Acta Chemica Scandinavica A 32 (1978) 345–352

Equilibrium Studies of the Pyruvate Protonation and Metal Pyruvate Complexes in Aqueous Solutions

OVE FORSBERG, BERTIL GELLAND, PER ULMGREN and OLOF WAHLBERG

Departments of Inorganic and Structural Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

The protonation of pyruvic acid (HPyr) has been studied in 3 M (Na)ClO$_4$ and in 0.3 M (Na)ClO$_4$ aqueous solutions at 25 °C. The total concentration of pyruvic acid varied between 0.01000 M and 1.000 M. The equilibrium data obtained from emf measurements could be described by a single dissociation step.

The cadmium(II) and iron(II) complexes of pyruvic acid have been studied in 3 M (Na)ClO$_4$ medium, while the calcium pyruvate equilibria have been studied both in 0.3 M and in 3 M (Na)ClO$_4$ media. Formation constants of the main species are given in this article. Altogether 428 equilibrium solutions are studied.

Pyruvate ion frequently occurs as a reactant or a reaction product of chemical reactions[1,2] in living organisms. Pyruvic acid, or 2-oxo-propanoic acid is a monobasic acid with $pK_a = 2.49$ in pure water at 25 °C.[3] Complexes of the type MPyr$^+$ and MPyr$_2$ have been reported (*cf.* Table 1). The crystal structure of the sodium salt has been determined by X-ray diffraction methods.[9] Pyruvic acid dimerizes in aqueous solution. In pure water this reaction is rather slow provided that pH ≤ 5. The presence of CN$^-$, OH$^-$ or metal ions speeds up the dimerization of HPyr.[7c] The dimer is a dibasic weak acid with $pK_{a1} = 1.73$ and $pK_{a2} = 3.72$ in 1 M NaCl medium.[7c]

The present study has been undertaken to determine the main complexes of Cd^{2+}, Ca^{2+} and Fe^{2+} with pyruvic acid. In man calcium and iron are two of the most abundant metals, while cadmium occurs only in very low concentrations. The complex formation with calcium has been investigated by Davies[4] and by Schubert and Lindebaum.[5] They found one complex, CaPyr$^+$, with $\beta \sim 10$ M^{-1}. The complex formation of the pyruvate ion with Fe^{2+} and Cd^{2+} does not seem to have been studied earlier. Part of this report concerns the protonation of pyruvic acid, which had to be determined in the ionic media used. The influence of the diffusion potentials and the activity coefficients on the measured data also had to be investigated. In order to determine the influence of Na$^+$ on the complex formation, measurements have been made both in 0.3 M NaClO$_4$ medium and in 3 M NaClO$_4$ medium.

The equilibria studied are generally written as

$$p\text{H}^+ + q\text{M}^{2+} + r\text{Pyr}^- \rightleftharpoons \text{M}_q\text{Pyr}_r\text{H}_p{}^{2q+p-r} \qquad (1a)$$

The equilibrium constant for the formation of a complex can be written as

$$\beta_{pqr} = h^{-p}b^{-q}c^{-r}c_{pqr} \qquad (1b)$$

where h, b, c and c_{pqr} are the concentrations of the species H$^+$, M^{2+}, Pyr$^-$ and M$_q$Pyr$_r$H$_p{}^{2q+p-r}$, respectively.

EXPERIMENTAL

Chemicals and analyses

Sodium perchlorate, *perchloric acid*, *sodium chloride* and *sodium hydroxide* were prepared and analyzed as described in Ref. 11. The preparations of cadmium perchlorate solutions, calcium perchlorate solutions and iron(II) perchlorate solutions have been described elsewhere.[11b,c,d]

Table 1. Metal-pyruvate complexes in aqueous solution. A literature survey. HPyr = pyruvic acid. Temperature is 25 °C.

Investigation	Notes	Species	log β_{pqr}
Method = Solubility			
Davies[4]	→ 0	$CaPyr^+$	1.08
Method = Ionic exchange			
Schubert and Lindenbaum[5a]	0.16 M NaCl	$CaPyr^+$	0.8
Schubert and Lindenbaum[5a]	0.16 M NaCl	$SrPyr^+$	0.5
Schubert, Russell and Meyers[5b]	0.16 M NaCl	$RaPyr^+$	0.89
Chibnall and Cannan[6]	0.16 M NaCl	$RaPyr^+$	1.9
Method = Potentiometry			
Leussing and Shultz[7a]	0.65 M KCl	$MnPyr^+$	1.26
Gelles and Hay[8]	→ 0	$CuPyr^+$	2.3
Gelles and Hay[8]	→ 0	$CuPyr_2$	4.9
Leussing and Shultz[7a]	0.65 M KCl	$NiPyr^+$	1.15
Leussing and Hanna[7b]	0.5 M KCl	$NiPyr^+$	1.12
Tallman and Leussing[7c]	1.0 M NaCl	$NiPyr^+$	0.883
Leussing and Hanna[7b]	0.5 M KCl	$NiPyr_2$	0.46
Tallman and Leussing[7c]	1.0 M NaCl	$NiPyr_2$	0.948
Leussing and Shultz[7a]	0.65 M KCl	$ZnPyr^+$	1.28
Leussing and Hanna[7b]	0.5 M KCl	$ZnPyr^+$	1.26
Tallman and Leussing[7c]	1.0 M NaCl	$ZnPyr^+$	1.16
Leussing and Hanna[7b]	0.5 M KCl	$ZnPyr_2$	1.98
Tallman and Leussing[7c]	1.0 M NaCl	$ZnPyr_2$	2.00

