# Adsorption of Adenine, Adenosine, and Adenosine Nucleotides on Nickel(II) Hexacyanoferrate(II)

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Nickel(II) hexacyanoferrate(II) has been found to be an effective adsorbent for number of biomonomers such as adenine, adenosine, 5′-AMP, 5′-ADP, and 5′-ATP. The adsorption is maximum at a p$K_a$ value specific to each biomonomer. The specific binding ability of adsorbate is related to the number of phosphate groups attached to an adenosine moiety. The overall adsorption trend is found to be in the order of 5′-ATP > 5′-ADP > adenine > 5′-AMP > adenosine. The infrared spectral studies of adsorption adducts suggested that adsorption takes place due to the complex formation between adsorbate molecule and outer divalent metal ion of nickel(II) hexacyanoferrate(II). Such adsorption processes could have helped in the protection of biomolecules from degradation on the primitive earth.

Little is known about how the biomonomers were concentrated from dilute aqueous solutions and condensed to biopolymers in the course of chemical evolution. However, one of the suggestions is that clays and other minerals may have provided surfaces onto which small molecules would have concentrated and subsequently polymerized. The condensation of alanine or glycine on homoionic bentonite clay minerals showed remarkable increase in the catalytic activity when substituted with divalent trace metal ions such as $Cu^{+2}$, $Ni^{+2}$, or $Zn^{+2}$ and when a cyclic process of drying and wetting was employed.[1] Some adsoption studies on other clay and clay minerals thus suggested strong adsorption of nucleotides,[2] amino acids,[3,4] peptides,[5,6] and sugars.[7—10] Recently, oligomerisation of nucleotides on the clays is also reported by Ferris et al.[11]

Kobayashi and Ponnamperuma[12] proposed that there exists a co-relationship between the concentration of chemical elements in the primordial sea and their biological behavior. Further, it is assumed that divalent transition metal ions which were in abundance in the primeval sea would have formed complexes with the simple molecules readily available to them.[12,13] It is therefore reasonable to assume that transition metal ions could easily have formed a number of soluble and insoluble complexes with abundant $CN^-$, in the primeval sea. The insoluble cyanometal complexes thus formed could have settled at the bottom of the sea or at the seashore and might have catalysed a number of reactions like condensation–oligomerisation, oxidation, and interaction reactions on their surfaces. The existence of ferriferrocyanide and metal ferrocyanide on the primitive earth has recently been reported by Arrhenius.[14]

Nickel(II) was selected with hexacyanoferrate(II) as trace metal ions for the present adsorption studies, mainly due to its presence in the primeval sea (100—1 nM),[12] as well as to study the possible role of nickel in the chemical evolution. A number of insoluble cyanometal complexes were prepared in this laboratory and their catalytic behavior has been studied from the viewpoint of chemical evolution.[15,16] The present communication deals with adsorption studies of adenine, adenosine, adenosine monophosphate (5′-AMP), adenosine diphosphate (5′-ADP), and adenosine triphosphate (5′-ATP) on nickel(II) hexacyanoferrate(II). Infrared spectral studies of adsorption adducts have indicated complex formation between adsorbate molecule and outer divalent nickel cation of metal hexacyanoferrate(II).

## Experimental

**1. Materials.** Potassium hexacyanoferrate(II) (B. D. H.), nickel chloride (B. D. H.), adenine (Sigma), adenosine (S. R. L.), 5′-AMP (Sigma), 5′-ADP (Sigma), and 5′-ATP (Sigma) were used as received. All other chemicals were of analytical reagent grade. Doubly distilled water was used throughout the studies.

**2. Methods.** Synthesis of nickel(II) hexacyanoferrate(II), $Ni_2[Fe(CN)_6]\cdot xH_2O$: Nickel(II) hexacyanoferrate(II) was synthesised according to a method reported by Kourim et al.[17] A solution of potassium hexacyanoferrate(II) (167 ml, 0.1 M, 1 M = 1 mol $dm^{-3}$) was slowly added to the solution of nickel chloride (500 ml, 0.1 M) with constant stirring. An excess of nickel chloride markedly improves the coagulation of the precipitate. The reaction mixture was then heated at 60 °C on a water bath for 2—3 h and kept as such for 24 h at room temperature. The precipitate was filtered and washed thoroughly with water and dried in an oven at 60 °C. The dried product was ground and sieved to 100 mesh size. A greenish powder thus obtained was quite stable in water. The purity of nickel(II) hexacyanoferrate(II) was checked by comparing X-ray diffraction data of the complex. The relative intensity data and interplanner spacing, $d$, were in good agreement with reported values.[18]

