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'On–off–on' fluorescent indicators of pH windows based on three separated components†

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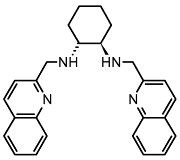
Received (in Cambridge, UK) 21st June 2002, Accepted 5th September 2002
First published as an Advance Article on the web 24th September 2002

'On–off–on' fluorescent indicators for pH windows are obtained with ternary systems in which the three separated components are Cu^{2+}, a tetraaza ligand and the fluorophore Coumarin 343: protonation of Coumarin 343, its coordination to Cu^{2+} and its displacement from Cu^{2+} by OH^- give an inverse bell-shaped variation of emission with pH.

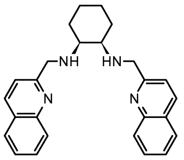
Systems displaying an 'on–off–on' or 'off–on–off' fluorescence emission upon variation of pH are of interest, as they allow facile visualization of pH windows.[1] These could be useful tools both for material sciences and for life sciences, at least provided that, for the latter case, they are fully soluble in water. A small number of multi-component molecules have been described in the literature, which display 'off–on–off' fluorescence emission with pH. Beside a very recent example based on the Eu^{III} luminescent complex of a tetra-quinoline-substituted cyclen ligand,[2] these molecules usually contain anthracene as the light-emitting component and one or more covalently linked pyridine and amine groups as pH-dependent quenching fragments.[1,3,4]

In this work we use a different approach, resembling the 'chemosensing ensemble' technique,[5] to obtain simple non-covalently bonded three-component systems capable of displaying an 'on–off–on' fluorescence emission with pH. We worked according to this scheme: the first component (**L**) is a tetraaza ligand, the second (M) is a five-coordinated metal cation, the third (F–AH) is a fluorescent molecule equipped with the acidic non-coordinating fragment –AH, which can undergo deprotonation and transform into the coordinating $-A^-$ fragment. At low pH values both the ligand and the fluorescent molecule are protonated, the system is in its fully separated form, $\{LH_n^{n+} + M + F{-}AH\}$ and the fluorescence of the free F–AH molecule is observed (fluorescence 'on'). At intermediate pH values the system is in its fully assembled form, $\{L\cdots M\cdots A{-}F\}$: M is bound to L and $F{-}A^-$ is bound to M, completing its coordination sphere and fluorescence is 'off', as it is quenched by the metal centre by means of Photoinduced Electron Transfer or Energy Transfer processes. Finally, at high pH values, the hydroxide anion displaces $F{-}A^-$ from the metal fifth coordination site, $\{L\cdots M\cdots OH + F{-}A^-\}$ is obtained and the system displays the full fluorescence of the $F{-}A^-$ free molecule (fluorescence 'on').

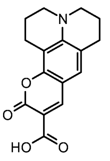
We have used the new molecules **tcq**‡ and **ccq**‡ as the tetraaza ligands, which contain two amino-quinoline bidentate units separated, respectively, by a *R*,*R*-*trans*-1,2-cyclohexyl and by a *cis*-1,2-cyclohexyl spacer. Cu^{2+} is used as the metal cation. The acid–base and coordinative properties of **tcq** and **ccq** have been examined in 4 : 1 dioxane–water as the solvent (due to the low solubility of the free ligands and of their metal complexes in pure water), by means of potentiometric titrations. Non-linear least squares treatment[6] of the obtained emf *vs.* added base data allowed us to determine the formation constants for the protonated and metal-containing species existing in the $2 < pH < 14$ range.[7] Only two protonation steps are observed for the ligands between pH 2 and 14 (formation of $\mathbf{L}H^+$ and $\mathbf{L}H_2^{2+}$ species) and only two metal-containing species form in the same pH interval, $[Cu(\mathbf{L})]^{2+}$ and $[Cu(\mathbf{L})(OH)]^+$ ($\mathbf{L}$ = **tcq**, **ccq**). From the obtained log*K* values,[7] it is possible to draw the distribution diagrams for the two $\mathbf{L}/Cu^{2+}$ systems, as % of species (with respect to total **L**) *vs.* pH (drawings are available in the ESI†). For $\mathbf{tcq}/Cu^{2+}$ in 1 : 1 molar ratio (total **tcq** and total $Cu^{2+} = 1 \times 10^{-3}$ M) $[Cu(\mathbf{tcq})]^{2+}$ and $[Cu(\mathbf{tcq})(OH)]^+$ exist as the prevalent species in well defined and separated pH ranges, *i.e.* they are ≥ 90% for $4.0 < pH < 9.1$ and for $pH > 10.9$, respectively. For the system containing **ccq** and Cu^{2+} (1×10^{-3} M concentration) $[Cu(\mathbf{ccq})]^{2+}$ and $[Cu(\mathbf{ccq})(OH)]^+$ are over 90% for $3.3 < pH < 8.4$ and for $pH > 10.3$, respectively.