Sodium pyruvate $NaC_3H_3O_3$. The analytical grade reagent from BDH was used. The molecular weight was determined by titration of solutions containing accurately weighed samples of sodium pyruvate acidified with perchloric acid. As a titrant, a standard sodium hydroxide solution was used. The experimental value, 110.1 ± 0.1 g/mol equals the theoretical value within the stated error. From proton resonance spectra it was found that 5 % of the pyruvate had formed pyruvate dimer.

Pyruvic acid solutions were prepared by adding perchloric acid to sodium pyruvate solutions.

Apparatus

The measurements of emf were performed with an *automatic titration system*, which includes a master digital voltmeter (Solartron Instruments) and a digital computer (Philips P855). A complete description will be given elsewhere.[10] The NMR measurements were made with a Varian model XL 100 Spectrometer. The saltbridge, electrodes and thermostat are described in Ref. 11. The glass electrodes (Beckman No. 39303) were checked against hydrogen electrodes.

THE TITRATION PROCEDURE

All solutions studied contained sodium perchlorate as an ionic medium to hold the activity coefficients approximately constant and to eliminate the influence of the diffusion potentials on the data. The concentration of ClO_4^- was kept constant either at 3 M or 0.3 M, while Na^+ was varied within the following ranges: $2.830 \leq [Na^+] \leq 3.160$ M or $0.270 \leq [Na^+] \leq 0.320$ M, respectively. From one or two burets, V_T ml of a solution with known analytical concentration of H^+ ($\equiv H_T$) were introduced into V_0 ml of a solution with an analyzed concentration of H^+ ($\equiv H_0$) to yield ($V_0 + V_T$) ml of a solution with

$$H = (V_0H_0 + V_TH_T)/(V_0 + V_T) \qquad (2)$$

The total concentrations of pyruvic acid ($\equiv C$) and metal ion ($\equiv B$) were held constant in each titration except in four experiments (see below). In some titrations, where an acidified solution of pyruvic acid was made gradually more alkaline, a sodium hydroxide solution and a pyruvic acid solution were introduced from two separate burets. The two solutions were immediately mixed by stirring to disperse locally concentrated solutions of hydroxide ions.

THE EXPRESSION FOR EMF

The solutions studied were analyzed for $[\mathrm{H}^+]$ by the following cells

$$-\mathrm{Ag, AgCl(s)} \left| [\mathrm{Cl}^-]=0.010\ \mathrm{M} \right| \begin{array}{l}\text{Equilibrium}\\ \text{solution}\\ \text{studied}\end{array} \left| \begin{array}{l}\text{Glass}\\ \text{elec-}\\ \text{trode}\end{array} \right. + \qquad (3a)$$

$$-\mathrm{Ag, AgCl(s)} \left| \begin{array}{l}\text{Equilibrium}\\ \text{solution contain-}\\ \text{ing 0.01 M NaCl}\end{array} \right| \begin{array}{l}\text{Glass}\\ \text{electrode}\end{array} + \qquad (3b)$$

Sodium perchlorate was added to all solutions so that $[\mathrm{ClO}_4^-]=3$ M or 0.3 M. Proton concentrations were calculated from

$$E=E_0'+(RT\ln 10/F)\lg h+E'_j,\ \text{where}$$
$$RT\ln 10/F=59.155\ \text{mV at } 25\ ^\circ\text{C} \qquad (3c)$$

The constant E_0' was determined for each titration from measurements in solutions with $0.8<\mathrm{pH}<1.4$ where $C\approx[\mathrm{HPyr}]$. The contribution of protons to h from dissociated HPyr was, however, taken into account. The variations of the activity coefficients and the diffusion potentials in the cell (3a) were for practical reasons expressed as two terms[11e] E_0' and E_j' where

$$E_0'=E_{00}+j_C C \ \text{and} \qquad (3d)$$

$$E_j'=\Sigma j_{pr}c_{pr}\ \text{(summation over all } p,r) \qquad (3e)$$

E_0' is a constant for each titration with constant C, and E_j' is expected to vary at most a few mV. As a first approximation we set $E_j'=j_{10}h$. From studies of $E=\mathrm{f}(C)_h$ in solutions dominated by HPyr or Pyr^-, respectively, two terms $j_{11}[\mathrm{HPyr}]$ and $j_{01}[\mathrm{Pyr}^-]$ in eqn. (3e) could be estimated. The term $j_{10}h$ is known from earlier experiments.[11] Our second approximation of E_j' is then

$$E_j'=-16.8[\mathrm{H}^+]+j_{01}[\mathrm{Pyr}^-]+j_{11}[\mathrm{HPyr}] \qquad (3f)$$

The computer program LETAGROP[12a,b] was used to refine the parameters j_{pr} in eqn. (3e) and if necessary to introduce new terms in the expression for E_j'.