**3. Spectral Studies.** Electronic spectra of adenine, adenosine, and adenosine nucleotides were recorded on a Beckman DU-6 spectrophotometer; the characteristic values of $\lambda_{max}$ are given in Table 1. Infrared spectra of the adsorbent, adsorbates and ad-

Table 1. Characteristic UV Spectral Bands (nm) of Adenine Adenosine, and Its Nucleotides at pH 7.0

| Biomolecules | $\lambda_{max.}$ (nm) |
|---|---|
| Adenine | 217, 263 |
| Adenosine | 219, 262 |
| 5′-AMP | 218, 262 |
| 5′-ADP | 220, 260 |
| 5′-ATP | 221, 262 |

sorption adducts were recorded in KBr discs on a Perkin Elmer FTIR spectrophotometer. Infrared spectral data are summarized in Table 2.

**4. Adsorption Studies.** Experimental conditions were optimized for the adsorption studies by carrying out a few preliminary experiments. The adsorption of adenine, adenosine, and adenosine nucleotides on nickel hexacyanoferrate(II) was studied as a function of pH (3.0—10.0) by adding 5.0 ml buffer of desired pH and 5.0 ml of biomonomer solution to 100 mg of nickel(II) hexacyanoferrate(II). Since we realize the abundance of phosphate ions in the primeval sea, the other adsorption studies were carried out at pH 7.0 using phosphate buffer at 30 °C. The adsorption of biomonomers on nickel(II) hexacyanoferrate(II) as a function of biomonomer concentration was also studied by the addition of 10.0 ml ($5.0\times10^{-5}$—$1.3\times10^{-3}$ M) solution to 100 mg of nickel-(II) hexacyanoferrate(II). In a typical experiment, a biomonomer-nickel(II) hexacyanoferrate(II) suspension was shaken initially for half an hour at 30 °C and was then allowed to equilibrate for 24 h with intermittent shaking and finally centrifuged. The supernatent liquid was decanted off and the concentration of biomonomer leftover after adsorption was determined spectrophotometrically.

**5. Surface Area Measurements.** A similar procedure to that described previously was adopted for the surface area determination[19,20]. A weighed amount of nickel(II) hexacyanoferrate(II) (100 mg) was equilibrated at 30 °C with a known volume (10 ml) of Methylene Blue ($0.5\times10^{-5}$—$4.0\times10^{-5}$ M) for 24 h. After equilibrium, the leftover Methylene Blue was measured spectrophotometrically at 661 nm.

## Results and Discussion

The surface area of nickel(II) hexacyanoferrate(II) was found to be 0.52 $m^2\,g^{-1}$ as determined using the relation given by Brindley and Thompson.[19,20]

$$\text{Specific surface area} = 6.02\times10^{-2}\,M_f\,A_m\ (m^2\,g^{-1}) \quad (1)$$

where,

$M_f$= Amount of Methylene Blue (mmole) adsorbed per 100 g of metal hexacyanoferrate-(II) when surface is completely covered with a monolayer of Methylene Blue dye.

$A_m$= Cross sectional area per molecule in $Å^2$ on the surface.

We assumed that normally Methylene Blue molecules will take up an orientation flat on the surface and we took the area per adsorbed molecule as 130 $Å^2$. The value of $M_f$ was obtained from the Methylene Blue adsorption isotherm corresponding to the monolayer capacity (Fig. 1). Substitution of $M_f$ value in the above formula gave the value of surface area of nickel(II) hexacyanoferrate(II).

Adsorption studies of adenine, adenosine, and adenosine nucleotides were carried out over a wide pH range of 3.0—10.0. The desired pH of the solution was maintained by using phosphate ($KH_2PO_4$, 0.01 M+$Na_2HPO_4$, 0.01 M) and borax (0.05 M) buffer. Figure 2 shows the variation in adsorption of biomonomers with increase in pH; the only exception is of adenosine. Overall adsorption of adenine, 5′-AMP, 5′-ADP, and 5′-ATP has been found to increase at respective $pK_a$ values of adsorbate.

