Complexes of Vitamin B_6.
VI*. Kinetics and Mechanisms of the Ternary Complex Formations of Iron(III) with Picolinic Acid and Pyridoxol

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Interactions of picolinic acid with iron(III) on one hand and with iron(III)–pyridoxol system on the other hand were studied in acidic solutions in the pH range 1–2.5. It was concluded that a simple binary 1:1 complex of iron(III) picolinate is formed in the pH range 1–2.0. The rate law was found to obey the following relation: $k'_{obs} = a + b(PC^-)$. The stability constant of the 1:1 complex has been calculated from the kinetic data to be 1.05×10^6 at $I = 0.5$ M and $T = 25\,°C$. It was also concluded that a ternary complex of iron(III) with singly protonated species of pyridoxol and picolinic acid, $FeHPHPC^{3+}$, is formed in the pH range 1–1.8. The rate law is $k''_{obs} = c + d(HPC)$. The stability constant of the ternary complex is found to be 4.70×10^2 at $I = 0.5$ M and $T =$ 25 °C. In the pH range ~1.8–2.5 the rate law is quite different. It is of the following form: $k'''_{obs} = m(PC^-)^2$. This was discussed in terms of the formation of a ternary complex species, $FeHP(PC)_2^+$.

Introduction

The metabolism of tryptophan, in presence of the cofactor vitamin B_6, yields a variety of important substances, including serotonin, indolacetic acid, niacin and others. Side products may also be formed such as kynurenic acid, xanthurenic acid and picolinic acid [1]. Binary complexes of Fe(III) with picolinic acid (HPC) were studied by Anderegg [2] and Timberlake [3] using potentiometric methods. They have concluded that several complexes may be formed in aqueous acidic solutions. Complexes of 1:1 and 1:2 as well as hydrolyzed products, $Fe(PC)_2OH$ and Fe_2-

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*Part V. Interaction of Pyridoxol with Pyridoxamine in Presence of Copper(II) ions, *Bioinorg. Chem.*, in press.
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TABLE I. The Dissociation Constants of P and PC and the Stability Constants of Their Binary and Ternary Complexes

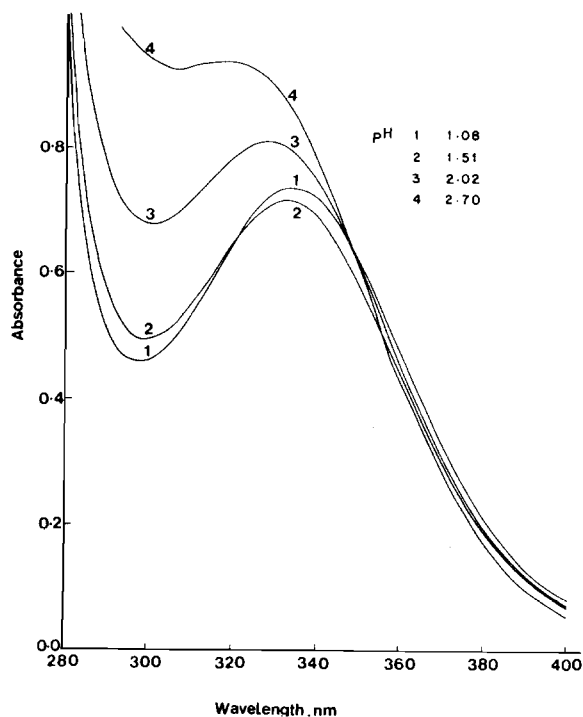| Reaction	log K	Ref.
$H_2P^+ \rightleftharpoons HP + H^+$	−4.85	6
$HP \rightleftharpoons P^- + H^+$	−8.92	6
$H_2PC^+ \rightleftharpoons HPC + H^+$	−1.60	7
$HPC \rightleftharpoons PC^- + H^+$	−5.40	7
$Fe + H_2O \rightleftharpoons FeOH^{2+} + H^+$	−2.70	4
$Fe^{3+} + HP \rightleftharpoons FeHP^{3+}$	4.47	4
$FeOH^{2+} + HPC \rightleftharpoons FePC^{2+} + H_2O$	3.32	This work
$Fe^{3+} + PC^- \rightleftharpoons FePC^{2+}$	6.02	This work
$Fe^{3+} + 2PC^- \rightleftharpoons Fe(PC)_2^+$	12.88	3
$Fe^{3+} + 2PC^- + OH^- \rightleftharpoons (Fe(PC)_2OH)$	23.92	3
$2Fe^{3+} + 4PC^- + 2OH^- \rightleftharpoons (Fe(PC)_2(OH))_2$	50.72	3
$Fe^{3+} + HP + HPC \rightleftharpoons FeHPHPC^{3+}$	2.67	This work
$Fe^{3+} + HP + 2PC^- \rightleftharpoons FeHP(PC)_2^+$	≥17	estimated

Fig. 1. The spectra of Fe(III)–picolinic acid system at various pH values: 1) pH = 1.08; 2) pH = 1.51; 3) pH = 2.02; 4) pH = 2.70; $T_{Fe} = 2 \times 10^{-4}$ M, $T_{pc} = 2 \times 10^{-4}$ M, optical path = 20 mm.