Attainment of equilibrium

The attainment of equilibrium between monomeric pyruvic acid and protons is rapid, while the formation of dimer is slow. For each measured data point a stable emf value was obtained within a few minutes after mixing of a solution. This value remained stable within ±0.2 mV for at least 8 h in acid solution and for a few hours in neutral solution. The emf values were read 10 min after the mixing of solutions for each experimental data point.

Each titration was completed within 6 h, when studying the systems $\mathrm{Ca}^{2+}-\mathrm{H}^+-\mathrm{Pyr}^-$ and $\mathrm{Cd}^{2+}-\mathrm{H}^+-\mathrm{Pyr}^-$, but within 3 h for the systems $\mathrm{H}^+-\mathrm{Pyr}^-$ and $\mathrm{Fe}^{2+}-\mathrm{H}^+-\mathrm{Pyr}^-$, thus before appreciable formation of pyruvate dimers. The concentration of pyruvate dimer was found to be approximately 5 % for all data points. No data points were measured at low Z-values for the iron(II) pyruvate system, since stable emf values could not be obtained within a few minutes. The Ca^{2+}-pyruvate data in Fig. 3b are restricted to $1.0>Z_{\mathrm{H/C}}>0.2$ representing the points used in the graphic treatment described below. The data at low Z-values were discarded in the graphic treatment since we wanted to keep the influence of pyruvate dimers low. However, all the data, even those at low Z-values, were used in the final refinement of the equilibrium model whereby the dissociation of the pyruvate dimer was taken into account. Back titrations show that the data can be explained by equilibrium models for reversible processes, within the limits of the experimental uncertainty (*cf.* Figs. 2, 3). The iron solutions used in the experiments were carefully reduced before each titration (*cf.* Ref. 11d).

SURVEY OF EXPERIMENTAL DATA*

The primary data consist of measured volumes, emf values and analyzed total concentrations. In four titrations (Fig. 1) the total concentration of pyruvic acid was varied at constant pH. For most measurements, however, both B and C were held constant for each titration. The emf of the cell (3a) or (3b) was measured and from eqn. (3c) h was calculated. These data were transformed to the formation functions $Z_{\mathrm{H/C}}$ and $Z_{\mathrm{C/B}}$ by eqns. (4a, b).

---

* The primary data are available on request to the Department of Inorg. and Structural Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden.

$$Z_{H/C} = (H-h)/C \tag{4a}$$

$$Z_{C/B} = (C-c)/B \tag{4b}$$

The data transformed to $Z_{H/C}(\log h)_{B,C}$ or $Z_{C/B}(\log c)_{B,C}$ are shown in Figs. 2, 3 and 4. E_0' (which is a function of C, *cf.* eqn. 3d) was estimated for each titration from five or six acid points by the computer program TRAVE[11f] which accounts for the dissociation of HPyr. E_j' has been estimated by separate experiments. In the refinement of the final equilibrium model we search for a better estimation of E_j'. The least-squares sum $(Z_{calc}-Z_{exp})^2$ has been minimized (*cf.* eqn. 3e). Z_{calc} was obtained from the eqns. (1b), (4a), (4c), (4d) and (4e). Z_{exp} was obtained from eqns. (2), (3c) and (4a).

$$H = h + \Sigma p c_{pqr} \tag{4c}$$

$$B = b + \Sigma q c_{pqr} \tag{4d}$$

$$C = c + \Sigma r c_{pqr} \tag{4e}$$

TREATMENT OF DATA

All solutions contained 5 % pyruvate dimer as measured by NMR. Calculations by the program HALTAFALL[12c] show that the influence of 5 % dimer on the data is at most 2 % of H. Since the effect of dimers is so small in the present case, the dimers were taken into account only in the final least-squares calculations. The data treatment is divided into two main sections: (1) Estimation of the relationship between activity coefficients and the liquid junctions as a function of the pyruvate concentration and the determination of pK_a for pyruvic acid. (2) Determination of the main metal complexes of pyruvic acid with Ca^{2+}, Cd^{2+} and Fe^{2+}.