tcq



ccq



Coumarin 343
(**C343**H)

UV-Vis spectra carried out at pH 7 and pH 11 display d–d bands centred at 642 nm ($\varepsilon = 120\ M^{-1}\ cm^{-1}$) and 613 nm ($\varepsilon = 125\ M^{-1}\ cm^{-1}$) for $[Cu(\mathbf{tcq})]^{2+}$ and $[Cu(\mathbf{tcq})(OH)]^+$ and at 665 nm ($\varepsilon = 95\ M^{-1}\ cm^{-1}$) and 612 nm ($\varepsilon = 106\ M^{-1}\ cm^{-1}$) for $[Cu(\mathbf{ccq})]^{2+}$ and $[Cu(\mathbf{ccq})(OH)]^+$, respectively. The spectra indicate that Cu^{2+} is five-coordinated in both forms and this is confirmed by the crystal structure[8] obtained for $[Cu(\mathbf{tcq})Cl]CF_3SO_3 \cdot 1.5H_2O$, *i.e.* the product precipitated by slow evaporation from a 1 : 1 solution of $Cu(CF_3SO_3)_2$ and $\mathbf{tcq} \cdot 2HCl$ at pH 7. Cu^{2+} assumes a roughly trigonal bipyramidal arrangement with the two quinoline nitrogens and the amine nitrogen N3 lying in the same plane and N2 and a Cl^- anion occupying the apical position of a distorted trigonal bipyramid, while the CF_3SO_3 anion is non-coordinating (a drawing and detailed distances and angles are available in the ESI†). According to these data, in the absence of coordinating anions the complexes $[Cu(\mathbf{tcq})]^{2+}$ and $[Cu(\mathbf{ccq})]^{2+}$ bind a water molecule in their fifth coordination position. In the perspective of our aims, this water molecule should be easily displaced by a stronger coordinating group. As the F–AH component we used the Coumarin 343 molecule (**C343**H), which features a –COOH moiety displaying a pK_a of 7.30 in 4 : 1 dioxane–water.[9] Irradiation of Coumarin 343 is carried out at 430 nm, *i.e.* on the isosbestic point observed in the visible spectra of this molecule undergoing the equilibrium $\mathbf{C343}H = \mathbf{C343}^- + H^+$ in 4 : 1 dioxane water mixture. The emission band displays a maximum at 490 nm for the **C343**H species, shifting with pH at 470 nm for the $\mathbf{C343}^-$ carboxylate species. Solutions containing only Coumarin 343 are thus

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† Electronic supplementary information (ESI) available: further experimental and structural details. See http://www.rsc.org/suppdata/cc/b2/b205951g/

DOI: 10.1039/b205951g

intensely fluorescent at any pH value, as shown by the profile I_f *vs.* pH at 473 nm, which is an isosbestic point in the emission spectra series obtained on changing pH (gray triangles in Fig. 1; the emission spectra series is available in the ESI†). Fluorescent intensity *vs.* pH has then been measured for the three-component system made by **L**/Cu^{2+}/**C343**H in 200:200:1 molar ratio (analytical concentrations: 4×10^{-4} M, 4×10^{-4} M, 2×10^{-6} M) with the usual excitation wavelength of 430 nm. The two orders of magnitude difference in the concentration of **C343**H with respect to the two other components has been chosen considering the strong emission intensity of Coumarin 343 and with the aim to let it adapt to the system, playing a true indicator role, without modifying the % of species *vs.* pH distribution of the **L**/Cu^{2+} complexes. What is found with **tcq** and **ccq** is similar, and only the first case is described in detail.

