

WAS FERROCYANIDE A PREBIOTIC REAGENT?

ANTHONY D. KEEFE* and STANLEY L. MILLER

*Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA
92093-0506, USA*

(Received 10 June, 1995)

Abstract. Hydrogen cyanide is the starting material for a diverse array of prebiotic syntheses, including those of amino acids and purines. Hydrogen cyanide also reacts with ferrous ions to give ferrocyanide, and so it is possible that ferrocyanide was common in the early ocean. This can only be true if the hydrogen cyanide concentration was high enough and the rate of reaction of cyanide with ferrous ions was fast enough. We show experimentally that the rate of formation of ferrocyanide is rapid even at low concentrations of hydrogen cyanide in the pH range 6–8, and therefore an equilibrium calculation is valid. The equilibrium concentrations of ferrocyanide are calculated as a function of hydrogen cyanide concentration, pH and temperature. The steady state concentration of hydrogen cyanide depends on the rate of synthesis by electric discharges and ultraviolet light and the rate of hydrolysis, which depends on pH and temperature. Our conclusions show that ferrocyanide was a major species in the prebiotic ocean only at the highest production rates of hydrogen cyanide in a strongly reducing atmosphere and at temperatures of 0 °C or less, although small amounts would have been present at lower hydrogen cyanide production rates. The prebiotic application of ferrocyanide as a source of hydrated electrons, as a photochemical replication process, and in semi-permeable membranes is discussed.

Key words: Ferrocyanide, ferricyanide, Prussian Blue, blueprint, hydrogen cyanide production rates, prebiotic synthesis

1. Introduction

Hydrogen cyanide is central to prebiotic syntheses being a precursor to both amino acids and purines (Miller and Orgel, 1974). However, little consideration has been given to the uptake of hydrogen cyanide by ferrous ions to give ferrocyanide and by ferric ions to give ferricyanide. Ferrocyanide has been mentioned as a possible abundant component of the primitive ocean (Orgel, 1974), as a potential prebiotic reagent by Arrhenius *et al.* (1994), and there are also some prebiotic synthesis experiments producing amino acids (Tiwari, 1983; Tiwari and Sharma, 1985). There is also work using ferrocyanide as a peroxidase model (Kamaluddin and Sushama, 1988), to develop light-dependent pH gradients (Deamer and Harang, 1990), and as semi-permeable membranes (Kulesza and Doblhofer, 1989; Samanta and Basu, 1989). Zinc and cupric ferrocyanides have been proposed as absorbents for nucleotide phosphates (Kamaluddin and Sharma, 1994) and ammonium ions (Braterman *et al.*, 1995).

* Present address: NASA Ames Research Center, M/S 239-4, Moffett Field, CA 94035.

Although ferrocyanide and ferricyanide are known to be very stable compounds and easily synthesized*, it is not clear whether they are stable under the dilute conditions of the primitive ocean. Their stabilities depend on the ferrous or ferric ion concentrations, cyanide concentrations, the pH, and the temperature. An equally important question is whether the rate of formation and decomposition of ferrocyanide is rapid enough on the geological timescale for equilibrium to be approximated. This is a crucial point which we have investigated experimentally.

A central issue is the concentration of ferrous ions in the primitive ocean. This is unknown, but it is likely to have been much higher than at present because anaerobic conditions would have prevented the formation of ferric ions by oxygen oxidation, although there are other processes which can oxidize ferrous ions. This would be followed by precipitation of insoluble ferric hydroxide. Hydrogen sulfide will precipitate ferrous sulfide and so maintain the ferrous ion concentration at low levels, so the presence of substantial concentrations of ferrous ions would depend on whether the ferrous ions were in excess concentration over hydrogen sulfide. We will assume that there was a surplus of ferrous ions. The exact concentration of ferrous ions does not much effect whether or not ferrocyanide is stable except at very high ferrous ion concentrations and low hydrogen cyanide concentrations.

We will show here that ferrocyanide is only stable under a narrow band of pH, temperature and hydrogen cyanide production rates. We also show that the rate of formation and decomposition of ferrocyanide is sufficiently rapid that these equilibrium calculations are applicable to the primitive ocean.

2. The Stability of Ferrocyanide

There has been considerable uncertainty in the equilibrium constant for the formation of ferrocyanide, shown in Equation (1), as it has never been measured directly.



$$K_{\text{eq}} = \frac{[\text{Fe}(\text{CN})_6^{4-}]}{[\text{Fe}^{2+}][\text{CN}^{-}]^6}$$

The data have been reviewed by Sharpe (1976) and by Beck (1987) who selected a value for the equilibrium constant of 2.5×10^{35} at 25 °C. This is based on the directly measured enthalpy of reaction of -85.8 kcal/mol and the third law entropy of ferrocyanide. This gives $\Delta G^{\circ} = -48.2$ kcal/mol.

* Prussian Blue, $(\text{Fe}^{\text{III}})_4(\text{Fe}^{\text{II}}(\text{CN})_6^{4-})_3$, is the oldest synthetic complex ion. It was first produced in Berlin in 1710 by Diesbach who heated blood, bone and other animal waste material with potash. The first published accounts are apparently those of Woodward (1724) and Brown (1724) who heated dried blood and meat with potassium nitrate and potassium tartrate.

The enthalpy of this reaction permits us to estimate K_{eq} at other temperatures using Equation (2) where I is the ionic strength.

$$\text{Log}_{10}K_{eq} = -27.49 + \frac{18,750}{T} \quad (I = 0). \quad (2)$$

We can also write the equilibrium as a function of pH as

$$\frac{[\Sigma\text{Fe}(\text{CN})_6^{4-}]}{[\text{Fe}^{2+}][\Sigma\text{HCN}]^6} = K_{eq} \frac{\left[1 + \frac{[\text{H}^+]}{K_{\text{HCN}}}\right]^6}{\left[1 + \frac{[\text{H}^+]}{K_4}\right]}$$

where

$$[\Sigma\text{HCN}] = [\text{HCN}] + [\text{CN}^-]$$

$$[\Sigma\text{Fe}(\text{CN})_6^{4-}] = [\text{Fe}(\text{CN})_6^{4-}] + [\text{Fe}(\text{CN})_6\text{H}^{3-}]$$

$$K_{\text{HCN}} = \frac{[\text{H}^+][\text{CN}^-]}{[\text{HCN}]}$$

$$K_4 = \frac{[\text{Fe}(\text{CN})_6^{4-}][\text{H}^+]}{[\text{Fe}(\text{CN})_6\text{H}^{3-}]}$$

The effect of ionic strength cannot be neglected in this reaction because of the four negative charges on the ferrocyanide and because the activity coefficient of cyanide enters as the sixth power.