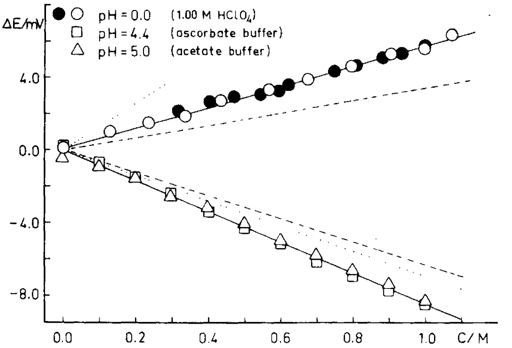

Fig. 1. E as a function of C at constant log h. The symbols represent experimental data points for pyruvic acid. The solid lines correspond to eqns. (5a) and (5b). The dotted lines refer to ascorbic acid [11e] and the broken lines refer to acetic acid (14).

1a. Estimations of $E_0'+E_j'$. The data have been plotted in Fig. 1 as $E = f(C)_{pH}$. Straight lines were obtained. Forward and backward titrations agree indicating that no irreversible reactions take place in the solutions studied. The eqns. (5a) and (5b) were calculated to describe these data in 3 M (Na)ClO$_4$:

$$E_0'+E_j' = E_{00} + (5.6 \pm 0.5)C \text{ at pH} = 0 \text{ where } C = [\text{HPyr}] \tag{5a}$$

$$E_0'+E_j' = E_{00} - (8.5 \pm 0.5)C \text{ at pH} = 4.4 \text{ and } 5.0 \text{ where } C = [\text{Pyr}^-] \tag{5b}$$

The measurements at pH = 0 were performed in 1.00 M HClO$_4$ solutions. The measurements at

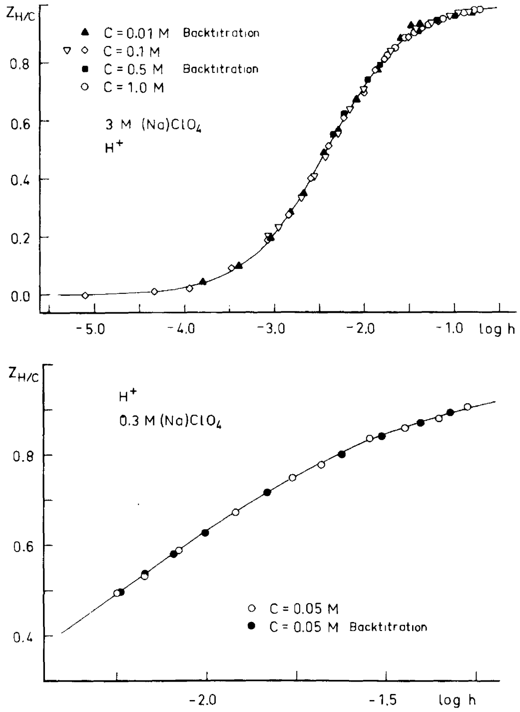

Fig. 2. Z(= the average number H^+ bound per C) as a function of log h. The solid curve corresponds to a normalized function and pK_a = 2.40 in 3 M (Na)ClO$_4$ and pK_a = 2.20 in 0.3 M (Na)ClO$_4$.

pH = 4.4 and 5.0 were performed in ascorbate and acetate buffers, respectively. Thus, using one value of E_0' for each titration with constant C, the variations of activity coefficients and liquid junction potentials for each titration are approximated by

$$E_j' = -14.1\,c - 16.8\,h \qquad (5c)$$

while now $E_0' = E_{00} + 5.6\,C$ (*cf.* eqns. (3d), (3e), (5a) and (5b))

1b. The protonation of the pyruvate ion. The formation function $Z_{H/C}$ (log h)$_C$ is represented in Fig. 2. All the experimental data could be explained by assuming the presence of HPyr and Pyr^- in the solutions. From eqns. (1b) and (4a), (4c) and (4e) one obtains

$$Z_{H/C} = (\beta_{101}h)/(1+\beta_{101}h) \qquad (5d)$$

The normalized function $u/(1+u) = f(\log u)$ was fitted to the experimental $Z(\log h)_C$. The "best" fit gave

$$pK_a = \log\beta_{101} = \log u - \log h = 2.4 \pm 0.1 \text{ for 3 M (Na)ClO}_4 \qquad (5e)$$

and $pK_a = 2.2 \pm 0.1$ for 0.3 M (Na)ClO$_4$.

1c. Least-squares treatment of the data. Least-squares refinements of pK_a by program LETAGROP[12a,b] (*cf.* Table 2a) yielded the values given below (*cf.* eqn. (7)).

No significant change was observed when models with different hypotheses for E_j' were refined. This fact was interpreted to mean that the errors caused

Table 2a. Least-squares treatment of data for equilibrium between pyruvic acid and protons. $U_Z = (Z_{calc} - Z_{exp})^2$. K_{D1} and K_{D2} are the acidity constants of the pyruvic acid dimer H_2Pyr_2. The systematic errors δE_0 and δH have been adjusted. n.v. = not varied.

