Accessory publication

Capillary electrophoresis study of iron(II) and iron(III) polyaminocarboxylate complex speciation

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Calculation of electrophoretic mobilities

The observed electrophoretic velocity of an analyte, v_{obs}, is the distance from the point of injection to the detector (L_d, cm) divided by the migration time (t_m, in s).

$$v_{obs} = \frac{L_d}{t_m} \quad \text{(A1)}$$

The applied potential (P, V, divided by the total capillary length (L_t, cm) is the electric field strength (E, V cm^{-1}), and indicates the voltage drop across the capillary.

$$E = \frac{P}{L_t} \quad \text{(A2)}$$

Analyte velocity divided by the electric field strength yields the observed electrophoretic mobility (μ_{obs}, cm^2 V^{-1} s^{-1}).

$$\mu_{obs} = \frac{v_{obs}}{E} = \frac{L_d}{t_m} \cdot \frac{L_t}{P} \quad \text{(A3)}$$

Using Eqn A3, the observed electrophoretic mobility can be empirically calculated for any peak from its migration time.[1] When the wall on the capillary bears a net charge, electroosmotic flow (EOF) causes movement of the electrolyte fluid within the capillary.[2] Thus, the electrophoretic mobility observed during a CE separation is the sum of the analyte mobility due to EOF (μ_{eof}) and the effective electrophoretic mobility (μ_{eff}).

$$\mu_{obs} = \mu_{eff} + \mu_{eof} \quad \text{(A4)}$$

The contribution of EOF to analyte mobility (μ_{eof}) is determined from the time required for a neutral analyte to reach the detector.[1] The migration time corresponding to the neutral marker 4-methyl-3-penten-2-one was used to calculate μ_{eof} according to Eqns A3 and A4.

Calibration curves for iron complexes with EGTA and TMDTA

Sample calibration curves for EGTA and TMDTA complexes with Fe^{II} and Fe^{III} are presented in Fig. A1. CE separations and detection of complexes was performed as described in the text.
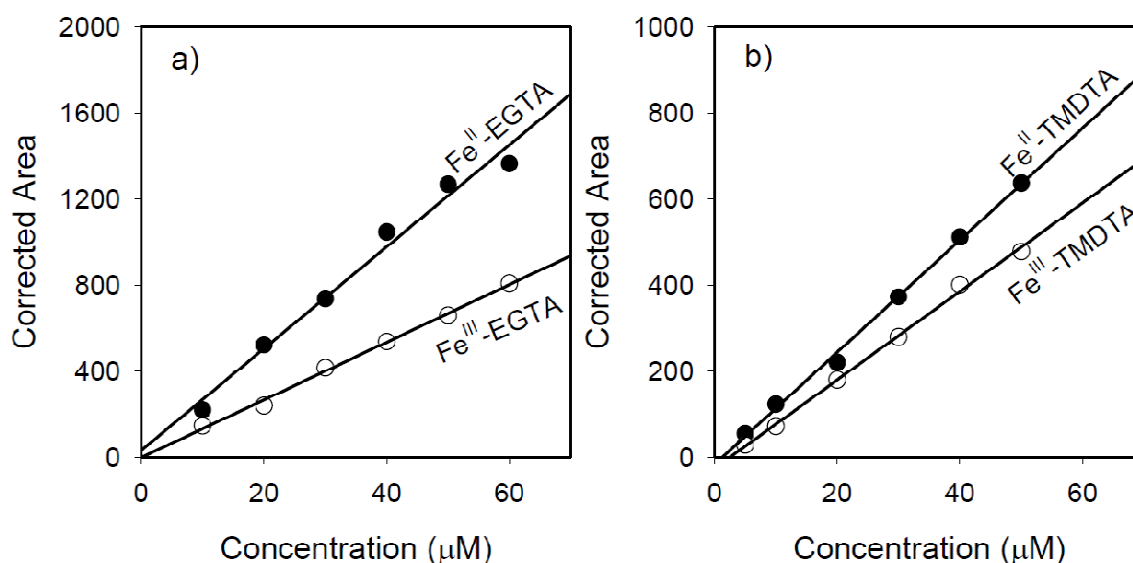

Fig. A1.   Calibration curves for Fe^{II}–EGTA, Fe^{III}–EGTA, Fe^{II}–TMDTA and Fe^{III}–TMDTA complexes using the MOPS BGE method. All standards solutions contained 10 mM NaCl and either 10 mM MES (a) or 10 mM MOPS (b) to keep pH constant. The MOPS BGE consisted of 50 mM MOPS (pH = 7.1) and 0.5 µM TTAB. Values of R^2 from all four curves were between 0.996 and 0.997.

