

Formation and stability of organic layers around inorganic particles in aqueous media: an introduction to origin of life

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Abstract

Formation of non bonded organic layer around inorganic particles like iron sulfide and zeolites, was hypothesized as starting point for one of the possible mechanisms of the origin of life on earth. In this study, the general circumstances needed for creation of such organic layer was investigated. An aliphatic hydrocarbon (hexane), an aromatic hydrocarbon (toluene), an alcohol (n-octanol) and an amino acid (phenylalanine) were used for simulation of the primordial soup. FeS, montmorillonite and clinoptilolite were used as insoluble inorganic particles. Non-bonded organic layer can be formed in both fresh and saline waters. Formation of organic layer is a pH sensitive process and the best pH for formation of this layer is around 6 (similar to the pH of primordial oceans of the earth). Low concentration of water in organic layer causes the less degradation rate of the polymers and various organic molecules and thus increases their stability.

Keywords: Organic Layer; Hydrogel Hypothesis; Origin of Life; Salinity Effect; pH Effect.

Introduction

A wide variety of theories have been proposed about the origin of life [1]. Perhaps the most investigated theory about life origins is the vesicular origin of life. Norris and Raine [2] proposed that protocells form spontaneously. The fitness of the protocells was determined as a result of non-covalent molecular associations between the molecules of the membrane and necessary macromolecules in protocells. Donaldson et al. [3] reviewed the possible scenario for the

transition from geochemistry to biochemistry. They suggested that the aerosols of amphiphiles in prebiotic earth are thermodynamically collapse into two asymmetric divisions. One of the daughter products has been bacterial sized and the other would have been the virally sized. These scientists show the evidence about the role of these aerosols in the origin of life.

Another interesting hypothesis about the life origins uses mineral surfaces as concentrating and catalyzing agent [4]. Bebie and Schoonen [5] examined the Wächtershäuser origin of life hypothesis and showed

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that pyrite (FeS_2) can adsorb anionic organic molecules such as adenosine and 5'- adenosine monophosphate. They also showed that existence of Fe^{2+} and phosphate ion in the solution can affect the adsorption phenomenon. A useful review about the effect of mineral surfaces and geochemical cycles in origin and development of life on surface and subsurface environments was written by Trevors [6]. Branciamore et al. [7] built a model for RNA world evolution within the pores of the honeycomb minerals. They show that the effect of group selection in a mineral honeycomb could have been efficient enough to protect prebiotic ribozymes of different types from environmental disasters. They also suggest that the mutations in this model can acquire useful functions such as replicase activity or the production of membrane compartments. Hansma [8] proposed that the space between mica sheets act as the first cell. In this scenario, mechanical energy has been used to form covalent bond.

Another hypothesis that recently was introduced by Trevors et al. [9] is the origin of life in hydrogel environment. Insoluble mineral particles in primordial oceans and lagoons act as adsorbent. This phenomenon can concentrate the molecules and then the chemical reaction between them can occur. Also, these adsorbed chemical species -small organic and inorganic molecules and polymers- can create a jelly environment that the activity of water in this media was smaller than the ocean or lagoon. This jelly media around small insoluble inorganic particles was named hydrogel environment by Trevors [9]. In a review article, Trevors and Pollack [10] introduced this hypothesis. They proposed that this hydrogel environment can retain water, hydrocarbons, soluble mineral salts, solutes and gases. In another paper, Trevorse [11] proposed that the first living cell on earth was created when environmental microscopic entropy was within a range suitable for origin of cells in hydrogel environment. Possible composition and organization of cytoplasm in prebiotic cells from a theoretical point of view was discussed by Trevors [12, 13]. In these papers, he proposed that an unstructured microscopic hydrogel (volume 0.1 to 0.2 μm^3) was changed to the first organized cytoplasm. Continuously, Trevorse published a paper and proposed that the origin of the first microbial cell occurred as a series of increasing levels of organization within the prebiotic hydrogel. He suggested that the hydrogel finally made a transition to the microbial mat capable of growth and division. After that, Trevors and Pollack [10] proposed that the prebiotic cells did not require the cytoplasmic membrane. They suggested that hydrogel environment have had a dual role as both a pre-cytoplasm and a

boundary layer.

Hydrogel hypothesis could answer a lot of questions about the prebiotic world. This hypothesis could be a possible explanation about the origin of cytoplasm, membrane and metabolism. The first step for appearance of hydrogel environment is the formation of non bonded organic layer around inorganic particles in aqueous media. But as we know until now, there are no experimental reports about production and stability of this structure in aqueous media. In this paper, we investigate the production and stability of this non bonded organic layer around inorganic particles (FeS , Montmorillonite and Clinoptilolite) in various conditions (various organic and inorganic molecules solutions) and examine the stability of the layer in various chemical and physical circumstances.