Fig. 2. a) The pH dependence of k_{obs} of binary system.

$(PC)_4(OH)_2$ were formed. On the other hand El-Ezaby *et al.* [4] has reported the formation of a 1:1 complex of Fe(III) with vitamin B_6 component, pyridoxol (P),
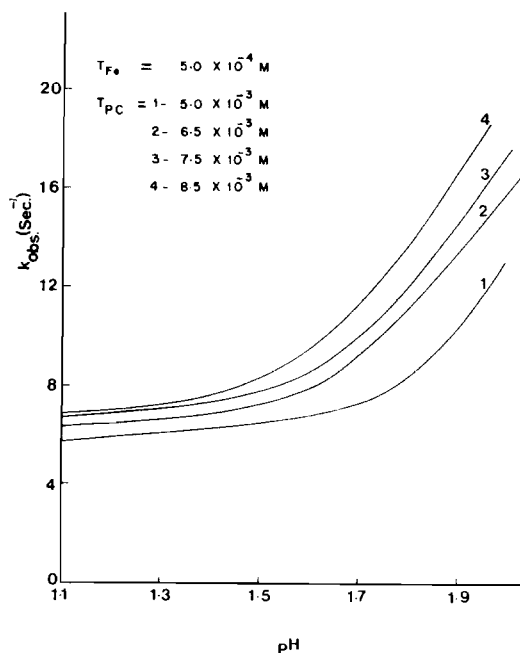
in acidic medium. Moreover, the kinetics of its formation have also been discussed. Table I lists the values of stability constants previously reported.

In the present work the kinetics of the interaction of Fe(III) with picolinic acid were studied. In addition, the kinetics of the interaction of the Fe(III)–pyridoxol system with picolinic acid are considered.
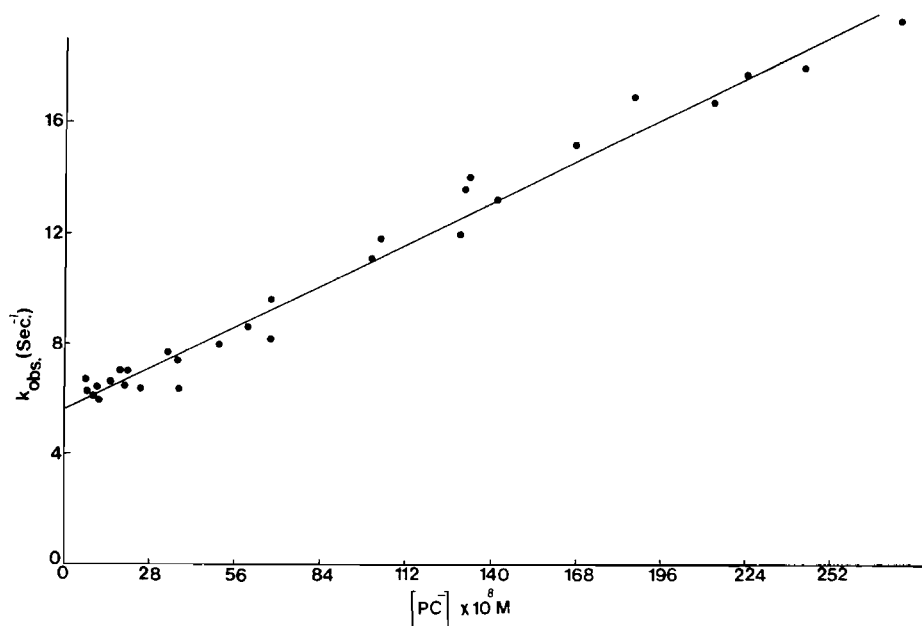

