A novel approach to monitoring of the diffusion junction potential in speciation studies by polarography under very acidic conditions. Part II: The quasi-reversible Cu(II)-picolinic acid system.

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Abstract

The use of polarography to accurately determine stability constants of complexes formed under very acidic conditions (below pH 2) is demonstrated. The diffusion junction potentials, which must be accounted for below pH 2, were evaluated by applying protocols developed where Tl(I) is used as an internal reference. The Cu(II)-picolinic acid (2-pyridinecarboxylic acid) system studied was chosen since the CuL^+ species only exists in solution below pH 2 under the conditions used and literature data exists to confirm the accuracy of procedure. Additionally, the reduction of Cu(II) was quasi-reversible and procedures to determine the reversible half-wave potentials were investigated. Average log β values of 7.75 ± 0.09 for CuL^+ and 14.8 ± 0.1 for CuL_2 were obtained, which compared well to literature data.

Keywords: Acidic conditions, diffusion junction potential, picolinic acid, quasi-reversible, stability constants.

1. Introduction

Determining stability constants of metal-ligand complexes under acidic conditions is not trivial. Any technique that employs a reference electrode (RE) has limited accuracy in determining formation constants for species formed below about pH 2. This is due to the large and pH-dependent diffusion junction potential (E_j) that is formed between the RE filling solution and the sample solution. Glass electrode potentiometry (GEP) has even further limitations under very acidic conditions because mass-balance equations, which are solved for the free hydrogen ion concentration from the deprotonation of the ligand caused by the complexation reaction, cannot be accurately solved when the background solution contains more than about 10^{-2} M strong acid. [1,2] Additionally, irrespective of the technique that is used, the pH of the solution has to be measured and the E_j constrains the accuracy with which this can be done.

When a metal ion is characterized by high affinity to a ligand then complexation can start at extremely low pH

and the copper(II)–picolinic acid (Cu–L) system is one among many such examples. Knowledge of chelating properties (which incorporates a metal-ligand model and formation constants of all identified metal species formed) is of importance in many fields [1], hence there were numerous attempts to model the Cu–L system [3-10] using a range of analytical techniques, as shown in Table 1. When using spectrophotometry [3-5], calorimetry [6] or a copper amalgam electrode [7], stability constants for CuL and CuL_2 were determined. However, a large spread in the reported data is observed exemplifying difficulty in studying metal complexes under highly acidic conditions. It is known that GEP is unparalleled in the determination of formation constants and this technique alone provided the majority of all known data in the field of metal ion complexation. [1] As can be seen in Table 1, however, stability constant only for CuL_2, which could be investigated only above pH ~2, was determined by GEP [8] which clearly illustrates intrinsic limitation of this technique as also reported by Suzuki *et al.* [3] who observed irreproducible

results generated from GEP. Furthermore, data for CuL, which is fully formed already in highly acidic medium, were not reported from voltammetry [5,6].

The CuL complex was found and formation constant determined in buffered sample solutions from ligand titrations employing a copper amalgam electrode [7]. This approach minimized errors coming from the junction potential at the reference electrode and we also explored the ligand titration method in this work but without the use of buffers. We avoid employing buffers because (i) our aim is to develop a general-purpose methodology which could also be suitable for highly acidic metal ions, such as bismuth(III), which can only be studied by acid-base titrations [11] and (ii) certain metal ions, and Cu(II) in particular, might be complexed by buffers; it has been reported that Cu(II) forms strong complexes even with monoprotonated ligands which are commonly used in preparing buffers for biological applications. [12]

Table 1. Literature formation constants for the copper(II)–picolinic acid system at the given ionic strength (μ) and temperatures.

log K_1	log K_2	log β_2	T /°C	μ /M	Technique	Ref
8.6	7.4	16.0	25	0.1	Spec	[3]
8.73	6.78	15.51	25	1	Spec	[4]
7.9	6.6	14.5	20	0.2	Spec	[5]
7.71	6.83	14.54	25	1	Cal	[6]
7.95	7.00	14.95	20	0.1	Cu-Hg	[7]
	6.0		25	0.02	GEP	[8]
		14.88	20	0.2	Voltam	[5]
		15.35	20	0.5	Voltam	[9]
		16.1	25	0.2	Voltam	[10]

Spec = Spectrophotometry; Cal = Calorimetry; Cu-Hg = Cu(II) amalgam electrode; Voltam = Voltammetry

In previous work, [13] the authors demonstrated that polarography could be used to determine formation constants in solutions at pH < 2, provided the diffusion junction potential was compensated for. The variation in E_j values was monitored in-situ as a function of pH by measuring the reduction potential of thallium(I) which was added to the test solution. It is important to stress that Tl(I) is not chelated by picolinic acid up to at least pH 8 [13] and its hydrolysis only occurs above pH 11 (log K = 0.30 for TlOH at 25 °C and 0.5 M ionic strength [14]). Because of that, any change in the reduction potentials for Tl(I) below pH ~2 could be attributed to changes in E_j. It was also demonstrated that the free or uncomplexed metal ion potential (E(M)) for the metal ion of interest, a critical parameter for calculating formation constants determined by polarography, could be more

accurately determined by relating it to the free Tl(I) potential (E(Tl)). [13]

When studying the Cd(II) picolinic acid system starting from pH 0.3, a novel MLH species was found to exist in the low pH region. Even though it was a minor species in solution under the conditions used which resulted in only about a 2 mV shift, with the accurate compensation for E_j (as well as taking the effect of the changing ionic strength on the reduction potentials of the metals ions into account) information about this species was not lost. To support the existence of this species, a crystal was grown from an acidic solution with Cd(II) and picolinic acid concentrations such that the MLH species would be dominant in solution. The crystal structure showed that the pyridine nitrogen was protonated and the Cd(II) was bound to the two carboxylate oxygen atoms, indicating the MLH species. [15] This demonstrates the robustness of the strategies developed and the protocols used to interpret polarographic data to study metal-ligand equilibria in general and under very acidic conditions in particular.

In order to fully test these procedures, the Cu(II)-picolinic acid system was selected because the CuL species exists at pH 0 already. Since log β for CuL has been established (by other techniques) and recommended values are quoted in the NIST database [14] containing critically assessed literature data, it was decided to evaluate the log β values for both CuL and CuL$_2$ by polarography and compare it to these values.

A further complication arose as Cu(II) was not reversibly reduced in nitrate solutions, the background anion utilised in this work. The kinetic properties of the electron transfer process had to be considered as the reversible reduction potentials are required to apply the theories leading to the rigorous evaluation of stability constants. [16, 17] The additional aim was thus to find the most suitable protocol to determine the reversible half-wave potential from quasi-reversible DC waves and several approaches were considered.

2. Experimental

2.1. Materials

All reagents were analytical grade. Stock solutions of 0.100 M Tl(I) nitrate and 0.100 M Cu(II) nitrate were both made up in 0.5 M HNO$_3$. Deionized water of resistivity 18 MΩ cm was always used.

2.2. Instrumentation

Polarographic experiments were carried out using the same cell and automated instrumental setup as previously described [13]. Sampled DC polarography was employed with a step time of 1 s, a current integration time of 60 ms and a step potential of 4 mV.

The kind of a glass electrode (GE) which was found to work exceptionally well in highly acidic medium (Metrohm cat. no. 6.0234.100) [2], was calibrated by the titration of standardised solutions of 0.5 M HNO$_3$ with 0.5 M NaOH and the calibration plot was calculated. Because the accurate measurement of pH is critical in this work, a deviation from linearity of the GE response in the very acidic region had to be accounted for. To this effect, a quadratic function was fitted to experimentally recorded GE potential readings in the highly acidic region to minimise E_j errors [2]. In this work pH signifies $-\log[H^+]$.