For reaction 1.

$$K_{eq} = \frac{a_{\text{Fe}(\text{CN})_6^{4-}}}{a_{\text{Fe}^{2+}} a_{\text{CN}^-}} = \frac{m_{\text{Fe}(\text{CN})_6^{4-}} \gamma_{\text{Fe}(\text{CN})_6^{4-}}}{m_{\text{Fe}^{2+}} \gamma_{\text{Fe}^{2+}} m_{\text{CN}^-}^6 \gamma_{\text{CN}^-}^6}$$

$$K_{app} = K_{eq} \frac{\gamma_{\text{Fe}^{2+}} \gamma_{\text{CN}^-}^6}{\gamma_{\text{Fe}(\text{CN})_6^{4-}}} = \frac{m_{\text{Fe}(\text{CN})_6^{4-}}}{m_{\text{Fe}^{2+}} m_{\text{CN}^-}^6} \quad (3)$$

where m is the molality and γ is the single ion activity coefficient. We use K_{app} rather than the thermodynamic K_{eq} because we are interested in concentrations rather than thermodynamic activities. We will use the single ion activity coefficient approach in these calculations (Kielland, 1937; Miller and Smith-Magowan, 1990). For potassium ferrocyanide activity coefficients we have

$$\gamma_{\pm} = (\gamma_+^4 \gamma_-)^{\frac{1}{5}} \quad \gamma_+ = \gamma_{\pm}^{\frac{1}{4}} \quad \gamma_- = \gamma_{\pm}^4$$

At the ionic strength of sea water ($I = 0.72$) the γ_{\pm} for K_4 interpolates to 0.158 (Robinson and Stokes, 1959). γ_- for $\text{Fe}(\text{CN})_6^{4-}$ is 6.23×10^{-4} and γ_+ for K^+ is

0.630. Similarly $\gamma_{\pm} = 0.450$ for ferrous chloride, so $\gamma_+ = \gamma_{\pm}^2 = 0.202$ and $\gamma_- = \gamma_{\pm}^{\frac{1}{2}} = 0.621$. For cyanide we use the values for sodium chloride $\gamma_{\pm} = \gamma_+ = 0.666$. Putting these single ion activity coefficients into Equation (3) gives

$$K_{\text{app}} = K_{\text{eq}} \frac{\gamma_{\text{Fe}^{2+}} \gamma_{\text{CN}^-}^6}{\gamma_{\text{Fe}(\text{CN})_6^{4-}}} = 28.3 K_{\text{eq}} = 7.1 \times 10^{36} \quad \text{at } 25^\circ \text{C.}$$

Assuming the activity coefficients do not vary significantly with temperature, Equation (2) becomes

$$\text{Log}_{10} K_{\text{app}} = -26.04 + \frac{18,750}{T} \quad (\mathbf{I} = 0.72).$$

3. The Rate of Formation of Ferrocyanide

Although the equilibrium constant for ferrocyanide formation is reasonably accurate, there appear to be no kinetic data for this reaction which are needed if the equilibrium calculation is to be applicable. Although ferrocyanide is rapidly formed in strong base, there are no kinetic data on its formation in the pH range 6–9.

There are some kinetic data on the decomposition of $[\text{Fe}(\text{CN})_6]^{4-}$ in the pH range 4–8 (Legros, 1964). The rate appears to be proportional to a protonated $[\text{Fe}(\text{CN})_6\text{H}]^{3-}$

$$\text{Rate}_{\text{reverse}} = k_r [\text{Fe}(\text{CN})_6\text{H}^{3-}]. \quad (4)$$

Therefore

$$\text{Rate}_{\text{reverse}} = \frac{k_r [\Sigma \text{Fe}(\text{CN})_6^{4-}]}{1 + \frac{K'_4}{[\text{H}^+]}} \quad (5)$$

where

$$K'_4 = \frac{[\text{H}^+][\Sigma \text{Fe}(\text{CN})_6^{4-}]}{[\text{Fe}(\text{CN})_6\text{H}^{3-}]}.$$

The value of K'_4 found by Legros to fit this equation is $\text{p}K'_4 = 6.4$ and the value of k_f is $2.7 \times 10^{-7} \text{ s}^{-1}$. The ratio of forward and reverse rates is the equilibrium constant, i.e.

$$k_f = K_{\text{eq}} k_r.$$

And

$$\text{Rate}_{\text{forward}} = k_f [\text{Fe}^{2+}] [\text{CN}^-]^6 [\text{H}^+].$$

Therefore

$$\text{Rate}_{\text{forward}} = \frac{k_f [\text{Fe}^{2+}] [\Sigma\text{HCN}]^6 [\text{H}^+]}{\left[1 + \frac{[\text{H}^+]}{K_{\text{HCN}}}\right]^6}.$$

From $k_r = 2.67 \times 10^{-7} \text{ s}^{-1}$ and $K_{eq} = 7.1 \times 10^{36}$, we calculate $k_f = 1.90 \times 10^{30} \text{ M}^{-7} \text{ s}^{-1}$. At pH 8 the forward rate would be

$$\text{Rate}_{\text{forward}} = 8.3 \times 10^{14} [\text{Fe}^{2+}] [\Sigma\text{HCN}^-]^6$$

These calculations suggest a rapid reaction at pH 8 with 10^{-3} M hydrogen cyanide, but a sluggish rate at pH 6 even on the geological time scale.

There are many uncertainties in this calculation, the most important being that there may be a different rate determining step at pHs higher than those at which the decomposition rates were measured (4–8). In addition the equation $K_{eq} = k_f/k_r$ does not always hold far from equilibrium. Other factors are the concentrations of various species of hydrated ferrous ions and the precipitation of ferrous hydroxide. Therefore, it was important to measure the kinetics of ferrocyanide formation at pH values closer to those in the ocean in order to determine whether or not equilibrium would be obtained.

4. Experimental

Measured amounts of a solution of potassium cyanide (100 mM) in three times freeze-degassed water were added to three times freeze-degassed buffered solutions of ferrous ammonium sulfate (2 mM) at pH 6 and 7 (buffered with 1 M morpholinethanesulfonate) and at pH 8 (buffered with 1 M tris (tris(hydroxymethyl)aminomethane)) in a sealed 1 cm path length quartz cuvette under nitrogen, and the absorbance was monitored at 250 nm with a Hewlett Packard 8452A Diode-Array Spectrophotometer. Care was taken to ensure the absence of oxygen which oxidizes ferrous ions to ferric ions followed by the precipitation of Prussian Blue, and the initial adjustment of buffer pH was accomplished with potassium hydroxide. The absorbance measurements for ferrocyanide were made at 250 nm rather than the λ_{max} of 210 nm to avoid absorption by ferrous ions.

5. Results

The rapid formation of ferrocyanide can be seen by the formation of a precipitate of ferrous ferrocyanide. This precipitate and solution turned blue on exposure to air by oxidation of ferrous ions to ferric ions and the formation of Prussian Blue