Adsorption isotherms were obtained at pH 7.0 by varying the concentration ($0.5\times10^{-4}$—$13\times10^{-4}$ M) of adsorbate. Adsorption isotherms thus obtained are depicted in Fig. 3.

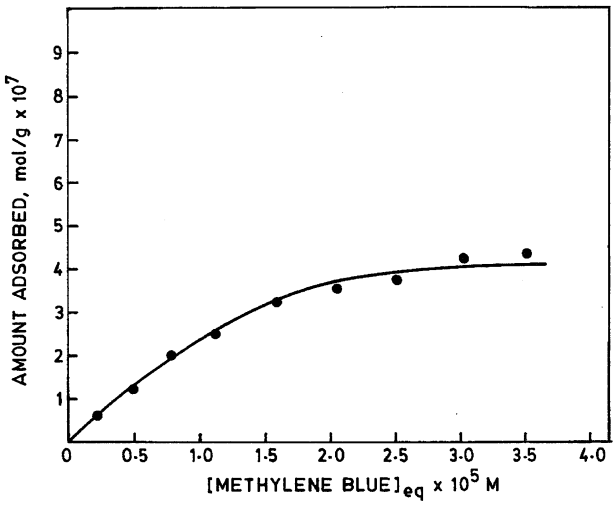


Fig. 1. Adsorption isotherm of Methylene Blue on nickel(II) hexacyanoferrate(II), pH=7.0; temp 30 °C.

Table 2. Characteristic Infrared Spectral Frequencies ($cm^{-1}$) of Adenine, Adenosine, and Its Nucleotides

| Biomolecule | $\nu_{N-H}$ | $\delta_{NH_2}$ | Typical of purine nucleus | Typical of sugar residue | $\nu_{P-O-C}$ | $\nu_{P-O-P}$ | $\nu_{P=O}$ |
|---|---|---|---|---|---|---|---|
| Adenine | 3159, 3280 | 1668 | 1554, 1605 | — | — | — | — |
| Adenosine | 3125, 3325 | 1663 | 1560, 1608 | 1063, 1107 | — | — | — |
| 5′-AMP | 3125, 3331 | 1663 | 1550, 1595 | 1030, 1130 | 1080 | — | 1370 |
| 5′-ADP | 3110, 3345 | 1690 | 1551, 1620 | 1035, 1120 | 1060 | 965 | 1310 |
| 5′-ATP | 3115, 3330 | 1696 | 1555, 1610 | — 1106 | 1080 | 963 | 1380 |

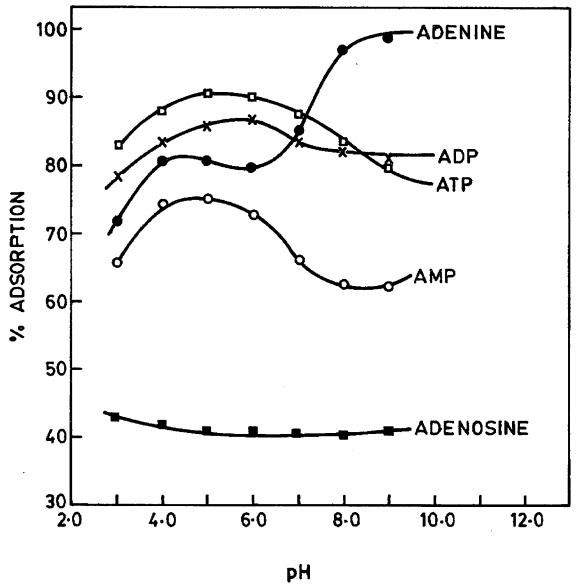


Fig. 2. Adsorption of adenine, adenosine, and adenosine nucleotides on nickel(II) hexacyanoferrate(II) as a function of pH, temp=30 °C.