2.3. Procedure

Three types of experiments were run as fully described before. [13] Firstly, to investigate the variation in E_j with pH, several polarographic pH titration experiments were performed in a solution containing 0.5 M HNO$_3$ and the two metals ions, 9.97×10^{-5} M Cu(II) and 1.99×10^{-4} M Tl(I), in the absence of ligand. Secondly, Cu(II)-picolinic acid equilibria were studied by polarographic pH titration, where initial solutions were the same as above. To this effect, solid picolinic acid was added such that the total ligand-to-Cu(II) concentration ratios were 32, 103 and 207 for three separate experiments. In all these polarographic pH titrations, the titrant was 0.5 M NaOH and the pH step was about 0.1. At each pH step a polarogram and the GE potential were recorded and selected polarograms are shown in Fig. S1a. Thirdly, Cu(II)-picolinic acid equilibria were studied by polarographic ligand titration in three separate experiments at pH 0.47, 0.90 and 1.37. In this case the initial solution consisted of a mixture of 0.5 M HNO$_3$, 0.5 M NaOH and 9.97×10^{-5} M Cu(II) such that the required pH was attained. The titrant was a 0.5 M picolinic acid solution adjusted to the same pH as the test solution and the total ligand-to-Cu(II) concentration ratios were varied stepwise between 20 and 200 with a polarogram recorded at each step (see Fig. S1b for selected polarograms). The pH was kept constant throughout the titration by adding a few μL of 0.5 M NaOH as required.

Experimental half-wave potentials ($E_{1/2}$) and diffusion limited currents (I_d) were obtained by fitting the DC reduction waves, recorded at each step in the titration experiments, using Eq. 1 [18]:

$$f(E_{appl}) = \frac{I_d}{\exp(n\delta F/RT)(E_{appl} - E_{1/2}) + 1} + I_b \quad (1)$$

where E_{appl} refers to the applied potential, I_b is the background current, n is the number of electrons transferred and δ measures the steepness of the reduction wave and should be unity for reversible electron transfer processes. The reduction waves for Cu(II) and Tl(I) were fitted separately. The function used to describe the background current was $I_b = a + bE_{appl} + c \exp(d \times E_{appl})$, where the exponential term accounted for the onset of mercury oxidation when fitting the Cu(II) wave or hydrogen evolution when fitting the Tl(I) wave. Mercury oxidation should ideally be avoided, but its close proximity to Cu(II) reduction made it impossible to evade completely. The reduction of Tl(I) was fully reversible across the pH range, so δ was set equal to one in this case. This was not so for the Cu(II) reduction with δ values varying between 1 and 0.6. The $E_{1/2}$ values obtained from Eq. 1 were thus for the quasi-reversible process ($E_{1/2}^q$).

Methods employed to determine the reversible $E_{1/2}$ values ($E_{1/2}^r$) from these quasi-reversible polarograms were investigated so that stability constants could be accurately calculated. Once reversible $E_{1/2}$ values were determined and were corrected for E_j where necessary, the polarographic data were treated as previously described in detail [13,17] to evaluate stability constants using the following relationship:

$$\left\{ E(M) - E(M_{comp})_{(i)} \right\} - \frac{RT}{nF} \ln \frac{I(M_{comp})_{(i)}}{I(M)_{(i)}} = \frac{RT}{nF} \ln \frac{[M_T]_{(i)}}{[M]_{(i)}} \quad (2)$$

where (i) indicates the experimental values at either each pH step or after each addition of ligand solution, depending on the type of titration performed. All other symbols used are listed and defined in the Appendix. The left side of the equation (also called the corrected potential shift) is calculated from the experimentally determined values derived from the measured polarograms. When plotted vs. pH or log[L] it gives the experimental complex formation curve (ECFC). The right side of the equation, called the calculated complex formation curve (CCFC) when plotted vs. pH or log[L], involves solving for mass balance equations to determine [M] at each point in the titration. The stability constants for the Cu(II)-picolinic acid species were refined such that the difference between the ECFC and CCFC is minimised, while those given in Table 2a were kept constant. This was achieved using dedicated software called 3D-CFC. [19]

3. Results and Discussion

3.1. Determining reversible half-wave potentials for Cu(II) from quasi-reversible polarograms

It is well known that the extent of reversibility of an electron transfer process can be assessed from a DC wave by the plot of log $\{I/(I_d - I)\}$ against E_{appl}. A fully reversible process would produce a straight line of slope -0.05916/n V at 25 °C and for an irreversible process the magnitude of the slope would be smaller. The plot would not be linear for a quasi-reversible electron transfer process. [18] Alternatively, the value of δ obtained from fitting the DC wave (Eq. 1) gives a good indication. It has been suggested that for $1 < \delta < 0.9$ the electron transfer process can be regarded as fully reversible for our application, for $0.9 < \delta < 0.5$ the process is quasi-reversible and for $\delta < 0.5$ the process is irreversible. [20]

The reduction of Cu(II) in nitrate and perchlorate background electrolytes depends on both the pH [21-23] and the ionic strength [23,24] of the solution. Studies have been performed using cyclic voltammetry [23,24], as well as alternating current (AC) [22] and direct current (DC) [21] polarography to explain these observations. It was demonstrated that as the pH was increased, the degree of reversibility also increased for the reduction of Cu(II) in both perchlorate and nitrate media by cyclic voltammetry. [23] Additionally, the reduction kinetics of Cu(II) in nitrate solutions is faster than that in perchlorate solutions and the reduction becomes less reversible in lower ionic strength solutions. [24]

In Fig. S2 it is seen that when picolinic acid is absent from the solution in a pH titration, the decrease in the value of δ for Cu(II) reduction follows a similar trend to the decrease in ionic strength during the titration (from about 0.5 M to about 0.25 M) even though the pH is increasing. This indicates that nitrate plays a role in the reduction process, although the exact mechanism is not fully understood, and that the effect of the ionic strength is greater than that of pH. Unfortunately working in very acidic solutions and at relatively low ionic strength does not allow for the addition of a supporting salt electrolyte to maintain a constant ionic strength. These titrations were terminated before hydrolysis of Cu(II) could take place, so hydrolysis products did not play a role in the extent of reversibility, as has been shown to occur. [21-23] After chelation of Cu(II) by picolinic acid, the electron transfer rate was slightly enhanced, but still remained quasi-reversible over much of the pH range (see Fig. S2). Various approaches to determine the $E_{1/2}^r$ from a quasi-reversible reduction wave were considered.

Using the curved plot of log$\{I/(I_d - I)\}$ vs. E_{appl} for a quasi-reversible DC polarogram, Koryta extrapolated the asymptote from the foot of the wave to the E_{appl}-axis and the value at the intercept corresponded to $E_{1/2}^r$. [25,26]

In this work, even after the background currents were subtracted, the clear curvature as presented in literature examples was not obvious. Deciding where to draw the asymptote was problematic and resulted in predicted $E_{1/2}^r$ values varying by more than 10 mV (see Fig. S3).

Cukrowski and Zhang [27] estimated the value of $E_{1/2}^r$ from the quasi-reversible polarogram itself. The polarogram was first fitted using Eq. 1 to obtain the values of I_d and the variables for the background current. (The half-wave potentials determined where $\delta < 0.9$ refer to $E_{1/2}^q$.) The polarogram was then refitted by first setting $\delta = 1$ and removing data points at the top part of the reduction wave where the current was still increasing (before the diffusion limited current was reached) so that the fitted curve passed through all remaining points, particularly those at the foot of the wave and where the current is diffusion limited. The only parameter refined in this case was $E_{1/2}$, which should now correspond to $E_{1/2}^r$ (see Fig. 1).
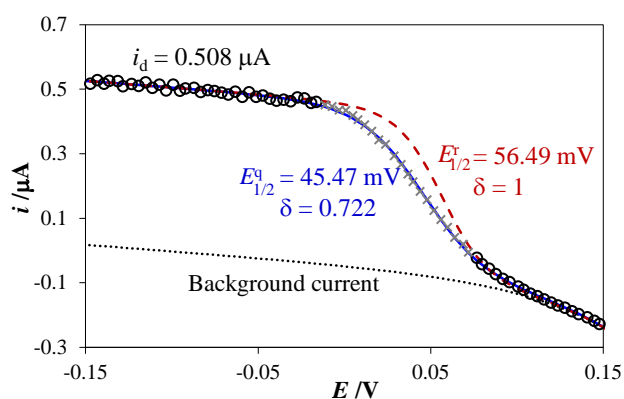

Fig. 1. Polarogram for the reduction of Cu(II) at pH 1.51. The polarogram was fitted using Eq (1) through (i) all points (—) and (ii) only points indicated by O while fixing $\delta = 1$ (---) to estimate $E_{1/2}^r$.