Fig. 2. b) The k_{obs} of binary system as function of PC^- concentration; $T_{Fe} = 5 \times 10^{-4}$ M, $T_{pc} = (5–8.5) \times 10^{-3}$ M.
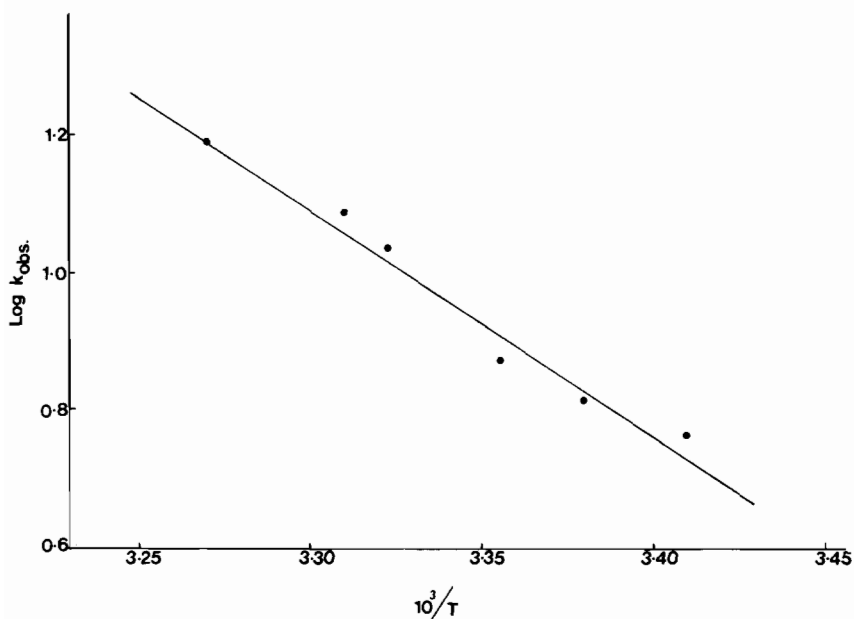
Fig. 3. Arrhenius plot of Fe(III)–PC$^-$ system. $T_{Fe} = 5 \times 10^{-4}$ M, $T_{pc} = 5 \times 10^{-3}$ M, at pH = 1.4.

Experimental

Materials

Picolinic acid (BDH) and pyridoxol hydrochloride (E. Merck) were analytically pure chemicals. They were used without further purification. Stock solutions of 0.1 M of the ligands were kept in the dark in cool place. Stock solution of iron(III) chloride (0.1 M) was prepared in 1.0 M HCl. Iron(III) concentration was checked by gravimetric methods.

Measurements of pH were carried out using a Radiometer pH-meter type 63 equipped with combined glass electrode (GK 2301 C). The pH meter was calibrated by Radiometer pH solutions at 4.00 ± 0.01 and 7.00 ± 0.01 at 25 °C. Universal buffers of different pH's were further used to calibrate the pH meter. Observed and calculated pH values were accurate within ±0.03 in the pH range 2.50–5.50. The pH region below 3.5 was also calibrated by measurements in standardized HCl solutions.

Kinetic measurements were done using a Durrum Stopped-flow apparatus. The optical path length is 20.00 mm. The mixing syringes and cuvette were thermostated at the required temperature in the range 20–30 °C. The observed rate constants were taken for 80% reaction completion.

Spectrophotometric measurements were carried out on a Cary 17 spectrophotometer.

In all measurements, the ionic strength was kept constant at 0.50 M KCl. The concentration range used in kinetic measurements was (2–5) × 10^{-4} M, (2–4) × 10^{-3} M and (2–8.5) × 10^{-3} M in case of Fe(III) ions, pyridoxol and picolinic acid, respectively.
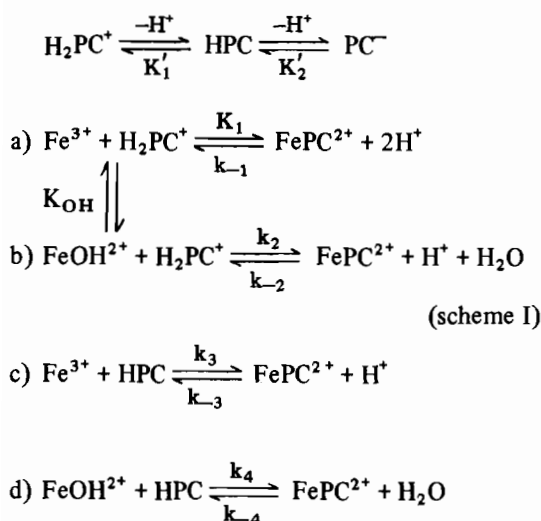
Results and Discussion

The Reaction of Fe(III) with Picolinic Acid

The spectra of Fe(III)–picolinic acid system are pH-dependent in the wavelength range 315–400 nm, with a maximum absorbance at $\lambda = 335$ nm. Typical spectra are shown in Fig. 1. The kinetic runs were carried in the pH range 1–2 with picolinic acid concentration (T_{PC}) in excess to that of Fe(III), (T_{Fe}). The pseudo-first order rate constants, k_{obs}, were obtained from the slopes of the plots of log (As_∞/ $As_\infty - As_t$) vs. time where As_∞ and As_t are the absorbance at infinite time and time t, respectively, obtained from the oscillographic trace. Figure 2(a) shows the dependence of k_{obs} on pH. The plots of k_{obs} vs. (PC$^-$), the deprotonated picolinic acid species concentration, lie on one and the same straight line for different initial concentrations of the picolinic acid, Fig. 2(b). Other plots of k_{obs} vs. (HPC) or (H$_2$-PC$^+$) species did not show the same behaviour. This conclusion indicated that Fe(III) forms mostly a chelate with PC$^-$ species. In other words, this linear relationship may be expressed explicitly as follows:

$$k'_{obs} = a + b \, (PC^-) \tag{1}$$

where a and b are the intercept and slope of the line. Their values* are (5.66 ± 0.17) sec^{-1} and (5.33 ± 0.14) × 10^6 l mol^{-1} sec^{-1}, respectively. The following scheme has been proposed to account for the observed rate data.

