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Luminescent chemosensors for transition metal ions

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Contents

Abstract

The need for selective and sensitive sensors able to monitor in real-time and real-space the concentration of analytes of biological, clinical, and environmental interest is nowadays generally accepted. Of the various kinds of chemosensors, luminescence-based chemosensors present many advantages, since they can take profit of the high sensitivity and versatility typical of photoluminescence spectroscopy. In this contest, special interest has been devoted to the development of luminescent chemosensors for transition metal ions, since some of them are present in biological systems in trace amounts and, at the same time, they all can represent an environmental concern when present in uncontrolled amounts. In this contribution we review the chemical systems able to act as luminescent chemosensors for this class of metal ions. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Chemosensors; Luminescence; Transition metal ions; Molecular recognition; Optodes

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1. Introduction

Typically, chemosensors are molecules of abiotic origin that are able to bind selectively and reversibly the analyte of interest with a concomitant change in one or more properties of the system, such as redox potentials, absorption or fluorescence spectra [1,2]. Because of the two different processes occurring during analyte detection, i.e. molecular recognition and signal transduction, chemosensors can usually be schematized [3] as made of three possible different components (Fig. 1): a receptor (responsible for the selective analyte binding), an active unity (whose properties should change upon complexation) and, eventually, a spacer that can change the geometry of the system and tune the electronic interaction between the two former moieties.

Among the different chemosensors, fluorescence-based ones present many advantages: fluorescence measurements are usually very sensitive (single molecule detection is possible), low cost, easily performed, and versatile, offering subnanometer spatial resolution with submicron visualisation and submillisecond temporal resolution [1,2]. Furthermore, many opportunities exist for modulating the photophysical properties of a luminophore, such as the introduction of proton-, energy- and electron-transfer processes, the presence of heavy-atom effects, changes of electronic density, and the destabilisation of a non-emissive $n\pi^*$ excited state [1]. This offers, as a consequence, a wide number of possibilities for tailoring efficient luminescent chemosensors.

An important feature of chemosensors is that signal transduction of the analyte binding offer the possibility to monitor its concentration in real-time and real-space. Chemosensors can and do find use in many disciplines; consequently, this area of research is attracting attention in the scientific community, especially among chemists, biologists, physicists and material scientists. In biochemistry, clinical and medical sciences, and cell biology, freely mobile sensor molecules are employed extensively in microscopy, a use that offers the possibility of performing real-space measurements. For analytical chemistry and environmental sciences, the need for real-time measurements can take advantage of the development of optical fibres. This technology has extended the possibility of performing remote real-time measurements with optical sensors (optodes) by monitoring changes in the absorption or luminescence spectra of chromogenic or fluorescent compounds immobilized on

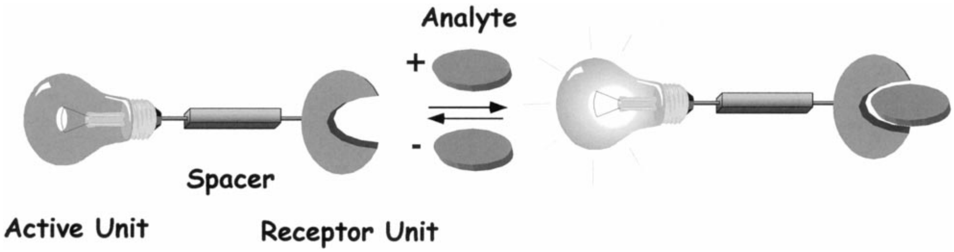
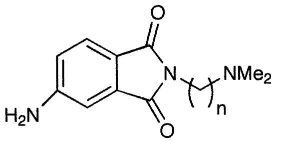


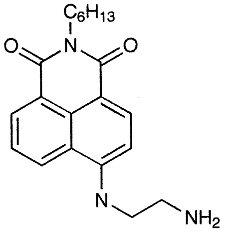
Fig. 1. Schematic representation of a luminescent chemosensor.

polymer matrix on the tip of a fibre. It is clear indeed that light is a very versatile signal, and micro sized optical fibres can allow analysis at almost any location. Two recent reviews [4,5] witness the tremendous research done in optode development foreseeing a rapid spread of optical sensors.

