Interaction of Thymine and Uracil with Copper, Cobalt and Silver Ferrocyanides and its Implications in Chemical Evolution and Origins of Life

Abstract

Effect on pH on adsorption of nucleic acid bases (thymine and uracil) at two different concentrations 1 x 10^{-4} M and 1 x 10^{-5} M on copper, cobalt and silver ferrocyanides was studied over a pH range (1.0 – 10.0) at room temperature 30 ± 1° C. The progress of adsorption was followed spectrophotometrically by measuring the absorbance of the nucleic acid base solutions at their corresponding λ_{max}. Maximum adsorption was found to occur at neutral pH for both thymine and uracil. Effect of concentration on adsorption of thymine and uracil on copper, cobalt and silver ferrocyanides was studied in a concentration range 10^{-4} – 10^{-5} M at neutral pH 7.0 ± 0.1 and at room temperature 30 ± 1° C. The interaction followed the Langmuir type of adsorption in general in the concentration range of 10-4 to 10-5 M of thymine and uracil solution. The uptake of nucleic bases on metal ferrocyanides follow the order CuFc > AgFc > CoFc for the adsorption of thymine and CuFc > CoFc > AgFc for the adsorption of uracil. Effects of presence of salts on the adsorption of thymine and uracil on metal ferrocyanides also been studied . Results on the present studies suggest the importance of metal ferrocyanides as possible condensing agents for biomolecules during the course of chemical evolution and the origin of life on prebiotic earth. The insoluble metal ferrocyanides on coming in contact with biomolecules must have either formed metal complexes or could have enhanced the formation of biopolymers in fluctuating environment.

Keywords: Interaction, thymine, uracil, nucleic bases, metal ferrocyanides, chemical evolution, origins of life

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1. Introduction

Due to easy of formation of cyanide under prebiotic conditions, cyanide ion might have formed stable complexes with transition metal ions on primitive earth. It is also thought that during the course of chemical evolution, cyanide could have also formed some insoluble double metal ferrocyanides of general formula M_2 [Fe $(CN)_6$] . xH_2O where M = Fe, Ni, Cu, Co, Mo etc. It is well established that metal ferrocyanides act as adsorbent (Tewari and Webster, 2006) and Photosensitizers [Tewari, 2005].

All organisms on Earth today use the same four bases in the same genetic code and the same twenty amino acids (out of the hundreds possible). Furthermore they all use the same basic mechanism of DNA-protein conversion. Even more surprising is that the genes that specify certain functions in vastly different creatures are very similar in their sequence of bases; that is, they are homologous. This research examines a specific route as to how the initial life-forms may have been produced on Earth that evolved into more complex creatures over time (Fitz et al., 2007).

Earth being bombarded with outer space elements and comets soon led to chemical reactions that created water on the surface of the planet. Colliding asteroids and the process of weathering and tectonic movements under the Earth's crust created craters and large geographical depressions. These huge gouges became lifeless seas and oceans with a mixture of elements and prehistoric rocks, silica, clays, iron-containing compounds, as well as carbon, nitrogen, oxygen and other life-essential elements. Simple organic molecules such as amino acids, nucleic acid bases, and simple sugars soon were formed from chemical reactions under appropriate conditions (Ali and Kamaluddin, 2006).

This research is specifically concerned with one of the essential life molecules which is nucleic acid base that forms links along the double helix structure of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) from which proteins are synthesised. These nucleic acid bases aligned themselves over decades under the appropriate chemical conditions in order to form a meaningful

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structure that soon evolved to be the first life form on Earth. This alignment was assisted by a chemical process of adsorption that allows an adsorbent to adsorb these nucleic acid bases onto their surfaces to facilitate bonding and closer interaction and over the years, this spontaneous alignment became biologically successful and gave rise to a very meaningful biomolecule that evolved into the first life form. The adsorbent that aided this proposed condensation of nucleic acid bases has to be a prebiotic molecule. Due to their solid surfaces, various clay minerals, silica and carbon have been proposed to play important roles in concentrating the biomolecules from dilute prebiotic soup in the primeval seas during the course of chemical evolution. Interaction with a wide variety of organic molecules including amino acids, peptides, nucleic acids and sugars with clay and clay minerals have been studied suggesting their possible role in chemical evolution (Balmaseda et al., 2002).

The adsorption of pyrimidine on the loughlinite Lewis acid cites through the pyrimidine lone pairs has been discussed (Akyuz and Akyuz, 2004). The electrochemical behaviour of purine bases and their derivatives is studied on electrodes modified with carbon nanotubes. The strongest adsorption and oxidations are observed at +0.8 V and +1.0 V for guanine and adenine, respectively, (Abdullin, 2008). The adsorption of purine on Ge(100) has been investigated using scanning tunnelling microscopy (STM) supported by ab initio theoretical calculations (Jung et al., 2012).

The adsorption behaviour of nucleosides (adenosine, guanosine and thymidine) on to mesoporous silica, porous carbons and carbon nanocage were studied (Datta et al., 2011) The plane wave density functional theory methods have used to explore the adsorption modes and configurations of uracil molecules on a gold surface to gain insight into the rational design of surface functionalization (Irrera and De Leeuw, 2011). The adsorption sites of adenine, adenosine, AMP,

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ADP, ATP, Poly A, uracil, uridine, UMP, UDP, UTP, and Poly U on sodium montmorillonite are investigated (Perezgasga et al., 2005).

The interaction of adenine and thymine with and adsorption on zeolites were studied by using FT-IR, EPR spectroscopy, X-RD and SEM Studies. It is observed from X-RD that thymine adsorption increased the decomposition of zeolites while adenine prevented it. Zeolite adsorbed some amount of adenine and thymine protected from hydrolysis and UR radiation and could be available for molecular evolution (Bau et al., 2012). Computational study performed using the density functional theory shows that hydrated and non-hydrated tetrahedral and octahedral kaolinite mineral surfaces in the presence of a cation adsorb the nucleic acid bases thymine and uracil well (Michalkova et al., 2011).