Materials and Methods

FeS powder, hexane, toluene, phenylalanine, n-octanol, methyl metacrylate, CaCl_2 , KCl and NaCl were purchase from Merck. All the chemicals used without further purification. Montmorillonite and clinoptilolite (from the deposits in the region of Semnan, Iran) were purchased from Aldrich and Afrand Tuska respectively. HPLC grade water (Millipore Q) was used for solution preparation.

General procedure: Our experiments divided into two categories. In one set of experiments, we examine the production and stability of the non bonded organic layer under various conditions such as various amounts of organic chemicals, various salinities (KCl , NaCl and CaCl_2), pH and wetting and drying cycles. In series two of the experiments, the adsorption isotherms of organic chemicals for creation of this layer were investigated. These experiments were done in presence of various organic chemicals and in various concentrations. Finally, amount of water in non bonded organic layer and the adsorption of mono and divalent cations on inorganic particles (KCl , NaCl and CaCl_2 with and without organic layer) were investigated.

In a typical category one experiment, 0.05g inorganic material (FeS , Montmorillonite or Clinoptilolite), 1 cm³ HPLC grade water and appropriate amount of organic material (for example hexane 39×10^{-5} mol, toluene 36×10^{-5} mol, n-octanol 25×10^{-5} mol and phenylalanine 1×10^{-5} mol) was added to a test tube. This mixture was allowed to evaporate in room temperature under inert atmosphere (CO_2 gas) for about 2 weeks (simulation of drying and wetting cycles in primordial earth). After drying, 100 μL water was added to the mixture and after one day, the microscopic

images were taken from the particles.

As an example for category two experiments, 0.05g inorganic material, 1cm³ HPLC grade water and various amounts of organic material (hexane, toluene, n-octanol and phenylalanine) were added to test tubes in room temperature and the remnant concentrations of toluene and phenylalanine in the tubes after 6 days were detected with an HPLC instrument. In these experiments no evaporation was allowed. Finally, the adsorption isotherms for toluene and phenylalanine were drawn.

Detection Method: Detection of water amount in organic layer was carried out as reference [14]. 0.5g inorganic material was mixed with 1 cm³ water and appropriate amount of organic mixtures (hexane 39×10⁻⁴mol, toluene 36×10⁻⁴mol, n-octanol 25×10⁻⁴mol and phenylalanine 1×10⁻⁴mol) in test tubes and kept for two weeks. After centrifugation, 1.5cm³ CaCl₂ solution in methanol and 1.5cm³ pure methanol (HPLC grade methanol with known water amount) was added to the test tubes and mixed with the precipitates in the tubes. Finally, after second time centrifugation, the absorbance of solution in 656nm was detected with a spectrophotometer.

Microscopic images were taken with an invert microscope (Olympus X7) and the concentration detections were carried out with an HPLC model Agilent series 1200 equipped with a C18 column in room temperature. A Perkin Elmer Lambda 25 instrument was used for spectrophotometric measurements. Concentration of Ca²⁺, Na⁺ and K⁺ were measured with titration method (EDTA) and flame photometer (Sherwood flame photometer 410) respectively.

Results

Formation of non bonded organic layer

The first question in hydrogel theory was the possibility of formation of the non bonded organic layer in presence of the various organic materials. For studying of the effect of organic materials, we choose fresh water media (without any inorganic salts) and four organic materials; A saturated hydrocarbon (hexane), an aromatic hydrocarbon (toluene), an alcohol (n-octanol) and an amino acid (phenylalanine). Amount of these materials were varied from 3.9×10⁻⁵ to 3.9×10⁻² mol in