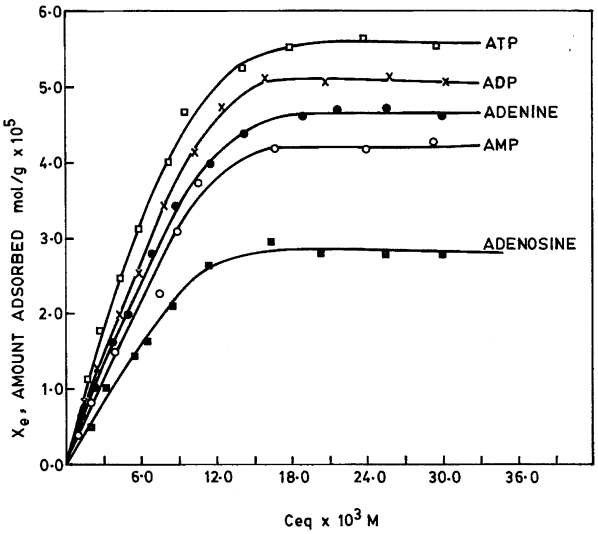


Fig. 3. Adosrption isotherms of adenine, adenosine, and adenosine nucleotides on nickel(II) hexacyanoferrate(II), pH=7.0; temp=30 °C.

The asymptotic nature of the curves suggest Langmuir type adsorption. At higher concentrations of adsorbate, a saturation limit of nickel(II) hexacyanoferrate(II) has been observed i.e. as equilibrium concentration $C_{eq}$ approaches infinity, the amount adsorbed $X_e$ approaches complete monolayer formation, i.e., $X_m$ asymptotically. The adsorption data can be represented through a Langmuir adsorption isotherm which deals with monolayer formation of solute molecules on the surface of adsorbent. It is given by Eq. 2,

$$\frac{C_{eq}}{X_e} = \frac{1}{K_L X_m} + \frac{C_{eq}}{X_m} \quad (2)$$

which can also be written as

$$\frac{1}{X_e} = \left(\frac{1}{C_{eq}}\right)\left(\frac{1}{K_L X_m}\right) + \frac{1}{X_m} \quad (3)$$

where

$X_e$= Amount (mmole) of solute adsorbed per gram of adsorbent,

$C_{eq}$= Equilibrium concentration of solute,

$X_m$= Moles of solute required per gram weight of metal hexacyanoferrate(II) for the formation of a complete monolayer on the surface,

$K_L$= Constant related to the heat of adsorption or enthalpy.

The values of $X_m$, monolayer capacity and $K_L$ are obtained from a plot of $X_e^{-1}$ vs. $C_{eq}^{-1}$ (Fig. 4) and are given in Table 3. From the trend of $X_m$ values, it is clear that the adsorption of adenine, adenosine, 5′-AMP, 5′-ADP, and 5′-ATP on nickel(II) hexacyanoferrate(II) follows a pattern:

5′-ATP > 5′-ADP > adenine > 5′-AMP > adenosine.

The adsorption ability of adsorbent is perhaps linked with its complexing ability and with the ligating nature of adsorbate molecules. Unlike the simple aromatic amines or amino acids[15,16] ambivalent properties of adenine, adenosine and its nucleotides render a number of sites for metal binding[21] (Fig. 5). Adenine commonly offers the N-7 and N-9 positions of imidazole ring or N-1 and N-3 positions of pyrimidine ring to metal ions for binding. In case of

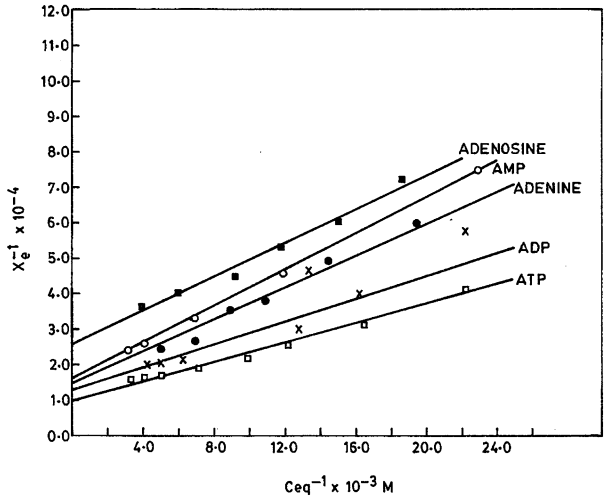


Fig. 4. Langmuir adsorption isotherms for adenine, adenosine, and adenosine nucleotides on nickel(II) hexacyanoferrate(II).

