Environ. Chem. **2013**, *10*, 409–416
doi:10.1071/EN13103_AC

Supplementary material

Capillary electrophoresis facilitates determination of metal complex stoichiometry by Job's method of continuous variation

Nathan E. Boland[A,B] *and Alan T. Stone*[A]

[A]Department of Geography and Environmental Engineering, Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21218, USA.

[B]Corresponding author. Present address: Whitman College, Hall of Science, 345 Boyer Avenue, Walla Walla, WA 99362, USA. Email: bolandne@whitman.edu

Proof of Job's method of continuous variation

Derivations provided here are based upon earlier treatments.[1–3] Suppose that the free metal ion and free ligand combine to form a single, simple complex with stoichiometry (M_pY_q) according to:

$$pM + qY \leftrightarrow M_pY_q \quad (1)$$

The equilibrium constant corresponding to this reaction is defined as:

$$K_{pq} = \frac{[M_pY_q]}{[M]^p[Y]^q} \quad (2)$$

Rearranging yields:

$$[M_pY_q] = K_{pq}[M]^p[Y]^q \quad (3)$$

It is assumed that the metal ion and ligand are either 'free' or part of this complex. Mass balance equations for total metal concentration (M_T) and total ligand concentration (Y_T) can be written as:

$$M_T = M + p[M_pY_q] \quad (4)$$

$$Y_T = Y + q[M_pY_q] \quad (5)$$

Job's method of continuous variation (hereafter Job's method) is based upon a series of experiments where (i) the sum of M_T and Y_T is a constant, which we will call X_T, and (ii) the ratio of M_T to X_T, which we will call R, is increased:

$$X_T = M_T + Y_T \quad (6)$$

$$R = \frac{M_T}{X_T} = \frac{M_T}{M_T + Y_T} \quad (7)$$

Eqns 4 and 5 can be rewritten using this ratio:

$$[\mathrm{M}] = X_\mathrm{T}R - p[\mathrm{M}_p\mathrm{Y}_q] \quad (8)$$

$$[\mathrm{Y}] = X_\mathrm{T}(1 - R) - q[\mathrm{M}_p\mathrm{Y}_q] \quad (9)$$

Next, absorbance or some other quantity proportional to $[\mathrm{M}_p\mathrm{Y}_q]$ is plotted as a function of R. The derivatives of Eqns 3, 8 and 9 with respect to R are relevant:

$$\frac{\mathrm{d}\left[\mathrm{M}_p\mathrm{Y}_q\right]}{\mathrm{d}R} = pK_{pq}\left[\mathrm{M}\right]^{p-1}\left[\mathrm{Y}\right]^q\frac{\mathrm{d}\left[\mathrm{M}\right]}{\mathrm{d}R} + qK_{pq}\left[\mathrm{M}\right]^p\left[\mathrm{Y}\right]^{q-1}\frac{\mathrm{d}\left[\mathrm{Y}\right]}{\mathrm{d}R} \quad (10)$$

$$\frac{\mathrm{d}\left[\mathrm{M}\right]}{\mathrm{d}R} = X_\mathrm{T} - \frac{p\mathrm{d}\left[\mathrm{M}_p\mathrm{Y}_q\right]}{\mathrm{d}R} \quad (11)$$

$$\frac{\mathrm{d}\left[\mathrm{Y}\right]}{\mathrm{d}R} = -X_\mathrm{T} - \frac{q\mathrm{d}\left[\mathrm{M}_p\mathrm{Y}_q\right]}{\mathrm{d}R} \quad (12)$$

We would like to find $R_{\max}$, the value of R that yields the maximum value of $[\mathrm{M}_p\mathrm{Y}_q]$. This maximum value should correspond to the point where $\mathrm{d}[\mathrm{M}_p\mathrm{Y}_q]/\mathrm{d}R$ is equal to zero. At this point in the 'Job's plot', Eqns 11 and 12 become:

$$\left[\frac{\mathrm{d}\left[\mathrm{M}\right]}{\mathrm{d}R}\right]_{R_{\max}} = X_\mathrm{T} \quad (13)$$

$$\left[\frac{\mathrm{d}\left[\mathrm{Y}\right]}{\mathrm{d}R}\right]_{R_{\max}} = -X_\mathrm{T} \quad (14)$$

Inserting these results into Eqn 10 and again setting $\mathrm{d}[\mathrm{M}_p\mathrm{Y}_q]/\mathrm{d}R$ is equal to zero yields:

$$pK_{pq}\left[\mathrm{M}\right]_{R_{\max}}^{p-1}\left[\mathrm{Y}\right]_{R_{\max}}^{q}X_\mathrm{T} - qK_{pq}\left[\mathrm{M}\right]_{R_{\max}}^{p}\left[\mathrm{Y}\right]_{R_{\max}}^{q-1}X_\mathrm{T} = 0 \quad (15)$$

Algebra then yields:

$$p\left[\mathrm{Y}\right]_{R_{\max}} - q\left[\mathrm{M}\right]_{R_{\max}} = 0 \quad (16)$$

Inserting Eqns 8 and 9 yields:

$$pX_\mathrm{T}\left(1 - R_{\max}\right) - pq\left[\mathrm{M}_p\mathrm{Y}_q\right]_{\max} - qX_\mathrm{T}R_{\max} + pq\left[\mathrm{M}_p\mathrm{Y}_q\right]_{\max} = 0 \quad (17)$$

Additional algebra yields:

$$pX_\mathrm{T} - pX_\mathrm{T}R_{\max} - qX_\mathrm{T}R_{\max} = 0 \quad (18)$$

$$p - pR_{\max} - qR_{\max} = 0 \quad (19)$$

$$R_{\max} = \frac{p}{p+q} \quad (20)$$

Eqn 20 can be rearranged to yield:

$$\frac{p}{q} = \frac{\mathrm{R}_{\max}}{1-\mathrm{R}_{\max}} \quad (21)$$

In summary, Job's method requires completing a set of experiments where $X_T = M_T + Y_T$ is kept constant while the quantity $R = M_T/X_T$ is increased. Plots of absorbance or some other quantity proportional to $[M_pY_q]$ as a function of R are then constructed. The maximum (or minimum) of these plots reveals R_{max}, the value of R where the concentration of the metal–ligand complex ($[M_pY_q]$) reaches its maximum value. Eqn 21 is then used to determine the stoichiometric ratio p/q of the complex.