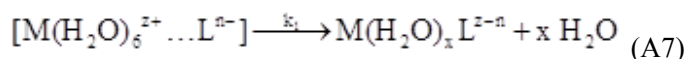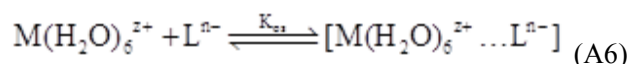**Dissociation of iron–polyaminocarboxylate complexes**

Metal ions are often classified as substitution-inert or substitution-labile based upon their characteristic rates of water exchange from their aquo-complex ions (Eqn A5).[3]

$$[M(H_2O)_n]^{z+} + H_2O^* \overset{k_{H_2O}}{\rightleftharpoons} [M(H_2O)_{n-1}(H_2O^*)]^{z+} + H_2O \quad (A5)$$
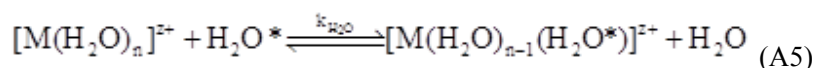
Half-lives for water exchange of metal ions span 20 orders of magnitude, ranging from 10^{-10} to 10^{10} s.[4] The water exchange rate constant, k_{H_2O}, for $[Fe^{III}(H_2O)_6]^{3+}$ is 1.6×10^2 s^{-1}.[5] This yields a half-life of $10^{-2.36}$ s (4.3 ms) which is in the middle of this range. Time scales for association and dissociation of Fe^{III} complexes with other monodentate ligands are comparable.[6] As ligand exchange of Fe^{III} monodentate complexes occurs much faster than the time required for an electrophoretic separation, these complexes

are expected to dissociate during electromigration and peaks for individual metal-complexes are not expected. However, when multidentate ligands (i.e. chelating agents) are complexed to metal ions, dissociation rates are considerably lower.[6]

Dissociation rate constants for the Fe^{II} and Fe^{III}-chelating agent complexes examined in this study have not been measured or reported in the literature. The following method was used to estimate dissociation rate constants of metal complexes. For a 1:1 metal ion-chelating agent complex, the complexation reaction is described by the Eigen–Wilkins mechanism:

$$M(H_2O)_6^{z+} + L^{n-} \xrightleftharpoons{K_{os}} [M(H_2O)_6^{z+} \ldots L^{n-}] \quad (A6)$$

$$[M(H_2O)_6^{z+} \ldots L^{n-}] \xrightarrow{k_1} M(H_2O)_x L^{z-n} + x\,H_2O \quad (A7)$$

where $[M(H_2O)_6^{z+} \ldots L^{n-}]$ represents an outer-sphere complex, K_{os} represents the outer-sphere association constant (in units of M^{-1}), and $M(H_2O)_x L^{z-n}$ represents the 1:1 complex. This mechanism assumes that formation of the first bond between metal and chelating agent is the rate limiting step for complex formation and is described by the rate constant k_1 (s^{-1}). The rate of complex formation (Rate$_f$) is:

$$Rate_f = k_1[M(H_2O)_6^{z+} \ldots L^{n-}] \quad (A8)$$

Using a pre-equilibrium assumption, Eqn A8 can be simplified to be written as the following expression:

$$Rate_f = K_{os}k_1[M(H_2O)_6^{z+}][L^{n-}] \quad (A9)$$

The association rate constant, k_a (L mol^{-1} s^{-1}) is therefore the following[6]:

$$k_a = K_{os}k_1 \quad (A10)$$

The value of K_{os} can be estimated using the Fuoss–Eigen equation,[7] and the value of k_1 (s^{-1}) can be estimated from the water exchange rate constant, which is known for many metal ions or can be estimated.[4]

Equilibrium occurs when the forward and backward reaction rates are equal. Thus,

$$k_a[M(H_2O)_6^{z+}][L^{n-}] = k_d[M(H_2O)_x L^{n-}] \quad (A11)$$

where k_d is the dissociation rate constant (s^{-1}). Simple rearrangement of A11 results in the following expression:

$$\frac{k_a}{k_d} = \frac{[M(H_2O)_x L^{n-}]}{[M(H_2O)_6^{z+}][L^{n-}]} = K_{ML} \quad (A12)$$

where K_{ML} is the formation constant for the 1:1 complex. The value of k_d can be estimated by rearrangement of Eqn A12 provided that k_a is known or can be estimated and that the proper value for the equilibrium constant, K_{ML} is employed.[6]