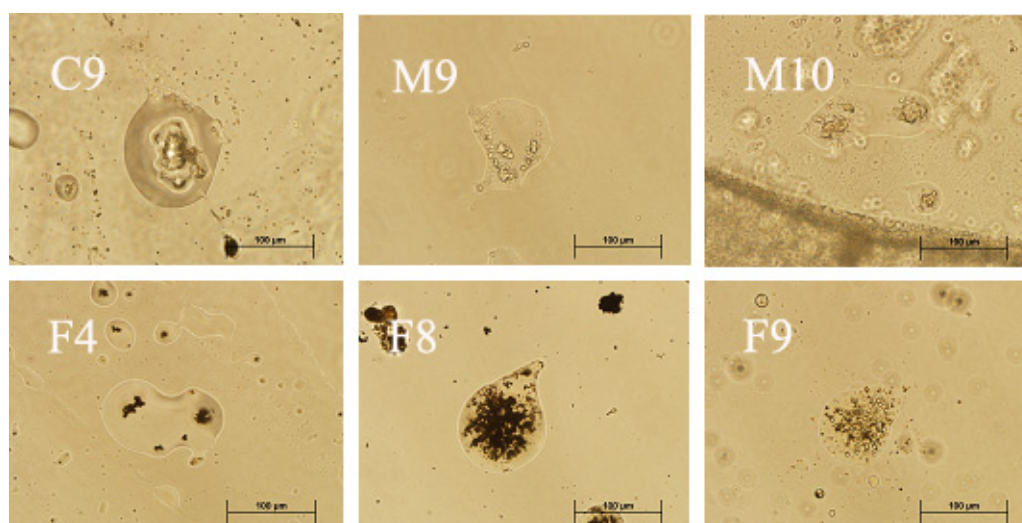


Figure 1. Microscopic images of formation of non bonded organic layer around inorganic particles. For abbreviations and concentrations of organic material see Table 1.

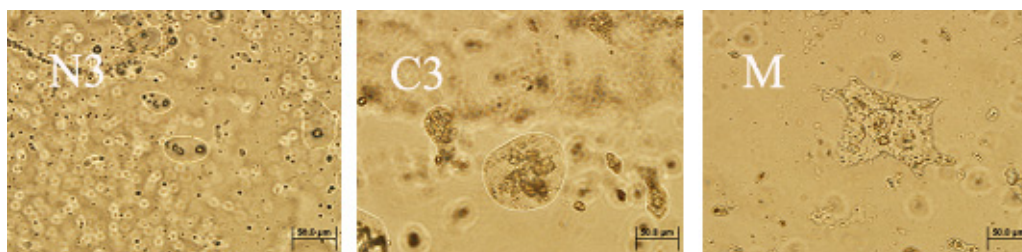


Figure 2. Effects of salinity in organic layer formation. Abbreviations and concentrations of organic and inorganic materials are given in Table 3.

Table 1. Detailed amount of organic materials in various experiments for investigation of the possibility of the formation of the non bonded organic layer. In all mixtures, the volume of water and weight of the inorganic materials were 1mL and 0.05g respectively. In “Organic Layer Formation” column, “Yes” indicates the successfully formation of organic layer and “No” indicated the negative response of the experiment.

Abbreviations C1 to C10: Clinoptilolite, M1 to M10 Montmorillonite and F1 to F10: FeS.

Sample name	Hexane $\times 10^5$ (mol)	Toluene $\times 10^5$ (mol)	n-Octane $\times 10^5$ (mol)	Phenilalanine $\times 10^5$ (mol)	Organic Layer Formation
C1	3.9	3.6	2.5	1	No
C2	7.8	7.2	5	1	No
C3	11.7	10.8	7.5	1	No
C4	15.6	14.4	10	1	No
C5	19.5	18	12.5	1	No
C6	23.4	21.6	15	1	No
C7	27.3	25.2	17.5	1	No
C8	31.2	28.8	20	1	No
C9	35.1	32.4	22.5	1	Yes
C10	39	36	25	1	Yes
M1	3.9	3.6	2.5	1	No
M2	7.8	7.2	5	1	No
M3	11.7	10.8	7.5	1	No
M4	15.6	14.4	10	1	No
M5	19.5	18	12.5	1	No
M6	23.4	21.6	15	1	No
M7	27.3	25.2	17.5	1	No
M8	31.2	28.8	20	1	No
M9	35.1	32.4	22.5	1	Yes
M10	39	36	25	1	Yes
F1	3.9	3.6	2.5	1	No
F2	7.8	7.2	5	1	No
F3	11.7	10.8	7.5	1	No
F4	15.6	14.4	10	1	Yes
F5	19.5	18	12.5	1	Yes
F6	23.4	21.6	15	1	Yes
F7	27.3	25.2	17.5	1	Yes
F8	31.2	28.8	20	1	Yes
F9	35.1	32.4	22.5	1	Yes
F10	39	36	25	1	Yes

1mL water. Amount of phenylalanine was kept constant (10^{-5} mol in 1mL water). Inorganic particles (FeS, clinoptilolite and montmorillonite) were 0.05g in all mixtures. Table 1 shows the detailed amounts of the materials in all mixtures. After drying for about 2 weeks and addition of 100 μ L water to the samples, the microscopic images were taken for proving the formation of non bonded organic layer. In figure 1, the microscopic images of some samples were shown.