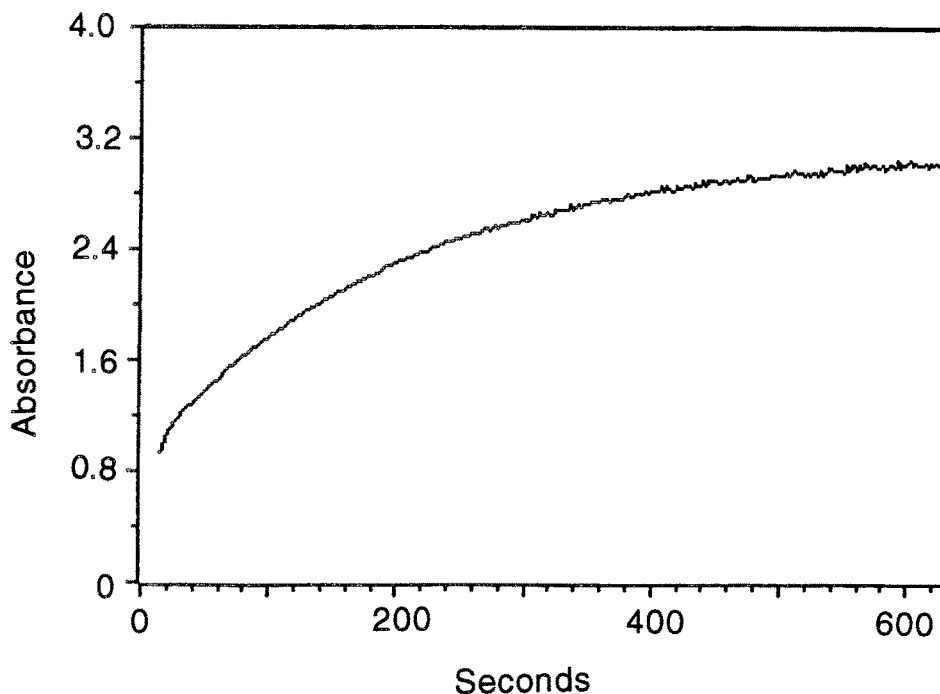


Figure 1. A plot of absorbance at 250 nm against time for a solution initially 1.67 mM in potassium cyanide and 16.7 mM in ferrous ammonium sulfate buffered at pH 6 with 1 M morpholinethanesulfonate. This shows the initial fast formation of the ferrocyanide, the plateau in absorption corresponding to the reaction going to completion. The initial rate of reaction was measured by drawing a tangent at $t = 0$.

$(\text{Fe}^{\text{III}})_4(\text{Fe}^{\text{II}}(\text{CN})_6^{4-})_3$.^{*} Identical observations were made upon mixing aqueous ferrous ammonium sulfate and aqueous potassium ferrocyanide. The absorbance curve taken immediately after the mixing of aqueous ferrous ammonium sulfate and aqueous potassium cyanide also corresponded to that of the ferrous ammonium sulfate and aqueous potassium ferrocyanide mixture.

A plot of the absorbance of the solution at 250 nm against time is shown in Figure 1. There is a rapid increase in absorbance due to the ferrocyanide formation, followed by levelling off of the curve as an off-white precipitate of ferrous ferrocyanide is formed so that some of the measured absorbance by the spectrophotometer is due to light scattering rather than absorbance by the ferrocyanide. In order to avoid complications due to scattering only the initial rates of reactions were

^{*} Prussian Blue is ferric ferrocyanide, $\text{Fe}_4^{\text{III}}(\text{Fe}^{\text{II}}(\text{CN})_6)_3$, and soluble Prussian Blue is $\text{KFe}^{\text{III}}\text{Fe}^{\text{II}}(\text{CN})_6$. Turnbull's Blue is frequently described as ferrous ferricyanide, $\text{Fe}_3^{\text{II}}(\text{Fe}^{\text{III}}(\text{CN})_6)_2$, but it has been shown to be identical to Prussian Blue since the electron transfer from the ferrous to the ferricyanide is rapid (Robin, 1962). Ferrous ferricyanide can be prepared from Prussian Blue by removing the water from the crystal lattice by vacuum pyrolysis at 400 °C (Cosgrove *et al.*, 1973) although this reverts to Prussian Blue on re-hydration. It may be useful to note that Prussian Blue is listed in Chemical Abstracts as *Color Index Pigment Blue 27*.

used. Since the ferrous ion concentration is in excess over the hydrogen cyanide concentration, the rate equation will be taken as

$$\frac{1}{[\text{Fe}^{2+}]} \frac{d[\text{Fe}(\text{CN})_6^{4-}]}{dt} = k_f [\Sigma \text{HCN}]^n$$

In log form this becomes

$$\log \frac{\text{rate}}{[\text{Fe}^{2+}]} = n \log[\Sigma \text{HCN}] + \log k_f$$

where

$$\text{rate} = \frac{d[\text{Fe}(\text{CN})_6^{4-}]}{dt}$$

Figure 2 shows the plot of these rates for pH 6. It can be seen that a straight line is obtained with a slope of 3.6. The value of k_f is $3.5 \times 10^3 \text{ M}^{-3.6} \text{ s}^{-1}$. A similar plot at pH 7 gives $n = 2.5$ and $k_f = 2.2 \times 10^2 \text{ M}^{-2.5} \text{ s}^{-1}$.

The order of the reaction with respect to hydrogen cyanide is not constant nor is it the value of six expected from the equilibrium constant and the ratio of the rates. We have not investigated this problem in detail because of the complexity of the reaction, but we think that the low value of n maybe due to the formation of FeOH^+ and to several different pathways for ferrocyanide formation. Alternatively 3.6 may be the average number of cyanide ligands needed for the complex to flip from high spin to low spin, this transition possibly being the rate determining step.

The experimental data demonstrate that ferrocyanide is formed rapidly even on the laboratory time scale and more than rapidly enough on the geological time scale for equilibrium calculations to be applicable.

6. Discussion

6.1. EQUILIBRIUM AS A FUNCTION OF pH AND TEMPERATURE

We wish to calculate the equilibrium at various pHs and temperatures. Since the pK_a of hydrogen cyanide is 9.2, at 25 °C most of the hydrogen cyanide will not be ionized. The ionization constant of hydrogen cyanide is given by Equation (4) (Schlesinger and Miller, 1973).

$$\text{pK}_a^{\text{HCN}} = -8.85 + \frac{3802}{T} + 0.01786T. \quad (6)$$

There is also the acid dissociation constant for ferrocyanic acid $\text{pK}_a = 6.4$. This will be disregarded in these calculations since it has a negligible effect at the pHs we shall consider.