Fig. 2a shows both $E_{1/2}^q$ and $E_{1/2}^r$ values determined for a pH titration without picolinic acid present. Additionally, since it has been shown that E_j values calculated using the Henderson equation approximated those determined polarographically fairly well [13], $E_{1/2}^r$ values for Cu(II) reduction were predicted as a function of pH by subtracting the calculated E_j values from the potential of free Cu(II) (E(Cu)) (determined as discussed in Section 3.2). Fig. 2a demonstrates that the $E_{1/2}^r$ values determined by the method proposed by Cukrowski and Zhang [27] follow this predicted trend. As expected from the decreasing δ values, there is only a small difference between $E_{1/2}^q$ and $E_{1/2}^r$ at the lowest pH where δ is

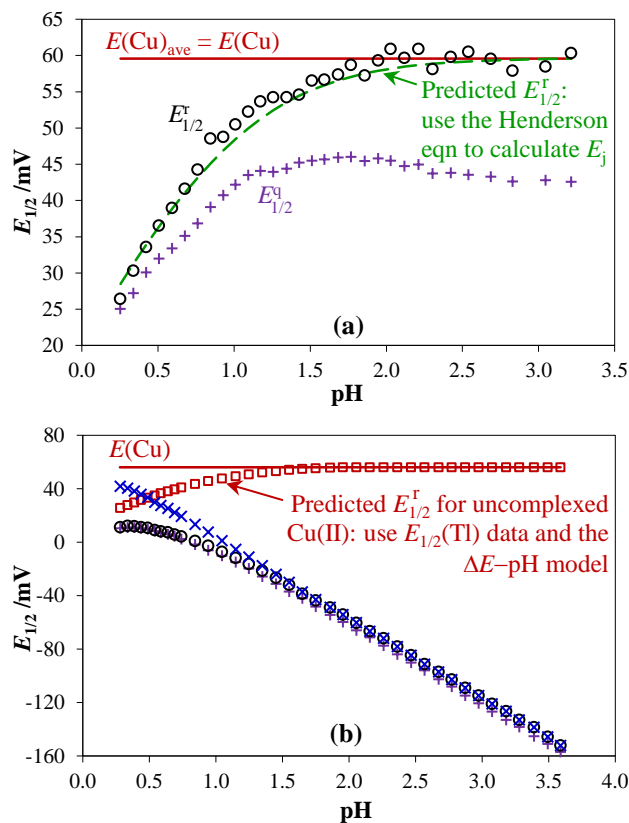closest to unity and the difference increases with increasing pH.

Fig. 2. Comparison of experimentally recorded $E_{1/2}^{q}$ values (+) and $E_{1/2}^{r}$ values (O), as determined using the procedure by Cukrowski and Zhang [23], for the reduction of Cu(II) in a pH titration in (a) the absence and (b) the presence of picolinic acid ([L]:[Cu(II)] = 103). The predicted $E_{1/2}^{r}$ values for uncomplexed Cu(II) without E_j correction are shown in (a) as (---) obtained by subtracting E_j, calculated using the Henderson equation, from $E(Cu)$ and in (b) as ($\square$) as determined from the $E(Tl)$ data and the $\Delta E - pH$ model. In (b) the $E_{1/2}^{r}$ values after accounting for E_j ($\times$) (as determined from the Tl(I) data) are also indicated.

Ružić *et al.* [26] also described how both $E_{1/2}^{r}$ and $E_{1/2}^{i}$ (the irreversible half-wave potential) could be determined graphically from the log plot described above. Since graphical analysis proved impossible here, the methodology used by Cukrowski *et al.* [20] was considered. They discuss how the expression derived by Ružić *et al.* [26] was rearranged and the background current included giving the relationship:

$$ I = \frac{I_d}{\exp(nF/RT)(E_{appl} - E_{1/2}^{r}) + \exp(\alpha nF/RT)(E_{appl} - E_{1/2}^{i}) + 1} + I_b $$
(3)

where α is the cathodic transfer coefficient. Eq. 3 was used to fit the quasi-reversible DC polarograms (using

non-linear curve fitting) to determine I_d and $E_{1/2}^{r}$ (as well as $E_{1/2}^{i}$, α and parameters for the background current). In order to reduce the number of variables when fitting these functions, the values of I_d and the background current variables were kept the same as those determined when applying Eq. 1. In this work it was found that the values of $E_{1/2}^{r}$ predicted by Eq. 3 followed no particular trend and were not meaningful (values of up to 15 V were found) and the values of $E_{1/2}^{i}$ were similar to those for $E_{1/2}^{q}$.

Matsuda and Ayabe [28-30] also used the log plot to determine both $E_{1/2}^{r}$, and in a similar fashion, Mkwizu [31] rearranged their derived expression to give:

$$ I = \frac{I_d}{\exp(nF/RT)(E_{appl} - E_{1/2}^{r}) + \Gamma\exp(\alpha nF/RT)(E_{appl} - E_{1/2}^{r}) + 1} + I_b $$
(4)

where $\Gamma = 1.13/\Lambda\tau^{1/2}$; τ is the drop life time and Λ is a kinetic parameter which includes the heterogeneous rate constants and is described for both simple or complexed metal ions. Eq 4 was used to fit the quasi-reversible DC polarograms (using non-linear curve fitting as before) to determine I_d and $E_{1/2}^{r}$ (as well as α, Γ and parameters for the background current). Unlike Matsuda and Ayabe [28-30], Γ was refined as a single value in this case and the I_d and the background current variables were kept the same as those determined when applying Eq. 1. The $E_{1/2}^{r}$ values predicted by Eq. 4 range between 50 mV and 135 mV, thus giving significantly higher values than expected and the $E_{1/2}^{r}$ –pH plot did not follow the trend predicted from calculated E_j values. The reason for these two fitting procedures failing in this case is unclear, but could be due to the close proximity of the mercury oxidation wave to the reduction wave of Cu(II).

The approach by Cukrowski and Zhang [27] was the most successful method for determining $E_{1/2}^{r}$ from quasi-reversible DC waves in this work and these values were used in further calculations throughout.

3.2. Modelling the difference in $E_{1/2}^{r}$ values for Cu(II) and Tl(I)

Using data from pH titrations, where the ligand was omitted, the difference in the $E_{1/2}^{r}$ values for Cu(II) and Tl(I) was modeled as a function of pH, as described before. [13] Firstly, the $E_{1/2}^{r}$ values in the pH region where E_j is constant (above about pH 2) were averaged to give $E(M)_{ave}$ which is equivalent to the free metal ion potential ($E(M)$). The difference $\Delta E(M)_{ave} = E(Cu)_{ave} - E(Tl)_{ave}$ was calculated as 487.7 ± 0.3 mV (for five experiments). For a titration including picolinic acid, $E(Cu)$ was then evaluated by adding $E(Tl)_{ave}$ (determined from the same experiment) to $\Delta E(M)_{ave}$. Interestingly,

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the reference electrode used to collect one of the data sets was problematic in that the potential was shifted almost 200 mV too positive, but the $\Delta E(M)_{ave}$ calculated was still within the range of the other experiments. This demonstrates how the Tl(I) potential data could act as an internal reference to the potential measurements of the metal ion of interest.