Figure 1 shows the microscopic images of the non bonded organic layer in clinoptilolite (C9; hexane 35.1×10^{-5} mol, toluene 32.4×10^{-5} mol, n-octanol 22.5×10^{-5} mol, phenylalanine 1×10^{-5} mol and 0.05g clinoptilolite in 1mL water), montmorillonite (M9; hexane 35.1×10^{-5} mol, toluene 32.4×10^{-5} mol, n-octanol 22.5×10^{-5} mol, phenylalanine 1×10^{-5} mol and 0.05g montmorillonite in 1mL water and M10; hexane 39×10^{-5} mol, toluene 36×10^{-5} mol, n-octanol 25×10^{-5} mol, phenylalanine 1×10^{-5} mol and 0.05g montmorillonite in 1mL water) and FeS

(F4; hexane 15.6×10^{-5} mol, toluene 14.4×10^{-5} mol, n-octanol 10×10^{-5} mol, phenylalanine 1×10^{-5} mol and 0.05g FeS in 1mL water, F8; hexane 31.2×10^{-5} mol, toluene 28.8×10^{-5} mol, n-octanol 20×10^{-5} mol, phenylalanine 1×10^{-5} mol and 0.05g FeS in 1mL water and F9; hexane 35.1×10^{-5} mol, toluene 32.4×10^{-5} mol, n-octanol 22.5×10^{-5} mol, phenylalanine 1×10^{-5} mol and 0.05g FeS in 1mL water). One can see in these images that the formation of organic layer around inorganic particles was occurred. This layer can encompass more than one particle and its diameter could be around 100 microns. As was proposed by Trevors, the organic layer has different refractive index from aqueous environment and can be seen in microscopic images. This difference was seen because of the concentrated organic materials around the particles.

Effects of salinity on organic layer formation

Table 2 shows the concentrations of main dissolved

Table 2. Main dissolved salts in archaean and modern oceans (in mmol/L) [15].

Cl ⁻	Br ⁻	I ⁻	SO ₄ ²⁻	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Sr ²⁺
Archaean Oceans								
920	2.25	0.037	2.3	789	18.9	50.9	232	4.52
Present Day Oceans								
556	0.86	0.0005	28.7	477	10.1	54.2	10.5	0.09

salts in present day and Archaean oceans¹⁵. As one can see in this table, Na⁺, Ca²⁺ and Cl⁻ were the common ions in Archaean oceans, thus we choose these ions for investigation of salt effects on organic layer formation. Three various concentrations of NaCl and CaCl₂ and one concentration of mixtures of these salts were chosen (Table 3). According to Table 2, concentration of Na⁺ and Ca²⁺ in archaean oceans could be around 0.8 and 0.25 mol/L respectively. We choose these concentrations and one tenth and hundredth of them for salt effect studies. In mixtures of two salts, we use only 0.8 and 0.25 mol/L for Na⁺ and Ca²⁺ respectively.

Table 3. Concentration of NaCl and CaCl₂ in mixture of organic material and salts for simulation of non bonded organic layer formation in saline water. Concentrations of materials was: hexane 39×10⁻⁵mol, toluene 36×10⁻⁵mol, n-octanol 25×10⁻⁵mol, phenylalanine 1×10⁻⁵mol and 0.05g inorganic particles in 1mL water.

Abbreviations: N: NaCl solution, C: CaCl₂ solution and M: mixed saline solution

Sample name	NaCl (mol/L)	CaCl ₂ (mol/L)	Non Bonded Organic Layer Formation
N1	0.8	0.0	Do not seen
N2	0.08	0.0	Do not seen
N3	0.008	0.0	Yes
C1	0.0	0.25	Yes
C2	0.0	0.025	Yes
C3	0.0	0.0025	Yes
M	0.8	0.25	Yes

Table 4. Adsorption of various cations on FeS, montmorillonite and clinoptilolite adsorbants in presence and absence of organic materials. Values are given in percent ($\frac{C_0-C}{C} \times 100$). Concentrations of materials was: hexane 39×10⁻⁵mol, toluene 36×10⁻⁵mol, n-octanol 25×10⁻⁵mol, phenylalanine 1×10⁻⁵mol, Na⁺ 8×10⁻⁴mol, K⁺ 2×10⁻⁵mol, Ca²⁺ 2.5×10⁻⁴mol and 0.05g inorganic particles in 1mL water.

Inorganic Material	K ⁺	Na ⁺	Ca ²⁺
With Organic Materials			
Clinoptilolite	19.7	6.4	6.0
Montmorillonite	36.8	22.5	1.4
FeS	36.2	22.0	5.7
Without Organic Materials			
Clinoptilolite	49.0	17.8	23.4
Montmorillonite	86.5	38.9	16.0
FeS	84.7	31.9	20.8

It is obvious from Figure 2 that in presence of NaCl, the organic layer was thin and has low quality but in CaCl₂ and mixtures of two salts, this layer is similar to fresh water environment.

