The Interaction of Ni^{2+} and Co^{2+} with Murexide

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In many instances the absorbance changes accompanying the reactions of metal ions with biologically significant molecules are of little use in determining the binding constants. In some cases, for example when a metal ion reacts exclusively with the phosphate moiety of a nucleotide, the absorbance changes are either zero or very small [1]. Alternatively, as in the reactions of transition metal ions with polynucleotides, the total absorbance change may be due to the sum of that due to complex formation and that due to changes in the stacking interactions [2, 3]. Consequently they cannot be used in the determination of binding constants. In such systems colorimetric reagents such as Eriochrome Black-T and Murexide which show significant colour changes in the visible region of the spectrum on complex formation have been used to determine equilibrium constants [4–6].

We have recently used Murexide as a metal ion indicator when determining the equilibrium constant for reaction of Ni^{2+} with poly(adenylic acid) at pH 7.0 [7]. As a consequence of this and other work in progress it has proved necessary to investigate more thoroughly the reactions of Ni^{2+} and Co^{2+} with Murexide in the pH range 5 to 7. Originally, the apparent equilibrium constants were determined at a single wavelength as described by McBryde [8]. We have now used the computer program SQUAD [9] to investigate the binding between both Ni^{2+} and Co^{2+} and Murexide. Additionally we have measured the rate of reaction of Ni^{2+} with Murexide in this pH range. Although kinetic data for this reaction have been previously reported [10, 11] the pH dependence of the rate constants at 25 °C has not been described. These data are necessary in order to facilitate the use of Murexide as an indicator in the study of Ni^{2+} complexation kinetics.

Experimental

Murexide (Merck) was used as purchased. Concentrations were determined using $\epsilon_{506} = 1.25 \times 10^4$ mol^{-1} cm^{-1} [12]. Due to the interactions of sodium ion with Murexide [7], KNO_3 was used as the supporting electrolyte. Solutions of $Ni(NO_3)_2 \cdot 6H_2O$(BDH) and $Co(NO_3)_2 \cdot 6H_2O$(BDH) were standardized by titration with ethylenediaminetetraacetate using Bromopyrogallol red and Xylenol Orange indicators respectively [13].

pH measurements were made using a Pye Model 290 pH meter equipped with a Pye Ingold E_o [7] combined pH electrode. Activity coefficients were calculated using the Davies equation [14]. The pH of all solutions was stabilized by the addition of cacodylate buffer. All measurements were carried out at 25.0 (±0.1) °C.

Spectrophotometric titrations were carried out by adding very small volumes of a concentrated metal solution to a thermostatted solution of Murexide contained in a cuvette in the cell compartment of a Cary 118 spectrophotometer. A calibrated micrometer syringe was used to effect the addition. Spectra were recorded in the range 460–560 nm using the Cary repetitive scan accessory. All measurements were completed within one hour of preparing the Murexide solutions. No appreciable decomposition had taken place within this time.

Equilibrium constants were calculated using the program SQUAD [9]. This program calculates the best set of equilibrium constants that will describe the data supplied. The absorbances at each of eleven different wavelengths from approximately ten spectra were used to calculate the apparent equilibrium constants at each pH. Subsequently all the data were combined to calculate the stability constants. The DIMENSION statements in SQUAD were modified in order to do this as the program as supplied can handle a maximum of twenty spectra.

Rate constants were measured using an Applied Photophysics stopped-flow device. The kinetic data were recorded using a DATALAB DL 901 transient recorder. These were then transferred to either the University DEC 2060 computer or a BBC microcomputer. Rate constants calculated from plots of $-\ln(A_t - A_\infty)$ against time using from 250 to 500 data points.