Adsorption of adenine was studied in the presence of montmorillonite clay with or without cations Ca^{2+}, Cu^{2+} and Mg^{2+}. Results of present study showed that adsorption trend largely depend on the nature of adsorbate and the absorbent (Gururani et al., 2012). Interaction of mono-, oligo-, and polynucleotides and their components on rutile (TiO_2) has been important for the origin of life. TiO_2 is a mineral common in many terrestrial crustal rocks (James Cleaves II et al., 2010). Several prebiotic experiments including (a) primary chondrite minerals (Olivine, Pyroxene etc.) and clay modified with metals (Cu, Fe, Ni, Mo, Zn, etc.) (b) using mineral with more organophilic characteristics (c) using sea water of different conc. of ions (i.e. Na^+, Cu^{2+}, Mg^{2+}, SO_4^{2-} and Cl^-) (d) determine possible products of decomposition due to interactions of amino acids and nucleic acid bases with minerals are reported to understand important issues related to origin of life on Earth (Zaia, 2012). The adsorption of purines, pyrinidines and nucleosides from aqueous solution to clay minerals is affected by suspension pH. With montmorillonite adsorption is also influenced by the nature of the exchangeable cations (Hashizume, 2015).

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Adsorptive interaction of nucleotides on the rutile (110) surface is investigated (Gemming et al., 2010). The possible role of montmorillonite clays in the prebiotic formation of RNA is discussed and it is also concluded from this study that ligation to the Cu^{2+} is a major force in the binding of nucleotides to Cu^{2+}montmorillonite and are not readily washed from the clay (Ferris et al., 1989). Adsorption of adenine on the surface of graphite crystals have been studied at different temperature. Results suggest that the effects of temperature and presence of inorganic solids must be included when assessing the prebiotic availability of adenine (Sowerby et al., 2001). Catalytic guanine oxidation using Ru $(bpy)_3^{2+}$ as mediator has been investigated electrochemically in viscous solutions containing high concentration of polyethylene glycol. DNA adsorption increase with increasing the viscosity of its solution and produces pre peaks in cyclic voltagrams (Szalai et al., 2002).Difference spectra have been observed in both adsorption and circular dichroism upon binding of various guanine nucleotides to ribonuclease F1 (Yoshida and Kanae, 1989). A search of literature indicated that very few reports available on interaction of aminoacids with metal ferrocyanides (MFcs) but no report available on interaction of nucleic acid bases with MFcs. In view of this attempt was made to study interaction of nucleic acid base with MFcs. In addition present work described interaction of thymine (Thy) and uracil (Ura) with copper ferrocyanide (CuFc), Cobalt ferrocyanide (CoFc) and silver ferrocyanide (AgFc) and their implications in chemical evolution.

2. Experimental

2.1 Chemicals

Copper nitrate, cobalt nitrate, silver nitrate, potassium ferrocyanide, thymine and uracil were obtained from BDH Poole, England. All chemicals were of analytical grade and used without further purification. Solutions were prepared in doubly distilled water.

2.2 Synthesis of metal ferrocyanides

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Copper and cobalt ferrocyanides were prepared by adding metal salts(500 mL, 0.1 M) and potassium ferrocyanide(167 mL, 0.1 M) with constant stirring (Kourim et al., 1964).A slight excess of the metal salt was added to the mixture to enhance coagulation of the precipitate. The reaction mixture was then heated at 60 ^{0}C in a water bath for 3 hours and was kept as such for 24 hours at room temperature. The precipitate was filtered using vacuum filtration and washed several times with distilled water. The precipitate was then kept in an oven pre-set at 60-65 ^{0}C for 2 hours until dried. The dried product was then grounded using a mortar and pestle and was sieved using a 125 µm mesh size sieve. The same procedure was used to produce silver ferrocyanide except that silver nitrate salt was used instead and the system was kept protected from light by being wrapped with aluminum foil and were performed in dark chamber.

2.3 Characteristics of metal ferrocyanides

Copper ferrocyanide is a rust brown, grainy insoluble solid. Cobalt ferrocyanide is grey blackish in colour and is an amorphous insoluble solid that possesses an octahedral structure with face centred cubic lattice. Silver ferrocyanide is a blue, powdery and insoluble solid. characterized on the basis of elemental and spectral studies. The percentage composition of metals was determined by VarianTechtron atomic absorption spectrophotometer (Vogel, 1978). Carbon, hydrogen, nitrogen analysis was performed on CEST – 118, CHN analyser. The elemental analysis percentage values metal ferrocyanides are given in Table 1.

Infrared spectra of compounds were recorded in potassium bromide disc on Beckmann IR-20 spectrometer. All three metal ferrocyanides show a broad peak at 3600 – 3625 cm^{-1} is characteristics of water molecules and OH group. Also a peak around 1600 – 1635 cm^{-1} due to H-O-H bending. Two sharp bands one at 2010 – 2080 cm^{-1} and other at 580 – 600 cm^{-1} in all three metal ferrocyanides are characteristics of cyanide stretching and Fe-C stretching frequencies (Nakamoto, 1963). Another sharp band at around 460 – 490 cm^{-1} probable shows the presence of metal (Cu, Co, Ag) – nitrogen band thus indicating a certain degree of

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polymerization in the product.   (Ratnasamy and Leonard, 1976; Nakamoto et al., 1958).
Infrared spectral data of copper, cobalt and silver ferrocyanides are given in Table 2.

2.4 Adsorption studies

2.4.1   *Effects of pH on adsorption of nucleic acid bases on metal ferrocyanides*