In spite of this large interest, however, only in the late 1970s, after the development of the supramolecular chemistry and the pioneering work of Pedersen, Cram and Lehn, a systematic research started on the synthesis of abiotic metal ions receptors. At the same time, the advancement of photochemistry gave the basis for designing systems in which changes in absorbance and/or fluorescence bands could signal analyte complexation. From then onwards, hundreds of possible sensory molecules were listed [1–15]. Among the different analytes, special interest is devoted to develop chemosensors for transition metal ions: usually they represent an environmental concern when present in uncontrolled amounts, but at the same time some of them such as iron, zinc, copper, and cobalt are present as essential elements in biological systems. In our contribution, we will review the chemical systems able to act as luminescent chemosensors for this class of metal ions. As a method, we will gather the various species in different classes, according to the receptor moiety present in the chemosensor, since it is usually this part that confers the required selectivity to the whole system. We have also inserted a paragraph dealing with sensors with peptide- and protein-based receptors. Although these systems are at the borderline between bio- and chemosensors, since also for them a synthetic effort is often required, we think that this paragraph can allow a better comprehension of the state-of-the-art in this field.



1a n = 2
b n = 3



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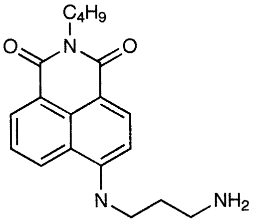
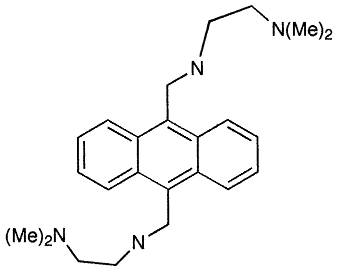


3

2. Chemosensors with acyclic receptor

Among the different receptor units, those consisting of only one aliphatic amine group are surely the simplest. Ramachandaram et al. [16] and Mitchell et al. [17] have recently used the possibility offered by aliphatic amines to form metal complexes with transition metal ions for developing fluorescent chemosensors. They obtained these molecules appending the luminophore to the amine by an alkyl chain. In the absence of metal ions and protons, the fluorescence of the active units is quenched by the occurrence of a photoinduced electron transfer (PET) process between the lone pair of the nitrogen and the appended chromophore. In the cases of **1a** and **1b** [16], a significant fluorescence enhancement (>30 times for **1a**) can be observed in acetonitrile solutions upon metal complexation with many transition metal ions, interestingly also with those that usually are reported to quench luminescence, such as Cr(III), Fe(III), Co(II), Ni(II) and Cu (II). The proposed mechanism for such an unusual enhancement is that complexation prevents the PET mechanism to occur, and this effect, that leads to an enhancement of the fluorescence, is only in part counterbalanced by the quenching effect due to the metal ion. This proposed mechanism is supported by the fact the greatest enhancement effect has been observed for the Zn^{2+} ion, which, having a d^{10} electronic configuration, does not usually introduce electron- or energy-transfer mechanisms for the deactivation of the excited state. A very similar effect, but with a greater selectivity for Cu^{2+} has been reported [17] for **2** and **3** in 2-propanol solution, where the fluorescence of the chemosensors (10 μM) is affected by sub-ppm levels of this ion, and only by much higher concentrations (10–100 ppm) of Ni^{2+} and Mn^{2+}. Zn^{2+}, Al^{3+}, and Ca^{2+} are reported not to alter the fluorescence spectrum of these two species. The enhancement factor in the case of **3** is about 3. de Silva et al. [9] have described a very similar compound as a PET pH sensor.



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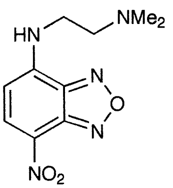
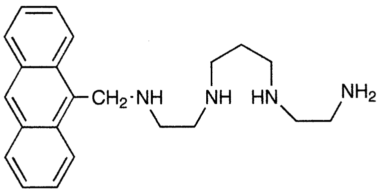