Secondly, the difference in the $E_{1/2}^{r}$ values for the two metal ions in the pH region where E_j varied with pH (below about pH 2) was determined for all five data sets (i.e. $\Delta E_{1/2}(M) = E_{1/2}^{r}(Cu) - E_{1/2}(Tl)$). This was necessary to account for slight differences in shifts in $E_{1/2}$ for Cu(II) and Tl(I) due to the decreasing ionic strength (from 0.5 M to about 0.25 M) during the titration. To test the effect of changing ionic strength on the reduction potentials, solutions containing Tl(I) and Cu(II) were made up in either 0.5 M or 0.25 M KNO₃, polarograms were recorded and the $E_{1/2}^{r}$ values were determined. The difference in these $E_{1/2}^{r}$ values for Cu(II) (i.e. $\Delta_\mu E_{1/2}(Cu)_{0.5-0.25}$) was −0.8 mV and $\Delta_\mu E_{1/2}(Tl)_{0.5-0.25}$ was −2.3 mV, indicating that $\Delta_\mu E_{1/2}(M)_{0.5-0.25}$ is different for the two metal ions. The average $\Delta E_{1/2}(M)$–pH relationship was modelled by a fifth order polynomial (see Fig. S4). Above pH 2.7, $\Delta E_{1/2}(M)$ was set equal to $\Delta E(M)_{ave}$, although no significant change in $\Delta E_{1/2}(M)$ was observed from pH 1.8 already.

Since pH titrations were started at pH 0.3, E_j had to be corrected in the very acidic region. Using the $\Delta E_{1/2}(M)$–pH model and the $E_{1/2}(Tl)$ data collected when ligand is present in the test solution, the magnitude of E_j can be calculated. An example of the measured and corrected $E_{1/2}$ values for a titration with picolinic acid present in the test solution is shown in Fig. 2b. The value of $E(Cu)$ can also be determined using $E(Tl)_{ave}$ and $\Delta E(M)_{ave}$.

3.3. Equilibria studies of the Cu(II)-picolinic acid system

When performing a pH titration experiment, the addition of picolinic acid to the test solution at pH 0.3 (containing and Cu(II) and Tl(I)), results in a negative shift in the reduction potential for Cu(II) demonstrating that complex formation takes place at this low pH already. When comparing the diffusion limited currents of the Cu(II)-picolinic acid complexes to that expected for uncomplexed Cu(II) throughout the entire titration, the values compared well indicating that the rates of diffusion are very similar. The difference between $E(Cu)$ and $E(Cu_{comp})$ (ΔE), that had been corrected for E_j where necessary, is thus the main factor in establishing the formation constants. It is clear that any error in (i) the $E_{1/2}^{r}$ values determined for a quasi-reversible process,

(ii) the magnitude of E_j or (iii) the value of $E(Cu)$, would affect the formation constants.

Slope analysis was done using the ECFCs to predict the metal-ligand species formed. A slope close to 60 mV/pH was found across the pH range. Given that the slope is approximately $60/n \times$ number of protons involved in the reaction, this indicates that CuL^+ forms at low pH where H_2L^+ is the dominant form of the ligand and CuL_2 forms where HL is predominant in solution. When both these species were included in the model and the stability constants were refined, the CCFCs fitted the experimental data well (see Fig. 3a).

The average log β values determined for the pH titrations are given in Table 2b. The larger standard deviation noted for the CuL^+ species was probably due to its formation in the pH region where the junction potential had to be accounted for. As seen from the species distribution diagram in Fig. 3c for [L]:[Cu(II)] = 100, CuL^+ is only present under very acidic conditions with insignificant concentrations existing above pH 2. Additionally, data carries more weight in the refinement process when the concentration of a species increases with increasing pH; for CuL^+ this occurs below pH 0.3 (the pH from where measurements were started). That being said, the values compared very well to the critically assessed literature values given in the NIST Database. [14] The CuL_2 species predominates over a wide pH range and is the only species present from about pH 2.5 to pH 14 under these conditions. The larger difference between the literature log β value for CuL_2 and that determined here is most likely due to more inaccurate $E_{1/2}^{r}$ values being estimated where δ is at its lowest, especially considering the rudimentary method used to calculate these values.

Table 2. (a) The dissociation constant for water, the stepwise protonation constants for picolinic acid and the overall stability constants for Cu(II) hydroxides at 25°C and 0.5 M ionic strength. [14] (The value in brackets is uncertain.) (b) Stability constants for Cu(II)-picolinic acid complexes determined using polarography at 25°C and ionic strength 0.25 – 0.5 M. Literature data are reported at 25°C and the indicated ionic strength.

(a) Equilibrium	log K	
$H_2O \leftrightarrow H^+ + OH^-$	−13.74	
$L^- + H^+ \leftrightarrow HL$	5.18	
$HL + H^+ \leftrightarrow H_2L^+$	(0.86)	
$Cu^{2+} + OH^- \leftrightarrow Cu(OH)^+$	6.1	
$2Cu^{2+} + OH^- \leftrightarrow Cu_2(OH)^{3+}$	7.7 [a]	
$2Cu^{2+} + 2OH^- \leftrightarrow Cu_2(OH)_2^{2+}$	16.7	
$3Cu^{2+} + 4OH^- \leftrightarrow Cu_3(OH)_4^{2+}$	33.7 [b]	
(b) This work	**log β_1**	**log β_2**
pH titration	7.75 ± 0.14	14.88 ± 0.05
Ligand titration	7.74 ± 0.05	14.69 ± 0.06
Literature [14]	**log β_1**	**log β_2**

$\mu = 0.1$ M	7.87	14.78
$\mu = 0.5$ M		14.70
$\mu = 1.0$ M	7.7	14.5

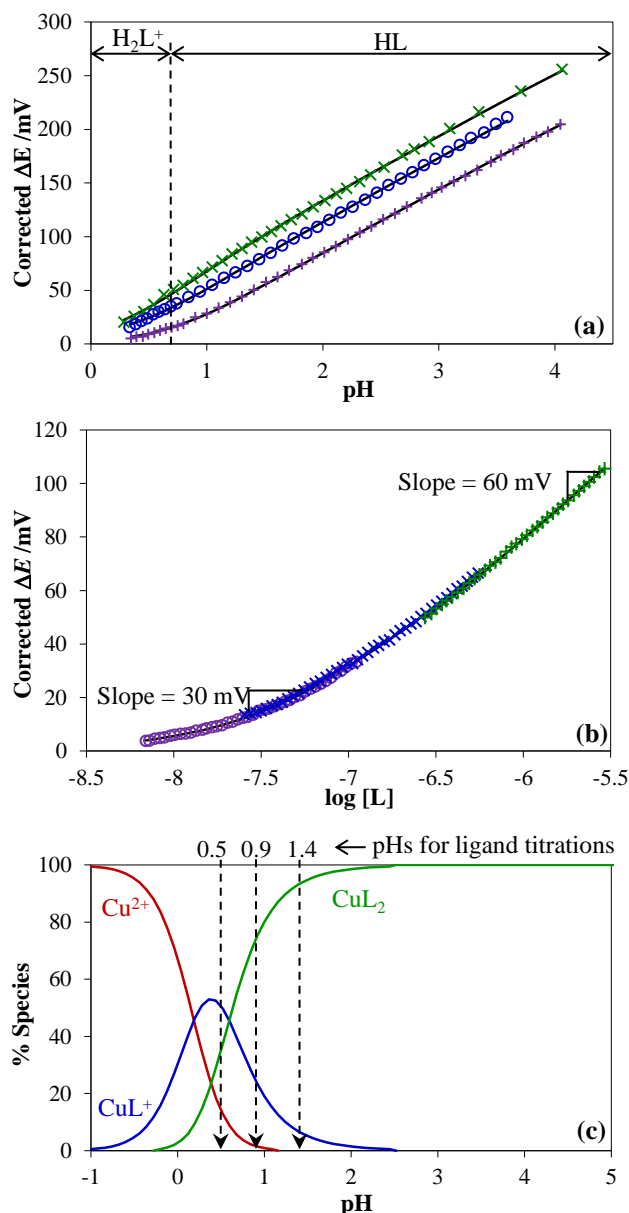[a] log K value for 3 M ionic strength. [b] log K value for 0.1 M ionic strength.