Buffer solutions for pH 1.0-10.0 were prepared using the following reagents for each
respective pH:pH 1.0: 0.2 M KCl + 0.2 M HCl; pH 2.0: 0.2 M KCl + 0.2 M HCl; pH 3.0:
0.1 M HOAc + 0.1 M NaOAc; pH 4.0: 0.1 M HOAc + 0.1 M NaOAc; pH 5.0: 0.1 M
HOAc + 0.1 M NaOAc; pH 6.0: 0.1 M HOAc + 0.1 M NaOAc; pH 7.0: 0.1 M KH_2PO_4 +
0.1 M NaOH; pH 8.0: 0.1 M Borax + 0.1M HCl; pH 9.0: 0.025 M Borax + 0.1 M HCl; pH
10.0: 0.025 M Borax + 0.1 M NaOH. The pH was verified with a Fisher Scientific pH
meter. Effect on pH on adsorption of thymine and uracil on metal ferrocyanides was
studied at two different concentrations 1 x 10^{-4} M and 1 x 10^{-5} M over pH range 1.0 – 10.0
and at room temperature 30 ± 1° C. Two different concentrations 10^{-4} M and 10^{-5} M of
thymine and uracil solutions were prepared and buffered with each of the buffer made. 10
ml of buffered thymine and uracil were added to their respective labelled cuvettes and the
initial absorbance of the solutions was taken using a Hach DR 5000 UV Spectrometer at
wavelengths 264.5 nm and 258.0 nm for thymine and uracil, respectively. A 100 mg of
each of the three metal ferrocyanides was then added to each cuvette and the mixture was
placed on a Stuart Scientific Flask Shaker at speed 10 at room temperature for 8 hours with
manual shaking at a regular 1 hour interval. The mixtures were then centrifuged using an
Eppendorf Centrifuge and the supernatant was decanted and collected. The final
absorbance of the supernatant was measured using the UV spectrometer at their respective
λ_{max} values. The percentage uptake of the nucleic acid bases by metal ferrocyanides were

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calculated for each of the pH. It was found that species of buffers used do not adsorb on the surface of metal ferrocyanides. It was checked by conductivity measurements.

2.4.2 *Effect of concentration on the adsorption of thymine and uracil on copper, cobalt and silver ferrocyanides*

The adsorption of thymine and uracil on metal ferrocyanides as a function of concentration range 10^{-4} - 10^{-5} M was studied at pH 7.0 ± 0.01 and at ~~room~~ temperature $30 \pm 1\ ^0$C. A buffer of pH 7.0 was made using potassium dihydrogen phosphate and sodium hydroxide. Stock solutions of concentration 1x 10^{-4} M of thymine and uracil were prepared. These two standard solutions were further diluted using the buffer solution to give the range of concentration from 10^{-4} M – 10^{-5} M. A 10 ml of buffered thymine and uracil solutions at each respective concentration were added to a series of test tubes and the initial absorbance was measured using the Hach DR 5000 UV spectrophotometer. A 100 mg of each of the three metal ferrocyanides was then added to each cuvette and the mixture was placed on a shaker at speed 10 at room temperature for 8 hours with manual shaking at a regular 1 hour interval. The mixtures were then centrifuged and the supernatant was decanted and collected. The final absorbance of the supernatant was measured using the UV spectrometer at their respective, λ_{max} values. The amount of thymine and uracil adsorbed on the surface of three metal ferrocyanides were calculated for each of the concentration.

2.4.3 *Effect of salts on the adsorption of thymine and uracil on copper, cobalt and silver ferrocyanides*

The effects of ~~salts~~ copper nitrate , cobalt nitrate and silver nitrate on the adsorption of thymine and uracil nucleic bases on metal ferrocyanides was studied by carrying out separate experiments at varying concentrations in the presence of 1.5×10^{-3} M of each salt used to prepare the three metal ferrocyanides. This study was conducted at pH 7.0 ± 0.01

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and room temperature. A buffer of pH 7.0 was made using potassium dihydrogen phosphate and sodium hydroxide. A known calculated mass of each of the three metal sal copper nitrate, cobalt nitrate , and silver nitrate was measured and dissolved in separate quantities of the pH 7.0 buffer to acquire a salt concentration of 1.5×10^{-3} M in each case. Stock amount of 10^{-4} M of thymine and uracil solutions were prepared using the salt-buffer solution. These two standard solutions were further diluted using the buffer solution to give a range of concentration of thymine and uracil from $1x\ 10^{-4}$ M – $1x\ 10^{-5}$ M.

A 10 ml of buffered thymine and uracil salt solutions at each respective concentration were added to a series of test tubes and the initial absorbance was measured using the UV spectrometer. A 100 mg of each of the three metal ferrocyanides was then added to each cuvette containing its own respective salts solutions and the mixture was placed on a shaker at speed 10 at room temperature for 8 hours with manual shaking at a regular 1 hour interval. The mixtures were then centrifuged at 1/2000 rpm and the supernatant was decanted and collected. The final absorbance of the supernatant was measured ~~using the UV spectrophotometer~~ at their respective, λ_{max} values. The amount of the thymine and uracil adsorbed on metal ferrocyanides was calculated for each of the concentration.

At salt concentration $1.5\ x\ 10^{-3}$ mol / L and pH 7.0 ± 0.01, the copper, cobalt and silver hydroxides not get adsorbed on the surface of metal ferrocyanides. This was verified by conductivity measurements of salt solution with and without metal ferrocyanides.

3   Results and Discussion

3.1 *Effect of pH on the adsorption of thymine and uracil on metal ferrocyanides*

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The percentage uptake of thymine and uracil as a function of pH was studied over the pH range of 1.0 – 10.0. Two different concentrations of the nucleic acid bases were used in this part of the investigation 10^{-4} M and 10^{-5} M. The effects of pH on the adsorption of thymine and uracil on copper, cobalt and silver ferrocyanides are shown on Figures 1 and 2. The percentage uptake was calculated using the following formula.

$$\text{Percentage Uptake} = \frac{\text{Difference in absorbance before and after adsorption}}{\text{Absorbance before adsorption}} \times 100$$

For both concentrations of thymine and uracil used, the maximum uptake occurred at neutral pH. According to Figure 1 that shows the percentage uptake of thymine and uracil at an initial concentration of 1×10^{-5} M, the most effective metal ferrocyanide in adsorbing thymine and uracil was found to be Cobalt ferrocyanide (66.0 % for Thymine and 65.0 % for Uracil). Copper ferrocyanide also was very effective in adsorbing thymine and uracil at this concentration. The least effective metal ferrocyanide was found to be silver ferrocyanide. For a higher initial concentration of thymine and uracil (1×10^{-4} M as shown in Figure 2), the overall percentage uptake was found to have been elevated. The most effective metal ferrocyanide in adsorbing thymine and uracil was found to be cobalt ferrocyanide (76.0 % and 96.0 %, respectively). The percentage uptake values of thymine and uracil at concentrations 10^{-4} M and 10^{-5} M on copper, cobalt and silver ferrocyanides are given in Table 3. Generally, it can be seen that as the concentration of the nucleic acid bases increases, the percentage uptake also increases. A high concentration means that more of the nucleic bases' molecules are available to bind to active sites on the adsorbent. Sufficient adsorbent molecules are available to occupy the active sites unlike in the situation where a deficient of such molecules are used leading to a low uptake. The percentage uptake of uracil is overall higher compared to that of thymine. This is solely due to the chemical structure of the two molecules.
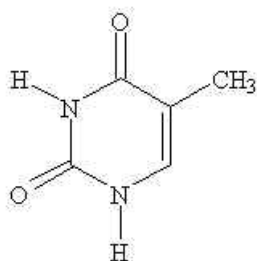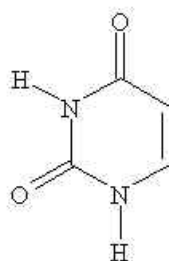
Image.1 Thymine   Image 2.Uracil