[(Na)ClO$_4$]	Np	$U \times 10^4$	$(\sigma Z) \times 10^3$	log($\beta_{101} \pm 3\sigma$)	−log($K_{D1} \pm 3\sigma$)	−log($K_{D2} \pm 3\sigma$)
3 M	89	8.64	8.64	2.415 ± 0.009	–	–
		8.62	8.63	2.403 ± 0.009	2.2 ± 0.4[a]	3.6 ± 0.4[a]
0.3 M	20	1.61	2.94	2.244 ± 0.005	–	–
		1.39	2.70	2.202 ± 0.004	1.73 n.v.[b]	3.72 n.v.[b]
		1.39	2.69	2.196 ± 0.004	1.9 ± 0.7[a]	3.7 ± 1.1[a]

[a] The dimers were included only to show the very small effect that 5 % pyruvate dimer has on the measured emf. [b] Values determined by Leussing and Tallman [7c] in 1 M NaCl at 25 °C.

Table 2b. Least-squares treatment of data for equilibria between Cd(II), Ca(II) or Fe(II) ions, pyruvic acid and protons. $U_Z = (Z_{calc} - Z_{exp})^2$. K_{D1} and K_{D2} are the acidity constants of the pyruvic acid dimer H_2Pyr_2. The systematic errors δE_0 and δH have been adjusted. n.v. = not varied.

M^{2+}	[(Na)ClO$_4$]	Number of points	$U \times 10^4$	$(\sigma Z) \times 10^3$	log($\beta_{101} \pm 3\sigma$)	log($\beta_{011} \pm 3\sigma$)	−log K_{D1}	−log K_{D2}
Cd^{2+}	3 M	159	118	8.4	2.415 n.v.	0.961 ± 0.015	–	–
Ca^{2+}	3 M	102	55.2	7.4	2.415 n.v.	0.571 ± 0.012	–	–
			49.1	7.0	2.403 n.v.	0.554 ± 0.011	1.73 n.v.[b]	3.72 n.v.[b]
			44.0	6.6	2.403 n.v.	0.581 ± 0.010	2.2 n.v.[a]	3.6 n.v.[a]
Ca^{2+}	0.3 M	20	0.42	1.5	2.244 n.v.	0.619 ± 0.014	–	–
			0.37	1.4	2.202 n.v.	0.596 ± 0.013	1.9 n.v.[a]	3.7 n.v.[a]
Fe^{2+}	3 M	38	12.9	5.9	2.403 n.v.	0.69 ± 0.04	2.2 n.v.[a]	3.6 n.v.[a]
			12.6	5.8	2.415 n.v.	0.67 ± 0.04	–	–

[a] The dimers were included only to show the very small effect that 5 % pyruvate dimer has on the measured emf. *Cf.* Table 2. [b] Values determined by Leussing and Tallman [7c] in 1 M NaCl at 25 °C.