Electromigration in capillary electrophoresis (CE)

The discussion provided below is based on prior publications.[4,5] Electroosmotic flow (EOF) is the net movement of the buffer solution (BGE) through the capillary under the influence of an applied electric field. When running in 'cation mode', the capillary wall comprises silanol groups that, owing to deprotonation, yield a net negative wall charge above pH 3. According to the Principle of Electroneutrality, the charge on the capillary wall must be counterbalanced by a layer of cationic counterions in overlying solution. Applying an electrical potential causes these cations to electromigrate towards the cathode. Through friction, the electromigrating cations entrain the BGE solution, yielding a net 'electroosmotic' flow (μ_{EOF}) of the BGE solution towards the cathode. When running in cation mode, sample is injected at the anode end of the capillary and the detector is placed near the cathode end of the capillary.

'Anion mode' involves addition of a cationic surfactant such as tetradecyltrimethylammonium bromide (TTAB) to the BGE solution, which adsorbs to the capillary wall, giving it a net positive charge. That charge, in turn, is counterbalanced by a layer of anions in the BGE solution. Applying an electrical potential causes these anions to electromigrate towards the anode, yielding an electroosmotic flow, μ_{EOF}, in that direction. When running in anion mode, the sample is injected at the cathode end of the capillary and the detector is placed near the anode end of the capillary.

The migration velocity of an anionic analyte (v_{obs}) through a capillary is found by dividing the length of capillary from the point of injection to the point of detection (L_d) by the time required for the analyte to reach the detector (t_m). Dividing the entire length of the capillary (L_T) by the applied potential (P) yields the electric field gradient (E). In order to compare runs performed using different capillary lengths and different applied potentials, it is first necessary to calculate an observed electrophoretic mobility (μ_{obs}):

$$\mu_{obs} = \nu_{obs} = \left[\frac{L_T}{P}\right]\left[\frac{L_d}{t_m}\right] \quad (22)$$

Next, we must subtract the electroosmotic flow contribution to mobility (μ_{eof}), which yields an effective electrophoretic mobility (μ_{eff}):

$$\mu_{eff} = \mu_{obs} - \mu_{EOF} \quad (23)$$

Electroosmotic flow acts equally on all solutes, and hence μ_{EOF} can be calculated from values of t_m obtained with a neutral chromophoric analyte. Effective electrophoretic mobilities are indicative of the charge and hydrodynamic radii of analytes, but are also influenced by the viscosity and ionic strength of the BGE.

Modelling Ni–DTPA speciation

Calculations including equilibrium constants for Ni–hydroxo species and Ni-containing hydroxide and oxide precipitates[6] indicate that the sum of Ni–hydroxo species is 0.1 % or less of Ni_T at pH 6.9 and neither $Ni(OH)_2(s)$ nor NiO(s) is predicted to form. These species are neglected in subsequent calculations.

With equilibrium constants X_T and Ni_T/X_T as inputs, equilibrium concentrations of each Ni–DTPA complex were calculated (at pH 7 and 25-mM ionic strength). The mass balance equations for all Ni-containing species (Ni_T) and DTPA-containing species ($DTPA_T$) are relevant:

$$Ni_T = [Ni^{2+}] + [NiDTPA] + 2[Ni_2DTPA] + 3[Ni_3DTPA_2] \quad (24)$$

$$DTPA_T = [DTPA]_{free} + [NiDTPA] + [Ni_2DTPA] + 2[Ni_3DTPA_2] \quad (25)$$

At pH 7, $[DTPA]_{free}$ is present almost entirely as $H_2(dtpa)^{3-}$. NiDTPA has two relevant protonation levels, $Ni(dtpa)^{3-}$ and $NiH(dtpa)^{2-}$. At pH 7, both $Ni(dtpa)^{3-}$ and $NiH(dtpa)^{2-}$ are present in large percent amounts. However, during an electrophoretic separation, these species will interchange rapidly and will co-electromigrate and we need only consider the sum of the two species (simply NiDTPA). As a result, we can write more specific forms of the mass balance equations above:

$$Ni_T = Ni^{2+} + [NiDTPA] + 2[Ni_2(dtpa)^{1-}] + 3[Ni_3(dtpa)_2^{4-}] \quad (26)$$

$$DTPA_T = [H_2(dtpa)^{3-}] + [NiDTPA] + [Ni_2(dtpa)^{1-}] + 2[Ni_3(dtpa)_2^{4-}] \quad (27)$$

Using appropriate equilibrium expressions from Table 1, we can write Eqns 26 and 27 in terms of equilibrium constants, $[Ni^{2+}]$ and $[dtpa^{5-}]$

$$\begin{aligned} Ni_T = {} & \left[Ni^{2+}\right] + K_1\left[Ni^{2+}\right]\left[dtpa^{5-}\right] + K_{1H}K_1\left[Ni^{2+}\right]\left[H^+\right]\left[dtpa^{5-}\right] \\ & + 2K_1K_2\left[Ni^{2+}\right]^2\left[dtpa^{5-}\right] + 3K_1K_2K_3\left[Ni^{2+}\right]^3\left[dtpa^{5-}\right]^2 \end{aligned} \quad (28)$$

$$\mathrm{DTPA_T} = \frac{\left[\mathrm{dtpa^{5-}}\right]\left[\mathrm{H^+}\right]^2}{\left[\mathrm{H^+}\right]^2 + \left[\mathrm{H^+}\right]K_{a1} + K_{a1}K_{a2}} + K_1\left[\mathrm{Ni^{2+}}\right]\left[\mathrm{dtpa^{5-}}\right] + K_{1H}K_1\left[\mathrm{Ni^{2+}}\right]\left[\mathrm{H^+}\right]\left[\mathrm{dtpa^{5-}}\right] + K_1K_2\left[\mathrm{Ni^{2+}}\right]^2\left[\mathrm{dtpa^{5-}}\right] + 2K_1K_2K_3\left[\mathrm{Ni^{2+}}\right]^3\left[\mathrm{dtpa^{5-}}\right]^2 \quad (29)$$

Rearranging Eqn 29 gives a quadratic equation:

$$a\left[\mathrm{dtpa^{5-}}\right]^2 + b\left[\mathrm{dtpa^{5-}}\right] + c = 0 \quad (30)$$

where,

$$a = 2K_1K_2K_3\left[\mathrm{Ni^{2+}}\right]^3,$$

$$b = K_1\left[\mathrm{Ni^{2+}}\right] + K_{1H}K_1\left[\mathrm{Ni^{2+}}\right]\left[\mathrm{H^+}\right] + K_1K_2\left[\mathrm{Ni^{2+}}\right]^2 + \frac{\left[\mathrm{H^+}\right]^2}{\left[\mathrm{H^+}\right]^2 + \left[\mathrm{H^+}\right]K_{a1} + K_{a1}K_{a2}} \text{ and}$$

$$c = \mathrm{DTPA_T}$$

Using the quadratic formula, we can solve for $[\mathrm{dtpa^{5-}}]$.

$$\left[\mathrm{dtpa^{5-}}\right] = \frac{-b \pm \sqrt{\left(b^2 - 4ac\right)}}{2a} \quad (31)$$

The flowchart in Fig. S1 describes how the equations above were implemented in a Microsoft Excel spreadsheet with iterative calculations enabled. The resulting equilibrium model allowed us to converge on the value of $[\mathrm{Ni^{2+}}]$ and $[\mathrm{dtpa^{5-}}]$ at each X_T and $\mathrm{Ni_T}/X_T$, and calculate the equilibrium concentration of each Ni–DTPA complex. The results of this model are plotted as dashed lines in Fig. 5 (and Fig. S6, see *Influence of differences in ionic strength on Job's plot maxima*).

