycerol-3-phosphate; (b) the product *sn*-glycerol-3-phosphate (reaction performed at 180°C in the presence of perlite).

flow rates were 50, 50, and 25 units, respectively. The ion spray voltage (IS) and temperature were −1500 V and 500°C, respectively (Analyst software version 1.4, Applied Biosystems, Foster, USA).

Effect of Minerals on the Synthesis of Phosphate Esters

Our major goal was to synthesize our aim products; besides, we were also interested to know about the effect of minerals on the yield of our products and to know whether these inhibit or catalyze the reaction. Hence, keeping this in mind, we used different minerals at different temperatures in our one set of experiment and compared the results with the other set of experiment that was carried out at the same temperatures and in which catalysts were not used.

We selected our products of Scheme 1 (glycerol phosphates) as a model compound to determine the effect of minerals as catalysts. We tested all of the minerals used in our experiment at different possible temperatures. However, the specific minerals at specific temperatures, given in Table 2, gave the best results.

ACKNOWLEDGMENTS

Gull Maheen is highly obliged to Chinese Scholarship Council and Ministry of Education Pakistan, for providing the opportunity to carry out Ph.D. study in China. She also thanks Nasim, Rahat, Mariam, and Shani for support and to Shafiq-ur-Rehman for taking pictures of the minerals.

REFERENCES

[1] Holm, N. G. Origins Life Evol Biosphere 1992, 22, 5.
[2] Daniel, I.; Oger, P.; Winter, R. Chem Soc Rev 2006, 35, 858.

[3] Rushdi, A. I.; Simoneit, B. R. T. Origins Life Evol Biosphere 2006, 36, 93.
[4] Feng, S.; Tian, G.; He, C.; Yuan, H.; Mu, Y.; Wang, Y.; Wang, L. J Mater Sci 2008, 43, 2418.
[5] Tsukahara, H.; Imai, E.-I.; Honda, H.; Hatori, K.; Matsuno, K. Origins Life Evol Biosphere 2002, 32, 13.
[6] Tian, G.; Yuan, H.; Mu, Y.; He, C.; Feng, S. Org Lett 2007, 9, 2019.
[7] Corliss, J. B.; Dymond, J.; Gordon, L. I.; Edmond, J. M.; Von Herzen, R. P.; Ballard, R. D.; Green, K.; Williams, D.; Bainbridge, A.; Crane, K.; van Adel, T. H. Science 1979, 203, 1073.
[8] Amils, R.; Gonzalez-Toril, E.; Fernandez-Remolar, D.; Gomez, F.; Aguilera, A.; Rodriguez, N.; Malki, M.; Garcia-Moyano, A.; Fairen, A. G.; Fuente, V. D. L.; Sanz, J. L. Planet Space Sci 2007, 55, 370.
[9] Cockell, C. S. Phil Trans R Soc B 2006, 361, 1845.
[10] Benison, K. C.; Bowen, B. B. Icarus 2006, 183, 225.
[11] Van Kranendonk, M. J. Earth-Sci Rev 2006, 74, 197.
[12] West, M. D.; Clarke, J. D. A.; Thomas, M.; Pain, C. F.; Walter, M. R. Planet Space Sci 2010, 58, 447.
[13] Simeone, R.; Dilles, J. H.; Padalino, G.; Palomba, M. Econ Geol 2005, 100, 115.
[14] Neil, J. O.; Carlson, R. W.; Francis, D.; Stevenson, R. K. Science 2008, 321, 1828.
[15] Javaux, E. J. Res Microbiol 2006, 157, 37.
[16] Fernandez-Remolar, D. C.; Morris, R. V.; Gruener, J. E.; Amils, R.; Knoll, A. H. Earth Planet Sci Lett 2005, 240,149.
[17] Doyle, M. G. Econ Geol 2001, 96, 1133.
[18] Ozawa, K.; Nemoto, A.; Imai, E.-I.; Honda, H.; Hatori, K.; Matsuno, K. Orig Life Evol Biosph 2004, 34, 465.
[19] Pasek, M. A. PNAS 2008, 105, 853.
[20] Arrhenius, G.; Sales, B.; Mojzsis, S.; Lee, T. J Theor Biol 1997, 187, 503.
[21] Graaf, R. M. D.; Schwartz, A. W. Orig Life Evol Biosph 2000, 30, 405.
[22] Yamagata, Y.; Watanabe, H.; Saitoh, M.; Namba, T. Nature 1991, 352, 516.
[23] Schwartz, A. W. Phil Trans R Soc B 2006, 361, 1743.
[24] Schwartz, A. W. Orig Life Evol Biosph 1997, 27, 505.
[25] Epps, D. E.; Nooner, D. W.; Eichberg, J.; Sherwood, E.; Oro, J. J Mol Evol 1979, 14, 235.
[26] Keefe, A. D.; Miller, S. L. J Mol Evol 1995, 41, 693.
[27] Schoevaart, R.; Rantwijk, F. V.; Sheldon, R. A. Chem Commun 1999, 24, 2465.
[28] Crans, D. C.; Whitesides, G. M. J Am Chem Soc 1985, 107, 7019.
[29] Rios-Mercadillo, V. M.; Whitesides, G. M. J Am Chem Soc 1979, 101, 5828.
[30] Holm, N. G.; Dumont, M.; Ivarsson, M.; Konn, C. Geochem Trans 2006, 7, 7.
[31] Galtier, N.; Tourasse, N.; Gouy, M. Science 1999, 283, 220.
[32] Forterre, P. Curr Opin Microbiol 2002, 5, 525.
[33] Gogarten-Boekels, M.; Hilario, E.; Gogarten, P. J. Orig Life Evol Biosph 1995, 25, 251.
[34] Forterre, P.; Brochier, C.; Philippe, H. Theor Pop Biol 2002, 61, 409.
[35] Giulio, D. M. J Theor Biol 2005, 237, 219.