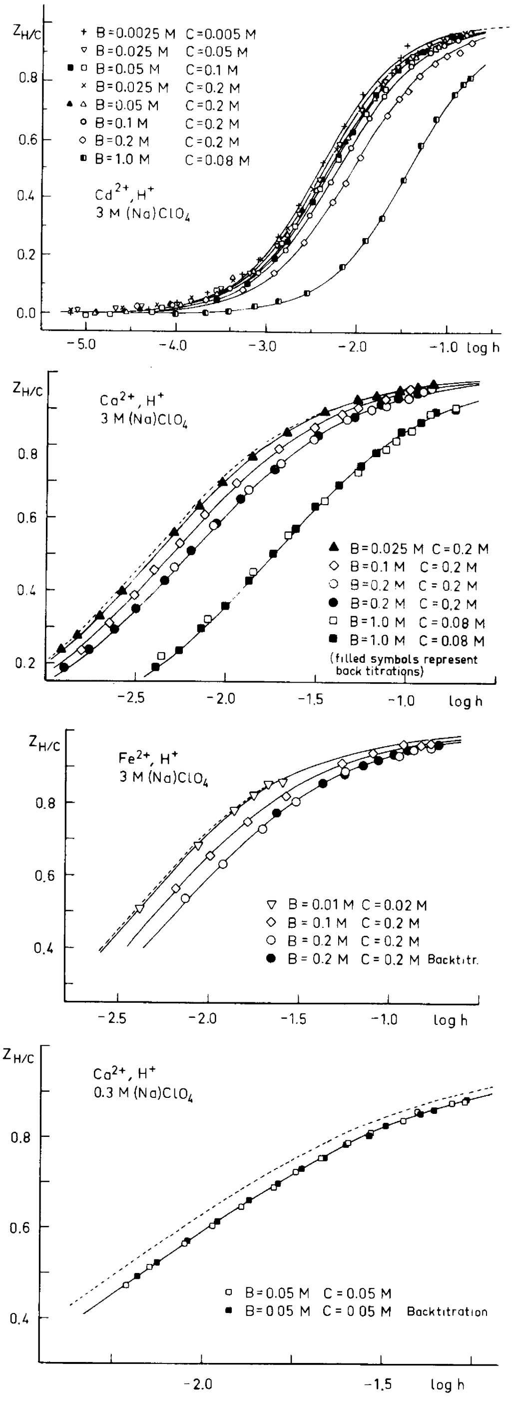

Fig. 3. $Z = Z_{H/C}$, = the average number of H^+ bound per C, as a function of log *h*. The solid curves have been calculated by the program HALTAFALL[12c] using the final equilibrium constants. The broken curves correspond to pyruvate solutions with metal ions absent.

by activity coefficients and diffusion potentials have been accounted for by E_0'. The fit of the model with the data was improved very little when the dimers were taken into account. Rough estimates of pK_a for the dimer are $pK_{a1} = 2.2 \pm 0.5$ and $pK_{a2} = 3.6 \pm 0.5$ (*cf.* Table 2a). Certainly, the dimers have very little influence on the present data.

2. *Determination of the equilibrium constants for the formation of metal pyruvate complexes.* In this equilibrium analysis we have first deduced a model by studying the graphs of $Z_{H/C}$ (log $h)_{B,C}$ and $Z_{C/B}$ (log $c)_{B,C}$ in Figs. 3 and 4. The least-squares program LETAGROP[12a,b] was then used for refinement of the model. The hydrolysis of the metal ion could be neglected in the pH-regions studied.[3] All equilibrium solutions studied contained 5 % pyruvate dimer, which caused the small deviation at $Z_{H/C} \approx 0.01$ evident in Fig. 3a. In the final least-squares refinement we have taken into account also the pyruvate dimers.

2a. *Graphic analysis of the data.* In Figs 3a – d the average number of H^+ bound per Pyr^- has been plotted as a function of log $[H^+]$ for several total concentrations of the metal ion. The influence of the metal ion on the curves $Z_{H/C}(\log h)_{B,C}$ is larger for pyruvate than for ascorbate for pH ≤ 5 (*cf.* Ref. 11). For the pyruvate metal complexes $Z_{H/C} \geq 0$ for all data (Fig. 3), in contrast to the ascorbate – metal complexes, which form chelated species at pH ≥ 5 giving $Z_{H/C} < 0$ (Ref. 11b).

As a first approximation we have assumed that the complexes are of the type $M_q(Pyr)_r^{2q-r}$ (thus with $p = 0$). Using this assumption we can easily calculate the average number of Pyr^- bound per M^{2+} as a function of $c = [Pyr^-]$ (*cf.* Ref. 11a). The function $Z_{C/B}(\log c)_{B,C}$ is shown in Figs. 4a – c. The theoretical expression for $Z_{C/B}$ is

$$Z_{C/B} = \Sigma r\beta_{qr}b^q c^r/(b + \Sigma q\beta_{qr}b^q c^r) \qquad (6a)$$

All the experimental points in Figs. 4a – c can be fitted by the normalized function $[u/(1+u)] = f(\log u)$, assuming one complex with $q = r = 1$. The translation of the normalized curve to the "best" fit with the experimental curves $Z_{C/B}(\log c)_{B,C}$ gives $\log \beta_{11} = \log u - \log c = 1.0 \pm 0.1$ for Cd^{2+}. The corresponding value for Fe^{2+} was 0.7 ± 0.1 and for Ca^{2+} 0.6 ± 0.1 (both in 3 M $NaClO_4$ and in 0.3 M $NaClO_4$).

2b. *Least-squares treatment by the LETAGROP method.*[12a,b] Models with additional species were tried, but no significant improvement of the fit with