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*R = 0.98.

$$H_2PC^+ \xrightleftharpoons[K_1']{-H^+} HPC \xrightleftharpoons[K_2']{-H^+} PC^-$$

a) $Fe^{3+} + H_2PC^+ \xrightleftharpoons[k_{-1}]{K_1} FePC^{2+} + 2H^+$

$K_{OH} \updownarrow$

b) $FeOH^{2+} + H_2PC^+ \xrightleftharpoons[k_{-2}]{k_2} FePC^{2+} + H^+ + H_2O$

(scheme I)

c) $Fe^{3+} + HPC \xrightleftharpoons[k_{-3}]{k_3} FePC^{2+} + H^+$

d) $FeOH^{2+} + HPC \xrightleftharpoons[k_{-4}]{k_4} FePC^{2+} + H_2O$

The reactions of iron(III) species with PC^- were not considered since the concentration of the latter is of small magnitude in the pH range used. The rate equation describing the mechanism shown in scheme I may be expressed as follows:

$$\frac{d(FePC^{2+})}{dt} = (Fe^{3+})(H_2PC^+) \left(k_1 + k_2 \frac{K_{OH}}{(H^+)} + k_3 \frac{K_1'}{(H^+)} + k_4 \frac{K_1'K_{OH}}{(H^+)^2} \right) -$$

$$(FePC^{2+})(k_{-1}(H^+)^2 + k_{-2}(H^+) + k_{-3}(H^+) + k_{-4}) \quad (2)$$

where k_1, k_2, k_3 and k_4 are the forward rate constants, k_{-1}, k_{-2}, k_{-3} and k_{-4} are the backward rate constants, K_{OH} (2.0×10^{-3}) [4] is the hydrolysis constant of Fe^{3+} and K_2' (3.98×10^{-6}) and K_1' (2.50×10^{-2}) are the dissociation constants of picolinic acid [5]. At constant hydrogen ion concentration eqn. 2 may be integrated, taking in consideration that T_{Fe} at any time has the form:

$$T_{Fe} = (Fe^{3+}) + (FeOH^{2+}) + (FePC^{2+}); (T_{pc} \gg T_{Fe}).$$

The integrated form may be expressed as follows,

$$\ln \frac{(FePC^{2+})_{eq}}{(FePC^{2+})_{eq} - (FePC^{2+})_t} = ((PC^-) U + W)t \quad (3)$$

where

$$U = \frac{(H^+)^2}{\left(1 + \dfrac{KOH}{(H^+)}\right) K_1'K_2'} \times$$
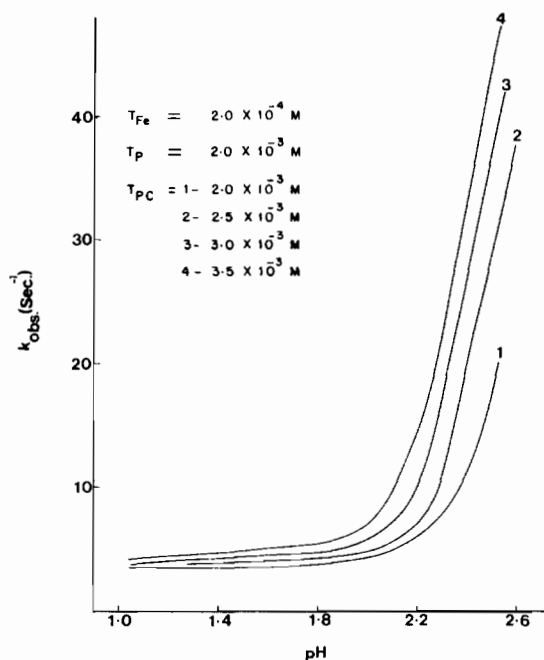
Fig. 4. The k_{obs} as function of pH for ternary systems $T_{pc} = (2-4) \times 10^{-3} M$, $T_P = 2 \times 10^{-3} M$, $T_{Fe} = 2 \times 10^{-4} M$.

$$\left(k_1 + k_2 \frac{K_{OH}}{(H^+)} + k_3 \frac{K_1'}{(H^+)} + k_4 \frac{K_1'K_{OH}}{(H^+)^2} \right)$$

and $W = k_{-1}(H^+)^2 + k_{-2}(H^+) + k_{-3}(H^+) + k_{-4}$. The observed rate constant may be equated to $(PC^-)U + W$ where the constants a and b are correlated to W and U, respectively.