Due to the absence of the methyl group in uracil, the double bond becomes more electron-deficient since methyl groups are strong electron donors. As a result, it is with much ease that a bond can result between uracil and a metal ferrocyanide than between the latter and thymine. As a result, the metal ferrocyanides are more prone to bond to the electron-deficient uracil resulting in a higher percentage uptake than that of thymine.

The adsorption of thymine and uracil occurred at neutral pH for all the systems investigated. The characteristic tautomeric nature of bases provides some insight into the pH effect (Krishnamurthy et al., 1999; Smith 1998; Luther et al., 1998, Greaves and Wilson, 1969; Hesselink, 1983). Under acidic conditions, bases occur as the cations and as neutrality is approached, they tend to shift to the more stable tautomeric forms. When the pH is greater than 7.0, this gives an indication that adsorption is taking place in the form of anions. In acidic conditions, the carbonyl form dominates; at neutral pH, the more stable tautomeric form results and beyond pH 7.0, the tautomeric form changes to give the phenolic structures. Adsorption of bases on metal ferrocyanides could be due to base interactions with replaceable divalent metal ions such as Cu (II), and Co (II) present outside the coordination sphere of the metal ferrocyanides (Dunicz, 1961). Considerable decrease in adsorption of bases on the metal ferrocyanide for both of the concentrations investigated (10^{-4} M and 10^{-5} M) may be because at higher pH, the coordination of available OH^{-} ions with divalent cations (Cu^{2+}, Co^{2+}) and

monovalent Ag^+ becomes competitive with that of the thymine and uracil molecules. Thymine and uracil are adsorbed best onto metal ferrocyanides at neutral pH.

3.2 Effect of concentration on the adsorption of thymine and uracil on metal ferrocyanides

This part of the experiment was carried out at pH 7 ± 0.01 since it was seen from the latter section that adsorption of thymine and uracil occurs best in a pH-neutral system. Room temperature (30 ± 1^0C) was also employed to carry out this part of the investigation. A series of standard solutions of concentration ranging from 10^{-5} M to 10^{-4} M were prepared using the two thymine and uracil separately and the system solvent (pH 7.0 ± 0.01 buffer).

It is clear from Figure 3 that at lower concentrations of thymine and uracil , the adsorption is rapid but at greater concentrations the uptake becomes almost constant. This holds true for all the system investigated with a positively sloping line and a flat tail. This phenomenon is quite adhering to the adsorption theory. As the number of adsorbate (bases) molecules increases, more will be available to be bound to the adsorbent (metal ferrocyanide) molecules. The adsorbent usually have a fixed number of adsorption sites and when all of these sites are occupied by the adsorbate molecules, a saturation point is reached where even increasing the adsorbates concentration will lead to no further binding (Lawless and Edelson, 1980). The latter statement holds true for a Langmuir adsorption process where monolayer coverage occurs and no other molecules can bind after all the sites are occupied. The values of maximum uptake of thymine and uracil on the different metal ferrocyanides are given in Table 4. The orders of maximum uptake of thymine and uracil observed on metal ferrocyanides:

Thymine:

Cobalt ferrocyanide < silver ferrocyanide < Copper ferrocyanide

Uracil:

Silver ferrocyanide < Cobalt ferrocyanide < Copper ferrocyanide

Langmuir plot ($1/Q_{eq}$ versus $1/C_{eq}$) for the adsorption of thymine and uracil on copper, cobalt and silver ferrocyanides are shown on Figure 4. The linearized form of the Langmuir equation was considered to develop the linear plots. The adsorption isotherms for thymine and uracil have been analysed in terms of the Langmuir adsorption equation (Dada et al., 2012):

$$\frac{1}{Q_{eq}} = \frac{1}{Q_0} + \frac{1}{Q_0 b} \cdot \frac{1}{C_{eq}}$$

where Q_{eq} is the amount of solute adsorbed per unit mass of adsorbent, C_{eq} the equilibrium concentration of solute in solution, Q_0 the limiting amount of adsorbate that can be taken up by unit mass of adsorbent, b the constant related to equilibrium constant or bonding energy or enthalpy (ΔH) of adsorption (b α $e^{-\Delta H/RT}$ the parameter b reflects the steepness of the approach to saturation; more precisely, the b value is the reciprocal of concentration at which half of the saturation of the adsorbent is attained). Actually "b" is a constant, which is function of adsorption energy. The appropriate Langmuir constants b and Q_0 were calculated from the slope and intercept of the Langmuir plots respectively. The values of Langmuir constants are given in Table 5.

3.3 Effect of salt on the adsorption of thymine and uracil on metal ferrocyanides

Effect of salts on the adsorption of thymine and uracil on Copper ferrocyanide , Cobalt ferrocyanide and silver ferrocyanide at their equilibrium concentrations are shown in Figure 5. The values of maximum uptake of thymine and uracil are given in Table 6. The orders of maximum adsorption of nucleic acid bases on metal ferrocyanides in the presence of salts are as follows:

Thymine:

 Cobalt ferrocyanide < silver ferrocyanide < Copper ferrocyanide

 Uracil:

 Silver ferrocyanide < Copper ferrocyanide < Cobalt ferrocyanide

In each experiment the values of maximum adsorption was found to be higher in the presence of salts in comparison to absence of salts.