The relevant reaction for the association/dissociation of Fe[III]–TMDTA at pH 7.1 is the following:

$$Fe(OH)_2^+ + H_2tmdta^{2-} \rightleftharpoons Fe^{III}(tmdta)^- + 2H_2O \qquad\qquad K_{ML} \quad (A13)$$
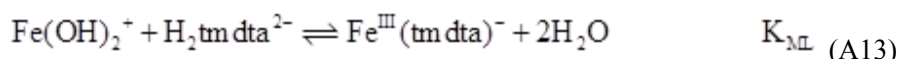
where K_{ML} is the equilibrium constant:

$$K_{ML} = \frac{[Fe^{III}(tmdta)^-]}{[Fe(OH)_2^+][H_2tmdta^{2-}]} \quad (A14)$$

The reactants and products shown in Eqn A13 are the predominant forms of the metal ion, chelating agent and metal ion-chelating agent complex at pH 7.1.

To calculate the value of K_{ML}, the equilibrium reaction shown in Eqn A13 was broken up into the following individual equilibrium reactions:

$$Fe^{3+} + tmdta^{4-} \rightleftharpoons Fe^{III}(tmdta)^- \qquad\qquad K_1 = 10^{21.61} \ (I = 0.1 \text{ M}) \quad (A15)$$

$$FeOH^{2+} + H^+ \rightleftharpoons Fe^{3+} + H_2O \qquad\qquad 1/{}^*K_1 = 10^{2.19} \ (I = 0.0 \text{ M}) \quad (A16)$$

$$Fe(OH)_2^+ + H^+ \rightleftharpoons FeOH^{2+} + H_2O \qquad\qquad 1/{}^*K_2 = 10^{5.6} \ (I = 0.0 \text{ M}) \quad (A17)$$

$$Htmdta^{3-} \rightleftharpoons tmdta^{4-} + H^+ \qquad\qquad {}^*K_1 = 10^{-10.3} \ (I = 0.1 \text{ M}) \quad (A18)$$

$$H_2tmdta^{2-} \rightleftharpoons tmdta^{3-} + H^+ \qquad\qquad {}^*K_2 = 10^{-7.9} \ (I = 0.1 \text{ M}) \quad (A19)$$

Equilibrium constants shown in these equations were taken from the NIST CRITICAL database.[8] The equilibrium constant K_{ML} (Eqn A14) was calculated by taking the product of the series of equilibrium constants. Ionic strength corrections were made using the Davies equation. Following the procedure outlined above, we obtained a value of $10^{12.2}$ for K_{ML} ($I = 0.01$ M).

We must now estimate the value of k_a from k_1 and K_{os}. The rate of water exchange of $Fe(OH)_2^+$ has not been measured, however we estimate it at a value of s^{-1} using the value for $FeOH^{2+}$ (1.2×10^5 s^{-1})[5] and applying a 10^3-fold increase in lability due to incorporation of the additional hydroxide ion into the inner coordination shell of Fe^{3+}.[6]

Values of K_{os} can be estimated by using the Fuoss–Eigen[7] equation:

$$K_{os} = \frac{4000\pi N_A r^3}{3} \exp\left(-\frac{U}{RT}\right) \quad (A20)$$

where U is the Debye–Huckel interionic potential, and r is the separation between reactants. The Debye–Huckel interionic potential is given by the following[6,9]:

$$U = \frac{N_A z_M z_L e^2}{4\pi\varepsilon\varepsilon_0 r} - \frac{N_A z_M z_L e^2 \kappa}{4\pi\varepsilon\varepsilon_0 (1+\kappa r)} \quad (A21)$$
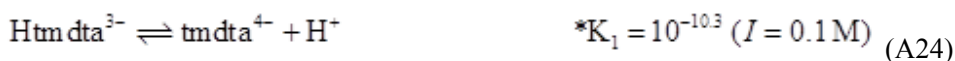
where z_M is the charge on the metal ion, z_L is the charge on the oxygen atom, e is the charge of the electron, N_A is Avogadro's number, ε_0 is the permittivity of free space, ε is the dielectric constant of the medium, and κ is the inverse Debye length. The outer-sphere association constant calculated using the Fuoss–Eigen equation[7] for interaction of +1 and –2 ions at a separation distance of 5 Å and an ionic strength of 0.01 M is 4.0 M^{-1}. Plugging this result into Eqn A10, we estimate a value of k_a equal to 5 × 10^8 s^{-1}.