Table 3. Langmuir Constants for Adenine, Adenosine, and Its Nucleotides Obtained after Adsorption on Nickel(II) Hexacyanoferrate(II)

| Biomolecules | $K_L \times 10^5$ | $X_m \times 10^5$(mol g$^{-1}$) |
|---|---|---|
| Adenine | 2.857 | 7.142 |
| Adenosine | 1.623 | 3.846 |
| 5′-AMP | 2.410 | 6.250 |
| 5′-ADP | 4.615 | 7.692 |
| 5′-ATP | 7.555 | 11.111 |

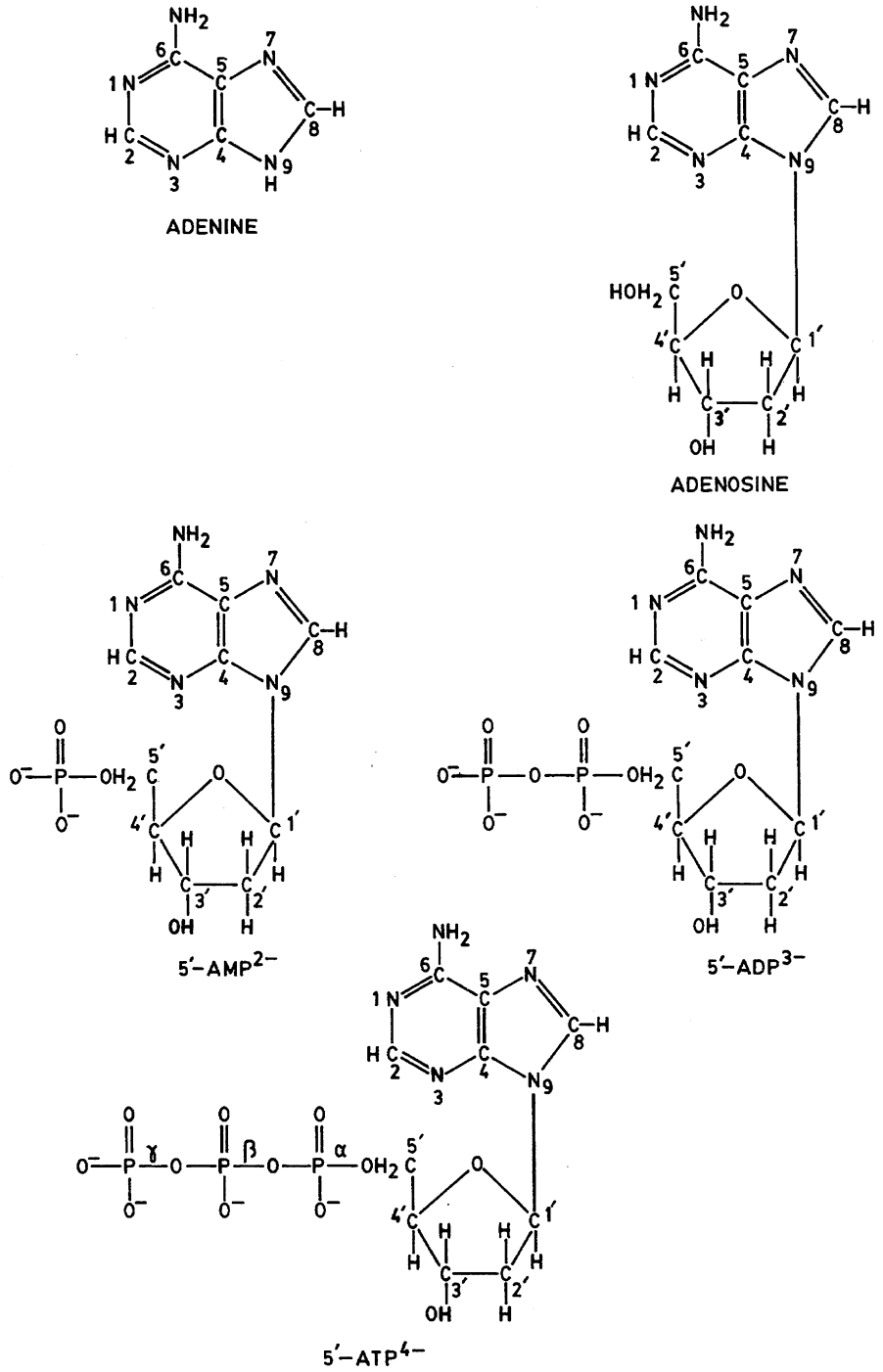


Fig. 5. Structures of adenine, adenosine, and adenosine nucleotides.

