The pH controlled uptake/release of citrate by a tri-copper(II) complex†

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The pH controlled movement of three copper ions inside a carefully designed receptor allows the recognition and uptake/release of citrate in aqueous solution in a manner that can be followed visually.

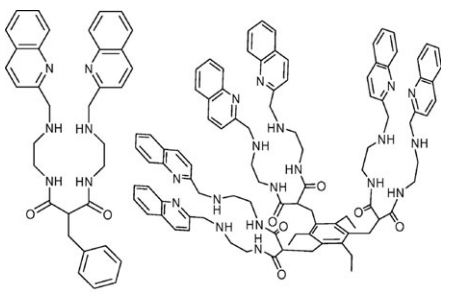
Artificial molecular machines are multi-component systems that are able to undergo large amplitude motions under the action of an external input, which can be chemical, electrochemical or physical in nature. Recently, a lot of attention has been focused on such systems, mainly from the point of view of developing molecular devices capable of information processing and data storage.[1] However, molecular machines could also act as smart receptors when a particular function is implemented, meaning that binding to a specific target takes place only when the proper command is given.[2]

According to the well established lock-and-key[3] concept, the shape of a molecule, and in particular the shape of a cavity inside a large molecule, is connected to its ability to act as a selective receptor. Given an external stimulus, an overall rearrangement of molecular systems may take place, and its shape and features may change dramatically. Let's imagine that only one of the possible shapes assumed by a given molecular system presents a cavity into which a chosen substrate can selectively fit. In this case, a molecular machine can advantageously enter the field of molecular recognition by acting as a "switchable receptor".[4] Some such examples have been published, in which the stimulus required to make the system change (towards the correct shape and features to recognize the substrate) is provided by light,[4c–e] pH changes[5] and cation complexation.[6] Recently, we have reported a series of systems, in which a pH change produces a double Cu^{II} translocation inside macrocyclic molecules.[7–9] This molecular movement transforms the binding abilities of the metal ions, and the chemical stimulus can switch the molecular system between two completely different shapes. In the first example reported,[9] when the system has an acidic pH, two copper ions are coordinatively unsaturated, and the molecular machine is able to selectively bind an imidazole residue in a bridging fashion. At a higher pH, the two copper ions move into two "saturated" coordination environments (as a consequence of the pH change) and are not able to bind anions. This system profits from a particular feature of the binding components, a so-called "coordinative bistability".[10] A bistable ligand contains two (or sets of two) compartments that have different binding tendencies towards metal cations, with one of the two compartment binding tendencies being strongly pH dependent. Thus, pH can be used to invert the binding affinities of the ligand compartments, and cations can be moved from different positions into the ligand with a simple change of pH.

In the context of extending this approach to the recognition of polyanions of biological importance, such as citrate,[11] and with the aim of exerting chemical control over the recognition process, we have designed a molecular device containing three Cu^{II} centers that can be reversibly moved into different positions by changes in pH. To do this, we decided to profit from a polyamido-polyamino fragment (**L′**, Scheme 1) we used extensively to demonstrate pH-controlled Cu^{II} motion and to organize three of these moieties into a suitable geometry. In particular, we synthesized system **L**, in which three subunits were implanted onto a 1,3,5-triethylbenzene platform.[11c,d,12] Next, the corresponding tri-copper(II) complex was synthesized *via* an *in situ* reaction of **L** with 3 equivalents of copper triflate.

After determining the protonation constants of **L** and its relative distribution diagram (see the ESI†) in an aqueous environment (water/dioxane 1 : 4), its complexation tendencies towards Cu^{II} were investigated.