A variation of the equilibrium model was used to calculate equilibrium concentrations of NiDTPA and $\mathrm{Ni_2DTPA}$ when formation of $\mathrm{Ni_3DTPA_2}$ is not considered. Terms for $\mathrm{Ni_3DTPA_2}$ were omitted from the mass balance equations. The results of this model are plotted as solid lines in Fig. 5 (and Fig. S6, see *Influence of differences in ionic strength on Job's plot maxima*).

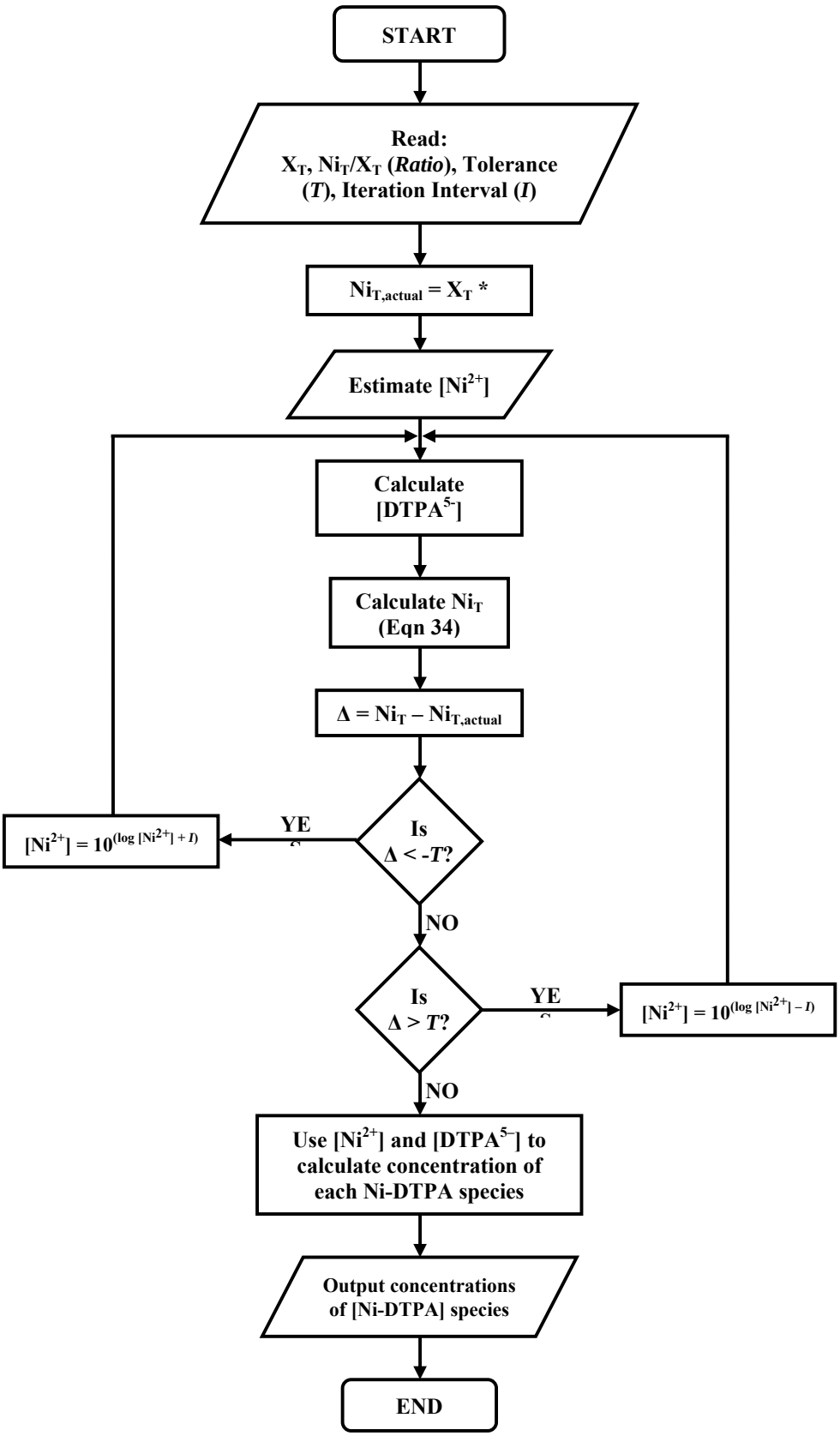

Fig. S1. Flowchart for calculating Ni–diethylenetriaminepentaacetate (DTPA) speciation.

Environ. Chem. **2013**, *10*, 409–416
doi:10.1071/EN13103_AC

Table S1. Consideration of stoichiometries for possible Ni–diethylenetriaminepentaacetate (DTPA) complexes

$DTPA_{free}$, Ni_{free}, NiDTPA, Ni_2DTPA, p, q and R_{max} have already been described in the literature. Positive values for Coordinative unsaturation indicate excess DTPA Lewis base groups and negative values indicate excess Ni coordinative positions. Bold entries indicate known (1:1 and 2:1) and proposed (3:2) stoichiometries.