Another experiment was planned to investigate the effect of organic layer on adsorption capacity of the mineral surfaces. Table 4 shows the results of this experiment. Concentrations of salts in this experiment (based on Table 2) were K⁺ 0.019mol/L, Na⁺ 0.8 mol/L and Ca²⁺ 0.25 mol/L. Percent of adsorbed ions were

calculated using $\frac{C_0-C}{C} \times 100$ formula. In this formula C₀ is the initial concentration of the ion and C is the concentration after equilibrium.

Effects of pH on non bonded organic layer formation

For investigating of the pH effects on organic layer formation, we plan a series of experiments at pH 3, 6, and 9. The concentration of other chemicals were as previous experiments (hexane 39×10⁻⁵mol, toluene 36×10⁻⁵mol, n-octanol 25×10⁻⁵mol, phenylalanine 1×10⁻⁵mol and 0.05g inorganic particles in 1mL water). Figure 3 shows the results of the experiments. As one can see, at pH 6 (proposed pH for primordial oceans) the non bonded organic layer was formed around all inorganic particles (FeS, montmorillonite and clinoptilolite) but at pH 3, we couldn't see this layer. At pH 9, similar to pH 3, the organic layer was not formed (do not shown at the Figure 3).

Effects of drying and wetting cycles on organic layer stability

In continuing our experiments, we investigate the effects of number of drying and wetting cycles on non bonded organic layer stability. Three mixtures of hexane 39×10⁻⁵mol, toluene 36×10⁻⁵mol, n-octanol 25×10⁻⁵mol, phenylalanine 1×10⁻⁵mol and 0.05g inorganic particles (FeS, montmorillonite and clinoptilolite) in 1mL water was dried and wetted (with 1mL water) for five times. Figure 4 shows the microscopic images of the mixtures.

Water content in non bonded organic layer

Organic layer is a mixture of organic and water molecules and thus can act as suitable environment for organic synthesis. For this reason, we investigate the

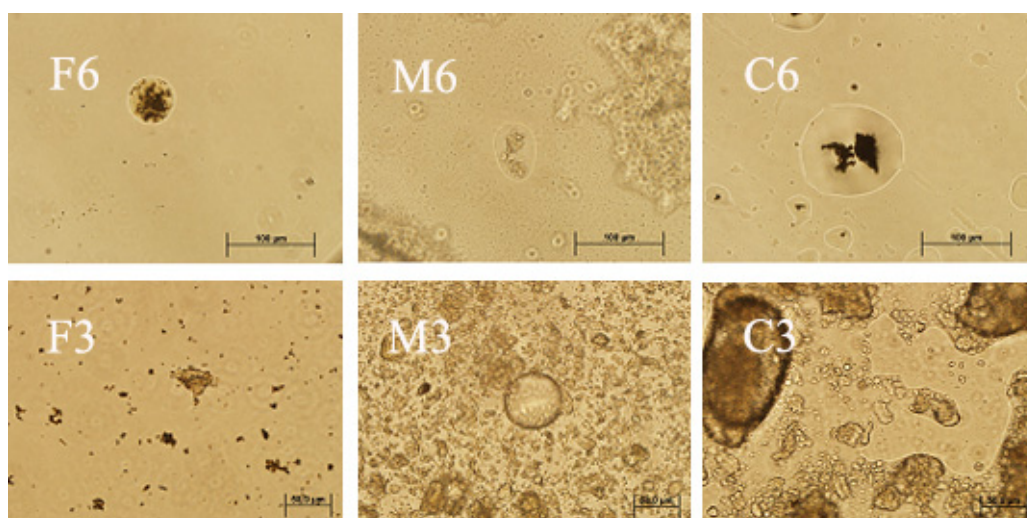


Figure 3. Effect of pH on organic layer formation. F6: FeS at pH=6, F3: FeS at pH=3, M6: montmorillonite at pH=6, M3: montmorillonite at pH=3, C6: clinoptilolite at pH=6 and C3: clinoptilolite at pH=3. For more details see the main text.

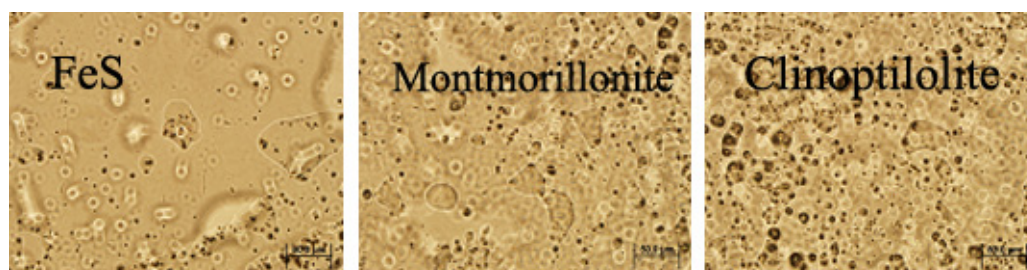


Figure 4. Effects of drying and wetting cycles on organic layer. All samples were dried and wetted five times.

