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Complexation of zinc(II) and other divalent metal ions by the fluorophore 2-methyl-8-(toluene-*p*-sulfonamido)-6-quinolyloxyacetic acid in 50% aqueous ethanol

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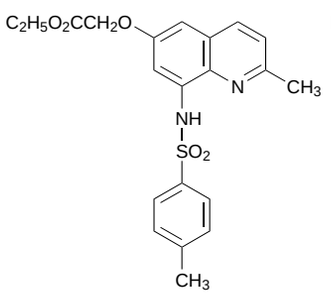
A potentiometric and fluorescence study of the complexation of divalent metal ions (M^{2+}) by the fluorophore 2-methyl-8-(toluene-*p*-sulfonamido)-6-quinolyloxyacetic acid (H_2L) in 50% (v/v) aqueous ethanol at 298.2 K and $I = 0.10$ mol dm^{-3} ($NaClO_4$) showed that the [ML] and $[ML_2]^{2-}$ complexes are characterised by $\log(\beta_1/dm^3\ mol^{-1})$ and $\log(\beta_2/dm^6\ mol^{-2})$ values of 8.12 ± 0.20 and 17.06 ± 0.11, respectively, when $M^{2+} = Co^{2+}$ and corresponding values of < 8 and 15.73 ± 0.03 when $M^{2+} = Ni^{2+}$, 11.96 ± 0.02 and 21.40 ± 0.03 when $M^{2+} = Cu^{2+}$, 9.65 ± 0.02 and 19.11 ± 0.06 when $M^{2+} = Zn^{2+}$ and 8.44 ± 0.50 and 15.38 ± 0.40 when $M^{2+} = Cd^{2+}$. In the cobalt(II) system, $[CoL_3]^{4-}$, where $\log(\beta_3/dm^9\ mol^{-3}) = 25.56 \pm 0.11$, was also detected. The pK_a values for H_3L^+ are 1.87 ± 0.10, 3.72 ± 0.03 and 10.01 ± 0.02. These data are discussed together with the fluorescence characteristics of HL^-, [ZnL] and $[ZnL_2]^{2-}$.

Zinc(II) is the second most abundant transition-metal ion after $Fe^{2+/3+}$ in humans and other mammals.[1–6] It is found either at the active site or as a structural component of numerous enzymes, and is important in many processes associated with cell activation and growth which include neurotransmission,[7,8] apoptosis,[9,10] signal transduction[11] and gene expression.[12,13] Thus, there is considerable interest in the distribution of intracellular Zn^{2+}, the concentration of which ranges from 10^{-9} mol dm^{-3} in the cytoplasm to 10^{-3} mol dm^{-3} in some vesicles. While the total amount of intracellular Zn^{2+} is readily measured by standard analytical techniques, the simultaneous detection of Zn^{2+} in a range of intracellular sites *in vivo* is more challenging. Potentially, such detection is offered by a fluorophore which fluoresces strongly upon selective complexation of Zn^{2+}.

A basic requirement of such a fluorophore is not only that it should be selective for Zn^{2+}, but also that it should readily traverse the cell membrane and not leak from the cell. Our new fluorophore, ethyl 2-methyl-8-(toluene-*p*-sulfonamido)-6-quinolyloxyacetate **1**, fulfils these requirements and is effective in the detection of Zn^{2+} in a range of mammalian cells.[14–19] Thus, lipophilic **1** readily traverses the cell membrane and is then hydrolysed to 2-methyl-8-(toluene-*p*-sulfonamido)-6-quinolyloxyacetic acid, H_2L **2**, which at the cell pH ≈ 7 exists in the less lipophilic and charged HL^- carboxylate form which is less likely to traverse cell membranes, but which is capable of complexing Zn^{2+}.† (A similar ester hydrolysis appears to prevent leakage of the Ca^{2+}-selective fluorophores Quin-2[20] and Fura-2[21] from cells).