adenosine, however, the presence of a sugar ring reduces the available coordination sites. N-9 position is blocked due to presence of sugar moiety and N-3 position, which is not a very strong ligating site initially, is made even less attractive by bulky sugar moiety. Thus only N-1 and N-7 positions are left effective for binding in adenosine.[22] Further, N-7 is less basic than N-1 and therefore the preferred coordination in $Ni^{+2}$ and $Cu^{+2}$ complexes is approximately 70% via N-7 site only. Nucleotides are of special interest, since phosphate groups are major binding sites available for metal ions.[23] In a case when a metal ion is coordinated through phosphate groups of nucleotide simultaneously, it may interact with N-7 of adenine moiety.[21] In the present adsorption studies, the bulky sugar residue in 5′-AMP apparently affects the adsorption by sterically hindering the possible coordination sites of phosphate moiety. If the terminal phosphate group is farther from a sugar group, as in the case of 5′-ADP and 5′-ATP, the rate of adsorption would be high.[24] The observed values of $X_m$ and $K_L$ (Table 3) support the above idea and the adsorption rate of 5′-AMP is less than that of adenine, 5′-ADP and 5′-ATP. The adsorption ability is greatly enhanced from 5′-ADP to 5′-ATP, suggesting that the adsorption is perhaps

Table 4. Shift in Typical Infrared Spectral Frequencies ($cm^{-1}$) of Adenine, Adenosine, Obtained after Adsorption on Nickel(II) Hexacyanoferrate(II)

| Biomolecule | $\nu_{N-H}$ | $\delta_{NH_2}$ | Typical of purine nucleus | Typical of sugar residue | $\nu_{P-O-C}$ | $\nu_{P-O-P}$ | $\nu_{P=O}$ |
|---|---|---|---|---|---|---|---|
| Adenine | 3123, 3273 | 1668 | 1554, 1614 | — | — | — | — |
| Adenosine | 3147, 3345 | 1664 | 1558, 1610 | 1057, 1107 | — | — | — |
| 5′-AMP | 3112, 3352 | 1664 | 1552, 1609 | 1032, 1135 | 1094 | — | 1394 |
| 5′-ADP | 3113, 3352 | 1695 | 1553, 1622 | 1031, 1123 | 1072 | 984 | 1329 |
| 5′-ATP | 3120, 3338 | 1700 | 1555, 1615 | — 1101 | 1094 | 978 | 1393 |

taking place through the binding of phosphates groups and nickel ion of metal hexacyanoferrate(II).

The nature of adsorption of adenine, adenosine, and its nucleotides on nickel(II) hexacyanoferrate(II) has been investigated by infrared spectral studies. For the infrared spectral studies, biomonomer-nickel(II) hexacyanoferrate(II) adduct was washed several times with water and infrared spectrum of the adduct was then noted. The characteristic infrared spectral frequencies of adenine, adenosine and its nucleotide were observed in the respective adducts (Table 4). A shift towards higher wavelength in the characteristic frequencies of adenine, adenosine, 5′-AMP, 5′-ADP, and 5′-ATP after adsorption supports the idea of coordination of adsorbate molecules with outer nickel cations of the adsorbent material.

Typical strong bands in the region 950—1150 $cm^{-1}$ are due to the presence of ribose residue; these remained mostly unaffected after adsorption in the case of adenosine, 5′-AMP, 5′-ADP, and 5′-ATP. This result suggests that the ribose residue is noninteracting. A notable shift is observed in the characteristic frequencies of purine nucleus and phosphate groups of nucleotides, pointing out a probable involvement of N-7 and phosphate groups in the coordination. In the case of adenine and adenosine, however, it could be N-7 site taking part in the coordination as a results of adsorption on the nickel(II) hexacyanoferrate(II). X-ray crystallographic studies of adsorption of nucleotides on homoionic bentonite clays by Liebmann et al.[2] have also shown cation-nucleotide complex formation via N-7 and phosphate groups. The typical cyanide stretching $\nu_{C\equiv N}$ band of nickel(II) hexacyanoferrate-(II) did not show any remarkable shift and remained mostly unaffected, which suggests that adsorbate molecules do not enter into the coordination sphere of iron by replacing strong ligand-like cyanide.