water content of this layer. As previous experiments, three mixtures of organic and inorganic materials (hexane 39×10^{-4} mol, toluene 36×10^{-4} mol, n-octanol 25×10^{-4} mol, phenylalanine 1×10^{-4} mol and 0.5g inorganic particles (FeS, montmorillonite and clinoptilolite) in 1mL water) were prepared. After equilibrium in two weeks, the mixtures were centrifuged and the precipitates (inorganic particles surrounded with organic layer) were extracted with HPLC grade methanol (with known amount of water content 6.61×10^{-5} mol (H₂O)/mL (CH₃OH)). Table 5 shows the results of this experiment. The mole percent of water for organic layers on clinoptilolite, FeS and montmorillonite are 0.63, 0.84 and 2.94 respectively.

Adsorption isotherms

It is obvious from Figure 1 that formation of non bonded organic layer in such diameter (about 100 micrometers) need multi layer adsorption of organic material on the inorganic particle surfaces. For investigation of this case, we drew the adsorption isotherms of phenylalanine and toluene in aqueous media. Figure 5 shows the adsorption isotherm for phenylalanine in water. In Figure 6, adsorption isotherm of phenylalanine is shown in presence of organic materials (hexane 39×10^{-5} mol, toluene 36×10^{-5} mol and n-octanol 25×10^{-5} mol). Figure 7 shows the adsorption isotherm of toluene in presence of hexane 39×10^{-5} mol, n-octanol 25×10^{-5} mol and phenylalanine 1×10^{-5} mol on FeS, montmorillonite and clinoptilolite.

Table 5. Water content of the organic layer around various inorganic particles. All chemicals are mixed in 1mL water in room temperature.

Inorganic Particle	Hexane $\times 10^{-4}$ (mol)	Toluene $\times 10^{-4}$ (mol)	n-Octane $\times 10^{-4}$ (mol)	Phenylalanine $\times 10^{-4}$ (mol)	Water $\times 10^{-5}$ (mol)
Clinoptilolite	39	36	25	1	6.40
Montmorillonite	39	36	25	1	30.55
FeS	39	36	25	1	8.57

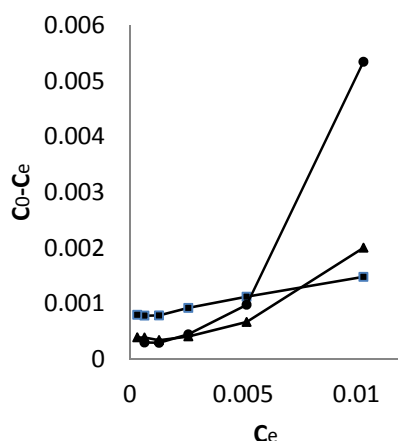
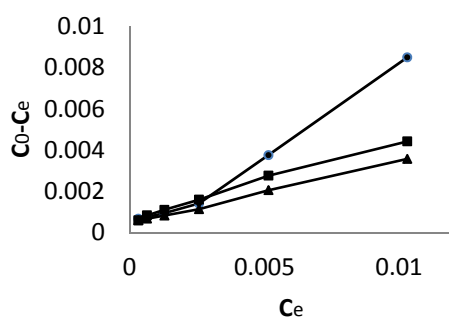


Figure 5. Adsorption isotherm for phenylalanine in water on FeS (●), montmorillonite (■) and clinoptilolite (▲) (C_0 : initial concentration if organic materials; C_e : concentration of organic materials at equilibrium)



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Figure 6. Adsorption isotherm for phenylalanine in water (in presence of hexane 39×10^{-5} mol, toluene 36×10^{-5} mol and n-octanol 25×10^{-5} mol) on FeS (●), montmorillonite (■) and clinoptilolite (▲) (C_0 : initial concentration if organic materials; C_e : concentration of organic materials at equilibrium)

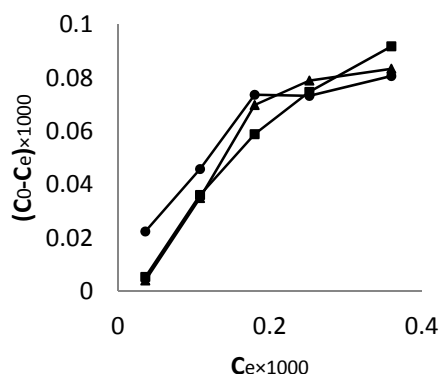


Figure 7. Adsorption isotherm for toluene in water (in presence of hexane 39×10^{-5} mol, n-octanol 25×10^{-5} mol and phenylalanine 1×10^{-5} mol) on FeS (●), montmorillonite (■) and clinoptilolite (▲) (C_0 : initial concentration if organic materials; C_e : concentration of organic materials at equilibrium)