A series of superimposable full-emission spectra is observed at low pH values ($2 < pH < 4$, $\lambda_{max,emission} = 490$ nm, typical of **C343**H), while on increasing pH from 4 to 7 a series of emission spectra with decreasing intensity is observed. Superimposable spectra with almost nil emission intensity are found at $7 < pH < 9.5$. From pH 9.5 to pH 11 spectra of increasing intensity are again observed, and at $pH > 11$ superimposable, full-emission spectra are recorded, with $\lambda_{max,emission}$ shifted at 470 nm, as typical of the free **C343**$^-$ carboxylate species. Noticeably, the reverse series of spectra is observed on going back from basic to acidic solution and the I_f values are reproducible, at the same pH values, after any change of pH. By plotting the I_f *vs.* pH profile at 473 nm, what is obtained with this system is thus a reverse bell-shaped curve, which is shown in Fig. 1 (black circles), corresponding to an 'on–off–on' fluorescence-readable pH window. The observed I_f *vs.* pH data fit with a constant of 4.8 (±0.1) log units for the equilibrium [Cu(**tcq**)]$^{2+}$ + **C343**$^-$ = [Cu(**tcq**)(**C343**)]$^+$. With this further data, a distribution diagram for the species containing the Coumarin 343 molecule can be drawn, which is shown in Fig. 1 (solid lines). The diagram clearly shows that, as expected, fluorescence emission is guaranteed at low pH values by the free **C343**H molecule (state **a** in Scheme 1). At intermediate pH values I_f is quenched due to the formation of the ternary complex [Cu(**tcq**)(**C343**)]$^+$ (state **b**) in which PET or ET processes involving Cu^{2+} and the excited **C343**$^-$ molecule are effective. At higher pH values, the formation of the hydroxide complex [Cu(**tcq**)(OH)]$^+$ promotes the release of the free **C343**$^-$ anionic species, and as a consequence fluorescence is revived (state **c**). An analogous 'on–off–on' fluorescent indicator for the pH window is obtained in the case of ligand **ccq**/Cu^{2+}/Coumarin 343 in 200:200:1 molar ratio. In this case, a log$K$ of 4.5 (±0.1) is determined for the [Cu(**ccq**)]$^{2+}$ + **C343**$^-$ = [Cu(**ccq**)(**C343**)]$^+$ equilibrium. The obtained reverse dumb-bell shaped I_f *vs.* pH profile presents a narrower non-fluorescent zone ($7.5 < pH < 9$) with respect to the system with **tcq**, due to the combination of the lower log$K$ value for the [Cu(**ccq**)]$^{2+}$/**C343**$^-$ interaction and of the more favourable pK_a value[7] for the coordinated water molecule in [Cu(**ccq**)]$^{2+}$. According to our results, 'on–off–on' fluorescent indicators of pH are easily obtained from three-component systems based on tetra-aza ligands, Cu^{2+} and a fluorescent fragment featuring a protonable binding unit. Unfortunately, their use in biological systems is prevented by their poor solubility in pure water and by the toxicity of the coordinatively labile Cu^{2+} cation. On the other hand, their use in material science can be easily envisaged. Moreover, it can be foreseen that on variation of (i) the fluorophore and its pK_a; (ii) the ligand and the pK_a of the water molecule coordinated to the copper complex; (iii) the ligand and the log$K$ value for the association with the fluorophore in its binding form, it can be possible to fine tune the amplitude and the position along the pH-axis of the fluorescence-signalled window.