Fig. 3. Comparison of the ECFCs and corresponding CCFCs (—) calculated using the species model CuL$^+$ and CuL$_2$ for **(a)** pH titrations where [L]:[Cu(II)] is 32 (+), 103 (○) and 207 (×) (the pH region with the major form of the ligand is also indicated); and **(b)** ligand titrations at pH 0.47 (○), 0.90 (×) and 1.37 (+) and slopes are indicated. **(c)** Species distribution diagram for the Cu(II)-picolinic acid system where $[Cu(II)] = 1 \times 10^{-4}$ M and $[L] = 1 \times 10^{-2}$ M. The arrows demarcate the pHs at which ligand titrations were performed.

Three ligand titrations were performed at the pHs indicated in Fig. 3c. The very acidic conditions were used in order to glean information about both the ML and ML$_2$ species. A small drift in pH is often observed during a ligand titration and is generally tolerable, but since a fairly big change in E_j occurs with a small change in pH at these very low pHs, the variation was not allowed and the pH was adjusted as necessary. In hindsight, the $E_{1/2}$(Tl) values could have been used to account for variations in E_j. When the ΔE values are calculated to determine stability constants, E_j will be negated provided the solution pH remains constant throughout the experiment. The extent of reversibility did not change much during a titration as the ionic strength remained fairly constant due to very little dilution taking place. Also the δ values were close to 0.9 and the $E_{1/2}^{r}$ values determined were thus close to the $E_{1/2}^{q}$ values.

Slope analysis of the ECFCs are displayed in Fig. 3b where the slope is approximately $60/n \times$ number of free ligand molecules involved in the reaction for ligand titrations. This analysis again indicated that CuL$^+$ and CuL$_2$ were the major species formed. The calculated curves fitted the experimental data well and the refined log β values, presented in Table 2b, compared well to the literature values.

3.4. Evaluation of errors had corrections implemented been ignored

In this work, two main sources of error were considered, namely the diffusion junction potential and the quasi-reversible nature of the Cu(II) reduction waves. To assess the effects when neglecting to correct for these factors in pH titration experiments, stability constants were calculated where (i) the $E_{1/2}^{r}$ (Cu) values were determined but E_j was not accounted for and (ii) E_j was corrected for but the $E_{1/2}^{q}$ values were used.

In the first case, when ignoring E_j, this would not only affect the $E(M_{comp})$ values determined below pH 2 where E_j varies with pH, but since the free Cu(II) potential is measured in the most acidic solution before ligand is added, E(Cu) would also be underestimated by about 30 mV (see Fig. 2b). Because the shift in potential due to complexation ($E(M) - E(M_{comp})$) is used in determining stability constants (see Eq. 2), it implies that not only will the stability constants for species formed below pH 2 be affected, but all stability constants will contain error. Using the $E_{1/2}^{r}$ (Cu) values, data was analysed without making corrections for E_j and the average log β obtained are given in Table 3 (For a more detailed analysis see Table S1). The very large standard deviation for the

CuL$^+$ species is mainly due to the apparent onset of complex formation occurring at much higher pHs. This is especially so for the [L]:[Cu(II)] = 32 experiment where the corrected ΔE values are below zero up to pH 0.90 due to the underestimated E(Cu) value and the smaller potential shifts observed for the lower [L]:[Cu(II)] ratio (see Fig 4a). The log β values for both species are thus significantly lower than those quoted in Table 2b.

Table 3. Log β values calculated for CuL$^+$ and CuL$_2$ when correcting for either the non-reversible reduction of Cu(II) species or E_j or both. When $E_{1/2}^q$ values were used, the CuL$_3^-$ species could also be included in the model.

Corrections	log β_1	log β_2	log β_3
Use $E_{1/2}^r$ (no E_j correction)	7.26	13.84	
	± 0.69	± 0.12	
Account for E_j (use $E_{1/2}^q$)	7.46	15.04	
	± 0.65	± 0.08	
Account for E_j (use $E_{1/2}^q$)	7.57	15.01	18.35
	± 0.51	± 0.08	± 0.49
Account for E_j and use $E_{1/2}^r$	7.75	14.88	
	± 0.14	± 0.05	

In the second case, after accounting for E_j, the effect of using the $E_{1/2}^q$ (Cu) values rather than the $E_{1/2}^r$ (Cu) values when determining stability constants was considered. Because the deviation from reversibility increased as the titration proceeded (and hence as the pH increased), it was expected that larger errors would be obtained for the CuL$_2$ species in this case. Additionally, since the $E_{1/2}^q$ values are more negative than the $E_{1/2}^r$ values, it was envisaged that the the stablity constants would be higher due to larger calculated ΔE values. The results in Table 3 show that the log β value for CuL$_2$ was larger, but that for CuL$^+$ was smaller. This is due to the steeper slope of the ECFC predicting that CuL$_2$ forms at lower pHs that is really the case. The large standard deviation for the CuL$^+$ species was due to the low value obtained for the [L]:[Cu(II)] = 207 experiment. From Fig. S5 it can be seen that the δ values for this experiment were lower than that for the other two experiments, thus indicating that larger errors would be expected. Although the ECFC could be fitted when including only the CuL$^+$ and CuL$_2$ species, points at the highest pHs could be better fitted by incorporating the CuL$_3^-$ species (see Fig 4b) and the results are given in Table 3. The CuL$_3^-$ species is clearly an artefact from the overestimation of the $E_{1/2}$ values when the $E_{1/2}^q$ (Cu) values were used.

It is only after both E_j and the non-reversible behaviour of the reduction of Cu(II) was accounted for that log β values comparable to those obtained in literature were obtained (see Table 2b).
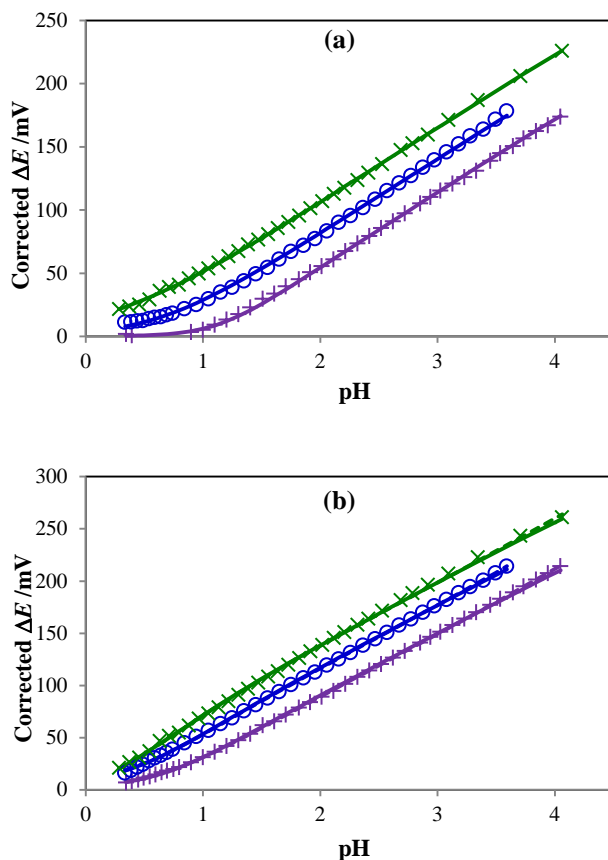

Fig. 4. Comparison of the ECFCs and corresponding CCFCs (—) calculated using the species model CuL$^+$ and CuL$_2$ for pH titrations where [L]:[Cu(II)] is 32 (+), 103 (○) and 207 (×) where (a) $E_{1/2}^r$ values were used but no E_j correction was made and (b) E_j correction was made but $E_{1/2}^q$ values were used. The CuL$_3^-$ species could also be included in the model in (b) as indicated by the additional CFCCs (---).

