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Using micelles for a new approach to fluorescent sensors for metal cations†

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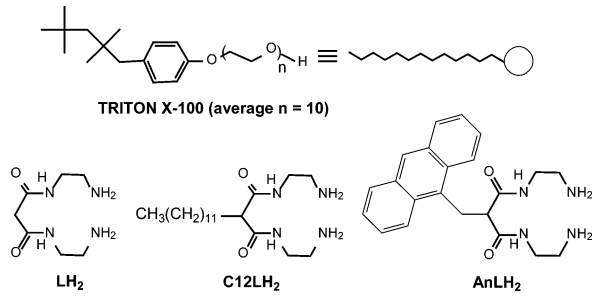
A new approach based on self-assembly inside micelles has been individuated to prepare a system behaving as a water-operating selective fluorescent sensor for Cu^{2+} and Ni^{2+}.

Fluorescent sensors for metal cations are commonly based on the covalent approach, *i.e.* they are multi-component molecules containing a fluorophore and a receptor covalently linked by a spacer.[1] In this communication we introduce a new approach, that makes use of components similar to those employed with the covalently linked molecules, but that is instead fully supramolecular, takes advantage of self-assembly and thus requires almost no synthetic effort. With this approach, a receptor is lipophilized with an alkyl chain which makes it insoluble in pure water. However, if water contains a suitable quantity of a surfactant, micelles are formed and the lipophilized receptor is solubilized by inclusion inside them.[2] Then, as it is well known from literature,[3] addition to the micellar water solution of a hydrophobic fluorescent aromatic hydrocarbon (*e.g.* pyrene) results in its inclusion inside the

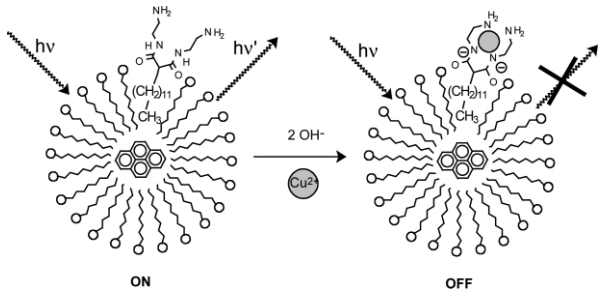


micelle. Under these conditions, if the lipohilized ligand offers no quenching mechanisms, the fluorescence of the aromatic hydrocarbon is full. Quenching can then be observed only if a metal cation is bound by the receptor, and the obtained complex offers intramicellar electron-transfer[4a,b] or energy-transfer processes, as is commonly found in particular for transition metal complexes.[4c] Thus, the whole system works as an ON–OFF fluorescent sensor. As a first example, we chose Cu^{2+} and Ni^{2+} as the target metal cations, pyrene as the fluorophore, dioxo-2,3,2 as a selective receptor (lipophilized with a linear C_{12} chain, *i.e.* ligand **C12LH$_2$**[5]) and the well studied neutral surfactant Triton X-100 for micelle formation. Plain dioxo-2,3,2 (**LH$_2$**) is known to selectively bind Cu^{2+} and Ni^{2+} in water,[6,7] at pH >7.5 and >8.5, respectively, thanks to the release of its two amido protons and the formation of the neutral [**L**Cu] or [**L**Ni] complexes. Accordingly, its methylanthracene derivative, **AnLH$_2$**, has been used as an ON–OFF fluorescent sensor for Ni^{2+} and Cu^{2+}, with enhanced selectivity for Cu^{2+}. However, for **AnLH$_2$** it has been necessary to use dioxane-enriched aqueous solutions (4 : 1 v/v $C_4H_8O_2$: H_2O) as the solvent.[8]

We now present evidence that our system also works as an ON–OFF sensor for Ni^{2+} and Cu^{2+} but *in water*, as described by Scheme 1, and it is also capable of selectively signalling Cu^{2+} in the presence of Ni^{2+}.