We now report a potentiometric titration and UV/VIS and fluorescence spectroscopic study of the complexation of Zn^{2+} by HL^-. Since ligands which complex Zn^{2+} usually also complex Co^{2+}, Ni^{2+} and Cu^{2+}, the interactions of HL^- with these ions, together with Mg^{2+}, Ca^{2+} and Cd^{2+} have also been examined. The use as histological stains for Zn^{2+} of 8-hydroxyquinoline[22] and *N*-(6-methoxy-8-quinolyl)toluene-*p*-sulfonamide[23] which bear structural similarities to HL^- has been reported.



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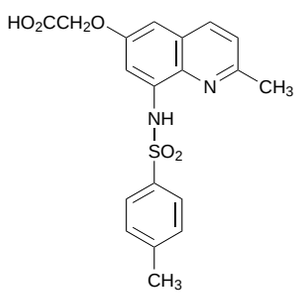

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Experimental

Materials

2-Methyl-8-(toluene-*p*-sulfonamido)-6-quinolyloxyacetic acid was prepared as described elsewhere.[24] Metal perchlorates were either prepared from metal carbonates through reaction with the stoichiometric amount of perchloric acid, or were purchased. In either case they were twice recrystallised from water, and were dried and stored over P_2O_5 under vacuum. Deionised water was further purified using a MilliQ-Reagent system to produce water with a resistance of >15 MΩ cm. Analytical grade ethanol distilled from CaO was used in all solution preparations. The metal perchlorate, perchloric acid and sodium hydroxide (all with *I* adjusted to 0.10 mol dm^{-3} with $NaClO_4$) titration solutions were prepared in 50% aqueous ethanol by volume under nitrogen and were standardised by conventional methods. Solutions for spectroscopic study were buffered with 0.10 mol dm^{-3} pipes buffer [piperazine-*N*,*N*′-bis(ethane-2-sulfonate)] (Cal Biochem) adjusted to pH 6.6 with NaOH and $HClO_4$ (BDH Analar). Disodium methylenedinitrilo-

† Compounds **1** and **2** are commercially available as Zinquin E and Zinquin A.

tetraacetate, Na_2H_2edta, was used as a sequestering agent for Zn^{2+}.

Potentiometric titrations

Potentiometric titrations were carried out using a Metrohm E665 Dosimat autoburette interfaced to a Laser XT/3-8086 personal computer in conjunction with an Orion SA720 potentiometer and an Orion Ross Sureflow combination electrode. The electrode was calibrated using standard buffer solutions and no corrections were made to pH values determined in the 50% aqueous ethanol solutions. Titrations were carried out at 298.2 ± 0.05 K in a water-jacketed vessel which was closed apart from a small exit for the nitrogen stream which was bubbled through the magnetically stirred titration solutions to exclude atmospheric carbon dioxide. The instrumentation was calibrated by titration of 0.100 mol dm^{-3} NaOH (1.00 cm^3) from the autoburette against 0.004 mol dm^3 HNO_3 (10.00 cm^3). The protonation constants for H_3L^+, pK_{an}, were determined by titration of a solution (10.00 cm^3) 0.004 and 0.001 mol dm^{-3} in H_2L and $HClO_4$, respectively, with 0.100 mol dm^{-3} NaOH (1.00 cm^3). The complexation constants for [ML], $[ML_2]^{2-}$ and $[ML_3]^{4-}$ (M^{2+} = Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} or Cd^{2+}), β_1, β_2, and β_3, respectively, were determined by titrations of solutions (10.00 cm^3) 0.00067–0.00335, 0.002–0.010, and 0.0003–0.0022 mol dm^{-3} in H_2L, $HClO_4$ and $M(ClO_4)_2$, respectively with 0.100 mol dm^{-3} NaOH (1.00 cm^3). All titrations were carried out in triplicate at least. The pK_{an}, β_1, β_2 and β_3 values were determined using the program SUPERQUAD.[25] A typical speciation plot is shown in Fig. 1.