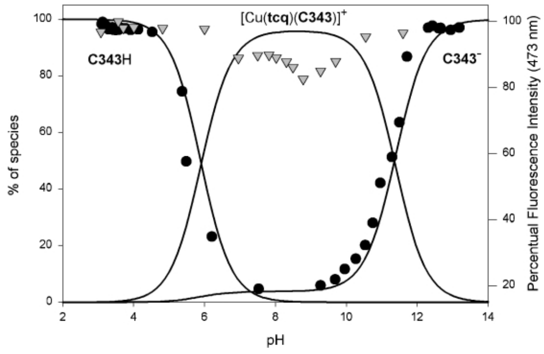
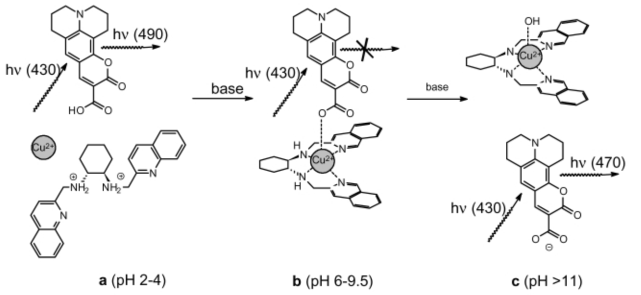


Fig. 1 Solid lines (left axis): % of Coumarin 343-containing species, with respect to total Coumarin 343, for a solution of **tcq**/Cu^{2+}/Coumarin 343 4×10^{-4} M/4×10^{-4} M/2×10^{-6} M. Gray triangles: I_f (percentual; right axis) *vs* pH for a 2×10^{-6} M Coumarin 343 solution, in the absence of any added species. Black circles: I_f (percentual; right axis) *vs* pH for a 2×10^{-6} M Coumarin 343 solution plus 4×10^{-4} M **tcq** and Cu^{2+}.



Scheme 1

This work was supported by the European Union (RT Network Molecular Level Devices and Machines—Contract HPRN-CT-2000–00029).

Notes and references

‡ **ccq** and **tcq** ligands have been synthesized as oils by reduction with $NaBH_4$ (hot methanol as solvent) of the parent bis-imino compounds.[10] Redissolution in methanol, treatment with 37% HCl and addition of excess diethyl ether gave the ammonium salts of the two ligands, which were found analytically pure (CHN analysis).

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7 Formation constants in log units (value uncertainty in parenthesis) [**TCQ**H]$^+$ 8.38 (0.01), [**TCQ**H_2]$^{2+}$ 11.75 (0.02), [Cu(**TCQ**)]$^{2+}$ 9.45 (0.01), [Cu(**TCQ**)(OH)]$^+$ −0.52 (0.02); [**CCQ**H]$^+$ 8.77 (0.01), [**CCQ**H_2]$^{2+}$ 12.47 (0.02), [Cu(**CCQ**)]$^{2+}$ 10.95 (0.01), [Cu(**CCQ**)(OH)]$^+$ 1.65 (0.01)]. The values for the pK_a of the coordinated water molecule can be calculated from these data, obtaining a pK_a of 9.97 (0.02) for the formation of [Cu(**TCQ**)(OH)]$^+$ and of 9.30 (0.01) for the formation of [Cu(**CCQ**)(OH)]$^+$.
8 Crystal data. $C_{27}H_{28}ClCuF_3N_4O_3S \cdot 1.5H_2O$, $M = 671.61$, monoclinic, $a = 29.723(5)$, $b = 8.506(5)$, $c = 11.882(5)$ Å, $U = 2995(2)$ Å^3, $T = 298$ K, space group $C2$, $Z = 4$, $\mu(MoK\alpha) = 0.948$, mm^{-1}, 10133 reflections measured, 6368 unique ($R_{int} = 0.0494$) were used in all calculations. Final R indices [$I > 2\sigma(I)$]. $R_1 = 0.0461$, $wR_2 = 0.0968$. R indices (all data). $R_1 = 0.1326$, $wR_2 = 0.1258$. CCDC 188466. See http://www.rsc.org/suppdata/cc/b2/b205951g/ for crystallographic data in CIF or other electronic format.
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