This indicate that added metal ions (Cu^{2+}, Co^{2+}, and Ag^+) either first make complexes with the nucleic acid bases and then get adsorbed on the metal ferrocyanides surface or that the increase in ionic strength upon addition of the metal salt solution lowers the repulsive forces between the organic molecules and the metal ferrocyanide or that both of these mechanism are operative. Studies on salt effects have confirmed the assumption that at normal conditions adsorption is due to the formation of nucleic acid base-metal complexes (Tewari and Hamid, 2007). This is also assumable since it is unlikely for the nucleic acid base (thymine and uracil) molecule to enter into the coordination sphere of metal ferrocyanides by replacing strong ligands like cyanide. As such, some form of complexation is very possible that ultimately increases the binding capacity of the adsorbate to the adsorbent.

Lahav and Chang proposed a model for a prebiotic environment in which concentration, condensation and chemical evolution of biomolecules could have taken place. In this model, however, high concentration of soluble salts, such as chlorides and sulphates, are

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taken into account, where as previously more or less salt-free system had been assumed. The proposed system emphasises the need to develop the theoretical background and experimental methods for the study of interactions among biomolecules in the presence of high salt concentrations and solid surfaces soluble salts, as well as interaction between the biomolecules and these surfaces (Shapiro, 1999; Pace, 1991; Lahav and Chang, 1976).

4. Concluding Remarks

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The result of the present study reveal that insoluble metal hexacyanoferrate (II) complexes present at the bottom and shores of primeval seas could have interacted with thymine and uracil in such a way as to concentrate them from the dilute prebiotic soup during the course of chemical evolution and the origin of life. Biomonomers , thus concentrated, are thought to have been protected from degradation and undergone a class of reactions of prebiotic relevance producing biopolymers essential for the formation of the first living cells on this planet.

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References

Abdullin, T. I., Nikitina, I. I. and Bondar, O. V. (2008) Adsorption and oxidation of purine bases and their derivatives on electrodes modified with carbon nanotubes. *Russ. J. Electrochem*. 44(12): 1345-1349.

Akyuz, S. and Akyuz, T. (2004) FT-IR spectra of natural loughlinite (Na-sepiolite) and adsorption of pysinidineon loughlinite. *J. Mol. Struct*. 705 (1-3): 147-151.

Ali, S. R. and Kamaluddin, (2006), Interaction of aromatic amino acids with metal hexacyanochromo(III) complexes: A possible role in chemical evolution. *Bull. Chem. Soc. Jpn* 79(10): 1541-1546.

Balmaseda, J., Raguera, E., Gomez, A. Diaz, B and Acutie. M., (2002) Evaluation of cadmium hexacyanoferrate(III) as a microporous material. *Microporous Mesoporous Mater* 54: 285-292.

Bau, J. P. T., Cristine, E. A. C., de Souza, I. G., de Souza, Cm M. D., da Costa A. C. S., di Mauro, E., Zaia, C. T. B.V., Coronos, J., Casado, C. and de Santana H. (2012) Adsorption of adenine and thymine on zeolites; FT-IR and EPR spectroscopy and X-ray diffractometry and SEM studies. *Orig. Life Evol. Biosph.* 42(1): 19-29.

Dada, A. O., Olalekan, A. P., Olatunya, A. M. and Dada, O. (2012) Langmuir, Freudlich, Temkin and Dubimn – Radushkevich Isotherms studies of equilibrium sorption of Zn^{2+} unto Phosphoricacid modified rice husk. *IOSR J. Appl. Chem*. 3(1): 38-45.

Datta, K. K. R., Vinu, A., Mandal, S., Al-Deyah, S., Hill, J. P andAriga K. (2011) Base – selective adsorption of nucleosides to pore-engineered nanocarbon, carbon Nanocage. *J. Nanosci. Nanotechnol*. 11(5): 3959-3964.

Dunicz, B. L. (1961), Surface area of activated charcoal by Langmuir adsorption isotherm, *J. Chem. Edu.* 38:357-361.

Ferris J. P., Ertem G. and Agarwal V. K., (1989) The adsorption of nucleotides and polynucleotides on montmorillonite clay. *Orig. Life Evol. Biosph.* 19(2): 153-164.

Fitz, D., Reiner, H. and Rode, B. M. (2007) Chemical evolution towards the origin of life. *Pure Appl. Chem.* 9: 2101 – 2117.

Gemming, S. Enyashin, A. N., Franzel, J., Seifert, G., (2010) Adsorption of nucleotides on rutile (110) Surface. *Int. J. Mater Res.* 101(6): 758-764.

Greaves, M .P. and Wilson, M. J. (1969) The adsorption of nucleic acids by montmorillonite, *Soil Biol. Biochem.*1: 317-323.

Gururani, K., Pant, C. K. and Pathak, H. D. (2012), Surface interaction of adenine on montmorillonite clay in present and absence of divalent cations in relevance to chemical evolution. *Int. J. Sci. Technol. Res.* 1(9): 106-109.

Hashizume, H. (2015) Adsorption of nucleic acid bases, ribose and phosphate by some clay minerals. *Life* 5(1): 637-650.

Hesselink, F. T. (1983) Adsorption of polyelectrolytes from dilute solution. In G. D. Parfitt and C. H. Rochesters (eds). Adsorption from solution at the solid / liquid interface. Academic Press Ltd. London, UK, pp. 377- 412.

Irrera, S. and De Leeuw, N. H. (2011) A density functional theory study of the adsorption of uracil on the Au (100) surface. *Proc. R. Soc. A*. pp 1-11.

James C. (II), H. Jonsson, C. M., Jonsson, C. L. Sverjensky, D. A. and Hazen R. M. (2010), Adsorption of nucleic acid components on rutile (TiO$_2$) surfaces. *Astrobiol.* 10(3): 311-323.

Jung, S. J., Kim, D. H. Lee, J. Y., Kim, S. and Horg, S. (2012) Adsorption structure and reaction mechanism of purine on Ge(100) studied by scanning tunnelling microscopy and theoretical calculations. *J. Phys. Chem. C.* 116(12): 6953-6959.

Kourim, V., Rais J. and Million, B. (1964) Exchange properties of complex cyanides – I. *J. Inorg. Nucl. Chem*. 26: 1111-1115.