It is clear that U and W are pH dependent in contradiction to what has been obtained empirically. In other words U and W should be approximately equal to $K_{OH}k_4K_2'^{-1}$ and k_{-4} respectively, if H^+ concentration is greater than K_{OH} (2×10^{-3}). This was the situation in the present study (1–2.2) pH range. This conclusion may indicate that reaction (d) in scheme (I) contributes significantly to the formation of $FePC^{2+}$ species. If this is taken in consideration, one can calculate the stability constant of reaction (d) in scheme (I). The value so obtained was 2.11×10^3 *. The calculated activation energy obtained from the Arrhenius plot, Fig. 3, was 15 ± 1.1 Kcal mol^{-1} and the entropy of activation was -5.2 e.u. at 25 °C.

Interaction of Picolinic Acid with Iron(III)–Pyridoxol System

In a previous report [4], it has been concluded that pyridoxol reacted with iron(III) in aqueous acidic medium (pH range 1–2.5) to form the complex species $FeHP^{3+}$. In the present investigation, it was found that the interaction of picolinic acid with

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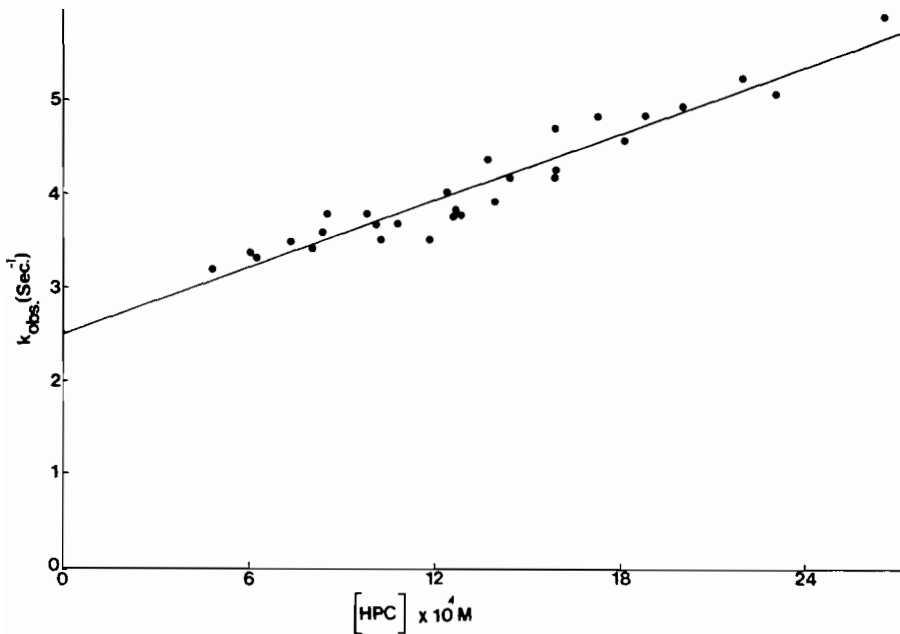*The formation constant of $FePC^{2+}$ is 1.06×10^6.

Fig. 5. The dependence of the k_{obs} of the ternary system on the concentration of HPC at pH's lower than 1.8, $T_{pc} = (2–4) \times 10^{-3}$ M, $T_P = (2–4) \times 10^{-3}$ M, $T_{Fe} = 2 \times 10^{-4}$ M.
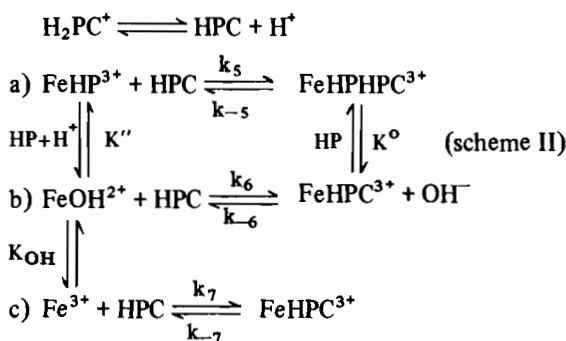
FeHP^{3+} system (where $T_{pc} \geqslant T_P \gg T_{Fe}$) showed two different rate processes depending on the pH of the medium, Fig. 4.

Kinetic study at pH values lower than 1.8

In the pH range 1–~1.8, the observed rate constant, k''_{obs}, is linearly dependent on the concentration of the singly protonated species of picolinic acid, HPC, Fig. 5. This may be expressed explicitly as follows:

$$k''_{obs} = c + d(HPC) \qquad (4)$$

where c and d are constants. Their values are (2.52 ± 0.1) sec^{-1} and (1.90 ± 0.01) 10^3 l mol^{-1} sec^{-1} * The reaction mechanism may be described by scheme II:

$$H_2PC^+ \rightleftharpoons HPC + H^+$$

a) FeHP^{3+} + HPC $\underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}}$ FeHPHPC^{3+}

HP+H$^+$ $\Big\Vert$ K″   HP $\Big\Vert$ K°   (scheme II)

b) FeOH^{2+} + HPC $\underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}}$ FeHPC^{3+} + OH$^-$

K$_{OH}$ $\Big\Vert$

c) Fe^{3+} + HPC $\underset{k_{-7}}{\overset{k_7}{\rightleftharpoons}}$ FeHPC^{3+}

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*R = 0.97 if the ligand species is (HPC); R = 0.88 if the ligand species is (PC$^-$).