Spectroscopy

The UV/VIS and fluorimetric measurements were carried out on a Cary 2200 and Perkin-Elmer LS50B instruments, respectively. The solutions studied were contained in 1 cm pathlength silica cells thermostatted at 298.2 K. Zinc(II) is environmentally ubiquitous and is present as a low-level impurity in some high-grade commercial chemicals as shown by our atomic absorption measurements. Accordingly, all components of a solution may either be further purified, at the risk of introducing other impurities, or the Zn^{2+} impurity level may be determined *in situ*. The second approach was adopted in this study where the $[Zn^{2+}]_{impurity}$ in two solution sets prepared with different batches of buffer and ethanol was found to be 7.0×10^{-7} and 1.03×10^{-6} mol dm^{-3} (which could be neglected in the potentiometric studies described above, but not in the UV spectrophotometric and fluorimetric measurements). Thus, the UV spectra of two sets of solutions of HL^- in the range 1.062×10^{-5}–5.311×10^{-5} mol dm^{-3}, one containing no edta and the other being 2.00×10^{-5} mol dm^{-3} in edta, were determined. (edta Complexes Zn^{2+} with $K = 10^{16.44}$ dm^3 mol^{-1} in aqueous solution[26] and is therefore a strong Zn^{2+} sequestering agent). In the absence of edta, UV absorbances attributable to the presence of $[ZnL_2]^{2-}$ (Fig. 2) were present, but were absent from the spectrum of HL^- alone determined from the solutions containing edta. The absorbance at 263 nm varied linearly with $[HL^-]$ and gave an intercept of 5.16×10^{-2} dm^3 mol^{-1} cm^{-1} at $[HL^-] = 0$, while in the presence of 2.0×10^{-5} mol dm^{-3} edta a parallel variation of lesser magnitude occurred and gave an intercept of zero at $[HL^-] = 0$. This is consistent with all impurity Zn^{2+} being complexed as $[ZnL_2]^{2-}$, as indicated by the β_1 and β_2 data in Table 1 in the absence of edta and $[Zn^{2+}]_{impurity} = 7.00 \times 10^{-7}$ mol dm^{-3}. The intensities of the impurity peaks were invariant with $[HL^-]$ in the range 4.25×10^{-6}–5.31×10^{-5} mol dm^{-3} consistent with HL^- being an insignificant contributor to the Zn^{2+} impurity level. This determination of $[Zn^{2+}]_{impurity}$ permitted the calculation of $[Zn^{2+}]_{total}$ and therefore of $[HL^-]$, [ZnL] and $[ZnL_2]^{2-}$ in solutions from which the UV/VIS spectra in Fig. 2 were determined. The $[Zn^{2+}]_{impurity}$ was determined in a different manner in the fluorescent studies as described below. The UV/VIS spectrophotometric data fitting to obtain the spectra in Fig. 2, and fluorescence data fitting discussed below, was effected on a AcerPower 466d computer using a non-linear least-squares regression analysis based on Method 5 of Pitha and Jones.[27]

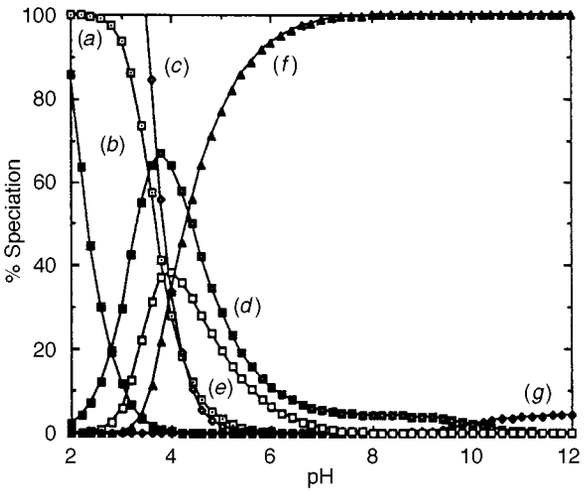


Fig. 1 Speciation plot for a 50% aqueous ethanol (v/v) solution 4.90×10^{-4} mol dm^{-3} in $[Zn^{2+}\ species]_{total}$ and 1.00×10^{-3} in $[H_nL^{(n-2)+}\ species]_{total}$ at 298.2 K and $I = 0.10$ mol dm^{-3} ($NaClO_4$). Species are shown as percentages where 100% = $[Zn^{2+}\ species]_{total}$. Species: (*a*) Zn^{2+}, (*b*) H_3L^+, (*c*) H_2L, (*d*) HL^-, (*e*) [ZnL], (*f*) $[ZnL_2]^{2-}$ and (*g*) L^{2-}