5

Many more examples of chemosensors for transition metal ions are reported whose receptor unit is composed of more than one amine unit, and that can take advantage of the chelating effect. The first example reported in the literature was the anthracene derivative **4**, from the laboratory of Czarnik [18,19]. In **4**, the appended amine groups quench the typical, intense fluorescence of the anthracene fragment, again by means of a PET process. Addition of zinc chloride to acetonitrile solutions of **4** drastically enhances the observed fluorescence more than a 1000-fold, since coordination makes PET processes thermodynamically inaccessible. In water solution the association constant was lower, and the same experiments failed; these results suggested however the use of stronger ligands, opening the way to the design of many new chemosensors. More recently, Ramachandaram and Samanta [20,21] have reported a fluorescence enhancement of **5** upon complexation with transition metal ions. Intramolecular PET from the amine groups is responsible for the quenching of the nitrobenzoxadiazole (NBD) luminescence in the free species. Addition of Cr^{3+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+} in acetonitrile leads to an enhancement of the fluorescence between 43- and 123-fold, accompanied by a blue shift of the spectrum. The receptor chelates the metal ion with the two amine groups; no selectivity is reported for a particular ion among the above mentioned species. At high metal ion concentration dynamic quenching reduces the intensity of the complexed species. In water solution the fluorescence enhancement factor is reduced to 1.3.



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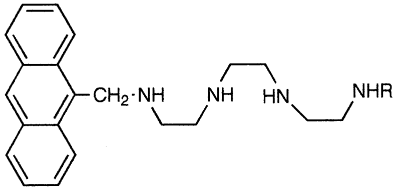
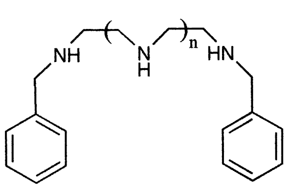
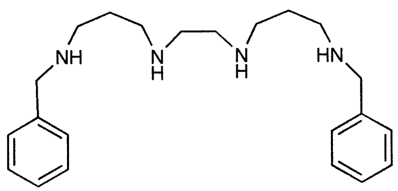


7 R = H
8 R = CH_2An

Chemosensors composed of polyamine receptors and an anthracene fragment were synthesized in different laboratories. Fabbrizzi et al. [22] have shown that the fluorescence of **6** in acetonitrile/water solutions depends on the pH conditions. In particular, the fluorescence decreases at pH values above 4, since when the amines are not protonated a PET process from the amines to the anthracene occurs and, as a consequence, the fluorescence intensity of the anthracene fragment is quenched. The pH dependence of the fluorescence intensity changes in the presence of Cu^{2+}, Ni^{2+}, and Zn^{2+}. For the two former ions, a decrease of the intensity is observed even at lower pH (< 3 for copper ions), since metal ion complexation in these conditions leads to the deactivation of the excited state via an energy transfer process from the chromophore to metal centred states. On the other hand, in the presence of Zn^{2+}, after an initial quenching on increasing pH, from pH 3.7 fluorescence began to revive to achieve, almost the original intensity at pH above 6. In the first part of the intensity versus pH profile, the ion does not interact with the ligand, and the progressive deprotonation of the ammonium ions allows PET to occur. At pH above 3.7, complexation with Zn^{2+} ions is achieved and, since this process increases the oxidation potentials of the amines, PET is not thermodynamically allowed anymore. A similar molecule, **7**, synthesized by Sclafani et al. [23], shows a similar behaviour with Zn^{2+} in pure water, although in this solvent the chelation enhanced fluorescence is lower (about sixfold). A different effect is observed for **8**, where two anthracene fragments are present. In the fluorescence spectrum of the free molecules there is evidence for excimer formation from a tail in the 450–600 nm region; addition of Zn^{2+} ions increases the fluorescence intensity in this part of the spectrum, indicating that ion complexation promotes the intramolecular excimer formation. The different response to Zn^{2+} at 414 nm (monomer emission) and at 495 nm (excimer emission) allows the fluorescence ratio at these two wavelengths to be used for a direct concentration determination.

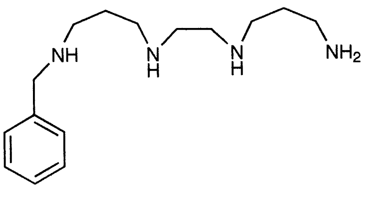


9a n = 1
b n = 2
c n = 3



10

Bernardo et al. [24] investigated the synthesis and the photochemical behaviour of a family of *N*,*N*′-dibenzylated open-chain polyamines **9**–**11**. They studied the effects on fluorescence of pH changes in aqueous solutions and chelation of metal ions such as Co^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+} and Zn^{2+}. In the free hosts PET can occur between the lone pair of the amines in the polyamine chain and the excited benzene moieties, while protonation or coordination with metal ion by the amines partially prevents the electron transfer to occur inducing an enhancement of fluorescence. All the species presented form complexes with a 1:1 stoichiometry and there is no experimental evidence for the formation of excimers.