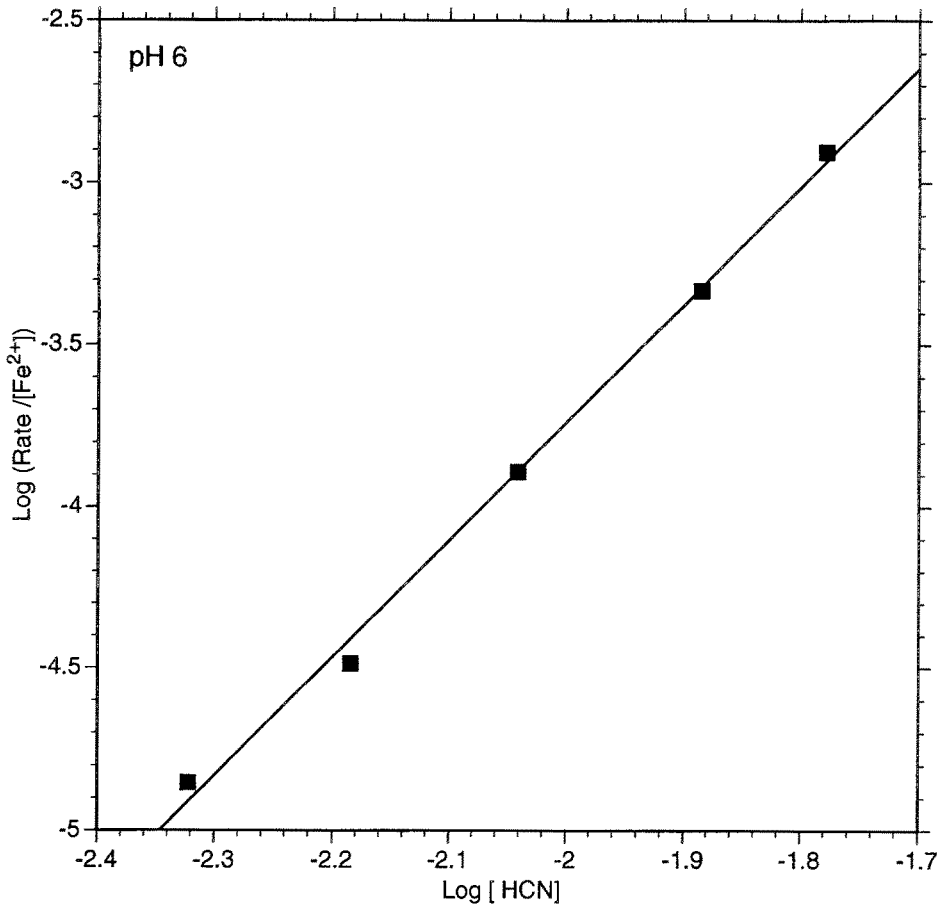


Figure 2. A plot of $\text{Log}_{10}(\text{Rate}/[\text{Fe}^{2+}])$ against $\text{Log}_{10}[\Sigma\text{HCN}]$. The slope gives the order of the reaction with respect to $[\Sigma\text{HCN}]$, and $\text{Log}_{10}k_f$ is the intercept. This experiment was performed at pH 6 in the presence of 1 M morpholinethanesulfonate buffer, potassium cyanide and ferrous ammonium sulfate.

6.2. CONDITIONS FOR THE FORMATION OF FERROCYANIDE

The first calculation will be the mole fraction of hydrogen cyanide tied up in ferrocyanide. Let $[\Sigma\text{HCN}] = [\text{HCN}] + [\text{CN}^-]$ noting that $[\Sigma\text{HCN}]$ does not include the cyanide in ferrocyanide. We have

$$[\text{CN}^-] = \frac{[\Sigma\text{HCN}]}{1 + \frac{[\text{H}^+]}{K_{\text{HCN}}}}$$

$$[\text{Fe}(\text{CN})_6^{4-}] = \frac{K_{\text{app}}[\text{Fe}^{2+}]K_{\text{HCN}}^6[\Sigma\text{HCN}]^6}{(K_{\text{HCN}} + [\text{H}^+])^6}$$

The mole fraction of cyanide in ferrocyanide $X_{\text{Fe}(\text{CN})_6}^{\text{CN}^-}$ is

$$X_{\text{Fe}(\text{CN})_6}^{\text{CN}^-} = \frac{6[\text{Fe}(\text{CN})_6^{4-}]}{6[\text{Fe}(\text{CN})_6^{4-}] + [\Sigma\text{HCN}]} \quad (7)$$

This mole fraction of cyanide is shown in Figure 3. It can be seen that the onset of ferrocyanide stability is very sharp, which is due to the sixth power of the cyanide in the equilibrium constant equation. This onset of ferrocyanide stability is also strongly dependent on temperature. Thus half the cyanide is in the form of ferrocyanide at 4×10^{-7} M hydrogen cyanide and 0 °C, this figure rises to 2×10^{-5} M hydrogen cyanide at 100 °C. The curves in Figure 3 have been calculated for 1 mM ferrous ion but they do not strongly depend on this concentration if $[\text{Fe}^{2+}] < [\Sigma\text{HCN}]$.

A similar calculation gives the mole fraction of ferrous ions in the form of ferrocyanide, $X_{\text{Fe}(\text{CN})_6}^{\text{Fe}^{2+}}$.

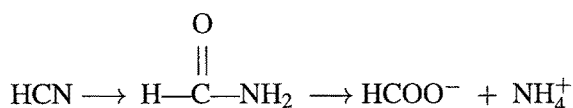
$$X_{\text{Fe}(\text{CN})_6}^{\text{Fe}^{2+}} = \frac{[\text{Fe}(\text{CN})_6^{4-}]}{[\text{Fe}(\text{CN})_6^{4-}] + [\text{Fe}^{2+}]} \quad (8)$$

This calculation is shown in Figure 4. It gives lines which are very similar to those of Figure 3 with the sigmoid curves displaced somewhat.

These two calculations were for pH 8. The effect of pH is shown in Figure 5 where $[\Sigma\text{HCN}]$ concentration is calculated for various pH values and temperatures with the constraint that $[\text{Fe}^{2+}] = [\text{Fe}(\text{CN})_6^{4-}]$. The results are not greatly different for ferrous ion: ferrocyanide concentration ratios of between 0.1 and 10. It can be seen that high temperatures and low pH values require high $[\Sigma\text{HCN}]$ values. Whether or not such high $[\Sigma\text{HCN}]$ values are feasible is taken up in the next section.

6.3. STEADY STATE HYDROGEN CYANIDE CONCENTRATIONS ON THE EARLY EARTH

There is no geological evidence for the atmospheric composition, the temperature or the pH of the ocean of the primitive Earth. The production rate of hydrogen cyanide is critically dependent on the atmospheric composition for electric discharge synthesis (Stribling and Miller, 1987), lightning synthesis (Chameides and Walker, 1981), and the presence of small amounts of methane for ultraviolet light synthesis via N atoms (Zahnle, 1986). The major path for loss of hydrogen cyanide is by hydrolysis to formate.



The steady state concentration, $[\Sigma\text{HCN}]$, has been calculated for various pH values, temperatures and hydrogen cyanide production rates (Stribling and Miller, 1987).