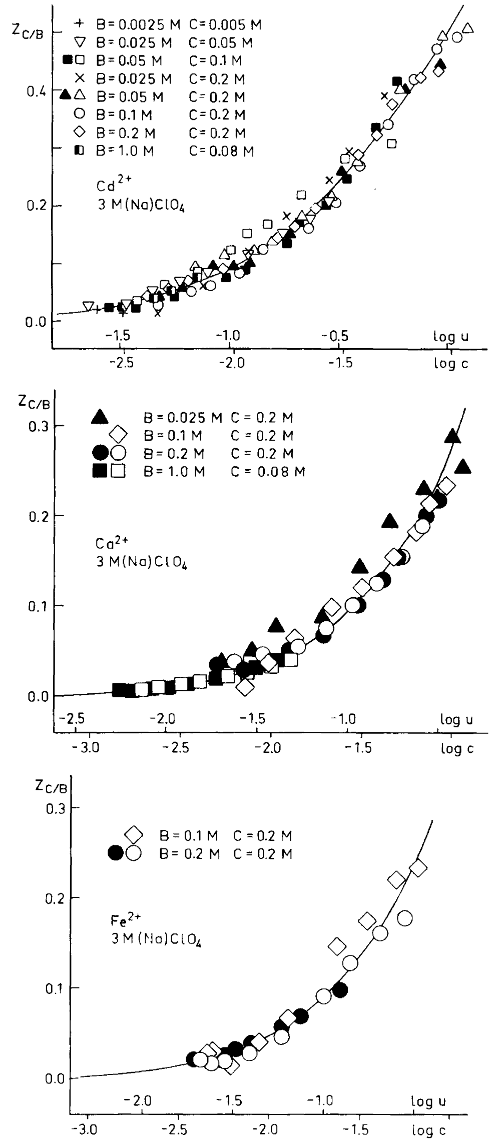

Fig. 4. $Z_{C/B}$ = the average number of C bound per B as a function of $c = [Pyr^-]$. $B \equiv Cd^{2+}, Ca^{2+}$ or Fe^{2+} and $C \equiv Pyr^-$. The solid curves correspond to the final values of the constants (Table 2b).

the data was obtained. Errors were assumed in the chemical analysis of H^+ in the stock solutions. The corresponding parameters suggest negligible errors; $|\delta Z| = 0.005-0.01$. The final equilibrium constants are given below.

FINAL RESULTS AND DISCUSSION

We propose the following reactions and constants valid at 25 °C

$$\begin{array}{ll} H^+ + Pyr^- \rightleftharpoons HPyr & \log \beta_{101} = 2.40 \pm 0.01 \\ & \text{in 3 M NaClO}_4 \\ & \log \beta_{101} = 2.20 \pm 0.01 \\ & \text{in 0.3 M NaClO}_4 \\ Ca^{2+} + Pyr^- \rightleftharpoons CaPyr^+ & \log \beta_{011} = 0.59 \pm 0.02 \\ & \text{in 3 M NaClO}_4 \\ & \log \beta_{011} = 0.56 \pm 0.02 \\ & \text{in 0.3 M NaClO}_4 \\ Fe^{2+} + Pyr^- \rightleftharpoons FePyr^+ & \log \beta_{011} = 0.69 + 0.04 \\ & \text{in 3 M NaClO}_4 \\ Cd^{2+} + Pyr^- \rightleftharpoons CdPyr^+ & \log \beta_{011} = 0.98 \pm 0.02 \\ & \text{in 3 M NaClO}_4 \end{array} \qquad (7)$$

The errors given are three times the standard deviations. The values of pK_a for pyruvic acid agree with those reported earlier, *viz.* $pK_a = 2.49(\mu \rightarrow 0)$,[13] 2.35-(0.5 M KCl),[7b] 2.39(0.65 M KCl),[7a] 2.10(1.0 M NaCl),[7c] all at 25 °C. The presence of 5 % dimer was taken into account. The roughly estimated values $pK_{a1} = 2.2$ and $pK_{a2} = 3.6$ for the dimers agree with those determined by Leussing and Tallman[7c] in 1 M NaCl, *viz.* $pK_{a1} = 1.73$ and $pK_{a2} = 3.72$. The expression used by us for description of the variation of activity coefficients and liquid junction potentials in 3 M (Na)ClO$_4$ is $E_j' = 5.6[HPyr] - 8.5[Pyr^-] - 16.8\ h$ [now with $E_0' = E_{00}$; *cf.* eqn. (3d)]. This expression may be compared with Persson's[14] expression for acetic acid in 3 M Na(ClO$_4$), *viz.* $E_j' = 3.40[HAc] - 6.32[Ac^-]$ and our[11e] expression for ascorbic acid in 3 M Na(ClO$_4$), *viz.* $E_j' = 13\ [H_2Asc] - 7[HAsc^-] - 16.8\ h$. The first term in E_j' is rather different for the three acids while the second term only differs slightly. If the medium is changed from 0.3 M NaClO$_4$ to 3 M NaClO$_4$ the value of pK_a changes 0.2 units, but the formation constant for CaPyr$^+$ remains the same within the limits of error. Our value of $\log \beta_{011}$ for CaPyr$^+$ is lower than Davies[4] value of 1.08 (extrapolated to zero ionic strength) and the value obtained by Schubert and Lindenbaum,[5] *viz.* 0.8 in 0.16 M NaCl. The only metal complexes formed in appreciable amounts in these systems over a time scale of a few hours seem to be MePyr$^+$. It should be noted that we also measured a few data points in more alkaline solutions than shown in Fig. 3a, which indicates a very low buffer capacity for pH ≥ 5.

Table 3. Formation constants, log β_{011} for $M^{2+} + X^- \rightleftharpoons MX^+$.