Results and Discussion

Equilibria

In 50% aqueous ethanol at 298.2 K and $I = 0.10$ mol dm^{-3} ($NaClO_4$) H_3L^+ undergoes three acid dissociations, equations (1) and (2) (where $n = 1$, 2 or 3) characterised by $pK_{a1} = 10.01 \pm 0.02$, $pK_{a2} = 3.72 \pm 0.03$ and $pK_{a3} = 1.87 \pm 0.10$ assigned to the sulfonamide, carboxylic and quinolinium protons, respectively. The complexation of M^{2+} by HL^- in 50% aqueous ethanol at 298.2 K, pH 6.6 and $I = 0.10$ mol dm^{-3} ($NaClO_4$), equation (3),

$$H_nL^{(n-2)+} \overset{K_{an}}{\rightleftharpoons} H_{n-1}L^{(n-3)+} + H^+ \quad (1)$$

$$K_{an} = [H^+][H_{n-1}L^{(n-3)+}]/[H_nL^{(n-2)+}] \quad (2)$$

$$M^{2+} + nHL^- \rightleftharpoons [ML_n]^{(2n-2)-} + nH^+ \quad (3)$$

occurs rapidly and is characterised by the complexation constants β_n, equation (4), where $n = 1$, 2 or 3 and $K_1 = \beta_1$, $K_2 = \beta_2/\beta_1$, $K_3 = \beta_3/K_2$, where K_n is a stepwise complexation constant (Table 1). A substantial variation in stability of [ML] occurs for Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} with [CuL] being the most stable as anticipated from the Irving–Williams series,[28] although the constraining nature of L^{2-} may be expected to accentuate small differences in the complexing characteristics of these metal ions. A similar variation occurs for the stability of $[ML_2]^{2-}$. (While a $[CoL_3]^{4-}$ species was detected, analogous species were not observed for the other metal ions.) A speciation plot for the Zn^{2+} system is shown in Fig. 1. The [MgL] and [CaL] species were not detected in this study consistent with $\log(\beta_1/dm^3\ mol^{-1}) < 2$ and the hard-acid character of Mg^{2+} (0.72) despite its similarity in size to the borderline hard-acid[29,30] Co^{2+} (0.745), Ni^{2+} (0.69), Cu^{2+} (0.73) and Zn^{2+} (0.74) ions. (The figures in parentheses are the six co-ordinate ionic radii in Å.[31]). This effect of the hardness on complex stability of