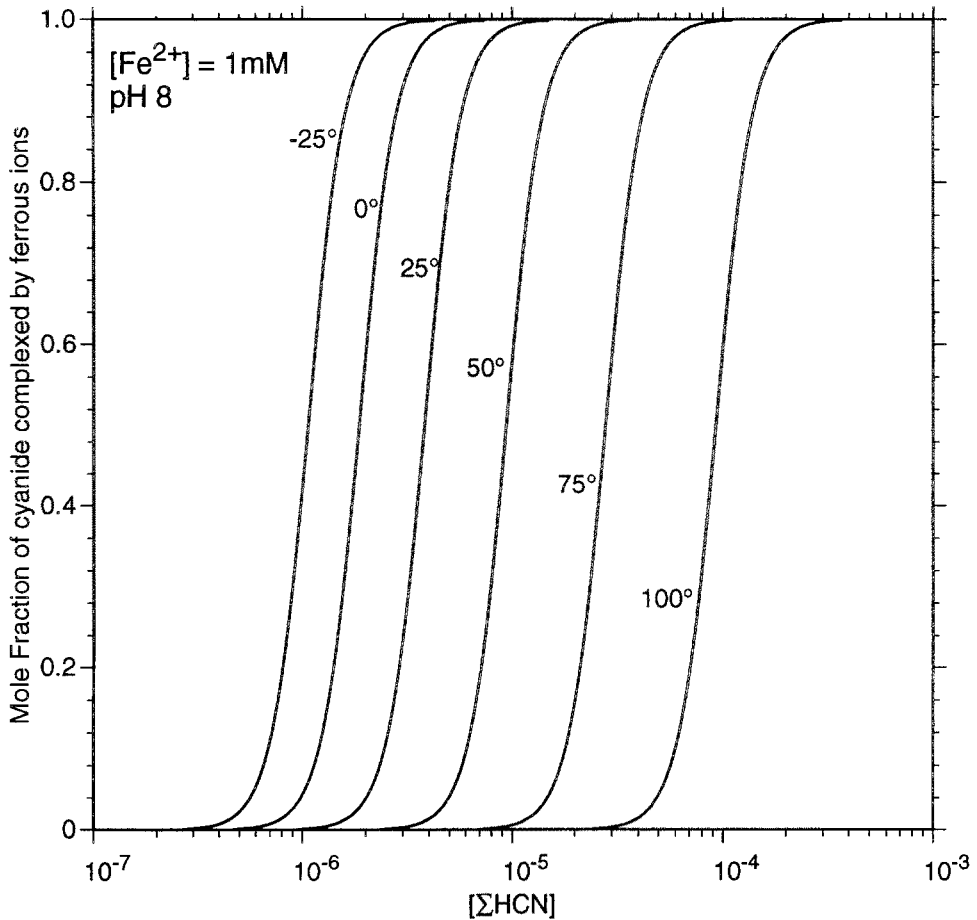


Figure 3. The mole fraction of cyanide complexed as ferrocyanide as a function of $[\Sigma\text{HCN}]$ ($[\Sigma\text{HCN}] = [\text{HCN}] + [\text{CN}^-]$) and temperature (Equation 7). The pH is fixed at 8 and the free ferrous ion concentration is fixed at $1 \times 10^{-3}\text{M}$. Higher ferrous ion concentrations increase the mole fraction of complexed hydrogen cyanide by a factor of approximately the increase in $[\text{Fe}^{2+}]$ (i.e. $[\text{Fe}^{2+}]/1 \times 10^{-3}$).

The major uncertainty in these calculations is the production rate of hydrogen cyanide from all sources. We will first assume a high value of $100 \text{ nmol cm}^{-2} \text{ yr}^{-1}$. This is the expected production rate for a reducing atmosphere or a favorable ultraviolet synthesis and can be considered as an upper end estimate, although not the highest reasonable value. Less reducing or neutral atmospheres would give lower figures with a carbon dioxide, nitrogen and water vapor atmosphere giving a figure of $0.01 \text{ nmol cm}^{-2} \text{ yr}^{-1}$ for spark discharge synthesis. Low temperatures and low pH values decrease the rate of hydrogen cyanide hydrolysis thereby increasing the steady state concentration of hydrogen cyanide. It is to be noted that the only sink for hydrogen cyanide which we have considered in our calculations is hydrolysis, but there are others such as use in amino acid and purine synthesis, polymerization,

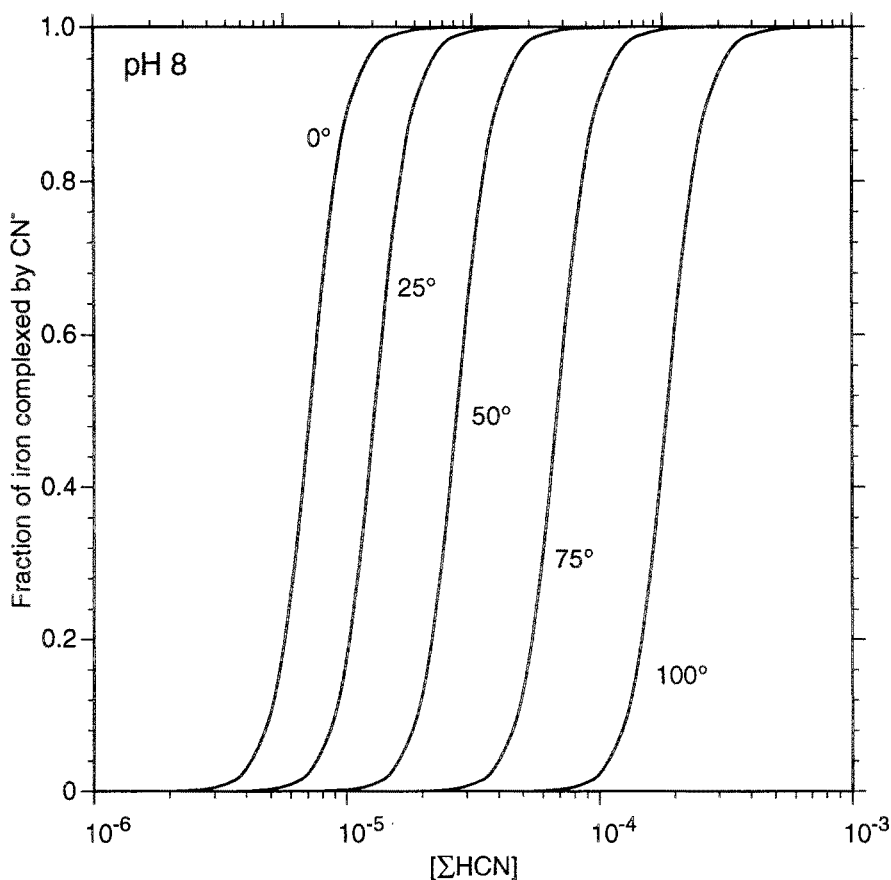


Figure 4. The mole fraction of ferrous ion complexed as ferrocyanide at pH 8 at various temperatures (Equation 8). This value is independent of the ferrous ion concentration.

reaction with itself or formaldehyde, and oxidation to cyanate. Passage through submarine hydrothermal vents is not a significant sink for hydrogen cyanide as the hydrolysis rate is much faster than the time for passage through the vents.

Also shown on Figure 5 are two dotted lines A and B indicating the steady state concentrations of hydrogen cyanide resulting from production rates of $1000 \text{ nmol cm}^{-2} \text{ yr}^{-1}$ at pH 8 (the line would be the same for a production rate of $100 \text{ nmol cm}^{-2} \text{ yr}^{-1}$ at pH 7) and $1000 \text{ nmol cm}^{-2} \text{ yr}^{-1}$ at pH 7 (the line would be the same for a production rate of $100 \text{ nmol cm}^{-2} \text{ yr}^{-1}$ at pH 6) respectively. Ferrocyanide is stable (i.e. the concentration of ferrocyanide \geq the concentration of ferrous ions) for conditions to the left of the dotted line, and unstable for conditions to the right.