4. Conclusion

This was an ideal metal-ligand system to test the protocols developed for working under very acidic conditions because CuL$^+$ only exists in solution below pH 2 for the concentration conditions used. Fig.3c highlights that, for the conditions used, at pH −0.3 about 10% of Cu(II) is complexed already and in a 1 M acid solution, about 30% of Cu(II) is in the CuL$^+$ form and CuL$_2$ has also started forming. An additional complication, however, was dealing with the quasi-reversible nature of Cu(II) reduction. The extent of irreversibility appears to be more strongly dependent on ionic strength than pH in nitrate solutions. The reduction of both uncomplexed Cu(II) and the Cu(II)-picolinic acid complexes was quasi-reversible at lower ionic strength (and higher pH in this case). In order to determine $E_{1/2}^r$ from quasi-reversible DC waves (as required for stability constant evaluation) several approaches were considered, but only the

rudimentary procedure by Cukrowski and Zhang [27] produced meaningful values. The $E_{1/2}(Cu)$–pH plot, predicted by subtracting E_j (determined using the Henderson equation) from $E(Cu)$, followed a similar trend to the $E_{1/2}^{r}$–pH plots for uncomplexed Cu(II), thus giving confidence to the $E_{1/2}^{r}$ values. In all three ligand titrations, the δ values from the Cu(II) reduction waves were all close to 0.9 indicating negligible deviation from a reversible reduction, hence the $E_{1/2}^{r}$ values were thus close to the $E_{1/2}^{q}$ values. The $E_{1/2}^{r}$ values were used in all further calculations.

When working under very acidic conditions, the $\Delta E_{1/2}(M)$–pH model was first established and then used together with the $E_{1/2}(Tl)$ data collected when ligand was present in the test solution, to accurately predict the magnitude of E_j and the $E(Cu)$ value. The reversible $E_{1/2}(Cu_{comp})$ values could then be corrected for E_j and the ECFC calculated.

Several factors thus had to be taken into account to produce Cu(II) potential data, as initially obtained from the pH titration experiments, for use in the calculation of stability constants. These included (i) the determination of reversible potential values from quasi-reversible reduction waves, (ii) correcting for the large junction potentials (E_j was about 30 mV at pH 0.3), (iii) the fact that E_j varies with pH, (iv) the slightly larger shift in $E_{1/2}$ for Tl(I) compared to that for Cu(II) due to the changing ionic strength and (v) the indirect determination of $E(Cu)$ using $E(Tl)_{ave}$ and $\Delta E(M)_{ave}$ data. Additionally, it is critical that pH be determined as accurately as possible, thus methodologies developed to calibrate the glass electrode under acidic conditions were also employed. [2] Taking all these into consideration, the fact that the stability constants compared very well to the literature data shows the rigor of these protocols as well as robustness of polarography as a technique for determining these values.

It was demonstrated that when evaluating log β values using potential data (from pH titrations) that had not been corrected for E_j, significantly smaller values were found as the both the $E(Cu)$ value and the reversible $E_{1/2}(Cu_{comp})$ values below pH ~2 were lower than if E_j were accounted for. It was furthermore shown that when simply using the quasi-reversible Cu(II) reduction potentials, rather than those for a reversible electron transfer, the log β values for CuL_2 were overestimated (leading to an underestimation of the log β values for CuL^+) and an additional artefact species (CuL_3^-) could even be included.

It was not necessary to compensate for E_j for the ligand titration data, provided $E(Cu)$ and $E(Cu_{comp})$ (as given in Eq. 2) are measured at the same pH. It may then be asked why it is necessary to do pH titration experiments which involve more correction factors. Since the type of metal-ligand species in solution depends on the pH, pH titrations provide an overall notion of the speciation across a wide pH range. Once that is established, it is easier to assess at which pH to run a ligand titration and additional stability constant data can then be determined. Furthermore it is not always possible to do ligand titrations as the solubility of the ligand may be too low to make up ligand solutions, there may be a limited amount of ligand if only small quantities were synthesised, the metal ion may be hydrolysed at the pH at which a ligand titration is to be run (this means the $E(M)$ cannot be determined), it is problematic to detect protonated or hydrolysed species that are pH dependant and so on. It can therefore be concluded that polarography can be used to accurately determine stability constants for metal-ligand complexes formed below pH 2 and this methodology can be applied to study unknown systems.

5. Appendix: List of Selected Symbols

E_j	Diffusion junction potential.
E_{appl}	Applied potential to the working electrode.
$E(M)$	Half-wave potential of the free or uncomplexed metal ion.
$E(M_{comp})$	Half-wave potential of the complexed metal ion.
ΔE	The shift in potential due to complex formation: $E(M) - E(M_{comp})$
$E_{1/2}^{r}$	Reversible half-wave potential.
$E_{1/2}^{q}$	Quasi-reversible half-wave potential.
$E_{1/2}^{i}$	Irreversible half-wave potential.
$E_{1/2}(M)$	Experimental half-wave potential.
$\Delta E_{1/2}(M)$	The difference in the reversible experimental half-wave potentials for Cu(II) and Tl(I): $E_{1/2}^{r}(Cu) - E_{1/2}(Tl)$
$E(M)_{ave}$	The average value of the experimental half-wave potentials for the free metal ion above pH 2 (= $E(M)$)
$\Delta_\mu E_{1/2}(M)_{0.5-0.25}$	The change in the reversible half-wave potential for a metal ion caused only by the change in ionic strength (from 0.5 to 0.25 M).
$I(M)$	Diffusion-limited current for the free or uncomplexed metal ion.
$I(M_{comp})$	Diffusion-limited current complexed metal ion.
$[L]$	Free ligand concentration.
$[M]$	Free metal ion concentration.
$[M_T]$	Total metal ion concentration.