We determined the protonation and Ni^{2+} and Cu^{2+} complexation constants for the water insoluble **C12LH$_2$** ligand, which dissolves easily in water containing 6.47 g l^{-1} of Triton X-100 (Caledon, average MW 647, corresponding to an average concentration of 0.01 M), thanks to the inclusion in the micelles of its hydrophobic chain. We worked with ligand **C12LH$_2$** concentrations of 0.001 M. Under these conditions, we carried out potentiometric titrations in water (0.05 M $NaNO_3$ as a supporting electrolyte). Interestingly, in agreement with what is already found in the literature for micelle-forming lipophilized ligands,[5] the found protonation and complexation constants[9] are very similar to those calculated for the plain **LH$_2$** ligand in water.[6,7] In particular, as found for **LH$_2$**, **C12LH$_2$** also forms only one significant M^{2+} complex species in the 2–12 pH range, *i.e.* the neutral [**C12L**M], that represents more than 95% of the system when the pH is raised over 7.05 and 8.75 (for Cu^{2+} and Ni^{2+}, respectively) and the two amido groups deprotonate according to the equilibrium **C12LH$_2$** + M^{2+} = [**C12L**M] + $2H^+$ (see Fig. 1 for species distribution for M = Cu; analogous data are available for M = Ni in the ESI†). The logarithmic formation constants for the mentioned equilibria are -5.20 and -9.64 for Cu^{2+} and Ni^{2+}, while -5.13[6] and -10.66[7] have been reported for **LH$_2$** under similar conditions. The Cu^{2+} complex is purple colored (λ_{max} = 516 nm, ε = 70 M^{-1} cm^{-1}) and the Ni complex yellow (λ_{max} = 460 nm, ε = 80 M^{-1} cm^{-1}). The λ_{max} and ε values are typical of these kinds of complexes.[6–8] Coupled spectrophotometric and pH-metric titrations confirm that the profile of absorbance *vs.* pH (absorbance taken at λ_{max}) superimposes the curve relating to the [**C12L**M] distribution (see Fig. 1 for M = Cu, black squares).

In a second step, pyrene was added to the surfactant–water solutions to obtain 9×10^{-6} M solutions of the fluorophore. AN (aggregation number) for Triton X-100 in pure water is reported to be in the range 104–111 (T = 298 K),[10] and we recalculated it under our operating conditions (0.001 M **C12LH$_2$** and 0.05M $NaNO_3$, T = 298 K) by means of the established technique based on TRF (time resolved fluorescence),[11] obtaining a value of AN ranging from 63 (pH = 2.5) to 74 (pH = 11.6). According to this, an average of 0.06 pyrene molecules reside in each micelle, while



Scheme 1

† Electronic supplementary information (ESI) available: distribution diagram for the surfactant/**C12LH$_2$** system in the presence of Ni^{2+} and series of pyrene emission spectra on changing pH. See http://www.rsc.org/suppdata/cc/b4/b404543b/

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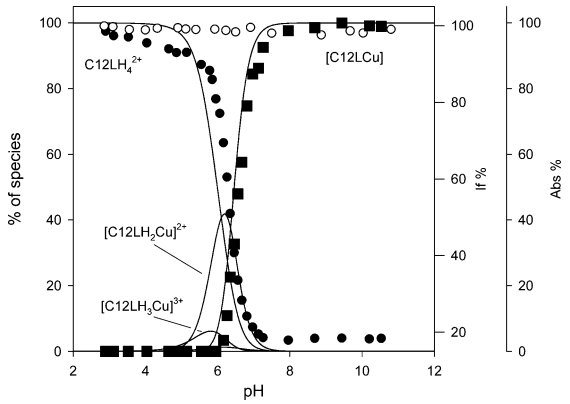

Fig. 1 Solid lines: distribution diagram (% of species *vs.* pH) for a water solution containing Triton X-100 (6.47 g l^{-1}), **C12LH$_2$** (0.001 M) and Cu^{2+}(0.001 M). The species corresponding to each profile are indicated on the diagram. ■: Absorbance (516 nm) *vs.* pH for the same solution; ●: I_f (393 nm) *vs.* pH for the same solution, containing also 9×10^{-6} M pyrene; ○: I_f (393 nm) *vs.* pH for a solution containing 9×10^{-6} M pyrene, Triton X-100 (6.47 g l^{-1}), **C12LH$_2$** (0.001 M), but no added Cu^{2+} cation.