The rate equation describing reactions a, b and c in scheme II is

$$(Y) \frac{d(FeHPHPC)^{3+}}{dt} =$$

$$= (HPC) \left(\frac{T_{Fe} - (FeHPHPC^{3+})(Y)}{(X)} \right) (L)$$

$$- (FeHPHPC^{3+}) (M) \qquad (5)$$

where
(L) $= k_5 + k_6/K''(HP)(H^+) + k_7/K°(HP)$,
(M) $= k_{-5} + k_{-6}(OH^-)/K°(HP) + k_{-7}/K°(HP)$,
K″ $= K_{FeHP}/K_{OH} = (FeHP^{3+})/((HP)(H^+)(FeOH^{2+}))$,
K° $= (FeHPHPC^{3+})/((FeHPC^{3+})(HP))$,
(Y) $= (K°(HP) + 1)^{-1}$,
(X) $= 1 + (K_{FeHP}(HP))^{-1} + (K''(HP)(H^+))^{-1}$
and
$T_{Fe} = (Fe^{3+}) + (FeOH^{2+}) + (FeHP^{3+}) +$
$+ (FePC^{2+}) + (FeHPHPC^{3+})$

The integrated form of eqn. 5 is written as follows:

$$\ln(Q_{eq}/(Q_{eq} - Q_t))* = ((HPC)(L)/(X) + (M)/(Y))t$$

In other words k''_{obs} may be written as follows:

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*Q_{eq} = (FeHPHPC^{3+}) at equilibrium; Q_t = (FeHPHPC^{3+}) at time t.
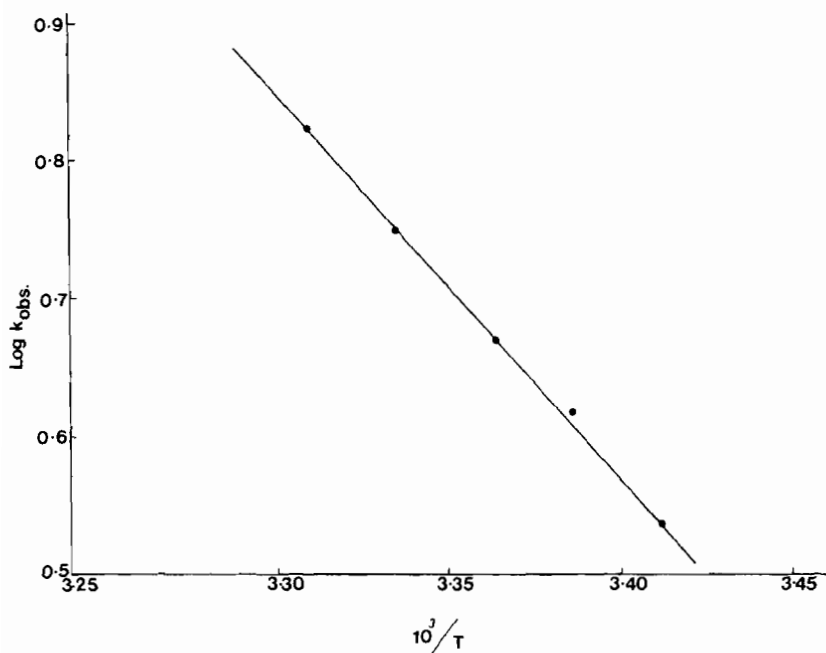
Fig. 6. Arrhenius plot of the ternary system. $T_{Fe} = 2 \times 10^{-4}$ M, $T_P = T_{pc} = 2 \times 10^{-3}$ M at pH = 1.5.

$$k''_{obs} = (HPC)(L)(X)^{-1} + (M)(Y)^{-1}$$
$$= (HPC)(k_5 + k_6(K''(HP)(H^+))^{-1} +$$
$$+ k_7(K^\circ(HP))^{-1}$$
$$(1 + K_{FeHP}(HP))^{-1} + (K''(HP)(H^+))^{-1})^{-1} +$$
$$[(k_{-5} + k_{-6}(OH^-)(K^\circ(HP))^{-1} +$$
$$k_{-7}(K^\circ(HP))^{-1}](1 + K^\circ(HP))^{-1})^{-1}$$

At constant pH, the observed rate constant is linearly dependent on (HPC) with a slope and intercept equal to $(L)(X)^{-1}$ and $(M)(Y)^{-1}$, respectively. However, it was found empirically (eqn. 4) that the slope and intercept are independent of H^+ ion concentration and are consequently equal to k_5 and k_{-5}. The stability constant of reaction a (scheme II) may now be calculated, $K_5 = k_5 k_{-5}^{-1} = 4.70 \times 10^2$.