X^-	M^{2+}		
	Ca^{2+}	Fe^{2+}	Cd^{2+}
Pyr^-	0.59	0.69	0.98
$HAsc^-$	0.00	0.20	0.45

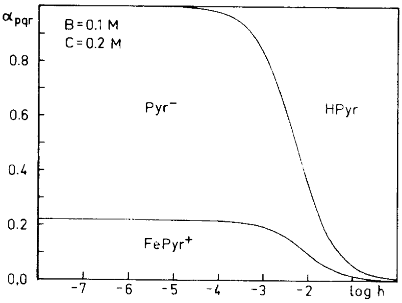

Fig. 5. The distribution of pyruvic acid among different species as a function of log *h*, calculated by means of HALTAFALL.[12c] The distribution curves for Fe^{2+} are very similar to those of Cd^{2+} and Ca^{2+}.

The metal–pyruvate complexes $MPyr^+$ are stronger than the corresponding metal–ascorbate complexes $MHAsc^+$ as can be seen in Table 3 of formation constants for metal–pyruvate and metal–ascorbate complexes.

The complexes $CdPyr^+$ and $CdHAsc^+$ are both weaker than the cadmium acetate complex $CdAc^+$. The value log $\beta_{011} = 1.30$ for $CdAc^+$ was determined by Leden[15] and later by Gerding[16] (log $\beta_{011} = 1.33$) in the same medium at the same temperature. The distribution of pyruvate on different species in an iron(II) pyruvate solution is demonstrated in Fig. 5.

Acknowledgements. We thank Professor Peder Kierkegaard and Professor Arne Magnéli for placing excellent facilities at our disposal. We are obliged to Miss Evelin Ehlers and Mr Ove Pettersson for their help with some of the experiments and calculations in connection with this work. Mr Anders Eriksson kindly helped us with the NMR-measurements. The English of this article was revised by Dr Don Koenig.

This investigation is financially supported by the Swedish Natural Research Council.

REFERENCES

1. Garfinkel, D., Anderson, J. and Achs, M. J. *Comput. Biomed. Res. 4* (1971) 1–125.
2. Lehninger, A. *Biochemistry*, Worth Publishers, New York 1970.
3. Sillén, L. G. and Martell, A. E. *Stability Constants, Chem. Soc. Spec.Publ. No. 17*, London 1964, and *Suppl. No. 1*, London 1969.
4. Davies, C. W. *J. Chem. Soc.* (1938) 277.
5. a. Schubert, J. and Lindenbaum, A. *J. Am. Chem. Soc. 74* (1952) 3529; b. Schubert, J., Russell, E. R. and Meyers, L. S. *J. Biol. Chem. 185* (1950) 387.
6. Chibnall, B. K. and Cannan, R. A. *Biochem. J. 27* (1933) 945.
7. a. Leussing, D. L. and Schultz, D. C. *J. Am. Chem. Soc. 86* (1964) 4846; b. Leussing, D. L. and Hanna, E. M. *J. Am. Chem. Soc. 88* (1966) 693; c. Tallman, D. E. and Leussing, D. L. *J. Am. Chem. Soc. 91* (1969) 6253.
8. Gelles, E. and Hay, R. W. *J. Chem. Soc.* (1958) 3673.
9. Tavale, S. S., Pant, L. M. and Biswas, A. B. *Acta Crystallogr. 14* (1961) 1281.
10. Forsberg, O., Johansson, K. E. and Kierkegaard, P. *To be published.*
11. a. Ulmgren, P. and Wahlberg, O. *Acta Chem. Scand. 25* (1971) 1000; b. Wahlberg, O. *Acta Chem. Scand. 25* (1971) 1045; c. Forsberg, O., Johansson, K., Ulmgren, P. and Wahlberg, O. *Chem. Scr. 3* (1973) 153; d. Ulmgren, P. and Wahlberg, O. *Acta Chem. Scand. A 28* (1974) 631; e. Ulmgren, P. and Wahlberg, O. *Chem. Scr. 8* (1975) 126; f. Ulmgren, P. and Wahlberg, O. *Chem. Commun. Univ. Stockholm* (1970) No. 4.
12. a. Arnek, R., Sillén, L. G. and Wahlberg, O. *Ark. Kemi 31* (1968) 353; b. Brauner, P., Sillén, L. G. and Whiteker, R. *Ark. Kemi 31* (1968) 365; c. Ingri, N., Kakolowicz, W., Sillén, L. G. and Warnqvist, B. *Talanta 14* (1967) 1261.
13. Pedersen, K. J. *Acta Chem. Scand. 6* (1952) 243.
14. Persson, H. *Acta Chem. Scand. 25* (1971) 1775.
15. Leden, I. *Sven. Kem. Tidskr. 58* (1946) 129.
16. Gerding, P. *Acta Chem. Scand. 22* (1968) 1283.

Received November 17, 1977.