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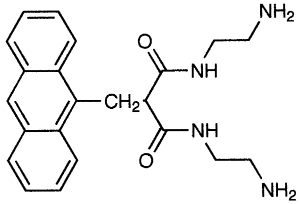

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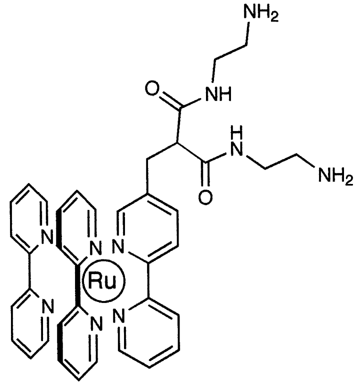
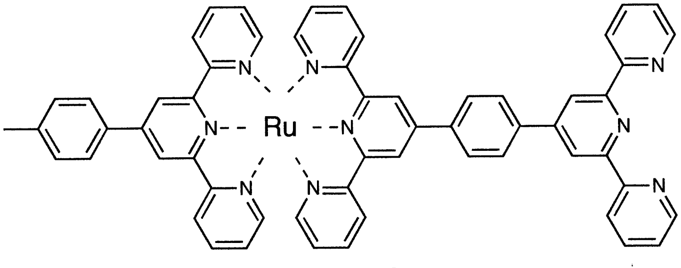


13

Fabbrizzi et al. have recently shown [25–27] that a well known category of ligands, dioxo-tetraamines (and in particular dioxo-2,3,2-tet, 1,4,8,11-tetraazaundecane-5,7-dione) can be successfully employed for signalling the presence of Ni^{2+} and Cu^{2+} if connected to chromophores such as anthracene (**12**) [25,26], or Ru(bpy)$_3^{2+}$ (**13**) [27]. For these chemosensors the complexation mechanism involves the deprotonation of the two amide groups. This very endoergonic process can take place only with metal ions which profit by a large ligand field stabilisation, in these cases only Ni^{2+} and Cu^{2+}. This makes ligands of this kind selective for these two cations, and their different ligand field stabilisation (higher for Cu^{2+} with respect to Ni^{2+}) also allows one to distinguish between them: complexation with Cu^{2+} takes place at lower pH values (it is complete at pH 7) than for Ni^{2+} (pH 9). Complexation leads to an almost complete quenching of the luminescence of the appended chromophore, most probably via an energy transfer mechanism to a metal-centred state. Compound **13** has also the advantage to be water soluble, and detection of concentrations as low as 10^{-7} M (in the ppb range) of Cu^{2+} has been reported in this solvent [27].



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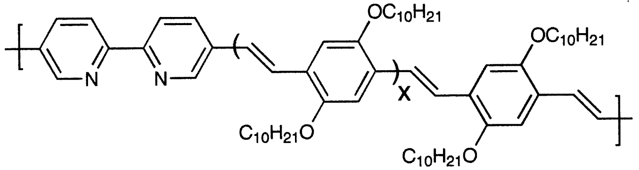
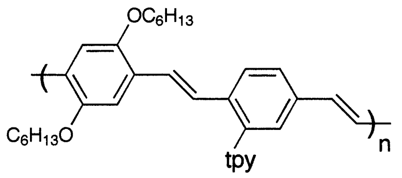