Krishnamurthy, R., Pitsch, S. and Arrhenius G. (1999) Mineral induced formation of pentose -2, 4 – bisphosphates. *Orig. Life Evol. Biosph.* 29: 139-152.

Lahav, N. and Chang, S. (1976) The possible role of solid surface area in condensation reactions during chemical evolution: Re-evaluation. *J. Mol. Evol*. 8: 357-380.

Lawless, J. G. and Edelson, E. H. (1980) in R. Holmquist (ed.) *Life Sci. Space Res.*, Pergamon Press, New York 18: 83-88.

Luther, A. , Brandsch, R. and von Kiedrowsky G. (1998) Surface – promoted replication and exponential amplification of DNA analogues. *Nature* 396:245-248.

Michalkova A., Robinson T. L.,Leszczynski, J. (2011) Adsorption of thymine and uracil on 1:1 clay mineral surfaces: comprehensive abinitio study on influence of sodium cation and water. *Phys. Chem. Chem. Phys*. 13(17): 7862 – 81.

Nakamoto, K., Fujilas, J. and Murata, H. (1958) Infrared spectra of metallic complexes – V. Theinfra-red spectra of nitro and nitrite complexes. *J. Am. Chem. Soc*. 80:4817-4823.

Nagamoto, K. (1963) *Infrared Spectra of Inorganic and Coordinate Compounds*. John Wiley, New York, p. 166.

Pace, N. R. (1991) Origins of life – facing up to the physical setting. *Cell* 65: 531-533.

Perezgasga, L., Serrato-Diaz, A., Negron – Mendoza A., De Pablo G. L. and Mosqueira, F. G. (2005). Sites of adsorption of adenine, uracil and their corresponding derivatives on sodium montmorillonite. *Orig. Life Evol. Biosph*. 35(2): 91-110.

Ratnasamy, P. and Leonard, A. J. (1976) Evolution of chromia. *J. Phys. Chem*. 76: 1938-1943.

Shapiro, R. (1999) Prebiotic cystosine synthesis: A critical analysis and implications for the origins of life. *Proc. Nat. Acad. Sci*. (USA) 96: 4396:4401.

Sowerby, S. J. Morth, C. M. and Holm N. G. (2001), Effect of temperature on the adsorption of adenine. *Astrobiol.* 1 (4): 481-487.

Smith, J. V. (1998) Biochemical evolution I. Polymerization on internal, organophilic silica surfaces of de-aluminate zeolites and feldspars. *Proc. Natl. Acad. Sci*. 3370- 3375.

Szalai, V. A., Jayawickamarajah, J. and Thorp, H. H. (2002) Electrocatalysis of guanine oxidation in polyethylene glycol solutions: The interplay of adsorption and reaction rate. *J. Phys. Chem. B*., 106: 709-716.

Tewari, B. B. (2005) Nickel ferrocyanide snesitized photooxidation of diphenylamine, *Rev. Soc. Quim Peru* 71(4): 273:277.

Tewari, B. B. and Hamid, N. (2007) Interaction of glycine and beta – alanine with nickel, cobalt and cadmium ferrocyanides. *Colloids Surf. A*, 296:264 – 269.

Tewari, B. B. and Webster, R. W. (2006) Life originate on primitive earth or on other plants is still a subject of controversy. *Bolivian J. Chem*. 23(1): 77-81.

Vogel, A. I. (1978) Vogels Text Book of Quantitative Inorganic Analysis, Including Elementary Instrumental Analysis, 4th (ed.) p. 827.

Yoshida H. and Kanae H. (1989) Interaction of guanine nucleotides with ribonuclease F1 as studied by adsorption and circular dichroism spectroscopies. *Chem. Lett*. pp. 177-180.

Zaia, D. A. M. (2012) Adsorption of amino acids and nucleic acid bases onto minerals: a few suggestions for prebiotic chemistry experiments, *Int. J. Astrobiol*. 11(04): 229-234.

TABLE 1: ELEMENTAL ANALYSIS OF COPPER, COBALT AND SILVER
            FERROCYANIDES

Metal ferrocyanides[a]	Percentage (%) found				
	Metal	Iron	Carbon	Hydrogen	Nitrogen
CuFc	28.30	12.40	16.03	2.56	18.32
CoFc	26.60	13.10	15.50	2.71	18.10
AgFc	40.22	8.75	11.47	2.75	13.77

[a]CuFc = Copper ferrocyanide

CoFc = Cobalt ferrocyanide

AgFc = Silver ferrocyanide

TABLE 2:   INFRARED SPECTRAL DATA OF COPPER, COBALT AND SILVER
FERROCYANIDES

Metal ferrocyanides	Absorption frequency (cm^{-1})				
	H$_2$O molecule OH group	HOH Bending	C $\equiv$ N Stretching	Fe – C	Metal - N[a]
CuFc	3700	1600	2080	580	490
CoFc	3510	1635	2070	595	460
AgFc	3800	1600	2010	600	490

[a] metal – nitrogen band shows degree

TABLE 3:   PERCENTAGE ADSORPTION OF THYMINE AND URACIL AT DIFFERENT INITIAL CONCENTRATIONS

ON THE COPPER, COBALT AND SILVER METAL FERROCYANIDES (Temperature $30 \pm 1°C$; Mass of MFC -= 100 mg;

Particle Size = 125 μm; Thymine λ_{max} = 264.5 nm; Uracil λ_{max}= 258.0 nm)

% Adsorption											
CuFc				CoFc				AgFc			
1×10^{-4} M		1×10^{-5} M		1×10^{-4} M		1×10^{-5} M		1×10^{-4} M		1×10^{-5} M	
Thy	Ura	Thy	Ura	Thy	Ura	Thy	Ura	Thy	Ura	Thy	Ura
0.13	1.98	3.39	5.06	0.26	0.24	2.91	0.00	0.24	5.96	9.38	1.23
0.26	10.48	8.75	14.80	1.29	6.51	4.05	1.29	6.15	0.13	2.56	3.45
0.33	9.31	5.88	1.00	5.82	3.13	6.56	0.98	9.61	3.91	10.91	1.39
4.47	1.13	5.80	2.82	1.64	0.74	2.99	2.99	0.33	0.74	7.46	10.61
1.06	7.17	4.08	10.53	3.32	3.53	22.73	13.73	1.98	4.22	4.00	1.75
0.67	14.62	13.73	26.92	0.93	57.12	17.31	3.33	0.50	0.41	8.70	26.32
37.87	85.35	31.31	64.77	85.53	96.04	65.56	54.12	84.91	87.07	13.28	30.68
0.58	4.15	0.99	9.21	76.04	1.74	0.93	4.05	64.32	0.50	9.01	9.33
0.41	12.37	1.22	4.17	0.41	2.82	1.22	8.22	3.18	23.73	4.97	22.45
3.58	5.30	1.43	6.54	3.38	3.48	3.28	8.43	11.39	5.55	3.08	1.30