Using the values for k_a and K_{ML}, we estimate the value of k_d for Fe^{III}–TMDTA to be ~3 × 10^{-4} s^{-1}. This indicates that dissociation is extremely slow at pH 7.1. The time scale of dissociation of this complex is much longer than time scales of CE separations. Estimates of the dissociation rate constants for the other Fe^{III} complexes employed in this study are also very low. Based on these calculations, Fe^{III} complexes with polyaminocarboxylate chelating agents are not expected to dissociate during electromigration.

Based upon water exchange from their aquo-complex ions, Fe^{2+} is usually assumed to be more labile than Fe^{3+}. The relevant reaction for the association/dissociation of Fe^{II}–TMDTA at pH 7.1, is the following:

$$Fe^{2+} + H_2 tmdta^{2-} \rightleftharpoons Fe^{II}(tmdta)^{2-} + 2H^+ \qquad\qquad K'_{ML} \quad (A22)$$
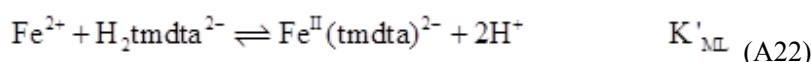
Using the same analysis as described above for Fe^{III}–TMDTA, we can calculate the value of K'_{ML}. The equilibrium constant shown in Eqn A22, K'_{ML}, can be calculated by considering the following individual equilibrium reactions:

$$Fe^{2+} + tmdta^{4-} \rightleftharpoons Fe^{II}(tmdta)^{2-} \qquad\qquad \log K_1 = 10^{13.42} \ (I = 0.1\,M) \quad (A23)$$

$$Htmdta^{3-} \rightleftharpoons tmdta^{4-} + H^+ \qquad\qquad {}^*K_1 = 10^{-10.3} \ (I = 0.1\,M) \quad (A24)$$

$$H_2 tmdta^{2-} \rightleftharpoons tmdta^{3-} + H^+ \qquad\qquad {}^*K_2 = 10^{-7.9} \ (I = 0.1\,M) \quad (A25)$$

After making the appropriate ionic strength corrections, the product of these two equilibrium constants yields a value of K'_{ML} equal to $10^{-4.6}$ ($I = 0.01$ M). Finally, the conditional equilibrium constant at pH 7.1 is obtained by incorporating $[H^+]$ into K'_{ML}:

$$K_{ML} = \frac{[Fe^{II}(tmdta)^{2-}]}{[Fe^{2+}][H_2tmdta^{2-}]} = \frac{K'_{ML}}{[H^+]^2} \quad (A26)$$

At pH 7.1, these results in a value of K_{ML} equal to $10^{9.6}$.

Table A1.   Thermodynamic data from the NIST Critical database[8] for Fe^{II} and Fe^{III} complexes with polyaminocarboxylate chelating agents