$$\beta_n = [ML_n^{(2n-2)-}]/[M^{2+}][L^{2-}]^n \quad (4)$$

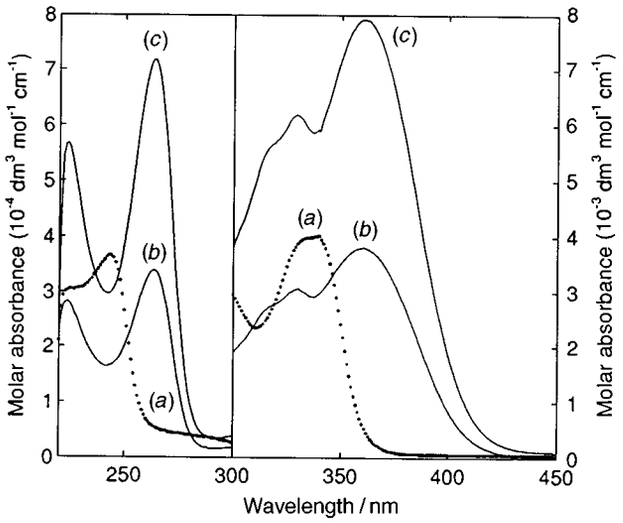


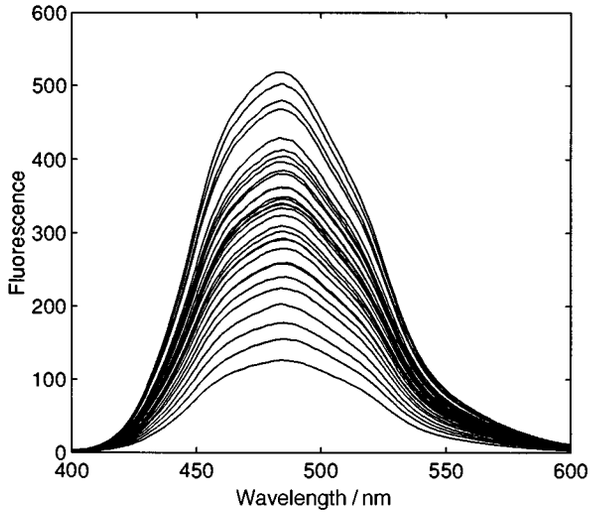
Fig. 2 Absorbance spectra of (*a*) HL^-, (*b*) [ZnL] and (*c*) $[ZnL_2]^{2-}$ in 50% aqueous ethanol solution 0.10 mol dm^{-3} in pipes buffer at pH 6.6 and 298.2 K. The spectrum of HL^-, (*a*) has maxima (nm), with molar absorbances (dm^3 mol^{-1} cm^{-1}) shown in parentheses, at 225 (3.40×10^4), 243 (3.66×10^4) and 336 (3.49×10^3). Analogous data for [ZnL], (*b*) are 224 (2.82×10^4), 263 (3.40×10^4), 318 (2.75×10^3), 330 (3.07×10^3) and 361 (3.81×10^3), and for $[ZnL_2]^{2-}$, (*c*) 224 (5.67×10^4), 263 (7.18×10^4), 318 (5.66×10^3), 329 (6.19×10^3) and 361 (7.92×10^3). The molar absorbtion for coefficients (*b*) and (*c*) are based on the complexes [ZnL] and $[ZnL_2]^{2-}$ and therefore to make comparisons on the basis of L^{2-} the molar absorbances of (*c*) must be halved. The absorbance data were derived from solutions where $[HL^-] = 1.27 \times 10^{-5}$ and [edta] = 1.00×10^{-5} mol dm^{-3}, respectively, for the left-hand section of the HL^- spectrum, and 1.06×10^{-4} and 1.00×10^{-5} mol dm^{-3}, respectively, for the right-hand section. For the [ZnL] spectrum data in the left-hand section were obtained from a solution for which $[HL^-]_{total} = 1.27 \times 10^{-5}$ and $[Zn^{2+}]_{total} = 2.47 \times 10^{-4}$ mol dm^{-3}, and for the right-hand section 1.06×10^{-4} and 2.00×10^{-3} mol dm^3. For the $[ZnL_2]^{2-}$ spectrum, data in the left-hand section were obtained from a solution for which $[HL^-]_{total} = 2.55 \times 10^{-5}$ and $[Zn^{2+}]_{total} = 1.27 \times 10^{-4}$ mol dm^{-3} and for the right-hand section 2.12×10^{-4} and 1.07×10^{-4} mol dm^{-3}

M^{2+} is also demonstrated by the larger hard acid Ca^{2+} (1.00) for which no complexation was detected, and the borderline hard acid Cd^{2+} (0.95) for which both [CdL] and $[CdL_2]^{2-}$ are quite stable (Table 1). The decrease in β_1 and β_2 characterising [CdL] and $[CdL_2]^{2-}$, by comparison with those characterising [ZnL] and $[ZnL_2]^{2-}$, reflects the greater size of Cd^{2+} and the consequent decrease in electrostatic interaction with L^{2-}, and possibly an increase in steric strain induced in bidentate L^{2-}.

UV/VIS and fluorescence spectra of HL^-, [ZnL] and $[ZnL_2]^{2-}$

The UV/VIS spectra of HL^-, [ZnL] or $[ZnL_2]^{2-}$ are shown in Fig. 2, from which it is seen that the displacement of H^+ from HL^- by Zn^{2+} produces substantial changes in the ligand spectrum. The spectra of the co-ordinated ligand in [ZnL] and $[ZnL_2]^{2-}$ are very similar.