TABLE 4: MAXIMUM UPTAKE OF THYMINE AND URACIL ON METAL FERROCYANIDES

(Temperature = 30 ± 1°C; pH 7.0 ± 0.01)

Nucleic acid base	Metal ferrocyanide	Particle size (μm)	Maximum Uptake, $\times 10^{-7}$ [mol/g]
Thymine	CuFc	125	34.25
	CoFc	125	22.50
	AgFc	125	33.94
Uracil	CuFc	125	28.28
	CoFc	125	15.97
	AgFc	125	13.53

TABLE 5: LANGMUIR CONSTANTS FOR THE ADSORPTION OF THYMINE AND URACIL ON METAL

FERROCYANIDES

(Temperature = 30 ± 1ºC; pH 7.0 ± 0.01)

Nucleic acid base	Metal ferrocyanide	Particle size (μm)	Langmuir Constants	
			b (Lmol^{-1}) $\times 10^{-11}$	Q_o (mg g^{-1}) $\times 10^{14}$
Thymine	CuFc	125	5.00	2.50
	CoFc	125	25.00	0.50
	AgFc	125	5.00	2.50
Uracil	CuFc	125	11.11	1.00
	CoFc	125	33.33	0.33
	AgFc	125	22.22	0.50

TABLE 6: MAXIMUM UPTAKE OF THYMINE AND URACIL ON METAL FERROCYANIDES IN THE PRESENCE OF

SALTS

(Temperature = 30 ± 1ºC; pH 7.0 ± 0.01)

Nucleic acid base	Metal ferrocyanide	Particle size (μm)	Salts [1.5×10^{-3} M]	Maximum Uptake, $\times 10^{-7}$ [mol/g]
Thymine	CuFc	125	$CuCl_2$	35.52
	CoFc	125	$Co(NO_3)_2$	21.97
	AgFc	125	$AgNO_3$	34.81
Uracil	CuFc	125	$CuCl_2$	32.21
	CoFc	125	$Co(NO_3)_2$	55.76
	AgFc	125	$AgNO_3$	14.75

Figure Captions
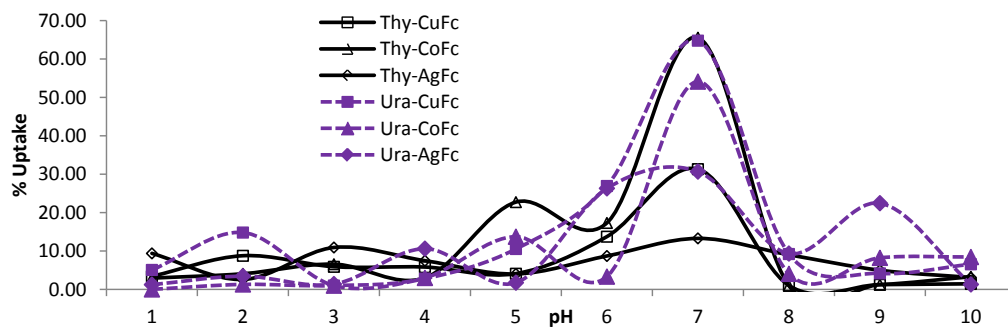
FIG.1. Effect of pH on the adsorption of thymine and uracil (concentration = 1×10^{-5} M) on metal ferrocyanides

(Temperature = $30 \pm 1°$ C; time = 8 hours; mass of MFc = 100 mg; particle size= 125 µm; thymine λ_{max} = 264.5 nm; uracil λ_{max} = 258.0 nm)

FIG.2: Effect of pH on the adsorption of thymine and uracil (concentration = 1×10^{-4} M) on metal ferrocyanides

(Temperature = $30 \pm 1°$ C; Time = 8 hours; Mass of MFc = 100 mg; Particle Size= 125 µm; Thymine λ_{max} = 264.5 nm; Uracil λ_{max} = 258.0 nm)

FIG.3.Adsorption isotherms of thymine and uracil on metal ferrocyanides

(Temperature = $30 \pm 1°$ C time = 8 hours; pH = 7.0 ±0.01; mass of MFc = 100 mg; particle size= 125 µm;

Thymineλ_{max} = 264.5 nm; uracil λ_{max} = 258.0 nm)

FIG.4.Langmuir plots of thymine and uracil on metal ferrocyanides

(Temperature = $30 \pm 1°$ C time = 8 hours; pH = 7.0 ±0.01; mass of MFc = 100 mg; particle size= 125 µm;

Thymineλ_{max} = 264.5 nm; uracil λ_{max} = 258.0 nm)

FIG.5. Adsorption isotherms of thymine and uracil on metal ferrocyanides in the presence of salt