On comparing the values of K_4 and K_5, one may conclude that Fe(III)—picolinate is more stable than the ternary complex, FeHPHPC^{+3}.
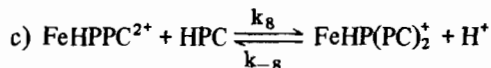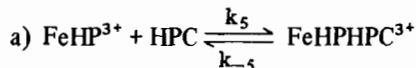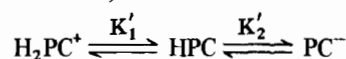
The variation of k''_{obs} with temperature is shown graphically in Fig. 6. From the regression analysis of the straight line, the slope was obtained corresponding to the activation energy, E_a. The value so obtained is equal to (11.53 ± 0.55) Kcal mol^{-1}. The calculated entropy of activation ΔS^* at 25 °C is equal to -18.9 e.u. Although the value of E_a of the ternary complex, FeHPHPC^{3+}, is less than that of the binary complex, FePC^{2+}, the rate of formation of the latter is faster than that of the former. This is due to the marked decrease in entropy of the formation of ternary complex with respect to that of the binary complex.

Kinetic study at pH values greater than 1.8

In the pH range ~1.8–2.5, the observed rate constant is dependent on the concentration of the unprotonated species of picolinic acid, PC$^-$, rather than that of HPC. Figure 7 shows the dependence of $k'''_{obs}/(H^+)$ vs. PC$^-$ concentration. This relationship is found to fit an equation of this form

$$k'''_{obs}/(H^+) = m(PC^-)^2 \qquad (6)$$

The regression analysis of eq. 6 yielded a value for m* equal to $(1.05 \pm 0.10) \times 10^{15}$. Different mechanistic models were tried to account for this experimental behaviour. The one which may be close enough, within experimental errors in the kinetic data, is shown in scheme III:

$$H_2PC^+ \underset{}{\overset{K'_1}{\rightleftharpoons}} HPC \underset{}{\overset{K'_2}{\rightleftharpoons}} PC^-$$

a) $FeHP^{3+} + HPC \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} FeHPHPC^{3+}$

b) $FeHPHPC^{3+} \overset{K}{\rightleftharpoons} FeHPPC^{2+} + H^+$ (scheme III)

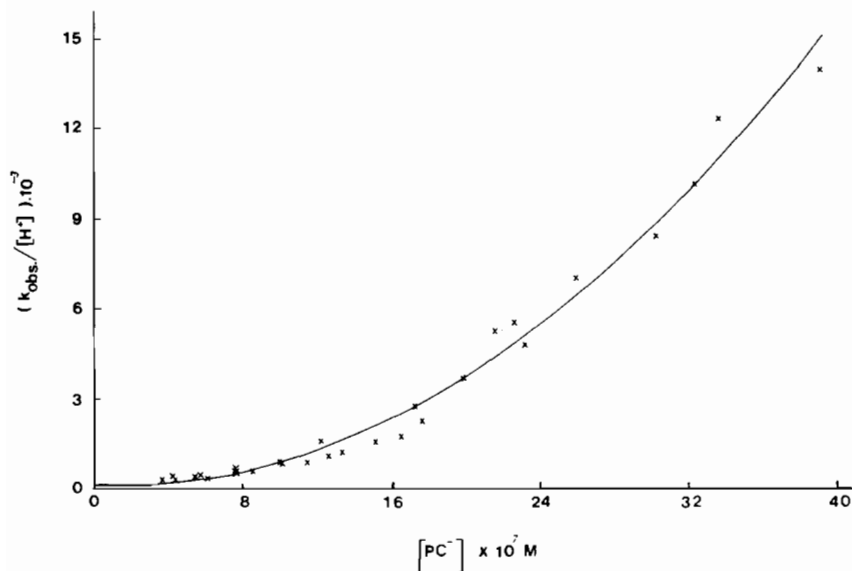c) $FeHPPC^{2+} + HPC \underset{k_{-8}}{\overset{k_8}{\rightleftharpoons}} FeHP(PC)_2^+ + H^+$

*R = 0.99.

Fig. 7. The dependence of the k_{obs} of the ternary system on PC^- at pH's higher than 1.8. $T_{pc} = (2-4) \times 10^{-3}$ M, $T_P = (2-4) \times 10^{-3}$ M, $T_{Fe} = 2 \times 10^{-4}$ M.