6. Acknowledgements

7. References

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Supplementary Information

Examples of experimental curves

A set of polarograms for both a pH and a ligand titration experiment is shown in Fig. S1a and S1b, respectively, to illustrate the change in the polarograms with changing solution conditions. In Fig. S1a it is clearly seen that as the pH was increased, the reduction wave for the Cu(II) species shifted to more negative potentials and the steepness of the wave decreased as reduction became less reversible. The reduction potential of Tl(I) shifted in a positive direction only below pH ~2 due to the changing E_j. The decrease in the current with increasing pH was mainly due to dilution on addition of the hydroxide solution. In Fig. S1b the reduction potential of Tl(I) was unchanged because the pH was kept constant throughout the titration and hence E_j was constant. The reduction wave for the Cu(II) species shifted to more negative potentials with increasing ligand concentration as expected. The decrease in current was far less in this case as very small volumes of the 0.5001 M picolinic acid solution were added.
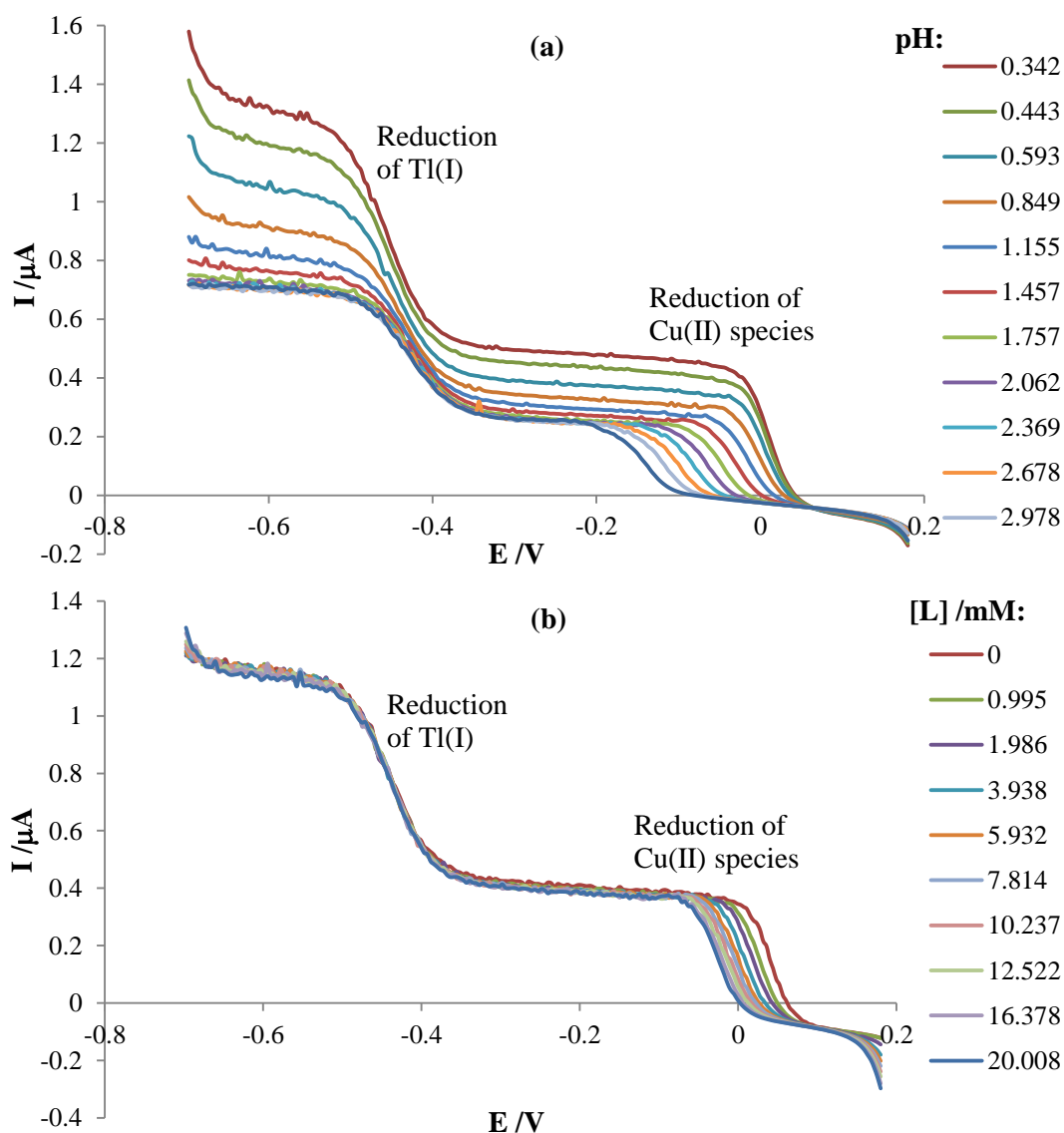

Fig. S1. Selected polarograms for (a) a pH titration where [L]:[M] = 103 and (b) a ligand titration at pH 0.90.

Variation in electrochemical reversibility of Cu(II) reduction

Fig. S2 gives examples of the change in the value of δ (which indicates the extent of reversibility) for the reduction of Cu(II) species in the absence and presence of picolinic acid. In the absence of ligand, a similar trend is noted between the change in ionic strength and the decrease in the extent of reversibility.
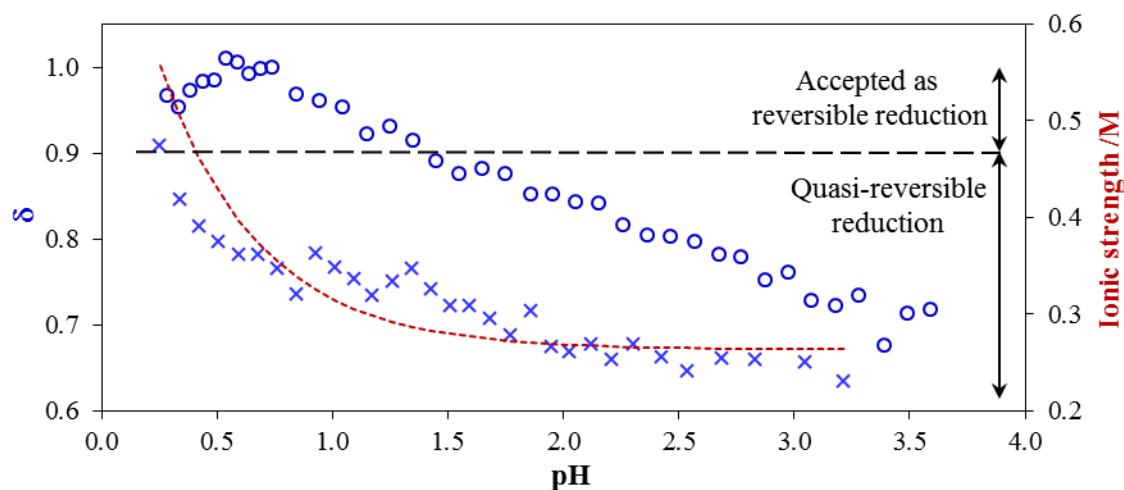

Fig. S2. The value of δ illustrates the change in the electron transfer rate for the reduction of Cu(II) in a nitrate background in the absence (×) and presence (○) of picolinic acid (where [Cu(II)]:[L] = 103). The variation in ionic strength of the solution is also indicated (---).

An example of logarithmic analysis of electrochemical reversibility

Due to the reduction of Cu(II) being quasi-reversible, various procedures were attempted to determine the reversible $E_{1/2}$ values as required for determining stability constants. Fig. S3 shows the attempted use of log analysis where asymptotes drawn from data at the foot of the wave, are exptrapolated to the E-axis where the intercept values correspond to the reversible $E_{1/2}$ values. [21,22] Here the difficulty in deciding where to draw the asymptotes is highlighted and the significant difference in the reversible $E_{1/2}$ values are shown.
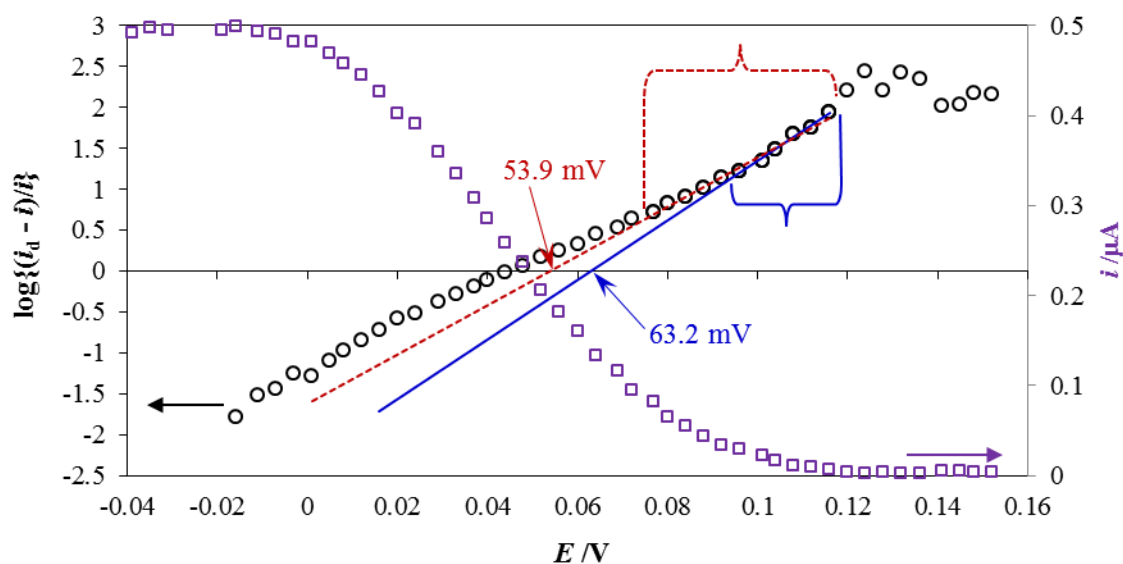

Fig. S3. Log analysis showing two possible asymptotes that could be extrapolated to determine $E_{1/2}^r$. The brackets indicate the data points used to calculate each of these. The DC wave with subtracted background current is also displayed (□).