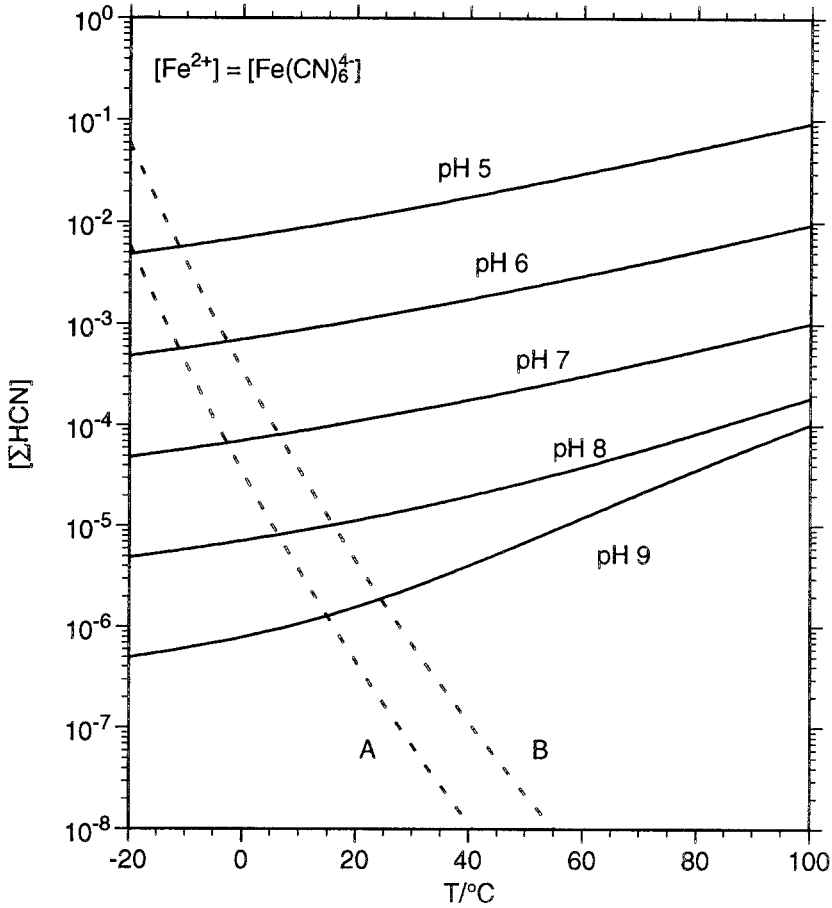


Figure 5. The $[\Sigma\text{HCN}]$ concentrations at which half of the ferrous ions are complexed as ferrocyanide (i.e. $[\text{Fe}^{2+}] = [\text{Fe}(\text{CN})_6^{4-}]$) as a function of pH and temperature. Curve A is the steady state oceanic concentration of $[\Sigma\text{HCN}]$ at pH 7 for a production rate of $100 \text{ nmol cm}^{-2} \text{ yr}^{-1}$ (or at pH 8 for a production rate of $1000 \text{ nmol cm}^{-2} \text{ yr}^{-1}$). Points much above curve A correspond to most of the ferrous ions in the form of ferrocyanide. Points much below curve A correspond to very little of the ferrous ions in the form of ferrocyanide. Curve B is the steady state concentration of $[\Sigma\text{HCN}]$ at pH 7 for a production rate of $1000 \text{ nmol cm}^{-2} \text{ yr}^{-1}$ (or $10\,000 \text{ nmol cm}^{-2} \text{ yr}^{-1}$ at pH 8).

It can be seen that ferrocyanide is only stable at low temperatures and high pH values. Lower values for the hydrogen cyanide production rate would only decrease the available conditions for ferrocyanide stability.

Figure 6 shows the stability conditions for ferrocyanide formation with several hydrogen cyanide production rates. It can be seen that the stability field of ferro-

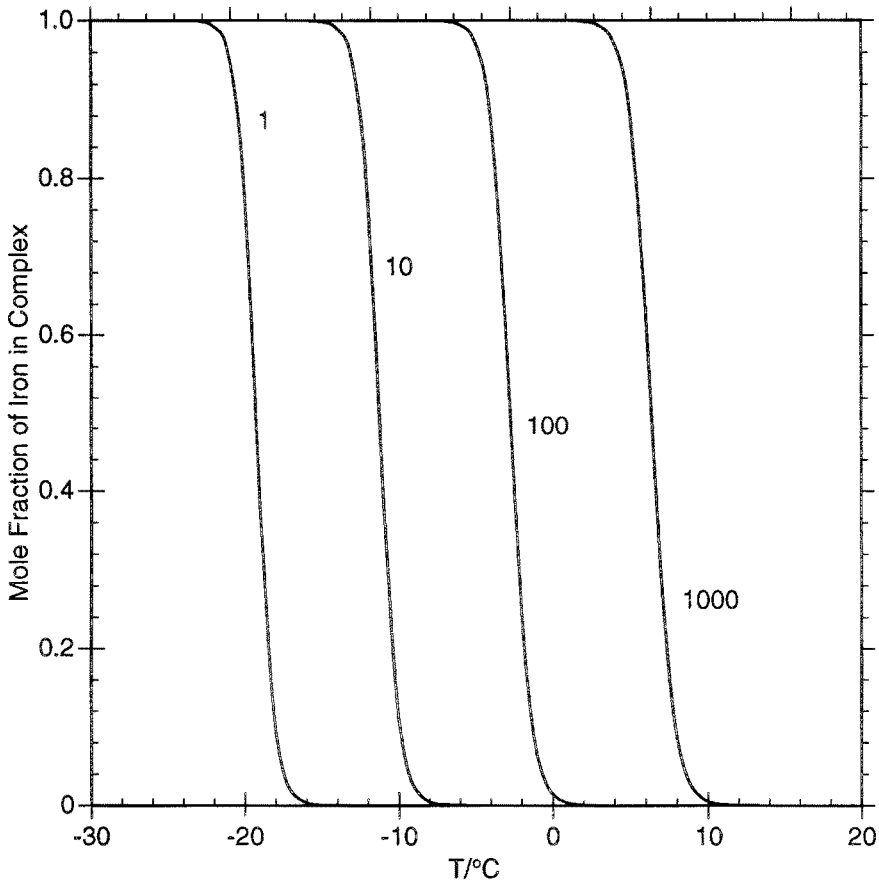


Figure 6. The mole fraction of ferrous ions complexed as ferrocyanide as a function of temperature and several production rates of hydrogen cyanide. The curves are independent of pH for pH values of 5 to about 8. The numbers on each curve are the production rates of hydrogen cyanide in $\text{nmol cm}^{-2} \text{yr}^{-1}$.

cyanide is very sensitive to temperature and hydrogen cyanide production rates. Even at hydrogen cyanide production rates of $1000 \text{ nmol cm}^{-2} \text{yr}^{-1}$, which is the highest reasonable value, ferrocyanide is stable only at temperatures close to or less than 0°C . In less reducing or neutral atmospheres only minor amounts of ferrocyanide can be formed even at temperatures as low as -40°C .

It can be shown that the production rate of hydrogen cyanide needed to complex half of the ferrous ions to form ferrocyanide is given by

$$S = \frac{V_o k_2^{\text{OH}^-} K_w}{K_{\text{HCN}} K_{6\text{app}}^{\frac{1}{6}}}$$

where

S = Production rate of hydrogen cyanide in moles $\text{cm}^{-2} \text{yr}^{-1}$.

V_o = Ocean volume (300 litres cm^{-2} at present).

K_w = Ionization constant of water.

$K_2^{\text{OH}^-}$ = Second order rate constant for hydrolysis of hydrogen cyanide (i.e. rate = $K_2^{\text{OH}^-} [\text{OH}^-] [\text{HCN}]$; $\log_{10} K_2^{\text{OH}^-} (\text{M}^{-1} \text{yr}^{-1}) = 19.18 - \frac{4440}{T}$) (Stribling and Miller, 1987).

Expressing this in terms of the known constants and T in $^{\circ}\text{C}$ gives

$$\log_{10} S = -6.76 + 0.1120 T - 3.10 \times 10^{-4} T^2.$$

This allows the calculation of the production rate at different temperatures necessary for half of the ferrous ions to be complexed as ferrocyanide. This production rate is independent of pH since increasing the pH by 1 unit increases the rate of hydrolysis of hydrogen cyanide by a factor of 10 and also increases the concentration of cyanide and the stability of ferrocyanide by the same factor.