Stoichiometry (p:q)	Empirical formula (Ni_pDTPA_q)	R_{max} ($p/(p+q)$)	Number of components ($p+q$)	Overall charge	Coordinative unsaturation ($8q-6p$)
0:1	$DTPA_{free}$	0	1	–5	8
1:6	$NiDTPA_6$	0.143	7	–28	42
1:5	$NiDTPA_5$	0.167	6	–23	34
1:4	$NiDTPA_4$	0.200	5	–18	26
2:7	Ni_2DTPA_7	0.222	9	–31	44
1:3	$NiDTPA_3$	0.250	4	–13	18
2:5	Ni_2DTPA_5	0.286	7	–21	28
1:2	$NiDTPA_2$	0.333	3	–8	10
3:5	Ni_3DTPA_5	0.375	8	–19	22
2:3	Ni_2DTPA_3	0.400	5	–11	12
3:4	Ni_3DTPA_4	0.429	7	–14	14
4:5	Ni_4DTPA_5	0.444	9	–17	16
1:1	**NiDTPA**	**0.500**	**2**	**–3**	**2**
5:4	Ni_5DTPA_4	0.556	9	–10	2
4:3	Ni_4DTPA_3	0.571	7	–7	0
3:2	**Ni_3DTPA_2**	**0.600**	**5**	**–4**	**–2**
5:3	Ni_5DTPA_3	0.625	8	–5	–6
2:1	**Ni_2DTPA**	**0.667**	**3**	**–1**	**–4**
5:2	Ni_5DTPA_2	0.714	7	0	–14
3:1	Ni_3DTPA	0.750	4	+1	–10
7:2	Ni_7DTPA_2	0.778	9	+4	–26
4:1	Ni_4DTPA	0.800	5	+3	–16
5:1	Ni_5DTPA	0.833	6	+5	–22
6:1	Ni_6DTPA	0.857	7	+7	–28
7:1	Ni_7DTPA	0.875	8	+9	–34
8:1	Ni_8DTPA	0.889	9	+11	–40
1:0	Ni_{free}	1.00	1	+2	–6

Job's plots developed using different detection wavelengths

If each CE peak corresponds to a unique chromophore, then moving to a different detection wavelength should alter the magnitude of the detector response at each value of Ni_T/X_T, reflecting different molar absorptivities at the different wavelengths. General trends in the Job's plot, and the position of the Job's plot maximum, should not change.

Recall that effective electrophoretic mobilities of free DTPA and NiDTPA are similar. With Peak A, at low values of Ni_T/X_T, both species contribute significantly to the CE response. In Fig. S2, the portion of

the Job's plots using 194, 214 and 254-nm detection when Ni_T/X_T is less than 0.3 are a reflection of this. At higher values of Ni_T/X_T, the contribution from NiDTPA is predominant. As a result, all three detection wavelengths yield the same trend, and indeed yield the same maximum.

The CE response for Peak B comes almost entirely from Ni_3DTPA_2, whereas the CE response for Peak C comes from Ni_2DTPA. As a result, all three detection wavelengths yield the same Job's plot trends and the same maxima.

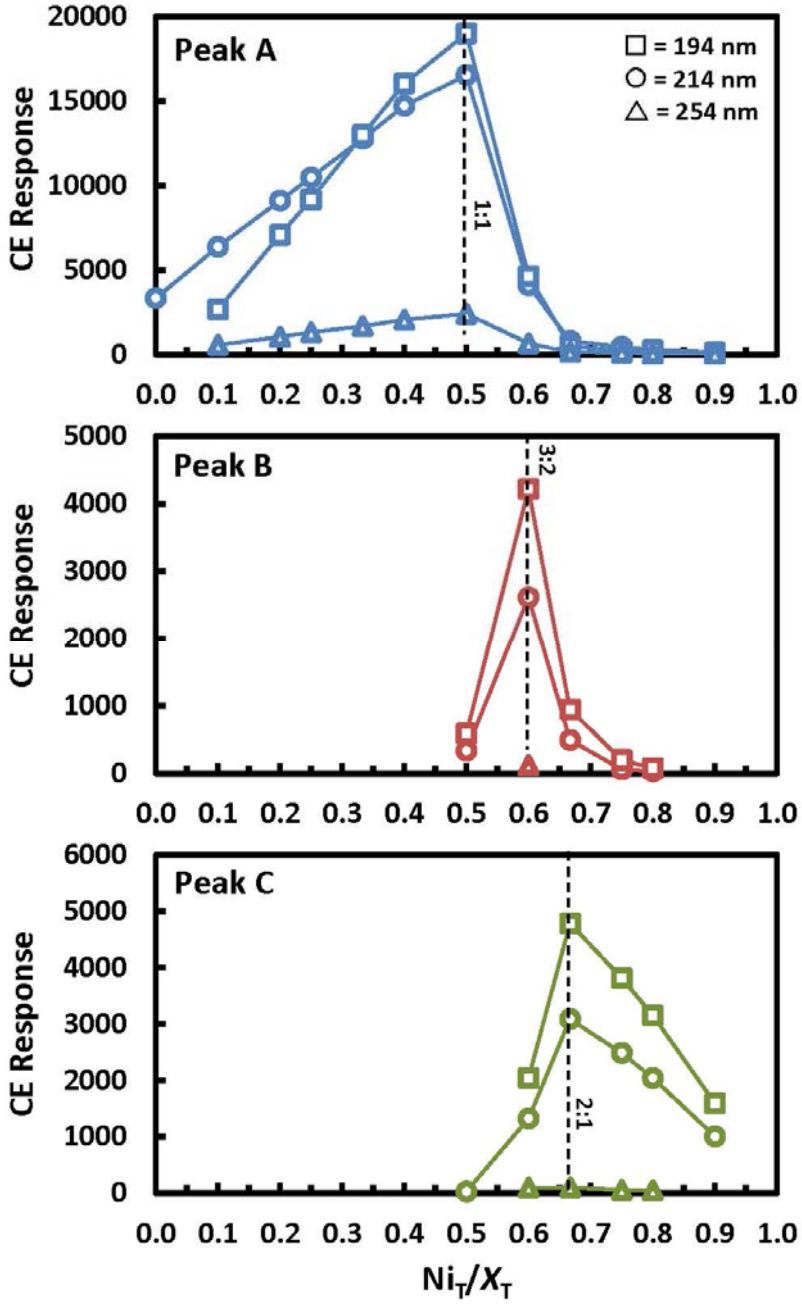

Fig. S2. Job's plots for electropherogram peaks at three different wavelengths (194, 214 and 254 nm) for solutions with X_T = 1000 μM. Capillary electrophoresis (CE) response corresponds to normalised absorbance output of the CE instrument.

Job's plots and assignment of Ni–DTPA complex stoichiometry

For each possible stoichiometry for a Ni–DTPA complex, denoted as Ni_pDTPA_q, an expected Job's plot maximum, R_{max}, can be calculated. As noted in *Proof of Job's method of continuous variation*, R_{max} corresponds to $p/(p + q)$. NiDTPA and Ni_2DTPA have already been described in the literature. They yield R_{max} values of 0.50 and 0.67, which coincide with our experimentally observed R_{max} values for Peaks A and C.

Twenty-seven possible stoichiometries are compiled in Table S1. DTPA possesses eight Lewis base groups and Ni has a coordination number of six. Hence, it was not necessary to consider stoichiometries requiring one Ni cation to coordinate more than six DTPA molecules, or one DTPA molecule to coordinate more than eight Ni cations.