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Protocol used to monitor $E_{1/2}$ of Cu(II) relative to Tl(I).

Fig. S4 shows the difference in the $E_{1/2}^{r}$ values between Cu(II) and Tl(I) (i.e. $\Delta E_{1/2}(M) = E_{1/2}^{r}(Cu) - E_{1/2}(Tl)$) determined at each pH for five data sets when using data from pH titrations in the absence of ligand. The average $\Delta E_{1/2}(M)$–pH relationship was modelled by a fifth order polynomial below pH 2.7. This difference was used to account for slight differences in shifts in $E_{1/2}$ for Cu(II) and Tl(I) due to the decreasing ionic strength (from 0.5 M to about 0.25 M) during the titration. Above pH 2.7 the value of $\Delta E_{1/2}(M)$ was taken as constant.
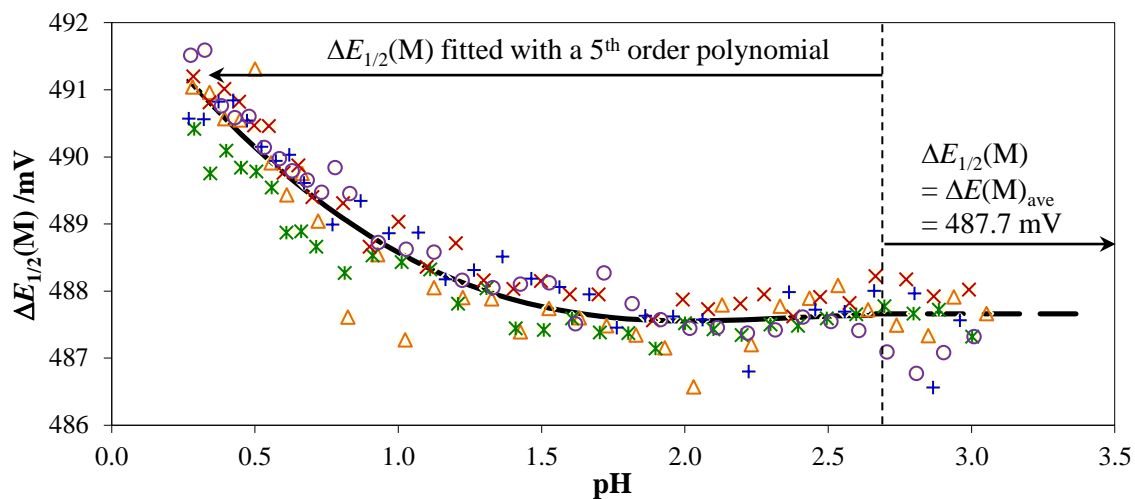

Fig. S4. Model of the reversible $E_{1/2}(Cu) - E_{1/2}(Tl)$ values as a function of pH for five data sets. Above pH 2.7, $\Delta E_{1/2}(M) = \Delta E(M)_{ave}$.

Variation in electrochemical reversibility of Cu(II) reduction in the presence of ligand

Fig. S5 shows the values of δ obtained for the three pH titration experiments, and particularly highlights the lower values obtained for the [L]:[Cu(II)] = 207 experiment.
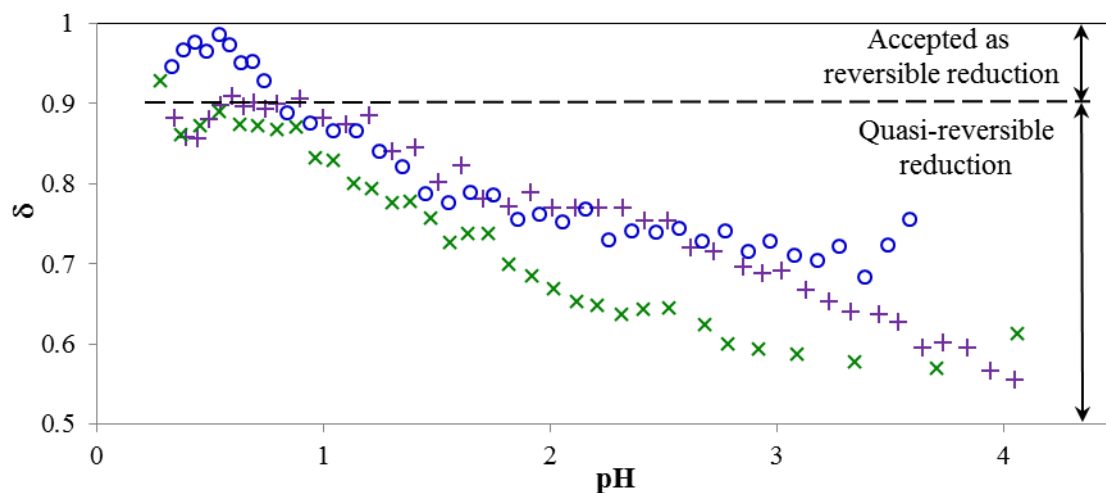

Fig. S5. Comparison of the δ values for pH titrations where [L]:[Cu(II)] is 32 (+), 103 (○) and 207 (×).

Error analysis in the computed formation constants

An expanded table of results obtained for the separate pH titration experiments is given in Table S1. The average values are quoted in Table 3. The standard deviation of fitting is calculated by $\sqrt{(SSE/(n - p))}$, where SSE is the sum of squares of error, n is the number of experimental points used and p is the number of parameters refined.

Table S1. Log β values calculated for CuL$^+$ and CuL$_2$ when using (a) $E_{1/2}^{r}$ values but no E_j correction; (b) $E_{1/2}^{q}$ values with E_j correction; (c) $E_{1/2}^{q}$ values with E_j correction, but including the CuL$_3^-$ species in the model; (d) $E_{1/2}^{r}$ values and E_j correction.

[L]:[Cu(II)]	32	103	207	Average
(a)				
log β_1	6.51 ± 0.08	7.40 ± 0.02	7.87 ± 0.01	7.26 ± 0.69
log β_2	13.881 ± 0.007	13.706 ± 0.008	13.942 ± 0.009	13.84 ± 0.12
S.D. of fitting	2.85	2.58	0.83	
(b)				
log β_1	7.81 ± 0.02	7.85 ± 0.02	6.71 ± 0.38	7.46 ± 0.65
log β_2	15.094 ± 0.007	14.994 ± 0.007	15.079 ± 0.007	15.04 ± 0.08
S.D. of fitting	1.93	1.40	3.54	
(c)				
log β_1	7.85 ± 0.02	7.88 ± 0.02	6.98 ± 0.21	7.57 ± 0.51
log β_2	15.050 ± 0.009	14.917 ± 0.009	15.064 ± 0.008	15.01 ± 0.08
log β_3	18.84 ± 0.06	18.34 ± 0.10	17.86 ± 0.10	18.35 ± 0.49
S.D. of fitting	0.38	0.77	2.86	
(d)				
log β_1	7.77 ± 0.02	7.88 ± 0.02	7.61 ± 0.05	7.75 ± 0.14
log β_2	14.895 ± 0.007	14.826 ± 0.007	14.915 ± 0.007	14.88 ± 0.05
S.D. of fitting	0.54	1.84	1.94	