(Concentration of Salt = 1.5×10^{-3} M; temperature = $30 \pm 1°$ C time = 8 hours; pH = 7.0 ±0.01; mass of MFc = 100 mg;

particle size= 125 µm; thymine λ_{max} = 264.5 nm; uracil λ_{max} = 258.0 nm)
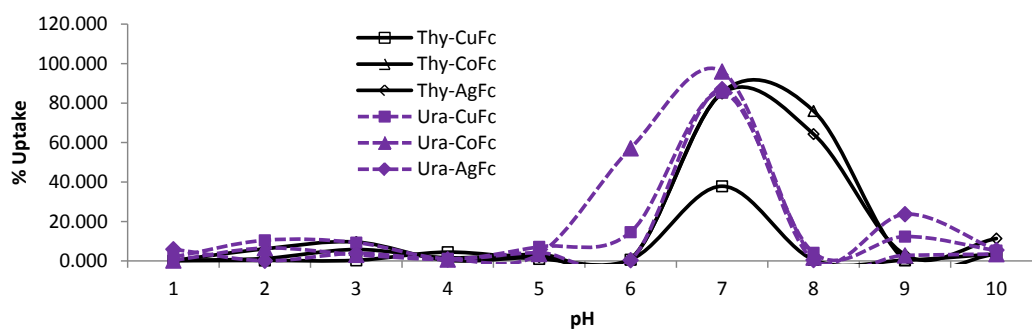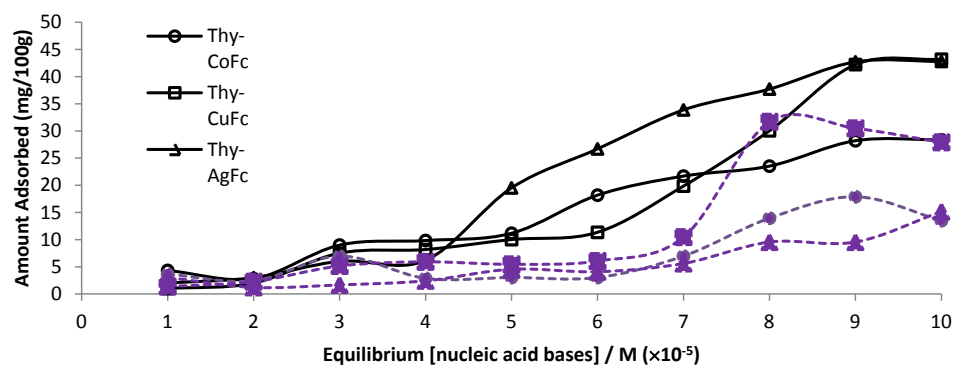
FIG.1. Effect of pH on the adsorption of thymine and uracil (concentration = 1×10^{-5} M) on metal ferrocyanides
(Temperature = $30 \pm 1^\circ$ C; time = 8 hours; mass of MFc = 100 mg; particle size= 125 µm; thymine λ_{max} = 264.5 nm; uracil λ_{max} = 258.0 nm)

FIG.2: Effect of pH on the adsorption of thymine and uracil (concentration = 1×10^{-4} M) on metal ferrocyanides
(Temperature = $30 \pm 1°$ C; Time = 8 hours; Mass of MFc = 100 mg; Particle Size= 125 µm; Thymine λ_{max} = 264.5 nm; Uracil λ_{max} = 258.0 nm)

FIG.3.Adsorption isotherms of thymine and uracil on metal ferrocyanides
(Temperature = 30 ± 1º C time = 8 hours; pH = 7.0 ±0.01; mass of MFc = 100 mg; particle size=
125 µm;
Thymineλ_{max} = 264.5 nm; uracil λ_{max} = 258.0 nm)

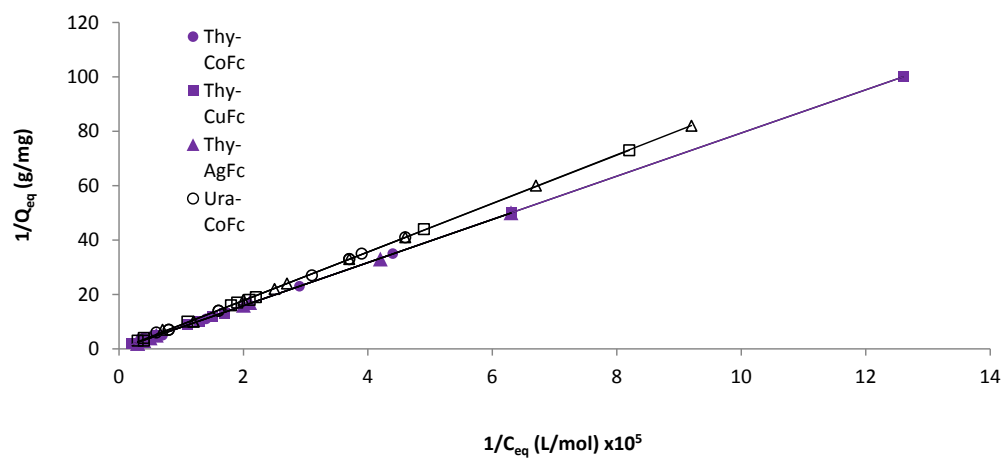FIG.4.Langmuir plots of thymine and uracil on metal ferrocyanides
(Temperature = 30 ± 1º C time = 8 hours; pH = 7.0 ±0.01; mass of MFc = 100 mg; particle size= 125 µm;
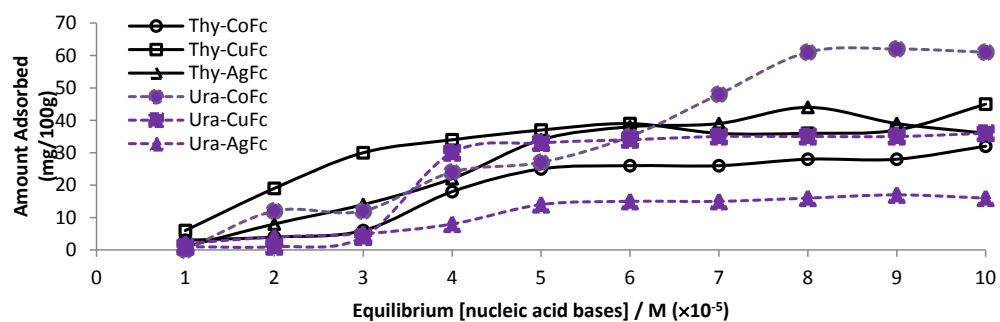Thymineλ_{max} = 264.5 nm; uracil λ_{max} = 258.0 nm)

FIG.5. Adsorption isotherms of thymine and uracil on metal ferrocyanides in the presence of salt (Concentration of Salt = 1.5×10^{-3} M; temperature = $30 \pm 1°$ C time = 8 hours; pH = 7.0 ± 0.01; mass of MFc = 100 mg;

particle size= 125 µm; thymine λ_{max} = 264.5 nm; uracil λ_{max} = 258.0 nm)