Discussion

As one can see in Table 1, formation of non bonded organic layer was affected by two parameters; concentration of organic materials and type of inorganic particles. Data reported in Table 1 shows that there is a threshold in concentration of organic chemicals for organic layer formation in fresh water media. In case of clinoptilolite and montmorillonite, when the amount of organic chemicals in 1mL water became 35.1×10^{-5} , 32.4×10^{-5} , 22.5×10^{-5} and 1×10^{-5} mol for hexane, toluene, n-octanol and phenylalanine respectively, the organic layer appeared. In solution containing FeS particles, organic layer appeared when the concentrations of hexane, toluene, n-octanol and phenylalanine were 15.6×10^{-5} , 14.4×10^{-5} , 10×10^{-5} and 1×10^{-5} mol respectively. These data indicate that without polymeric organic compound in solution, molecular organic chemicals adsorbed on the surface of the inorganic compounds and create organic layer around the particles.

Another parameter that can affect the formation of organic layer was the type of inorganic surface. As was shown in Table 1, organic layer around the FeS particles were formed easily in comparison with zeolite particles. Montmorillonite and clinoptilolite have similar behavior and the organic layer around these particles formed in equivalent concentration. This behavior probably returns to the similarity of the surface properties of these two zeolites. But in case of FeS particles, organic layer was formed very quickly because of the tendency of the organic materials to the surface of this sulfide.

We can propose from table 3 that presence of high concentration of monovalent cations (Na^+) have negative effects on non bonded organic layer formation but divalent cations (Ca^{2+}) have positive effects. In mixtures of mono and divalent cations, even in high concentration of these ions, the organic layer could be formed.

It is obvious from Table 4 that the adsorption percent of organic materials depends on type of inorganic particles. As one can see in this table, in absence of organic layer, FeS and montmorillonite are better adsorbents than clinoptilolite. This is due to the surface properties and charges of these materials. All inorganic particles adsorbed K^+ ion better than other ions. But in presence of organic layer, adsorption capacity of all mineral particles reduced, because of the minor solubility of these ions in organic layer. The non bonded organic layer can act as a barrier for transmission of the ions from bulk solution to the surface of the inorganic

particles. In presence of this layer, similar to absence of this layer, K^+ adsorbed more than other ions. This is probably the reason of the higher concentration of K^+ ion inside the modern cells compare to Na^+ ion.

As was known until now, the pH of primordial oceans (about 4 billion years ago) was around 6¹⁵. This pH was because of carbon dioxide solution in the oceans. Figure 3 shows that the formation of non bonded organic layer was pH sensitive and we could expect the formation of this layer only in neutral or slightly acidic environments. This phenomenon probably is because of the increase in solubility of n-octanol and phenylalanine in acidic and basic solutions (because of the protonation and deprotonation reactions). Thus, in pH 3 and 9 the organic material dissolved in water more than slightly acidic solutions (pH 6) and the non bonded organic layer wasn't formed.

Drying and wetting cycles were common event in primordial tidal zones and lagoons. In warm and sunny days, the water of these environments could be evaporated and with rain, the evaporated water could be replaced. As one can see in figure 4, the organic layer is stable after five times drying and wetting cycles but its diameter reduces and is around 50 μ m. This observation could be due to the decrease and increase of water content in environment or because of the evaporation of organic compound along with water.

One of the most important points in hydrogel theory is the content of water within non bonded organic layer. Because of the high activity of water in aqueous solutions, the stability of organic compounds in water isn't suitable and degradation reaction is important in this media. Thus, the synthesized organic materials were decomposed in aqueous solution over the time and couldn't aggregate to form supramolecular structures. Another effect of high content of water in aqueous solutions is the low chance of collision and reaction of organic molecules in this environment. Despite these effects, presence of water in appropriate amount is necessary for chemical synthesis. In presence of water, activation energy barrier of polar transition states are reduced and the kinetics of these reactions become very fast in comparison with pure organic media. As one can see in table 5, water content of each organic layer depends on inorganic particle type. Non bonded organic layer on montmorillonite absorbs maximum and on clinoptilolite absorbs minimum water molecules. This behavior is probably because of their surface hydrophilic properties.

As one can see from figure 5, the adsorption of phenylalanine on FeS, montmorillonite and clinoptilolite in low concentrations of phenylalanine are similar but in high concentrations, there are distinct difference

between FeS and the zeolites. Similarity in adsorption behavior of montmorillonite and clinoptilolite could be due to their surface properties. In case of phenylalanine adsorption in presence of organic materials (Figure 6), the difference between isotherms is less distinct and the curves are more similar to each other. This observation is probably because of the reduction of differences in surfaces of FeS and zeolites through adsorption of hexane, toluene and n-octanol.