Excitation at the absorbance maximum of HL^-, 336 nm, of a solution 5.00×10^{-6} mol dm^{-3} in HL^-, 1.00×10^{-5} mol dm^{-3} in edta and 0.10 mol dm^{-3} in pipes at pH 6.6 produced no detectable fluorescence in the range 400–600 nm. In the absence of edta, excitation at 358 nm, close to the absorbance maxima of [ZnL] and $[ZnL_2]^{2-}$, of solutions of HL^- showed systematic fluorescence increases with increasing total $[Zn^{2+}]_{added}$ over the lower part of the $[Zn^{2+}]_{added}$ range. The magnitude of this increase lessened at higher $[Zn^{2+}]_{added}$ consistent with the complexation of HL^- reaching completion (Fig. 3). In the absence of added Zn^{2+} a substantial fluorescence was observed for the 5.00×10^{-6} HL^- solution characterised by the lowest-amplitude spectrum in Fig. 3. This fluorescence results from the complexation of HL^- by the Zn^{2+} impurity (see Experimental section) common to this solution and the other 30 solutions whose spectra are shown in Fig. 3. Accordingly, it was decided simultaneously to determine the $[Zn^{2+}]_{impurity}$ and the fluorescence spectra of [ZnL] and $[ZnL_2]^{2-}$ by fitting equilibrium (3) to the montage of fluorescence spectra at 0.5 nm intervals over the range of 430–560 nm on the basis that $[Zn^{2+}]_{total} = [Zn^{2+}]_{added} + [Zn^{2+}]_{impurity}$. As HL^- is not excited by 358 nm radiation, its ground-state pK_{a3} and the β_1 and β_2 (Table 1) derived for the ground-state formation of [ZnL] or $[ZnL_2]^{2-}$ discussed above may be utilised in the analysis of the fluorescence variation with $[Zn^{2+}]_{total}$. This analysis produced the individual fluorescence spectra of L^{2-} in [ZnL] ($\lambda_{max} = 484$ nm, molar fluorescence = 9.86×10^7) and $[ZnL_2]^{2-}$ ($\lambda_{max} = 486$ nm, molar fluorescence = 6.67×10^7) shown in Fig. 4 and a determination of $[Zn^{2+}]_{impurity} = 1.03 \times 10^{-6}$ mol dm^{-3}. (The latter value compares with $[Zn^{2+}]_{impurity} = 7.0 \times 10^{-7}$ mol dm^{-3} deter-



Fig; 3 Variation of fluorescence with increasing $10^{-6}[Zn^{2+}]_{total} = 1.03$, 1.23, 1.43, 1.62, 1.83, 2.03, 2.23, 2.43, 2.63, 2.83, 3.03, 3.28, 3.53, 3.78, 4.03, 4.28, 4.53, 4.78, 5.03, 6.03, 7.03, 8.03, 9.03, 10.03, 11.03, 16.03, 21.03, 41.03, 61.03, 81.03 and 101.03 mol dm^{-3}. $[HL^-]_{total} = 5.00 \times 10^{-6}$ mol dm^{-3}. The first spectrum results from complexation of the 1.03×10^{-6} mol dm^{-3} impurity Zn^{2+} and the subtraction of $[Zn^{2+}]_{impurity} = 1.03 \times 10^{-6}$ mol dm^{-3} from the concentrations of all the solutions yields $[Zn^{2+}]_{added}$. Spectra were obtained in 50% aqueous ethanol solution 0.10 mol dm^{-3} in pipes buffer at pH 6.6 and 298.2 K