x = 0: **15a**
x = 2: **15b**

Another way to design chemosensors is using polypyridines. Among some recent examples, Barigelletti et al. [28] have shown the dependence of the photophysical properties upon Zn^{2+} concentration of a Ruthenium(II)-terpyridine chromophore functionalized with an uncoordinated terpyridine fragment **14**. In particular, in acetonitrile/water solution, addition of Zn^{2+} ions, forming a 2:1 (ligand:metal) complex, causes a gradual shift of the band maximum from 660 to 700 nm, and an increase of fluorescence intensity (with an enhancement factor ≥ 10) and lifetime (from 7.8 to 75.2 ns). These effects have been explained in terms of the delocalising ability of the added tpy moiety in the tpy–tpy ligand combined with the stabilising effect of the Zn^{2+} centre. The increase of delocalisation is also one of the effects responsible for pronounced changes in the absorption and luminescence spectra of the conjugated polymers **15** upon metal ion complexation [29]. When the bipyridines are not engaged in metal ion complexation, they assume a twisted conformation, allowing only a partial conjugation in the polymer backbone. Complexation with many transition metal ions forces the ligand to assume a planar configuration, allowing full conjugation, and this explains the observed red shifts in the absorption spectra. As far as the fluorescence spectra are concerned, Zn^{2+}, Cd^{2+}, Hg^{2+}, Ag^{+}, and Al^{3+} ions cause a pronounced red shift of the band, while Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, and Sn^{2+} quench the luminescence of the polymer. Another group of metal ions, namely Pb^{2+}, Fe^{2+}, Fe^{3+}, Cu^{+}, Sb^{3+}, causes a blue shift of the spectra, probably because some monodentate coordination in this case induces an even lower conjugation in the polymer backbone. Interestingly, these polymers do not respond to alkali or to alkaline earth metal ions. Conjugated polymers have also been used by Kimura et al. [30], who synthetized system **16**. In this case, Fe^{2+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, Cr^{2+}, Mn^{2+}, and Co^{2+}, completely quenched the fluorescence of the polymer, centred at 524 nm, while Pd^{2+}, Sn^{2+}, Al^{3+}, and Ru^{2+} caused a near complete quenching of the fluorescence with a slight blue shift of the spectrum. The fluorescence quenching of Fe^{2+} was linked to the formation of the bis-tridentate-type complex, as indicated by the absorption spectra. Addition of Zn^{2+} ions brought about a red shift of the emission peak to 563 nm, and this was attributed to an alteration of the electronic density of the polymer backbone.



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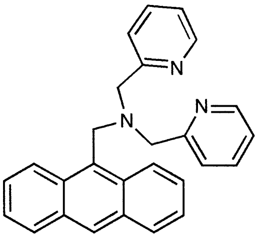
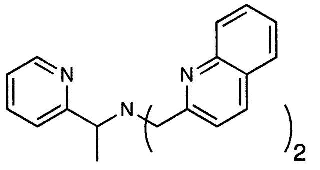


17

17 [31] was designed to function as an off–on–off switch for protons, and as an off–on fluorescent switch for post transition metal ions. Upon complexation with Zn^{2+} ions in acetonitrile, this sensor shows a maximum fluorescence enhancement factor of 77 (with a quantum yield of 0.77), and a log K_a value of 5.3. Also in this case, the fluorescence increase is due to prevention of PET processes from the amine and the pyridine units of the receptor.



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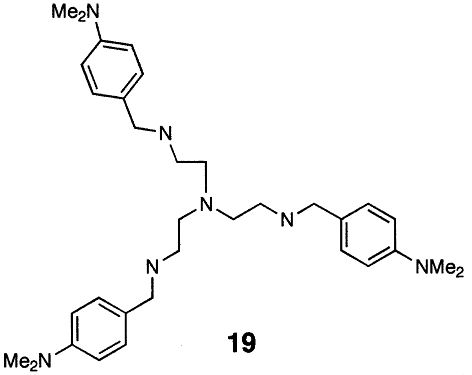
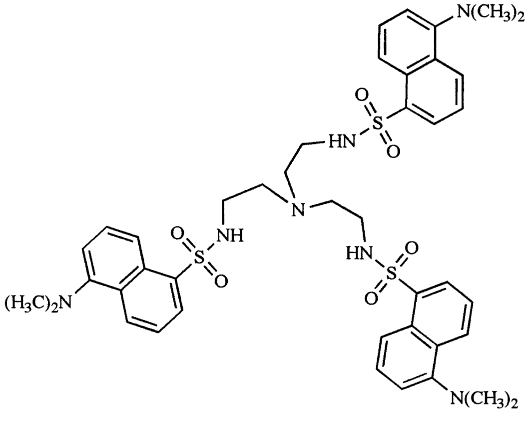