The rate equation describing the mechanism in scheme III may be written as follows:

$$\frac{d(FeHP(PC)_2^+)}{dt} = k_8 \frac{k_5(FeHP)^{3+}(HPC)^2K}{k_{-1}(H^+)} -$$

$$- k_{-8}(FeHP(PC)_2^+)(H^+) \qquad (7)$$

This equation has been derived if steady state approximation is applied on the species $FeHPHPC^{+3}$, $FeHPPC^{+2}$ is in fast equilibrium with $FeHPHPC^{3+}$, and

$$T_{Fe} = (FE^{3+}) + (FeHP)^{3+} + (FeHPHPC)^{3+} +$$

$$+ (FeHPPC)^{2+} + (FeHP(PC)_2^+)$$

The integrated form of equation 7 may be expressed as follows:

$$\ln\frac{(FeHP)(PC)_2^+)_{eq}}{(FeHP(PC_2^+)_{eq} - (FeHP(PC)_2^+)_t} = kt \qquad (8)$$

where

$$k = k_{-8}(H^+) + \frac{k_8k_5K(HPC)^2}{k_{-5}(H^+)Z} \qquad (9)$$

and

$$Z = 1 + k_5(HPC)((H^+) + K)/k_{-5}(H^+)$$

Equation 9 may be correlated with eqn. 6 if slight rearrangement is done:

$$\frac{k'''}{(H^+)} = k_{-8} + \frac{k_8k_5K(HPC)^2}{k_{-5}(H^+)^2 + k_5(HPC)((H^+) + K)(H^+)} \qquad (10)$$

If the term $k_5(HPC)(H^+)((H^+) + K)$ is ignored with respect to $k_{-5}(H^+)^2$ in eqn. 10, one may reach the conclusion that

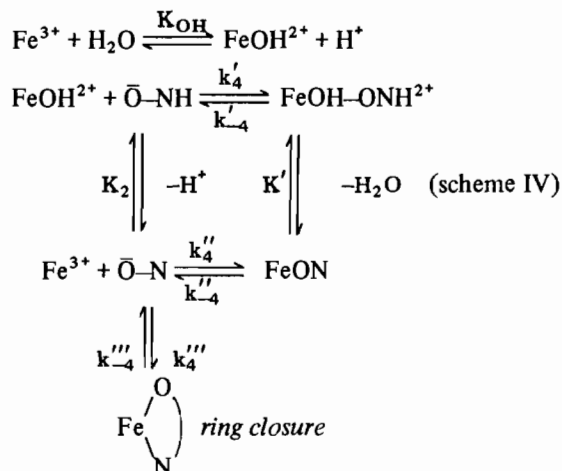$$\frac{k'''}{(H^+)} = k_{-8} + \frac{k_8k_5K(PC^-)^2}{k_{-5}K_2'^2} \qquad (11)$$

In such case k_{-8} must be of very small magnitude with respect to $k_8k_5K(PC^-)2/k_{-5}K_2'^2$ and may be neglected if the term $k'''(H^+)^{-1}$ is comparable with that of eqn. 6. Evaluation of the rate constant k_8, of reaction c (scheme III) may be achieved if it is assumed that $K \cong K_2'$. From the knowledge of the values of k_5, k_{-5} and K_2', the value of k_8 so obtained is 8.81×10^6, $1 \, mol^{-1} \, sec^{-1}$.

Conclusion

It is well known that both pyridoxol and picolinic acid form many species in aqueous solution, depending on the pH of the medium. In acidic solutions positively charged protonated species are in equilibrium with neutral species which are tautomeric with dipolar species. In the binary system of Fe(III)–picolinate, it seems that $FeOH^{2+}$ species rather than Fe^{3+} is the reactive species. This is in agreement with

many reports on the kinetics of the interaction of Fe(III) with many ligands [8].

The complexing of picolinic acid represented by $^-$O–N, where O^- is the carboxylate end and N is the pyridinic nitrogen end of the ligand, is given by the following reaction detailed scheme:

$$Fe^{3+} + H_2O \overset{K_{OH}}{\rightleftharpoons} FeOH^{2+} + H^+$$

$$FeOH^{2+} + \bar{O}\text{--NH} \underset{k'_{-4}}{\overset{k'_4}{\rightleftharpoons}} FeOH\text{--}ONH^{2+}$$

$$K_2 \Big\Updownarrow -H^+ \quad K' \Big\Updownarrow -H_2O \quad \text{(scheme IV)}$$

$$Fe^{3+} + \bar{O}\text{--N} \underset{k''_{-4}}{\overset{k''_4}{\rightleftharpoons}} FeON$$

$$k'''_{-4} \Big\Updownarrow k'''_4$$

$$\overset{O}{\underset{N}{Fe\big\langle \big\rangle}} \quad ring\ closure$$

Since the observed rate is a function of PC$^-$ one may conclude that ring closure occurred and a chelated complex of iron(III)–picolinate is formed. However, in the presence of pyridoxol, ring closure with pico-

linic acid is not allowed since the reactive species is FeHP^{3+} rather than FeOH^{2+} or Fe^{3+} in the same pH range. On the other hand raising pH increased the possibility of deprotonation of the ternary complex, enhancing ring closure and the formation of a chelated complex. The failure to obtain a 1:1:1 ternary complex of Fe(III): HP: PC$^-$ may be ascribed to the strong affinity of Fe^{3+} to PC$^-$ species and the higher stability of the second complex over the first complex.

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