The number of components refers to the sum $(p + q)$. Although it is true that the concentrations of all species decrease as X_T is decreased, the decrease becomes more pronounced as the number of components is increased. (Recall from Eqn 3 that $[M_pY_q]$ is proportional to $[M]^p[Y]^q$.) Table S1 is restricted to complexes where the number of components is nine or less, i.e. those most likely to be significant at the values of X_T investigated.

UV spectroscopy of Ni–DTPA solutions

Complex formation constants available in the literature for NiDTPA and Ni_2DTPA enabled us to calculate speciation for a wide range of Ni_T and Y_T conditions. All solutions contained 5.0 mM 3-morpholinopropane sulfonic acid (MOPS, pH 6.9) and enough NaCl to fix the ionic strength at 25 mM. When Ni_T is equal to 250 μM and $DTPA_T$ equal to 375 μM, NiDTPA represents nearly 100 % of all Ni–DTPA species. We prepared this solution and recorded the whole-sample spectrum shown in Fig. S3a using a conventional UV spectrometer. When Ni_T is equal to 1000 μM and $DTPA_T$ equal to 375 μM, Ni_2DTPA represents nearly 94 % of all Ni–DTPA species. We prepared this solution and recorded the whole-sample spectrum shown in Fig. S3c.

Using the CE results and Job's plots, we were able to estimate a complex formation constant for the species Ni_3DTPA_2, as described in the main text. Using this new complex formation constant, a new set of calculations can be performed. Calculations indicate that the concentration of Ni_3DTPA_2 is highest when Ni_T is equal to 750 μM and $DTPA_T$ equal to 500 μM. (Under these conditions, 146 μM NiDTPA, 100 μM Ni_2DTPA and 109 μM Ni_3DTPA_2 are calculated.) We prepared this solution and recorded the whole-sample spectrum shown in Fig. S3b.

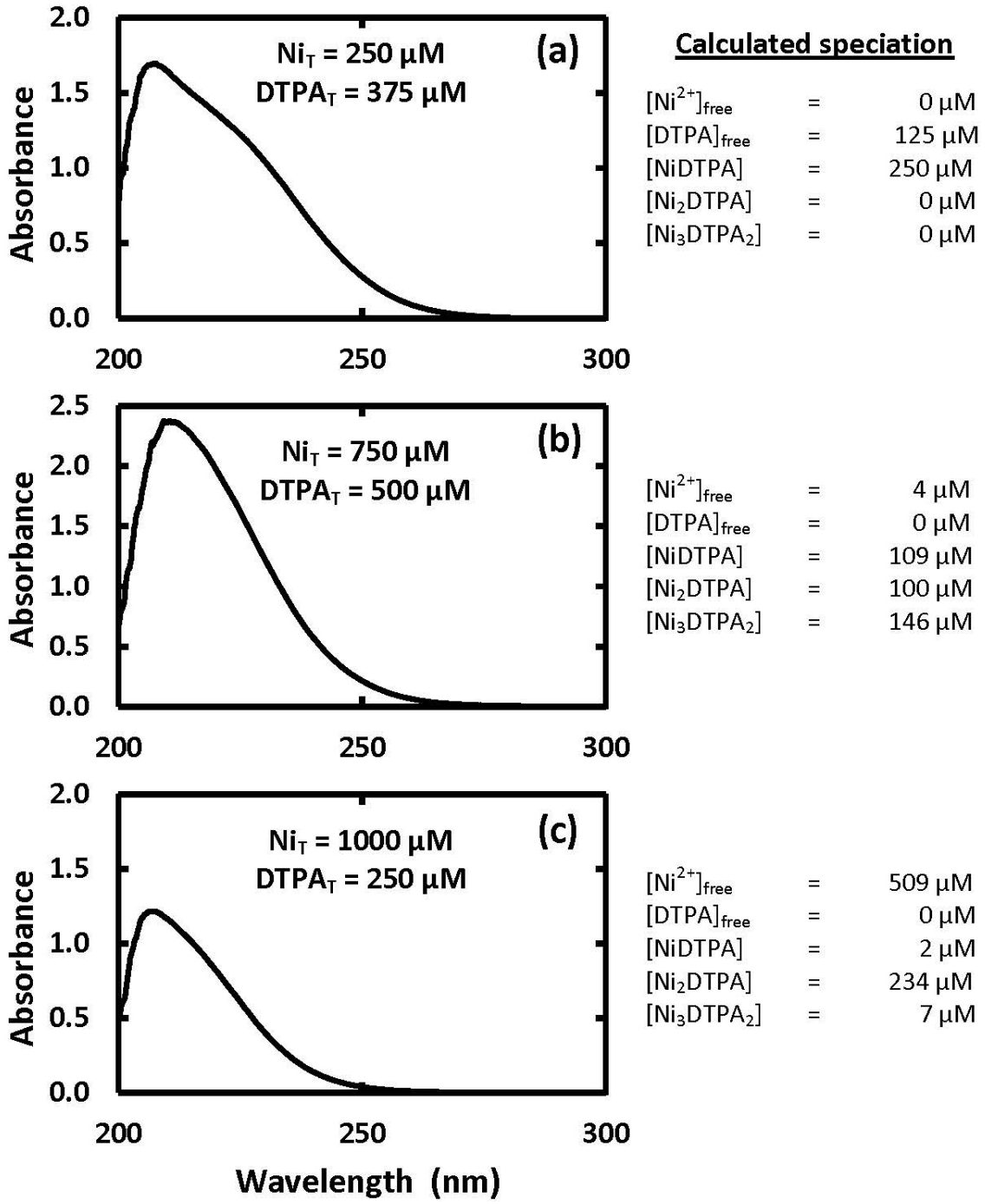

Fig. S3. Representative whole-sample spectra for solutions where (a) NiDTPA (DTPA, diethylenetriaminepentaacetate) predominates; (b) concentrations of NiDTPA, Ni_2DTPA and Ni_3DTPA_2 are all significant and (C) Ni_2DTPA predominates. All solutions contained 5.0 mM MOPS (pH 6.9) and enough NaCl to attain an ionic strength of 25 mM. Equilibrium calculations were performed using the equilibrium constants listed in Table 1.

Quantification of Ni–DTPA complex concentrations

Identification and quantification of the three Ni–DTPA complexes solely on the basis of whole-sample UV spectroscopy would be challenging given the similarity of the spectra in Fig. S2. Separation by CE is beneficial. A CE separation method for separating the three Ni–DTPA complexes is described in *Capillary electrophoresis* of the main text. Calibration curves (Fig. S4) were generated for free DTPA (as described in *Capillary electrophoresis peak assignments* in the main text), and for the three Ni–DTPA complexes (as described in *Quantification of Ni–DTPA complexes* in the main text).