19

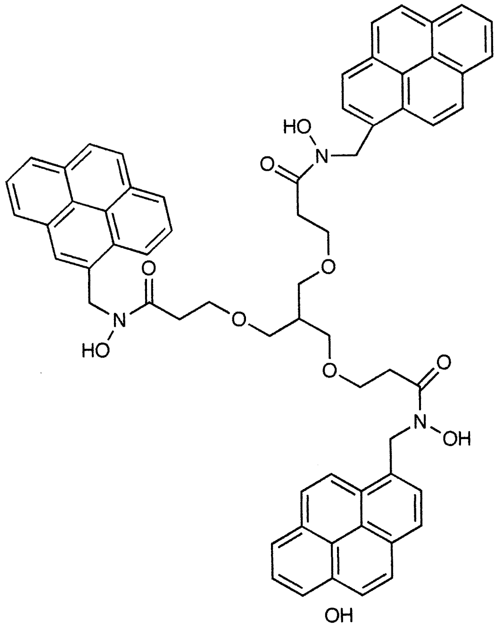
Tripodal ligands are also often used as receptor moieties. A very interesting example is that proposed by Castagnetto and Canary [32], who synthesized the chiroptically enhanced fluorescent sensor **18**. In this sensor, chelation with Zn^{2+} and Cd^{2+} ($K_a > 10^6$ M^{-1}) leads to an increase of the fluorescence intensity of the quinoline unit, with an observed fluorescence enhancement (378 nm, pH 7) of 30- and sixfold, respectively. In this case, complexation changes the nature of the lowest excited state from an $n\pi^*$ state (that usually tends to give intersystem crossing processes, and thus phosphorescence) to $\pi\pi^*$ (that usually gives a more intense fluorescence). Complexation with Fe^{2+} and Cu^{2+} did not result in a fluorescence increase. Complementary information can arise from looking at the exciton-coupled circular dichroism spectra (ECCD), where strong signals were observed upon complexation with trigonal bipyramidal metal ions (Zn(II) and Cu (II)), while weak signals could be detected with octahedral metal ions (Cd(II) and Fe(II)). Evaluation of both the fluorescence and ECCD properties of the complexes can lead to the identification of the metal. In fact these two techniques could distinguish, for example, Zn^{2+} (strong fluorescence and ECCD response), Cu^{2+} (strong ECCD but no fluorescence), Cd^{2+} (fluorescence but not ECCD), and Fe^{2+} (neither fluorescence nor ECCD). These observations show the principle that both isotropic and anisotropic detection may be utilized to maximise the information transmitted by a single sensor molecule.

Addition to each terminal amine nitrogen atom of tren of an *N*,*N*-dimethylaniline (DMA) subunit led to **19** [33]. Neutral DMA gives rise to a charge transfer excited state, which emits at 360 nm. When **19** is protonated (pH < 4 in water), the CT state can not be achieved, and the molecule is only poorly emissive. A maximum is reached at pH 5.3; progressive quenching (up to ca. 30% at pH 10) can be ascribed to the stepwise deprotonation of the amino groups of the tren subunit, which can quench DMA fluorescence by a PET mechanism. The intensity versus pH profile changes substantially upon addition of Cu^{2+}, Ni^{2+} and Zn^{2+}. When Cu^{2+} ions are present, the intensity reaches a maximum at pH 3.8, and then, when complexation occurs, the intensity decreases abruptly and full quenching is observed at pH 5.5. Therefore, **19**/Cu^{2+} equimolar system behaves as a very interesting efficient off–on–off pH sensor. A similar behaviour is observed when Ni^{2+} ions are present, but fluorescence decrease and quenching are observed at higher pH. This can also allow discrimination between the two metal ions, and experiments carried out at pH 6 demonstrated the selectivity towards Cu^{2+} ions. An energy transfer process is the proposed mechanism for the quenching observed upon metal ion complexation. Zn^{2+} ions behave quite differently: no decrease of the fluorescence intensity was observed even at pH > 6. In this case, metal coordination prevents amine-to-DMA PET to occur, without introducing energy- or electron-transfer processes involving the metal complex. Since the axial position opposite to the tertiary amine group is available for coordination, the **19**–Zn^{2+} complex has been also proposed as a chemosensor for carboxylate anions.