6.4. PHOTOCHEMICAL DECOMPOSITION OF FERROCYANIDE

Ferrocyanide is photochemically decomposed by ultraviolet light to $(\text{Fe}(\text{CN})_5 \text{H}_2\text{O})^{3-}$ which then rapidly loses the five cyanide ligands (Asperger, 1952). The $(\text{Fe}(\text{CN})_5 \text{H}_2\text{O})^{3-}$ can also react with a cyanide to regenerate the ferrocyanide. The net result is a photochemical steady state in which the ferrocyanide concentration is less than the equilibrium value. The wavelength for the photochemical dissociation is about 400 nm to 313 nm. At wavelengths below 313 nm hydrated electrons and ferricyanide are produced (Shirom and Stein, 1971a; b). Even in a well mixed ocean the photochemical steady state would be close to the equilibrium value except for the uppermost layers, because our kinetic data show that the rate of formation of ferrocyanide from ferrous ions and cyanide is rapid. The surface layer of the ocean would have lower than equilibrium concentrations of ferrocyanide, but this layer would not extend much below the depth to which ultraviolet light penetrates. This is not likely to be far as cyanide polymers and electric discharge tars strongly absorb ultraviolet radiation. Without knowing the concentration of absorbers this depth cannot be calculated, but we estimate that it would be less than a few centimeters and possibly much less. The photochemical dissociation of ferrocyanide would be more important in shallow lakes, lagoons and on beaches, possibly resulting in the release of considerable quantities of hydrogen cyanide as well as hydrated electrons. However, our kinetic data indicate that ferrocyanide would be re-synthesized at night except for pH values less than about 6.

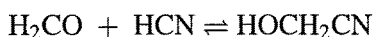
6.5. PRUSSIAN BLUE AND OTHER INSOLUBLE FERROCYANIDE COMPOUNDS

There are a wide range of insoluble ferrocyanide and ferricyanide compounds (Sharpe, 1976; Gmelin, 1932). The most insoluble of these compounds appears to be Prussian Blue, $(\text{Fe}^{\text{III}})_4(\text{Fe}^{\text{II}}(\text{CN})_6^{4-})_3$. The solubility product is given as $\text{pK}_{\text{sp}} = 40.52$, and the ferrocyanides of zinc, copper, nickel, cobalt and cadmium are nearly as insoluble (Tananaev, 1956). Thus if the ferric ion concentration was 10^{-5} M then the dissolved ferrocyanide concentration would be 1.4×10^{-7} M. This is to be compared with $\text{Zn}_2\text{Fe}(\text{CN})_6$ ($\text{pK}_{\text{sp}} = 15.39$), which gives 4.1×10^{-6} M ferrocyanide for 10^{-5} M zinc ions. Solid Prussian Blue is not as stable as this would imply as ferric ions are very insoluble as ferric hydroxide, with $\text{pK}_{\text{sp}} = 38$. Thus Prussian Blue is unstable with respect to ferric hydroxide formation at pH values higher than about 3. The presence of hydrogen sulfide would also precipitate much of the ferrous ions as well as the ferric. Thus Prussian Blue and other ferrocyanide compounds do not seem to be sufficiently insoluble to dominate the stability of dissolved ferrocyanide in the primitive ocean.

Prussian Blue has been reported to form in green rust double layer mineral structures (Arrhenius *et al.*, 1993). It is not clear whether Prussian Blue would form in the mineral under the more dilute conditions obtainable with reasonable atmospheric production rates of hydrogen cyanide. It would be interesting to investigate this point.

6.6. THE EFFECT OF FORMALDEHYDE ON HYDROGEN CYANIDE CONCENTRATIONS

It has been shown that formaldehyde reacts with hydrogen cyanide to form glycolonitrile



$$K_{\text{GN}}^{25^\circ} = 4.6 \times 10^5$$

where H_2CO refers to aqueous formaldehyde which is almost all present as the hydrate, $\text{H}_2\text{C}(\text{OH})_2$. It has been pointed out that glycolonitrile is sufficiently stable that the amount of free hydrogen cyanide in the presence of excess formaldehyde is too low to form purines, and conversely formaldehyde concentrations would be too low to form sugars in the presence of excess hydrogen cyanide (Schlesinger and Miller, 1973). If hydrogen cyanide is in excess over formaldehyde, then the excess concentration should be used in Equation (1). If formaldehyde is in excess over hydrogen cyanide then the value used in Equation (1) should be

$$[\text{HCN}] = \frac{[\text{HOCH}_2\text{CN}]}{K_{\text{GN}}[\text{H}_2\text{CO}]}$$

Thus if the concentrations of glycolonitrile and formaldehyde are 1mM and the concentration of hydrogen cyanide is 2.1×10^{-6} , then ferrocyanide will not form

except at pH values greater than about 9. Lowering the temperature increases the stability of both glycolonitrile and ferrocyanide and so the situation does not greatly change.

6.7. LOCAL HIGH CONCENTRATIONS OF HYDROGEN CYANIDE

The preceding calculations assumed a well mixed ocean and a uniform hydrogen cyanide concentration. However, there are always possible concentration mechanisms such as concentration by temperature gradients and by freezing (Miller and Orgel, 1974). Hydrogen cyanide does not concentrate by evaporation as it is more volatile than water at pH values below the pK_{HCN} of 9.2. At pH values above 9.2, which would have been unusual on the early Earth, the non-volatile cyanide anion predominates and it may be concentrated by evaporation. The hydrogen cyanide concentration in prebiotic lakes is unlikely to have been any higher than that of the ocean as the two systems are expected to have been in equilibrium with regard to volatile compounds. At high pH values where cyanide predominates over hydrogen cyanide, and may be concentrated by evaporation, the rate of hydrolysis of cyanide is much higher.

One source of relatively concentrated hydrogen cyanide would be a large addition of hydrogen cyanide to a local area by cometary impact (Clark, 1988), although formaldehyde is likely to be in excess over hydrogen cyanide in such an environment. If the hydrogen cyanide were confined to the area of impact, then the hydrogen cyanide concentration would be more than adequate to form ferrocyanide. But the ferrocyanide would decompose as the hydrogen cyanide dispersed.

6.8. FERROCYANIDE IN HYDROGEN CYANIDE CONCENTRATION MECHANISMS

Arrhenius *et al.* (1994) have suggested that ferrocyanide offers an interesting possibility for concentrating hydrogen cyanide that may be superior to the freezing of dilute aqueous solutions. If the hydrogen cyanide concentration is sufficient to form ferrocyanide and then precipitated by ferric ions to give Prussian Blue, the hydrogen cyanide could be subsequently released by the precipitation of the iron as ferric hydroxide on raising the pH by hydrogen sulfide precipitating FeS or FeS₂, or by a sufficiently strong complexing agent. It is not clear whether such strong complexing agents were available in sufficient concentrations even in local areas, but precipitation by hydrogen sulfide should be an efficient process. The process requires that the ferrocyanide and/or Prussian Blue are formed in the absence of hydrogen sulfide, the ferrocyanide is then concentrated or precipitated and hydrogen sulfide is added to the local system subsequent to this, and at the same time that the liberated hydrogen cyanide does not diffuse away. These are a specialized set of conditions, but are likely to have occurred in some local areas.

6.9. FERROCYANIDE COMPOUNDS AS USEFUL PREBIOTIC REAGENTS

There is an extensive chemistry known of ferrocyanide and ferricyanide, some of which may be relevant to prebiotic chemistry. Some of this is mentioned in the introduction, and we will briefly examine other possibilities.

Saygin (1981; 1983) has used ferricyanide as a photochemical source of CN^+ or NCO^- to produce carbamyl phosphate. This is a reasonable prebiotic process since ferrocyanide could be oxidized to ferricyanide, and most ferricyanide complexes are relatively soluble unlike ferric hydroxide. Ferricyanide is frequently used as an oxidizing agent although there are no prebiotic examples known (Thyagarajan, 1958).

Another role of ferrocyanides is the ability of some of them to absorb ions and other compounds. Thus zinc ferrocyanide has been shown to absorb ammonium ions quite efficiently, and this has been proposed as a prebiotic process (Braterman *et al.*, 1995).