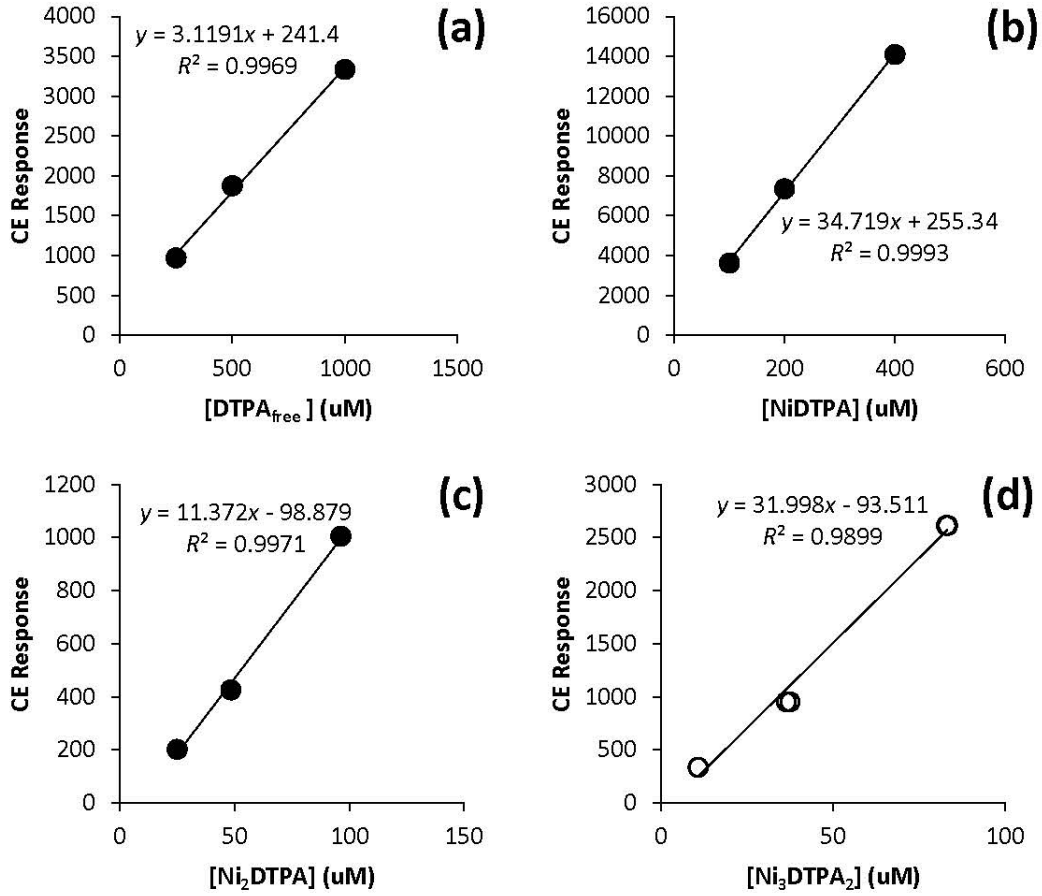

Fig. S4. Calibration curves for (a) $DTPA_{free}$, (b) NiDTPA, (c), Ni_2DTPA and (d) Ni_3DTPA_2 (DTPA, diethylenetriaminepentaacetate).

Influence of differences in ionic strength on Job's Plot maxima

Variations in ionic strength and pH influence chemical equilibria and cause deviations in Job's plot maxima.[7] Species with high charge may be influenced significantly by shifts in ionic strength. In Fig. S5, equilibrium concentrations of Ni–DTPA complexes in solutions with X_T = 5000 μM were calculated at 10 and 25-mM ionic strength. The concentration of $Ni_3(dtpa)_2^{4-}$ formed at 10-mM ionic strength is significantly lower than at 25-mM ionic strength. In addition, the Job's plot maxima of the $Ni_3(dtpa)_2^{4-}$ complex has deviated slightly to lower values of Ni_T/X_T.

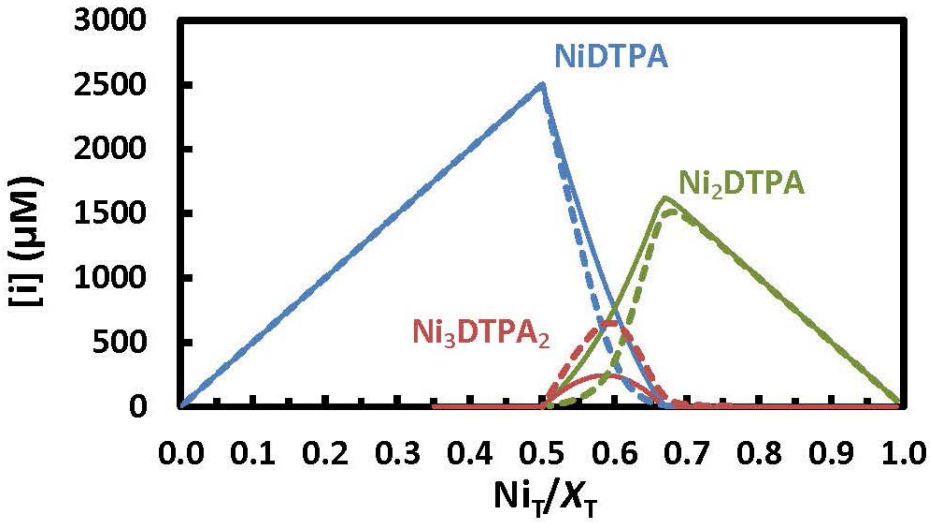

Fig. S5. Calculated concentrations of Ni–diethylenetriaminepentaacetate (DTPA) complexes at two different ionic strengths, 10 and 25 mM, represented by thin solid lines and dashed lines. X_T = 5000 μM. Activity coefficients calculated using the Davies equation were used to convert reported ^cK (Table 1 in main text) to the ^cK at the desired ionic strength.

All data used to determine stoichiometries and log *K*s in the main text are shown in Table S2. Table S3 shows data that was excluded due to insufficient pH buffer and indifferent electrolyte to fix the pH and ionic strength. For X_T = 2500 μM the pH was 6.94 ± 0.16 and the ionic strength was 20 ± 5 mM. For X_T = 5000 μM, the pH was 6.9 ± 0.3 and the ionic strength was 17 ± 7 mM. Deviations from predicted speciation (at pH 7 and 25-mM ionic strength) are apparent when the data from Table S3 are used to generate Job's plots (Fig. S6).