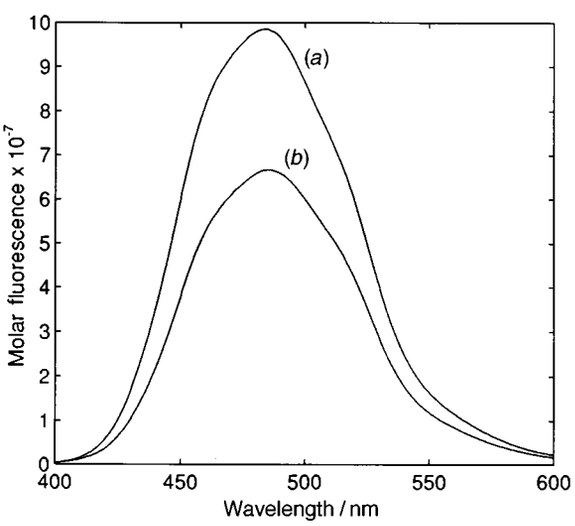


Fig. 4 Fluorescence spectra of (*a*) [ZnL] and (*b*) $[ZnL_2]^{2-}$ (per L^{2-}) derived from the data in Fig. 3 calculated from $\log(\beta_1/\text{dm}^3\ \text{mol}^{-1}) = 9.65$ and $\log(\beta_2/\text{dm}^6\ \text{mol}^{-2}) = 19.11$. For L^{2-} in [ZnL] $\lambda_{max} = 484$ nm and molar fluorescence = 9.86×10^7, and for $[ZnL_2]^{2-}$ $\lambda_{max} = 486$ nm and molar fluorescence = 6.67×10^7 (per L^{2-})

Table 1 Complexation constants[a] for $[ML_n]^{(2n-2)-}$ in 50% aqueous ethanol at 298.2 K, pH 6.6 and $I = 0.10$ mol dm^{-3} ($NaClO_4$)

M	$\log(\beta_1/dm^3\ mol^{-1})$	$\log(\beta_2/dm^6\ mol^{-2})$
Co[b]	8.12 ± 0.20	17.06 ± 0.11
Ni	<8	15.73 ± 0.03
Cu	11.96 ± 0.02	21.40 ± 0.03
Zn	9.65 ± 0.02	19.11 ± 0.06
Cd	8.44 ± 0.50	15.38 ± 0.40

[a] $K_1 = \beta_1$, $K_2 = \beta_2/K_1$ and $K_3 = \beta_3/K_2$; $\log(K_2/dm^3\ mol^{-1}) = 8.94$, >7.73, 9.44, 9.46 and 6.94 as the table is descended. [b] $\log(\beta_3/dm^9\ mol^{-3})$ 25.56 ± 0.11.

mined from UV absorption measurements discussed in the Experimental section, and is coincident with different batches of buffer being employed in the preparations of the solutions for UV and fluorescence spectroscopic study.) When $[Zn^{2+}]_{impurity}$ was set at 1.03×10^{-6} mol dm^{-3}, the $\log(\beta_1/dm^3\ mol^{-1}) = 9.34 \pm 0.01$ and $\log(\beta_2/dm^6\ mol^{-2}) = 19.29 \pm 0.01$ values, derived through fitting the variation of fluorescence with $[Zn^{2+}]_{total}$ in the range 430–560 nm at 0.5 nm intervals to equilibrium (3), compare reasonably with those derived potentiometrically. The corresponding molar fluorescences derived for L^{2-} in [ZnL] and $[ZnL_2]^{2-}$, 1.15×10^8 (484 nm) and 6.65×10^7 (486 nm) respectively, are reasonably similar to those derived in the first analysis of the fluorescence data.

The absence of detectable fluorescence for HL^- contrasts with the significant fluorescence observed for L^{2-} in [ZnL] and $[ZnL_2]^{2-}$. This is attributable to a restriction in the vibrational and internal rotational modes of bidentate L^{2-} and a consequent decrease in quenching efficiency by comparison with HL^-. The origin of the greater fluorescence of L^{2-} in [ZnL] than in $[ZnL_2]^{2-}$ is not obvious, but this environmental dependence of the fluorescence of co-ordinated L^{2-} has a potential application in differentiating between intracellular Zn^{2+} sites which is being explored.

No fluorescence of L^{2-} was detected in its complexes with Co^{2+}, Ni^{2+}, and Cu^{2+} and this is attributed to quenching of fluorescence through interaction with the unfilled d-orbital manifolds of these ions. Both [CdL] and $[CdL_2]^{2-}$ fluoresce less strongly than their Zn^{2+} analogues as is reported elsewhere.[16]

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