It is to be noted that nickel (II) cyanide amines form Hoffman-type clathrates with aromatic compounds (Iwamoto, 1984). Such stable clathrates might form with other types of compounds and could be of prebiotic importance.

We also note that blueprints, which were widely used before Xerox machines, consist of ferric citrate or ferric oxalate and ammonium ferricyanide (Kirk and Othmer, 1982). On photolysis the ferric citrate is converted to ferrous ions followed by electron transfer and the precipitation of ferric ferrocyanide (Prussian Blue). A replication process involving the blueprint process can be envisioned to take place under geological conditions. Errors in the replication process would also be replicated. This is analogous in some respects to the mineral origin of life theory of Cairns-Smith (1982), but it has the advantage of involving known and robust chemistry. The blueprint origin has the disadvantage of there being no obvious way to store genetic information on a molecular basis, nor is it clear how "mutations" in the blueprint could have selective pressures applied to them.

Another interesting compound is cupric ferrocyanide, which forms membranes semi-permeable to small molecules when absorbed on porous porcelain (Findlay, 1913). These membranes were used by Van't Hoff to test his theories of osmotic pressure near the turn of the century. Cupric ferrocyanide membranes might be more easily formed than lipid membranes under prebiotic conditions and may have useful selectivities for prebiotic processes. Important redox and photochemical processes might be possible with such membranes.

7. Conclusion

Ferrocyanides are potentially prebiotic reagents, but they are only stable in the oceans at temperatures of 0 °C or less and in the presence of relatively high concentrations of hydrogen cyanide. To have these concentrations of hydrogen

cyanide requires high hydrogen cyanide production rates and a strongly reducing atmosphere. Ferrocyanides and ferricyanides have interesting properties that may have been of prebiotic importance.

Acknowledgments

We thank the NASA Specialized Center of Research and Training (NSCORT) at the University of California San Diego for a post-doctoral fellowship (ADK) and grant support (SLM) and Jason P. Dworkin and Michael P. Robertson for technical assistance.

References

- Arrhenius, T., Arrhenius, G. and Paplawsky, W.: 1994, *Orig. Life Evol. Biosphere* **24**, 1–17.
- Arrhenius, G., Gedulin, B. and Mojzsis, S.: 1993, in: *Proceedings, Conference on Chemical Evolution and the Origin of Life*, Trieste, Italy, 1992, Ponnampertuma, C. and Chela-Flores, J. (eds.), A. Deepak Publishing, Hampton, Virginia, 25–49.
- Asperger, S.: 1952, *Trans. Faraday Soc.* **48**, 617–624.
- Beck, M. T.: 1987, *Pure and App. Chem.* **59**, 1703–1720.
- Braterman, P. S., Arrhenius, G., Hui, S. and Paplawsky, W.: 1995, *Orig. Life Evol. Biosphere* **25**, 531–538.
- Brown, J.: 1724, *Phil. Trans. Roy. Soc. London* **38**, 17–24.
- Cairns-Smith, A. G.: 1982, *Genetic Takeover and the Mineral Origins of Life*. Cambridge University Press, Cambridge, England.
- Chameides, W. L. and Walker, J. C. G.: 1981, *Orig. Life Evol. Biosphere* **11**, 291–302.
- Clark, B. C.: 1988, *Orig. Life Evol. Biosphere* **18**, 209–238.
- Cosgrove, J. G., Collins, R. L. and Murty, D. S.: 1973, *J. Am. Chem. Soc.* **95**, 1083–1086.
- Deamer, D. W. and Harang, E.: 1990, *Biosystems* **24**, 1–4.
- Findlay, A.: 1913, *Osmotic Pressure*, Longmans, Green and Co., London.
- Gmelin's Handbuch der Anorganischen Chemie*: 1932, **Eisen B**, 670–723.
- Iwamoto, T.: 1984, in: *Inclusion Compounds* **1**, Atwood, J. L., Davies, J. E. D. and MacNicol, D. D. (eds.), Academic Press, 29–57.
- Jordan, J. and Ewing, G. J.: 1962, *Inorg. Chem.* **1**, 587–591.
- Kamaluddin, M. N. and Sharma A.: 1994, *Orig. Life Evol. Biosphere* **24**, 469–477.
- Kamaluddin, M. N. and Sushama, D. S.: 1988, *Orig. Life Evol. Biosphere* **18**, 267–280.
- Kielland, J.: 1937, *J. Am. Chem. Soc.* **59**, 1675–1678.
- Kirk, R. E. and Othmer, D. E.: 1982, *Kirk-Othmer Encyclopedia of Chemical Technology* **20**, John Wiley and Sons, New York, 3rd edition, 134–135.
- Kulesza, P. J. and Doblhofer, K.: 1989, *J. Electroanal. Chem.* **274**, 95–105.
- Legros, J.: 1964, *J. Chim. Phys.* **61**, 909–922.
- Miller, S. L. and Orgel, L. E.: 1974, *The Origins of Life on The Earth*, Prentice Hall, Englewood Cliffs, NJ.
- Miller, S. L. and Smith-Magowan, D.: 1990, *J. Phys. Chem. Ref. Data* **19**, 1049–1073.
- Orgel, L. E.: 1974, in: *The Origin of Life and Evolutionary Biochemistry*, Dose, K., Fox, S. W., Deborin, G. A. and Pavlovskaya, T. E. (eds.), Plenum Publishing Corporation, New York, (1974), pp. 369–371.
- Robin, M. B.: 1962, *Inorg. Chem.* **1**, 337–342.
- Robinson, R. A. and Stokes, R. H.: 1959, *Electrolyte Solutions*, Butterworths, London.
- Samanta, T. and Basu, A. S.: 1989, *Z. Phys. Chemie* (Leipzig) **270**, 595–606.
- Saygin, Ö.: 1981, *Naturwiss.* **68**, 617–619.

- Saygin, Ö.: 1983, *Orig. Life Evol. Biosphere* **13**, 43–48.
- Schlesinger, G. and Miller, S. L.: 1973, *J. Am. Chem. Soc.* **95**, 3729–3735.
- Sharpe, A. G.: 1976, *The Chemistry of Cyano Complexes of the Transition Metals*, Academic Press, London, 115–120.
- Shirom, M. and Stein, G.: 1971a, *J. Chem. Phys.* **55**, 3372–3378.
- Shirom, M. and Stein, G.: 1971b, *J. Chem. Phys.* **55**, 3379–3382.
- Stribling, R. and Miller, S. L.: 1987, *Orig. Life Evol. Biosphere* **17**, 261–273.
- Tananaev, I. V., Glushkova, M. A. and Seifer, G. B.: 1956, *Zhur. Neorg. Khim.* **1**, 66–68.
- Thyagarajan, B. S.: 1958, *Chem. Rev.* **58**, 439–460.
- Tiwari, V. K.: 1983, *Nat. Acad. Sci. Letters (India)* **6**, 159–161. 1984, *Chem. Abstr.* **100**, 205196.
- Tiwari, V. K. and Sharma, R. K.: 1985 *Himalayan Chem. Pharm. Bull.* **2**, 32–33. 1986, *Chem. Abstr.* **105**, 6782.
- Woodward, J.: 1724, *Phil. Trans. Roy. Soc. London* **38**, 15–17.
- Zahnle, K. J.: 1986, *J. Geophys. Res.* **91**, 2819–2834.