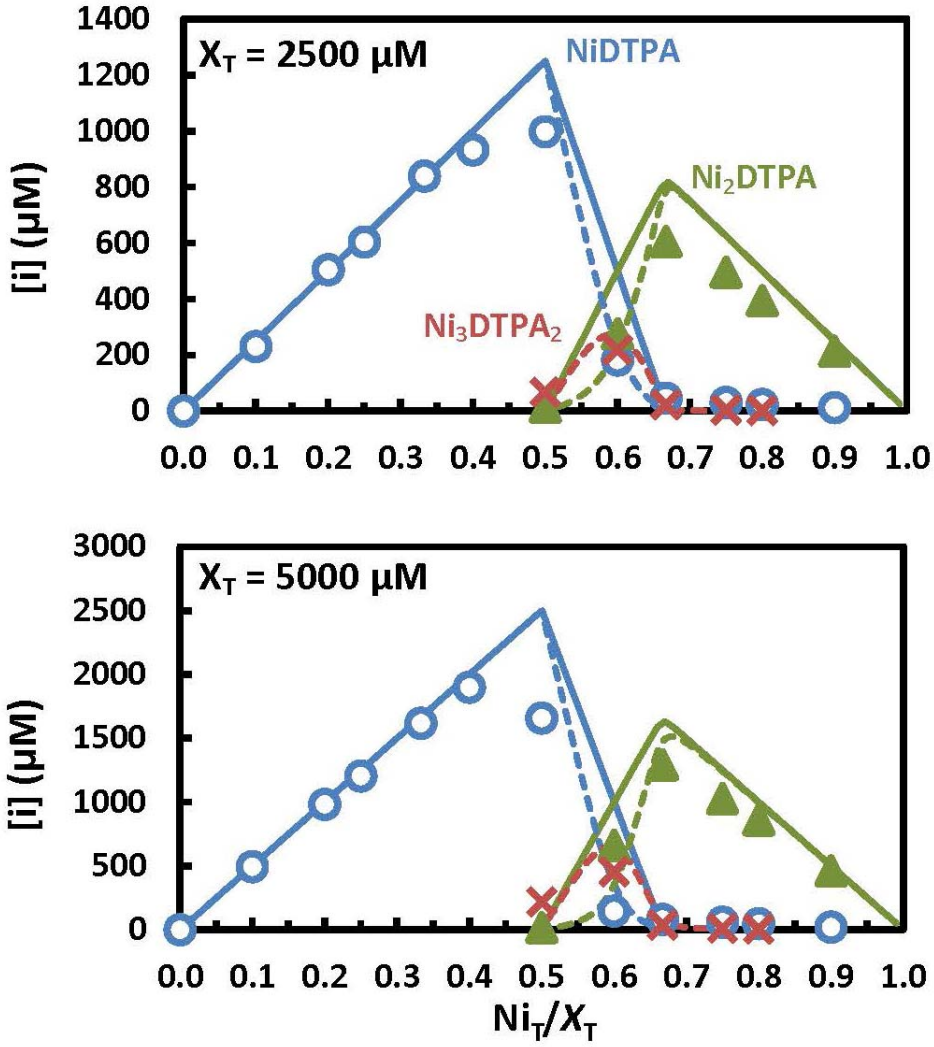

Fig. S6. Concentrations of Ni–diethylenetriaminepentaacetate (DPTA) complexes as a function of Ni_T/X_T at two different values of X_T. Symbols correspond to experimentally determined concentrations. Solid lines correspond to results from equilibrium calculations that consider only NiDTPA and Ni_2DPTA. Dashed lines correspond to results from equilibrium calculations that include the proposed Ni_3DTPA_2 species. Equilibrium calculations are described in the main text.

Table S2. Experimental data

All solutions contained 10 mM 3-morpholinopropane sulfonic acid (6.94 ± 0.07). n.d., not detected. DTPA, diethylenetriaminepentaacetate, $X_T = Ni_T + DTPA_T$

Solution composition				Capillary electorphoresis response (in absorbance units × 10^{-3})		
X_T (μM)	Ni_T (μM)	$DTPA_T$ (μM)	[NaCl] (mM)	Peak A	Peak B	Peak C
250	225.0	25.0	23.0	n.d.	n.d.	202
250	200.0	50.0	23.0	73	n.d.	526
250	187.5	62.5	23.0	109	n.d.	645
250	166.75	83.75	23.0	331	91	761
250	150.0	100.0	23.0	1365	337	445
250	125.0	125.0	23.0	4054	53	n.d.
250	100.0	150.0	23.0	3781	n.d.	n.d.
250	83.25	166.25	23.0	3312	n.d.	n.d.
250	62.5	187.5	23.0	2849	n.d.	n.d.
250	50.0	200.0	23.0	2269	n.d.	n.d.
250	25.0	225.0	23.0	1601	n.d.	n.d.
250	0	250.0	23.0	971	n.d.	n.d.
500	450.0	50.0	22.5	56	n.d.	426
500	400.0	100.0	22.4	156	n.d.	821
500	375.0	125.0	22.4	223	n.d.	1025
500	333.5	166.5	22.3	502	185	1250
500	300.0	200.0	22.3	2314	953	688
500	250.0	250.0	22.3	7758	153	n.d.
500	200.0	300.0	22.2	7665	n.d.	n.d.
500	166.5	333.5	22.2	6390	n.d.	n.d.
500	125.0	375.0	22.1	5336	n.d.	n.d.
500	100.0	400.0	22.1	4616	n.d.	n.d.
500	50.0	450.0	22.1	3352	n.d.	n.d.
500	0.0	500.0	22.0	1876	n.d.	n.d.
1000	900.0	100.0	20.9	127	n.d.	1005
1000	800.0	200.0	20.8	302	29	2040
1000	750.0	250.0	20.8	459	58	2486
1000	667.0	333.0	20.7	804	492	3087
1000	600.0	400.0	20.6	4094	2613	1322
1000	500.0	500.0	20.5	16533	333	26
1000	400.0	600.0	20.4	14715	n.d.	n.d.
1000	333.0	667.0	20.3	12766	n.d.	n.d.
1000	250.0	750.0	20.3	10467	n.d.	n.d.
1000	200.0	800.0	20.2	9112	n.d.	n.d.
1000	100.0	900.0	20.1	6370	n.d.	n.d.
1000	0.0	1000.0	20.0	3335	n.d.	n.d.

Table S3. Experimental data not used in determination of stoichiometries and log *K*s owing to greater variability in ionic strength and pH

All solutions contained 10 mM 3-morpholinopropane sulfonic acid (6.94 ± 0.07). n.d., not detected.