The formation constants obtained from potentiometric titrations (water/dioxane 1 : 4) conducted in presence of 1 equivalent of **L** (5×10^{-4} M) and 3 equivalents of Cu^{II} are shown in Table 1. As can be seen in the distribution diagram, upon raising the pH, up to three copper ions can be taken-up by the ligand, with species containing three copper ions reaching a concentration >95% at a pH of close to 5. Above this pH, deprotonation of the ligand starts to occur, with the consequential formation of di-, tetra- and hexa-deprotonated complex species. This suggests a stepwise rearrangement of the complex, with the movement of the three copper ions from



Scheme 1

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† Electronic supplementary information (ESI) available: Experimental details for the synthesis of ligand **L**, a graph of the spectrophotometric titration of $[Cu_3\mathbf{L}]^{6+}$ with phosphate at pH 5.8 and a distribution diagram of ligand **L**. See DOI: 10.1039/b813194e

Table 1 Logarithmic formation constants for species related to ligand **L** and Cu^{2+} (1 : 3 molar ratio)

Equilibrium and associated species	log K^a
[1,0,6] **L** + $6H^+ \leftrightharpoons \mathbf{LH_6}^{6+}$	40.6
[1,0,5] **L** + $5H^+ \leftrightharpoons \mathbf{LH_5}^{5+}$	37.1
[1,0,4] **L** + $4H^+ \leftrightharpoons \mathbf{LH_4}^{4+}$	30.7
[1,0,3] **L** + $3H^+ \leftrightharpoons \mathbf{LH_3}^{3+}$	23.4
[1,0,2] **L** + $2H^+ \leftrightharpoons \mathbf{LH_2}^{2+}$	16.1
[1,0,1] **L** + $1H^+ \leftrightharpoons \mathbf{LH_1}^{1+}$	8.4
[1,1,4] **L** + Cu^{2+} + $4H^+ \leftrightharpoons [Cu(\mathbf{LH_4})]^{6+}$	36.1
[1,2,2] **L** + $2Cu^{2+}$ + $2H^+ \leftrightharpoons [Cu_2(\mathbf{LH_2})]^{6+}$	32.2
[1,3,0] **L** + $3Cu^{2+} \leftrightharpoons [Cu_3(\mathbf{L})]^{6+}$	26.7
[1,3,−2] **L** + $3Cu^{2+} \leftrightharpoons [Cu_3(\mathbf{L_{-2H}})]^{4+}$ + $2H^+$	14.7
[1,3,−4] **L** + $3Cu^{2+} \leftrightharpoons [Cu_3(\mathbf{L_{-4H}})]^{2+}$ + $4H^+$	1.1
[1,3,−5] **L** + $3Cu^{2+} \leftrightharpoons [Cu_3(\mathbf{L_{-4H}})(OH)]^{+}$ + $5H^+$	−7.3
[1,3,−6] **L** + $3Cu^{2+} \leftrightharpoons [Cu_3(\mathbf{L_{-6H}})]$ + $6H^+$	−15.3

[a] All formation constants have a 0.1 uncertainty.

the three bis(aminoquinoline) compartments into the three bis(amino)-bis(amido) compartments. Additionally, a further penta-deprotonated species is hypothesized that corresponds to a complex in which two cations are placed in two deprotonated bis(amino)-bis(amido) compartments, while the one remaining into the bis(aminoquinoline) compartment coordinates one OH^- anion, as expected on the basis of reported data; at this pH, the deprotonation of a water molecule coordinated to a Cu^{II} ion in a similar coordinative environment is likely.[7]

A pH-spectrophotometric titration performed under the same conditions (Fig. 1) shows, in correspondence with the quantitative formation of $[Cu_3\mathbf{L}]^{6+}$ at pH 5, a band centred around 650 nm. Similar bands are observed when a Cu^{II} ion is coordinated by two amine and two quinoline nitrogens.[7,8] Upon increasing the pH, a progressive change in the visible spectrum is observed that can be perceived even with the naked eye.

A band centred at 594 nm is formed, with no further changes above pH 9. This value of λ_{max} compares well with those observed for several complexes of similar structure, and is typical of the coordination of a heterocycle in an apical