Fe^{II} complexes	
$Fe^{2+} + edta^{4-} = Fe^{II}$–EDTA^{2-}	log K_1 = 14.30 (25°C, 0.1 M)
Fe^{II}–(H)EDTA$^-$ = Fe^{II}–EDTA^{2-} + H$^+$	log K_{a1} = −2.0 (25°C, 0.1 M)
$Fe^{2+} + cdta^{4-} = Fe^{II}$–CDTA^{2-}	log K_1 = 18.9 (25°C, 0.1 M)
Fe^{II}–(H)CDTA$^-$ = Fe^{II}–CDTA^{2-} + H$^+$	log K_{a1} = −2.7 (25°C, 0.1 M)
$Fe^{2+} + tmdta^{4-} = Fe^{II}$–TMDTA^{2-}	log K_1 = 13.42 (20°C, 0.1 M)
Fe^{II}–(H)TMDTA$^-$ = Fe^{II}–TMDTA^{2-} + H$^+$	log K_{a1} = −3.34 (20°C, 0.1 M)
$Fe^{2+} + dtpa^{5-} = Fe^{II}$–DTPA^{3-}	log K_1 = 16.2 (25°C, 0.1 M)
Fe^{II}–(H)DTPA^{2-} = Fe^{II}–DTPA^{3-} + H$^+$	log K_{a1} = −5.30 (25°C, 0.1 M)
Fe^{II}–DTPA^{3-} = Fe^{II}–(OH)DTPA^{4-} + H$^+$	log K_{a2} = −8.77 (25°C, 0.1 M)
Fe^{II}–(OH)DTPA^{4-} = Fe^{II}–(OH)$_2$DTPA^{5-} + H$^+$	log K_{a3} = −9.41 (25°C, 0.1 M)
$Fe^{2+} + hedta^{3-} = Fe^{II}$–HEDTA$^-$	log K_1 = 12.2 (25°C, 0.1 M)
Fe^{II}–(H)HEDTA^0 = Fe^{II}–HEDTA$^-$ + H$^+$	log K_{a1} = −5.12 (25°C, 0.1 M)
$Fe^{2+} + egta^{4-} = Fe^{II}$–EGTA^{2-}	log K_1 = 11.8 (25°C, 0.1 M)
Fe^{II}–(H)EGTA$^-$ = Fe^{II}–EGTA^{2-} + H$^+$	log K_{a1} = −4.47 (20°C, 0.1 M)
Fe^{III} complexes	
$Fe^{3+} + edta^{4-} = Fe^{III}$–EDTA$^-$	log K_1 = 25.1 (25°C, 0.1 M)
Fe^{III}–(H)EDTA^0 = Fe^{III}–EDTA$^-$ + H$^+$	log K_{a1} = −1.3 (25°C, 0.1 M)
Fe^{III}–EDTA$^-$ = Fe^{III}–(OH)EDTA^{2-} + H$^+$	log K_{a2} = −7.39 (25°C, 0.1 M)
$Fe^{3+} + cdta^{4-} = Fe^{III}$–CDTA$^-$	log K_1 = 30.0 (25°C, 0.1 M)
Fe^{III}–CDTA$^-$ = Fe^{III}–(OH)CDTA^{2-} + H$^+$	log K_{a1} = −9.32 (25°C, 1.0 M)
$Fe^{3+} + tmdta^{4-} = Fe^{III}$–TMDTA$^-$	log K_1 = 21.61 (20°C, 0.1 M)
Fe^{III}–(H)TMDTA^0 = Fe^{III}–TMDTA$^-$ + H$^+$	log K_{a1} = −2.45 (20°C, 0.1 M)
Fe^{III}–TMDTA$^-$ = Fe^{III}–(OH)TMDTA^{2-} + H$^+$	log K_{a2} = −9.0 (25°C, 0.1 M)
$Fe^{3+} + dtpta^{5-} = Fe^{III}$–DTPA^{2-}	log K_1 = 27.7 (25°C, 0.1 M)
Fe^{III}–(H)DTPA$^-$ = Fe^{III}–DTPA^{2-}+ H$^+$	log K_{a1} = −3.56 (25°C, 0.1 M)
Fe^{III}–DTPA^{2-} = Fe^{III}(OH)DTPA $^{3-}$ + H$^+$	log K_{a2} = −9.66 (25°C, 0.1 M)
$Fe^{3+} + hedta^{3-} = Fe^{III}$–HEDTA^0	log K_1 = 19.7 (25°C, 0.1 M)
Fe^{III}–HEDTA^0 = Fe^{III}–(OH)HEDTA$^-$ + H$^+$	log K_{a1} = −3.88 (25°C, 0.1 M)
Fe^{III}–(OH)HEDTA$^-$ = Fe^{III}–(OH)$_2$HEDTA^{2-} + H$^+$	log K_{a2} = −8.83 (25°C, 0.1 M)
Fe^{III}–(OH)$_2$HEDTA^{2-} = Fe^{III}–(OH)$_3$HEDTA^{3-} + H$^+$	log K_{a3} = −10.0 (25°C, 0.1 M)
$Fe^{3+} + egta^{4-} = Fe^{III}$–EGTA$^-$	log K_1 = 20.5 (25°C, 0.1 M)
Fe^{III}–(H)EGTA^0 = Fe^{III}–EGTA$^-$ + H$^+$	log K_{a1} = −3.01 (25°C, 0.1 M)

Values for K_{os} and k_1 are required to calculate k_a. A value of K_{os} equal to is 50 M^{-1} was calculated using the Fuoss–Eigen equation[7] for interaction of +2 and −2 ions at a separation distance of 5 Å and an ionic strength of 0.01 M. The rate constant for water exchange for Fe^{2+} provides the estimate for k_1 (4.4×10^6 s^{-1}).[6] Plugging this result into Eqn A10, we estimate a value of k_a equal to 2×10^8 s^{-1}.

Using the values for k_a and K_{ML}, we estimate the value of k_d for Fe^{II}–TMDTA to be ~6×10^{-2} s^{-1} (0.2 min) which indicates that dissociation is occurring on the time–scale of electrophoretic separations. However, association of the complex is extremely rapid ($k_a = 2 \times 10^8$ s^{-1}) such that significant migration of free metal and free ligand in opposite directions is not expected. Based on these calculations, it is not surprising that Fe^{II} complexes with polyaminocarboxylate chelating agents were successfully separated using CE.

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