In an experimental approach towards the study of role of metal hexacyanoferrate(II) complexes, we have shown in the present work and in earlier work[15,16] that metal ions like copper, zinc, cobalt, and nickel can further attach with hexacyanoferrate(II). Such metal hexacyanoferrate(II) complexes have strong affinity for organic molecules of biological significance. The present study provides an information related to strong adsorption ability of nickel(II) hexacyanoferrate(II) for adenine, adenosine, and adenosine nucleotides. Thus, we presume that these adsorption processes may have protected biomolecules form degradation and further could have oligomerised them on their surface under the primitive earth conditions. But, a definite conclusion with respect to the exact coordination sites of adsorbate molecules can not be drawn strictly on the basis of infrared spectral data due to overlapping of large number of frequencies. However, only probable coordination sites could be proposed as discussed.

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## References

1) J. G. Lawless and N. Levi, *J. Mol. Evol.*, **13**, 281 (1979).
2) P. Liebmann, G. Loew, S. Burt, J. Lawless, and R. D. Maceroy, *Inorg. Chem.*, **21**, 1586 (1982).
3) S. C. Bondy and M. E. Harrington, *Science*, **203**, 1243 (1979).
4) C. Ponnamperuma, A. Shimoyama, and E. Friebele, *Origins Life*, **12**, 9 (1982).
5) D. J. Greenland, R. H. Laby, and J. P. Quirk, *Trans. Faraday Soc.*, **58**, 829 (1962).
6) D. J. Greenland, R. H. Laby, and J. P. Quirk, *Trans. Faraday Soc.*, **61**, 2013 (1965).
7) M. M. Mortland, *Adv. Agron.*, **22**, 75 (1970).
8) B. K. G. Theng, in "Chemistry of Clay of Organic Reactions," John Wiley and Sons, New York (1974).
9) A. Weiss, in "Organic Geochemistry," ed by G. Eglinton and M. T. J. Murphy, Springer-Verlag, New York (1969), p. 737.
10) N. Lahav and S. Chang, *J. Mol. Evol.*, **8**, 357 (1976).
11) J. P. Ferris, G. Ertem, and Z. P. Ding, "7th ISSOL Meeting and 10th International Conference on the Origin of Life," p. 63 (1993).
12) K. Kobayashi and C. Ponnamperuma, *Origins Life*, **16**, 41 (1985).
13) F. Egami, *J. Biochem.*, **77**, 1165 (1975).
14) G. Arrhenius, "Fourth Symposium on Chemical Evolution and Origin and Evolution of Life," NASA Am. Research Center, Moffet Field, CA (1990), July 24—27 (1990).
15) Kamaluddin, M. Nath, S. Deopujari, and A. Sharma, *Origins Life Evol. Biosphere*, **20**, 259 (1990).
16) Sushama Viladkar, T. Alam, and Kamaluddin, *J. Inorg. Biochem.*, **53**, 69 (1994).
17) V. Kourim, J. Raise, and B. Million, *J. Inorg. Nucl. Chem.*, **26**, 1111 (1964).
18) R. Rigamoti, *Gazz. Chim. Ital.*, **68**, 803 (1938).
19) G. W. Brindley and T. D. Thompson, *Clays Clay Miner.*, **18**, 203 (1970).
20) C. H. Giles, A. P. D'Silva, and A. S. Trivedi, *J. Appl. Chem.*,

**20**, 37 (1970).
21) H. Sigel, *Chem. Soc. Rev.*, **1993**, 255.
22) D. J. Hodgson, *Progr. Inorg. Chem.*, **23**, 211 (1977).
23) H. Sigel, *J. Inorg. Nucl. Chem.*, **39**, 1903 (1977).
24) G. Graf and G. Lagaly, *Clays Clay Miner.*, **28**, 12 (1980).