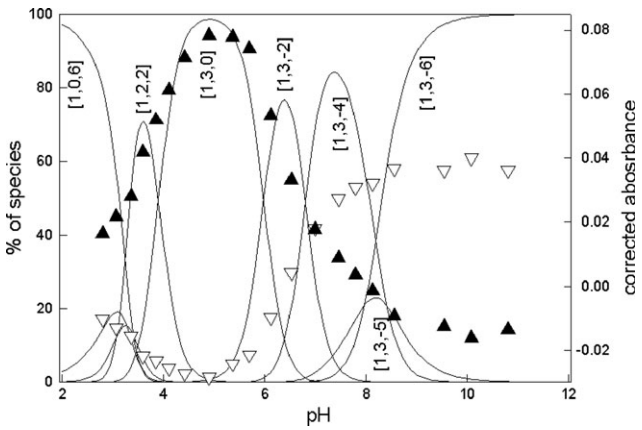


Fig. 1 Species distribution diagram for the system [**L**,Cu^{2+},H^+] obtained by potentiometric titration. The symbols represent data obtained from pH-spectrophotometric titrations. ▲ shows the absorbance of the band at 650 nm corrected for the values at 550 nm. △ shows the absorbance values at 594 nm corrected for the values at 650 nm.

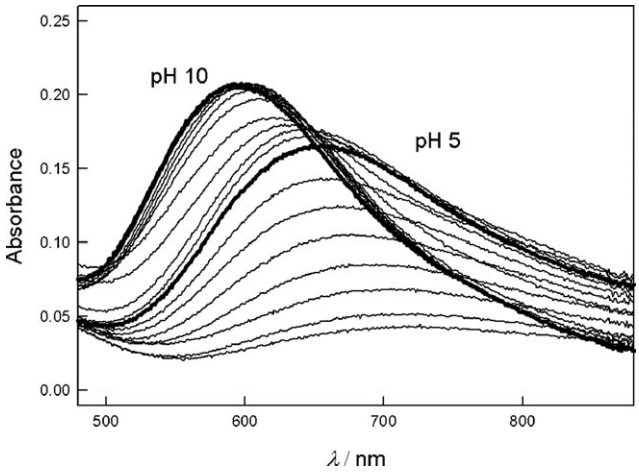


Fig. 2 The pH-spectrophotometric titration of a solution containing **L** (5×10^{-4} M) and 3 equivalents of Cu^{2+}. The bold lines represent the spectra obtained at pH 5 and 10.

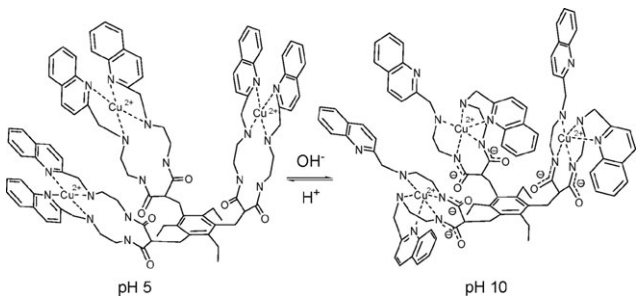
position of a copper cation in a bis(amino)-bis(amido) compartment.[7,8]

When corrected absorbance values for the bands at 594 and 650 nm are superimposed over the distribution diagram, it can be observed that the growth of the 650 nm band (▲) is coincident with the formation of $[Cu_3\mathbf{L}]^{6+}$, while the growth of the 594 nm band (△) is connected to the translocation of the copper ions into the bis(amino)-bis(amido) compartment.

The spectra obtained during the pH-spectrophotometric titration are shown in Fig. 2, with emphasis being placed on the spectra that represent the predominant species at pH 5 and 10, $[Cu_3\mathbf{L}]^{6+}$ and $[Cu_3(\mathbf{L_{-6H}})]$ respectively, as reported in Scheme 2.

It is evident that by changing the pH from acidic to basic, the three copper cations, each initially coordinated by two amine and two quinoline nitrogens, and with their coordination sphere completed by water molecules (band at 650 nm), can be translocated by the deprotonation of the six amido groups to give the neutral complex $[Cu_3(\mathbf{L_{-6H}})]$, which shows an absorption at 594 nm.

When the pH-spectrophotometric titration is repeated in presence of 1.5 equivalents of citrate, a different behaviour is observed (Fig. 3). On increasing the pH, a band with a maximum at around 700 nm is formed, and the absorption spectrum remains almost unchanged in the pH range 4.5–6.5, giving a pale green-coloured solution. Upon raising the pH



Scheme 2 The proposed rearrangement of the tri-copper complex occurring with pH change.