Figure 7 indicates that adsorption isotherms of toluene on FeS, Montmorillonite and Clinoptilolite are very similar. This phenomenon is due to molecular structure of toluene. Toluene molecule hasn't polar structure and then the properties of surfaces can't affect widely the adsorption of this molecule.

Mixture of organic and inorganic molecules (including an aliphatic hydrocarbon, an aromatic hydrocarbon, an alcohol, an amino acid and salts) in water could form a thick layer around unsolved inorganic particles. In this layer, there are high concentration of organic molecules and low activity of water molecules in comparison with aqueous solution media. In this manuscript, we show that this non bonded organic layer is formed easily in organic-water mixtures and could be a common phenomenon in primordial tidal zones and lagoons. Low activity of water in this layer cause the low degradation rate of organic polymers. This layer could be formed in fresh or saline water media. Formation of this layer are pH sensitive and in acidic or basic solutions (probably because of protonation of alcohol or deprotonation of amino acid in acidic and basic environments respectively) this layer couldn't be formed. The best pH range for creation of non bonded organic layer was neutral or slightly acidic environments (similar to pH of oceans in primordial earth). The organic layer is stable during drying and wetting cycles (as were done in aqueous environments of early earth) but its thickness is reduced after 5 drying and wetting cycles. Comparison of adsorption isotherm and formation of organic layer around inorganic particles shows that because of increase of adsorption with concentration, we can see the organic layer only when the concentrations reach a threshold quantity in aqueous solution.

It seems that the hydrogel layer could act as one of possible start points for emergence of precytoplasm and beginning of premetabolism in prebiotic world. Formation of non bonded organic layer around inorganic particles is the first step for hydrogel layer appearance. In this jelly like layer, the important polymers for future life could be formed and were protected from degradation. Finally, this layer could be

transformed to cellular mat by chemical evolution processes.

References

1. Walde P. Prebiotic Chemistry, From Simple Amphiphiles to protocell Models. Springer-Verlag, Berlin, 167 p. (2005).
2. Norris V., and Raine D.J. A Fission Fusion Origin for Life. *Orig. Life Evol. Biosph.*, **28**: 523-537 (1998).
3. Donaldson D.J., Tervahattu H., Tuck A.F., and Vaida V. Organic Aerosols and the Origin of Life: A Hypothesis. *Orig. Life Evol. Biosph.*, **34**: 57-67 (2004).
4. Cairns-Smith A. and Hartman H. *Clay Minerals and the Origin of Life*. Cambridge University Press, 10 p. (1986).
5. Bebie J., and Schoonen M.A.A. Pyrite and phosphate in anoxia and an origin-of-life hypothesis. *Earth Planet. Sci. Lett.*, **171**: 1-5 (1999).
6. Trevors J.T. The subsurface origin of microbial life on the Earth. *Res. Microbiol.*, **153**: 487-491 (2002).
7. Branciamore S., Gallori E., Szathmary E., and Czarán T. The Origin of Life: Chemical Evolution of a Metabolic System in a Mineral Honeycomb. *J. Mol. Evol.*, **69**: 458-469 (2009).
8. Hansma H. Possible origin of life between mica sheets. *J. Theor. Biol.*, **266**: 175-188 (2010).
9. Trevors J.T., and Pollack G. H. Hypothesis: the origin of life in a hydrogel environment. *Prog. Biophys. Mol. Biol.*, **89**: 1-8 (2005).
10. Trevors J.T. and Pollack G. H. Origin of microbial life hypothesis: A gel cytoplasm lacking a bilayer membrane, with infrared radiation producing exclusion zone (EZ) water, hydrogen as an energy source and thermosynthesis for bioenergetics. *Biochimie*, **94**: 258-262 (2012).
11. Trevors J.T. Suitable microscopic entropy for the origin of microbial life: Microbiological methods are challenges. *J. Microbiol. Methods*, **83** (3): 341-344 (2010).
12. Trevors J.T. Hypothesized origin of microbial life in a prebiotic gel and the transition to a living biofilm and microbial mats. *C. R. Biol.*, **334** (4): 269-272 (2011).
13. Trevors J.T. The Composition and Organization of Cytoplasm in Prebiotic Cells. *Int. J. Mol. Sci.*, **12**: 1650-1659 (2011).
14. Baia H.X., and Yang X.R. Spectrophotometric Determination of Water Content in Alcohol Organic Solvents. *J. Chin. Chem. Soc.*, **54**: 619-624 (2007).
15. Gargaud M., Barbier B., Martin H. and Reisse J. Lectures in Astrobiology. Springer-Verlag, Berlin, **1**, 105p (2005).