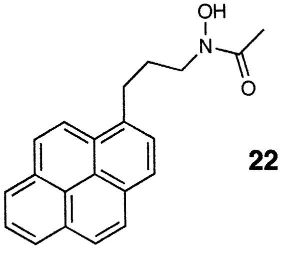


20

Another possibility was explored adding again three dansyl groups to a tren molecule to obtain **20** [34]. The dansyl chromophore is well known to give an intense fluorescence arising from a charge transfer state from the amine group of the dansyl to the naphthalenic ring. The fluorescence of **20** does not depend on the pH conditions in the 3–11 pH range. However, strong changes on its luminescence properties were indeed observed upon addition of Cu^{2+}, Co^{2+}, Zn^{2+}, and Cd^{2+} at pH 9.5. In particular, for the former two metal ions, a strong quenching ($I_{rel} = 1\%$) of the fluorescence intensity was observed, while for Zn^{2+} and Cd^{2+} a blue shift accompanied by an enhancement of the fluorescence quantum yield could be detected. The complexation mechanism involves for all metal ions the deprotonation of the sulphonamide groups. For Zn^{2+} and Cd^{2+} ions, the observed blue shift has been attributed to the increase of the electronic density on the naphthalene ring due to the deprotonation/complexation process, that moves the amine-to-naphthalene charge transfer state occurring in the dansyl chromophore towards higher energy. Cu^{2+} and Co^{2+} ions, together with this effect, introduce lower lying energy excited states centred on the metal, that deactivate the luminescent excited state centred on the dansyl chromophore. The complexation equilibrium is pH dependent since it involves deprotonation of the sulphonamide groups. It was observed that, while the complexation with copper ions is complete at pH 6, the complexation with the other metal ions requires higher pH values, and this makes **20** very selective for Cu^{2+} at pH conditions close to the physiological ones.

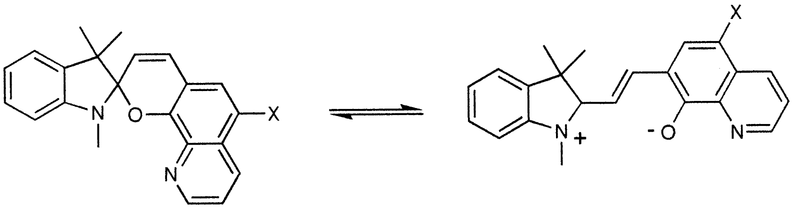


21



22

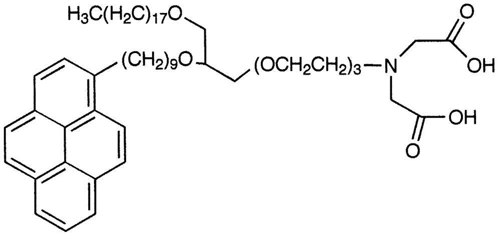
The fluorescence of the tripodal ligand **21** responds to the presence of Fe^{3+} and Ga^{3+} ions [35,36]. Compound **21** exhibits in MeOH or MeOH/H_2O a fluorescence spectrum composed of a very weak structured emission band, that can be associated with pyrene monomeric emission, and of a broad intense red-shifted band with an emission maximum at 485 nm. The latter band has been attributed to the emission of the pyrene excimer arising from intramolecular interactions. These two fluorescence bands are markedly quenched upon complexation of Fe^{3+} cations, because the occurrence of energy- or electron-transfer processes. Ga^{3+} ions caused instead a decrease of the excimeric component and a concomitant increase of the monomeric one. This ion causes completely different changes in the simpler **22**, which has the same chelating and chromophoric units [37]. Compound **22** shows an intense monomeric-like emission, that is partially quenched upon complexation with Ga^{3+}, while the intense excimer-like emission is promoted. In this case, metal ion complexation causes intermolecular interactions, and this explains such a behaviour. Again, Fe^{3+} almost completely quenches the fluorescence of the chemosensor, presumably via an energy-transfer mechanism.