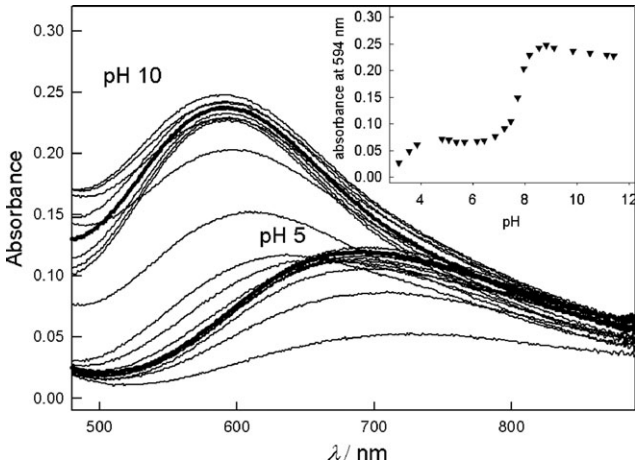


Fig. 3 The pH-spectrophotometric titration of a solution containing **L** (5×10^{-4} M), 3 equivalents of Cu^{2+} and 1.5 equivalents of citrate. Bold lines represents spectra obtained at pH 5 and 10.

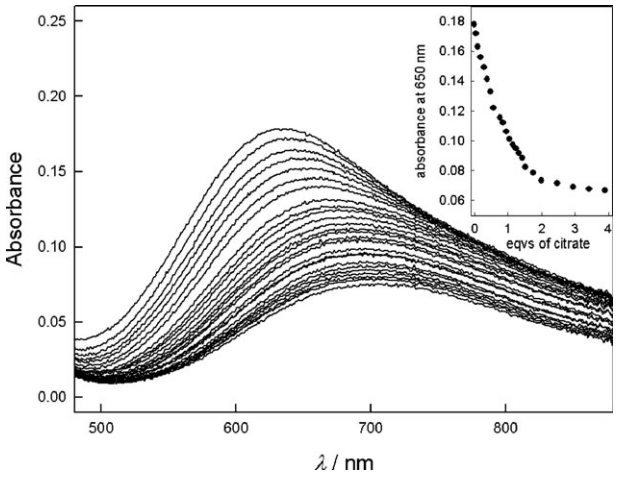


Fig. 4 The spectrophotometric titration of $[Cu_3\mathbf{L}]^{6+}$ with citrate at pH 5.8.

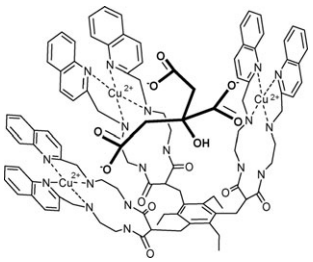
value above 6.5, the band centred at 594 nm starts to develop, reaches a maximum at around 8.5 and shows no further changes above this value. The titration profile obtained at 594 nm is shown in the inset of Fig. 3.

Thus, the major differences between the spectra in presence and absence of citrate are seen when the copper ions are located in the three bis(aminoquinoline) compartments under acidic conditions. A suggestion that comes from the profile is that, in presence of citrate, there is one species that is predominant and unchanging over the pH range 4.5–6.5. This demonstrates that in presence of citrate, the translocation of the three copper ions into the three dioxo compartments begins at a pH that is at least one unit higher than it is in absence of citrate. The obvious deduction is that the species observed in the pH range 4.5–6.5 is one in which citrate is coordinated to the three copper ions placed in the three bis(aminoquinoline) fragments, and that the presence of citrate stabilizes the aminoquinoline form of the complex. To check this, we performed a spectrophotometric titration of the copper complex (5×10^{-4} M) with citrate at pH 5.8 in a water/dioxane mixture (1 : 4) buffered with 0.05 M 2-(*N*-morpholino)ethanesulfonic acid (MES). At this pH, the distribution diagram shows that the $[Cu_3\mathbf{L}]^{6+}$ species has a relative concentration higher than 70% (while the rest is $[Cu_3(\mathbf{L}_{-2H})]^{4+}$, in which one of the copper ions is placed in the dioxotetramine compartment). When citrate is added, a sharp change in colour is observed: the blue solution turns to green. The titration profile is shown in Fig. 4, and its fitting according to a 1 : 1 stoichiometry gives a value of 3.9 log units for the formation constant of the 1 : 1 adduct between complex and citrate. Quite reasonably, complexation to the copper ions induces full deprotonation of citrate, which at this pH should be present in solution, mainly in its monoprotonated form (Scheme 3).[11]