~7 **C12LH$_2$** molecules are incorporated in each micelle (the low pyrene : micelle ratio has been chosen to avoid the formation of intramicellar excimers, which could easily form with Triton X-100 as surfactant, when an average of more than one pyrene per micelle is employed;[10c] the large excess of **C12LH$_2$** with respect to the fluorophore allows a significant fluorimetric response for small fractions of formed [**C12L**M]). With this concentration of surfactant, ligand, supporting electrolyte and fluorophore, we performed coupled pH-metric and fluorimetric titrations (λ_{exc} = 340 nm) and under these conditions full emission of the pyrene is observed over the 2 < pH < 12 interval (Fig. 1, white circles: $\lambda_{emission}$ = 393 nm, I_f *vs.* pH). The same titration but in the presence of 0.001 M $M(CF_3SO_3)_2$ (*i.e.* with Cu^{2+} or Ni^{2+} in a 1 : 1 molar ratio with respect to **C12LH$_2$**) showed a typical sigmoidal ON–OFF behaviour of the pyrene emission, as can be seen in Fig. 1 (black circles, $\lambda_{emission}$ = 393 nm, I_f *vs.* pH). In particular, I_f is quenched when the [**C12L**M] complex is formed (18% and 21% of the full intensity is reached in the maximum quenching zone, for Cu^{2+} and Ni^{2+}), so that our system signals the presence of Cu^{2+} and Ni^{2+} by switching off pyrene fluorescence in the micelle–complex–fluorophore self-assembled system, as described by Scheme 1. Interestingly, the energy-demanding deprotonation of the amido groups to form the [**C12L**M] complex can be promoted only by transition metal cations late in the first series, *i.e.* Cu^{2+} and Ni^{2+}.[8] Selectivity for these two cations was demonostrated with pH/fluorimetric titrations on water containing Triton X-100–pyrene–**C12L**H$_2$ and M^{2+} (M = Mn, Fe, Co, Zn), which did not result in any fluorescence quenching, as they do not form the [**C12L**M] complex. Moreover, selectivity of Cu^{2+} with respect to Ni^{2+} is possible by working at selected pH values, *i.e.* 7.0–7.5, in which [**C12L**Ni] is not formed, while [**C12L**Cu] reaches 95%. Addition of Ni^{2+} to the sensor solution buffered at pH = 7.2 (HEPES buffer; up to 2 : 1 Ni^{2+} : **C12LH$_2$** molar ratio) did not result in any variation of fluorescence. Further addition of Cu^{2+} resulted instead in the expected fluorescence quenching.‡ Finally, the intramicellar nature of the quenching mechanism is fully supported by parallel pH-metric, spectrophotometric and fluorimetric titrations run on solutions containing surfactant, pyrene, Cu^{2+} and the plain ligand **LH$_2$** in the same concentrations used in our system. In this case, the formed [**L**Cu] complex (purple, λ_{max} = 516 nm) is water-soluble and it is not included in the micelles. Accordingly, no quenching mechanism can set on, and full pyrene emission is observed over the whole 2–12 pH range.§

Beyond these results, the general advantages of this kind of approach include the easy change of the used fluorophore (allowing a wide choice of reading wavelengths), the easy change of the target metal cation (provided that a selective ligand is available in water, and a lipophilization route can be individuated) and the use of pure water, instead of prevalently organic solvent mixtures. Moreover, micelles are also known to allow separation of included species and our approach could thus lead to separation after signalling of the target species. In this respect, preliminary studies have shown that it is possible to treat with an ultrafiltration apparatus a solution at pH 10 of the Triton X-100/**C12LH$_2$**/Cu^{2+}/pyrene system, concentrating in 1/10 of the starting volume more than 95% of Cu^{2+} and more than 99.5% of pyrene. Studies are in course on optimization of this procedure and on Ni^{2+}/Cu^{2+} separation.

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Notes and references

‡ Additions of substoichiometric quantities (with respect to **C12LH$_2$**) of Cu^{2+} to solutions containing surfactant, pyrene and **C12LH$_2$** in the usual concentrations have been carried out also at pH 9, 10, 11 and 12, observing the expected fluorescence quenching. It has to be stressed that all the added Cu^{2+} is bound to the **C12LH$_2$** receptor, thus obtaining clear (*i.e.* non-turbid) solutions, up to 1 : 1 ligand : metal molar ratio.

§ Moreover, additions of Cu^{2+} to a solution containing only Triton X-100 and pyrene (*i.e.* with no **C12LH$_2$** ligand; pH 7.5) did not result in any I_f variation

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9 Ligand protonation: **C12LH$_2$** + H^+ = [**C12LH$_3$**]$^+$, log*K* = 8.73 (±0.01); **C12LH$_2$** + $2H^+$ = [**C12LH$_4$**]$^{2+}$, log*K* = 16.35 (±0.01). Copper complexes: **C12LH$_2$** + Cu^{2+} = [**C12LH$_2$**Cu]$^{2+}$, log*K* = 7.52 (±0.02); **C12LH$_2$** + H^+ + Cu^{2+} = [**C12LH$_3$**Cu]$^{3+}$, log*K* = 12.71(±0.03); **C12LH$_2$** + Cu^{2+} = [**C12L**Cu] + $2H^+$, log*K* = −5.20 (±0.03). Nickel complexes: **C12LH$_2$** + Ni^{2+} = [**C12LH$_2$**Ni]$^{2+}$, log*K* = 5.55 (±0.03); **C12LH$_2$** + Ni^{2+} = [**C12LH**Ni] + H^+, log*K* = −2.28 (±0.04); **C12LH$_2$** + Ni^{2+} = [**C12L**Ni] + $2H^+$, log*K* = −9.64 (±0.02). These data have been calculated from potentiometric titrations data using the Hyperquad suite (see ref. 12).
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