Results and Discussion

The reaction of metal ion with Murexide may be represented by equations

$$M^{2+} + H^+ + L^{2-} = MHL^+(\beta_{MHL}) \quad (1)$$

$$M^{2+} + L^{2-} = ML(\beta_{ML}) \quad (2)$$

$$K_{app} = ([ML] + [MHL^+])/[M^{2+}]([HL^-] + [L^{2-}]) \quad (3)$$

(1), (2) and (3) [15, 16] so that

$$K_{app}(1 + [H^+]/K_a) = \beta_{ML} + \beta_{MHL}[H^+] \quad (4)$$

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where K_a is the dissociation constant of Murexide. This was found to be 7.047×10^{-10} using SQUAD. A plot of the left-hand side of eqn. 4 against $[H^+]$ should yield a straight line of slope β_{MHL} and intercept β_{MHL}. Table I shows the results obtained from SQUAD for reaction of both Co^{2+} and Ni^{2+} with Murexide. The only species formed are the MHL^+ and ML species. The inclusion of $M(HL)_2$ species resulted in non-convergence of SQUAD. Consequently the presence of this species under the experimental conditions used must be discounted.

TABLE I. Stability Constants for Reaction of Ni^{2+} and Co^{2+} with Murexide at 25 °C and $I = 0.1$ mol dm^{-3} KNO_3.

Metal	log β_{ML}	log β_{MHL}
H^+	9.152 ± 0.015^a	–
Ni^{2+}	$6.34 \pm 0.02(6.33)^b$	$12.25 \pm 0.03(12.32)$
CO^{2+}	$5.81 \pm 0.04(5.85)$	$11.81 \pm 0.05(11.82)$

[a] Values presented are standard deviations of the constants as calculated by the program. [b] The values calculated from an unweighted linear least-squares fit of eqn. 4 are in brackets.

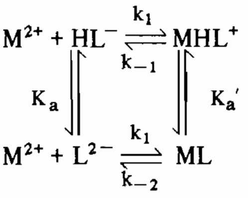
TABLE II. Kinetic Data for Reaction of Ni^{2+} with Murexide in Aqueous Solution at 25 °C and $I = 0.1$ mol dm^{-3} KNO_3.

10^3 $[Ni^{2+}]$/ mol dm^{-3}	$[H^+]$/mol dm^{-3}	k_{obs}/s^{-1}	k_{calc}/s^{-1} [a]
0.49	1.27×10^{-5}	4.13	4.40
0.98	1.27×10^{-5}	5.85	6.15
1.47	1.27×10^{-5}	7.49	7.91
1.96	1.27×10^{-5}	9.17	9.65
2.45	1.27×10^{-5}	11.12	11.41
0.49	1.27×10^{-6}	2.88	3.24
8.98	1.27×10^{-6}	4.79	5.01
1.47	1.27×10^{-6}	6.55	6.77
1.96	1.27×10^{-6}	9.75	8.54
2.45	1.27×10^{-6}	11.21	10.30
0.49	1.27×10^{-7}	2.27	2.20
0.98	1.27×10^{-7}	4.13	4.10
1.47	1.27×10^{-7}	6.01	6.00
1.96	1.27×10^{-7}	7.80	7.91
2.45	1.27×10^{-7}	9.71	9.81

[a] Calculated from eqn. 5.

Any mechanism for reaction of metal ions with Murexide at pH $\geqslant 5$ must take account of the pressence of the two species MHL^+ and ML. The mechanism shown in Scheme 1 does this. For this reaction scheme one obtains eqn. 5 where

$$k_{obs} = (k_1[H^+] + k_2K_a)\{[M]/(H^+ + K_a) + 1/\beta_{MHL}K_a([H^+] + K_a')\} \qquad (5)$$



Scheme 1

$K_a' = \beta_{ML}/\beta_{MHL} = 1.26 \times 10^{-6}$. Using a least squares fitting routine [17] values for k_1 and k_2 are readily found from the kinetic data (Table II). These give values of $3.57\ (\pm 0.08) \times 10^3$ and $6.1\ (\pm 2.8) \times 10^4$ dm^3 mol^{-1} s^{-1} for k_1 and k_2 respectively. The relatively high uncertainty in the value for k_2 is due to the fact that it is only at pH $\geqslant 7$ that the k_2 pathway is significant. Unfortunately however, kinetic data using Ni^{2+} as reactant species cannot be obtained at pH values significantly above 7 due to hydrolysis of the metal ion. The mechanism of the reactions of Ni^{2+} with Murexide and the location of the rate-determining step has been previously discussed [18].

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