The titration at this pH was repeated with acetate, nitrate and chloride. No change in colour was seen and no absorbance variations were registered. In the case of a dicarboxylic anion like succinate, a slight variation in the spectrum was observed with increasing anion concentration up to several equivalents, but no formation constant was detectable, probably because its value is very small. Only in the case of a phosphate titration was a clear change in the spectrum observed; fitting of the titration data gave a value of 3.18 log units for the formation constant of the 1 : 1 adduct between complex and phosphate. Under our experimental conditions, the complex specifically binds tricarboxylates such as citrate.

The titration with citrate was repeated at pH 12, and in this case no changes in spectral features pertinent to the $[Cu_3(\mathbf{L}_{-6H})]$ species were observed; in this form, the complex is not able to bind anions. The complex thus acts as a citrate receptor only when it is in the open form, with the three copper ions placed in the bis(amino)quinoline compartments at pH values below 6. When the pH is raised to basic values (pH > 7.5), the $[Cu_3(\mathbf{L}_{-6H})]$ species becomes predominant, and in this form the coordination of further anions to the copper centres is precluded.[13]

The system presented here can be seen as a prototype for the controlled uptake/release of biologically important polyanions; at basic pH values, the binding of citrate is not allowed, while the complex becomes a specific receptor for citrate when the pH is lowered to below 6, and the anion can then be taken-up. Release can be achieved simply by raising the pH once again. In addition, the system undergoes a change in colour that can be perceived by the naked eye upon citrate binding, providing a clear and efficient way of signalling the presence of the anion in solution.



Scheme 3 Proposed arrangement of citrate within the receptor cavity.

Experimental

All commercially available compounds were purchased from Aldrich or Fluka and used as received. ^{1}H NMR spectra were

recorded on a Bruker AMX 400 instrument. The UV/vis spectroscopic studies were performed using a Varian Cary 100 UV-Vis spectrophotometer. Mass spectra were recorded using a Thermo-Finnigan LCQ Advantage Max electrospray ionization instrument. For pH-spectrophotometric titrations, an ORION 420A pH meter equipped with a HANNA Instruments electrode was used. For protonation and complexation equilibrium constant determination an automated Radiometer Titralab TIM 900 titration apparatus was used. The synthesis of ligand **L** starting from 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene[12a] is described in the ESI.†

pH-spectrophotometric titrations

Spectra were recorded in the range 300–800 nm. pH variations were obtained by the addition of a standard 0.1 M NaOH solution (μL quantities) to a solution (20 mL, 5×10^{-4} M in water/dioxane 1 : 4, 0.05 M $NaNO_3$ as the ionic medium) of the fully protonated ligand (obtained by adding a known excess of acid), with the addition of 3 equivalents of $Cu^{II}(OTf)_2$ and 1.5 equivalents of sodium citrate trihydrate when necessary. The pH of the solutions in water/dioxane solvent mixtures were measured using the Nernst equation: $E = E^{\circ} + 59 \log [H^+]$, where E° was determined using Gran's method.[14]

Potentiometric titrations

Equilibrium constants for the protonation and complexation reactions were determined by pH-spectrophotometric measurements in water/dioxane 1 : 4 solutions at 25 °C, with 0.05 M $NaNO_3$ as the ionic medium, using the fully automatic equipment that has already been described.[15] The HYPERQUAD software package[16] was used to process the potentiometric data.

Spectrophotometric titrations with anions at fixed pH

Spectra were recorded in the range 300–800 nm. To a 5×10^{-4} M solution of **L** in water/dioxane 1 : 4 and 0.05 M MES were added 3 equivalents of $Cu^{II}(OTf)_2$. The pH was then corrected to pH 5.8 with NaOH. The resulting solutions were titrated with standard solutions of anions (obtained from the corresponding sodium salt). The HYPERQUAD software package[16] was used to process the spectrophotometric data.

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