DTPA, diethylenetriaminepentaacetate, $X_T = Ni_T + DTPA_T$

Solution composition					Capillary electorphoresis response (in absorbance units × 10^{-3})		
X_T (μM)	Ni_T (μM)	$DTPA_T$ (μM)	[NaCl] (mM)	Ionic strength (mM)	Peak A	Peak B	Peak C
2500	2250.0	250.0	16.6	25.9	414	n.d.	2130
2500	2000.0	500.0	16.1	22.6	816	48	3934
2500	1875.0	625.0	15.9	20.9	1079	129	4953
2500	1667.5	832.5	15.5	18.4	1619	757	6040
2500	1500.0	1000.0	15.2	20.8	6435	7438	2754
2500	1250.0	1250.0	14.8	22.6	35308	2292	129
2500	1000.0	1500.0	14.3	23.3	34720	n.d.	n.d.
2500	832.5	1667.5	14.0	23.8	32562	n.d.	n.d.
2500	625.0	1875.0	13.6	24.3	25657	n.d.	n.d.
2500	500.0	2000.0	13.4	24.7	23022	n.d.	n.d.
2500	250.0	2250.0	13.0	25.3	15027	n.d.	n.d.
2500	0.0	2500.0	12.5	26.0	8288	n.d.	n.d.
5000	4500.0	500.0	8.2	24.7	734	n.d.	4608
5000	4000.0	1000.0	7.4	18.2	1668	195	8590
5000	3750.0	1250.0	7.0	14.9	2091	363	10254
5000	3335.0	1665.0	6.3	9.9	2851	1257	12900
5000	3000.0	2000.0	5.8	15.0	5154	15544	6596
5000	2500.0	2500.0	5.0	18.5	58673	7500	171
5000	2000.0	3000.0	4.2	20.0	70655	n.d.	n.d.
5000	1665.0	3335.0	3.6	21.0	62994	n.d.	n.d.
5000	1250.0	3750.0	3.0	22.1	51206	n.d.	n.d.
5000	1000.0	4000.0	2.6	22.9	45091	n.d.	n.d.
5000	500.0	4500.0	1.8	24.3	31225	n.d.	n.d.
5000	0.0	5000.0	1.0	25.8	16298	n.d.	n.d.

Proof that concentrations of NiDTPA and Ni_2DTPA are equal when Ni_T/X_T is equal to 0.6

In *Quantification of Ni–DTPA complexes* of the main text it is asserted that when Ni_T/X_T = 0.6, the concentrations of NiDTPA and Ni_2DTA are equal. This is true because of the correlation between the ratios of Ni_T/X_T in the stoichiometry of each species at Ni_T/X_T = 0.6. Let us first examine the speciation of NiDTPA and Ni_2DTPA while ignoring the formation of Ni_3DTPA_2. At Ni_T/X_T = 0.6, the mass balance of total nickel (Ni_T) and total DTPA ($DTPA_T$) are defined by

$$\mathrm{Ni_T} = 0.6X_\mathrm{T} = \left[\mathrm{Ni^{2+}}\right] + \left[\mathrm{NiDTPA}\right] + 2\left[\mathrm{Ni_2DTPA}\right] \quad (33)$$

$$\mathrm{DTPA_T} = 0.4X_\mathrm{T} = \left[\mathrm{DTPA}\right]_\mathrm{free} + \left[\mathrm{NiDTPA}\right] + \left[\mathrm{Ni_2DTPA}\right] \quad (34)$$

At Ni_T/X_T = 0.6, several Ni–DTPA complexes form so it is reasonable to assume that $[Ni^{2+}]$ and $[DTPA]_{free}$, the sum of concentrations of all DTPA species not bound to Ni, are both small. Solving Eqns 33 and 34 simultaneously gives:

$$0.6\left[\mathrm{NiDTPA}\right] + 0.6\left[\mathrm{Ni_2DTPA}\right] = 0.4\left[\mathrm{NiDTPA}\right] + 0.8\left[\mathrm{Ni_2DTPA}\right] \quad (35)$$

which simplifies to

$$\left[\mathrm{NiDTPA}\right] = \left[\mathrm{Ni_2DTPA}\right] \quad (36)$$

Thus, at Ni_T/X_T = 0.6, the concentrations of NiDTPA and Ni_2DTPA are the same.

The result remains the same if we include Ni_3DTPA_2. Here the mass balance equations for Ni_T and $DTPA_T$ are:

$$\mathrm{Ni_T} = 0.6X_\mathrm{T} = \left[\mathrm{Ni^{2+}}\right] + \left[\mathrm{NiDTPA}\right] + 2\left[\mathrm{Ni_2DTPA}\right] + 3\left[\mathrm{Ni_3DTPA_2}\right] \quad (37)$$

$$\mathrm{DTPA_T} = 0.4X_\mathrm{T} = \left[\mathrm{DTPA}\right]_\mathrm{free} + \left[\mathrm{NiDTPA}\right] + \left[\mathrm{Ni_2DTPA}\right] + 2\left[\mathrm{Ni_3DTPA_2}\right] \quad (38)$$

Again, it is reasonable to assume that both $[Ni^{2+}]$ and $[DTPA]_{free}$ are small. Solving Eqns 37 and 38 simultaneously gives:

$$0.6\left[\mathrm{NiDTPA}\right] + 0.6\left[\mathrm{Ni_2DTPA}\right] + 1.2\left[\mathrm{Ni_3DTPA_2}\right] = 0.4\left[\mathrm{NiDTPA}\right] + 0.8\left[\mathrm{Ni_2DTPA}\right] + 1.2\left[\mathrm{Ni_3DTPA_2}\right] \quad (39)$$

which, again, simplifies to:

$$[\mathrm{NiDTPA}] = [\mathrm{Ni_2DTPA}] \quad (40)$$

Thus, at Ni_T/X_T = 0.6, the concentrations of NiDTPA and Ni_2DTPA are also the same even when formation of Ni_3DTPA_2 is included.

References

[1] P. Job, Formation and stability of inorganic complexes in solution. *Annales de Chimie* **1928**, *9*, 113.

[2] F. Woldbye, On the method of continuous variations. *Acta Chem. Scand.* **1955**, *9*, 299. doi:10.3891/acta.chem.scand.09-0299.

[3] M. T. Beck, I. Nagypál, *Chemistry of Complex Equilibria*. (Eds D. R. Williams, D. A. Durham) **1990** (Halsted Press: New York).

[4] D. R. Baker, *Capillary Electrophoresis, 1st edn* **1995** (Wiley: New York).

[5] A. Jouyban, E. Kenndler, Theoretical and empirical approaches to express the mobility of small ions in capillary electrophoresis. *Electrophoresis* **2006**, *27*, 992. doi:10.1002/elps.200500696.

[6] A. E. Martell, R. M. Smith, R. Motekaitis, *NIST Critically Selected Stability Constants of Metal Complexes Database v. 8.0* **2004** (National Institute of Science and Technology: Gaithersburg, MD).

[7] M. M. Jones, K. K. Innes, Restrictions on the use of Job's method. *J. Phys. Chem.* **1958**, *62*, 1005. doi:10.1021/j150566a031.