23a x = H
b x = NO_2

24a x = H
b x = NO_2

Systems **23** [38] are very interesting since they can be interconverted into the merocyanine isomers **24** by means of light irradiation and/or metal ion complexation. In particular the form **23a** is the predominant one in the absence of added metal ions or UV light, and has a very low fluorescence quantum yield. Addition of Zn^{2+} to an ethanol solution of **23a** lead to a 14-fold increase of the luminescence at 610 nm. This enhancement is due to the formation of the **24a** isomer engaged in the metal ion complexation. Interestingly, metal free **24** is not luminescent, since proton transfer can occur in the excited state, deactivating it to the ground state. This process is prevented by metal ion complexation. The sensitivity of **23a** for Zn^{2+} in ethanol/water solutions was found to be around 3 ppb. Some enhancement was also found upon addition of Cd^{2+} and Mg^{2+}. Non-luminescent chelates were observed with Cu^{2+}, Ni^{2+}, Co^{2+}, and Fe^{3+} monitoring the absorbance increase in the long-wavelength maxima, typical of the merocyanine form. For this system, irradiation of the metal chelate did not cause the release of the metal ion nor regeneration of **23a**. The insertion of the nitro group in the quinoline moiety to give **23b** induces two important changes in the system, namely (i) the shift of the equilibrium towards the open form even in the absence of added metal ions and (ii) a decrease in the complexation ability of the quinoline moiety. For **23b**, a ninefold increase of the fluorescence intensity was observed upon addition of zinc ions, only a three to fourfold increase upon addition of magnesium or cadmium ions and, very promisingly, a 20-fold increase upon addition of one equivalent of mercury ions. For this system, expulsion of the metal ion to give the closed form was observed upon irradiation with visible light.



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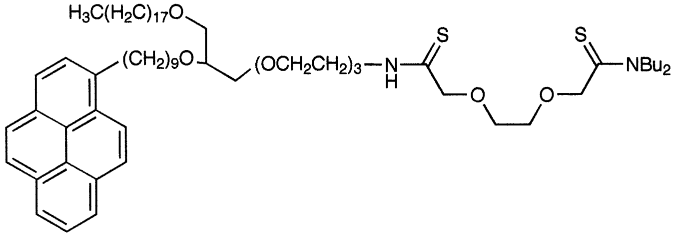
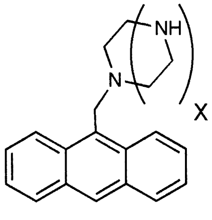
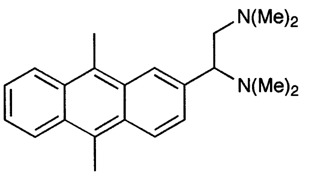


26

Compounds **25** [39] and **26** [40] are very similar, and show a remarkably high affinity for Cu^{2+} and Hg^{2+} ions, respectively. They can be co-sonicated in aqueous solution with phosphatydilcoline lipids to prepare stable mixed bilayer vesicles. In the absence of metal ions the vesicles show two distinct fluorescence bands: a weak band at 377 nm due to pyrene monomer and a broad band at 470 nm attributed to pyrene excimers. Metal ion complexation causes a reorganisation of the lipids, dispersing the aggregates of **25** and **26**, and this results in an increase of the monomer to excimer fluorescence ratio. Complexation of both chemosensors with the metal ions are always fast and reversible, and a detection limit of 5 nM for Cu^{2+} with **25** and of 100 nM for Hg^{2+} with **26** was reached.



27a (x = 1), **b** (x = 2), **c** (x = 3), **d** (x = 4), **e** (x = 5)



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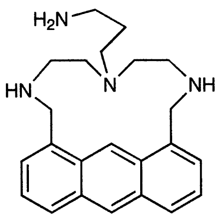
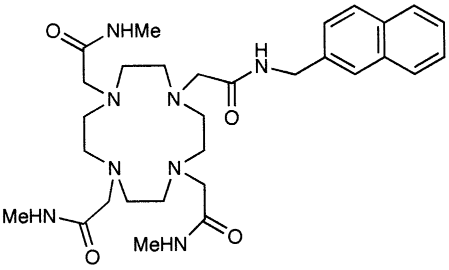


29

3. Chemosensors with cyclic receptor

One of the first examples of fluorescent chemosensors for transition metal ions possessing polyazamacrocycles, **27a–e**, was reported by Czarnik's group [41–43]. The luminescence of the appended anthracene is pH dependent, all compounds displaying low fluorescence under basic conditions. This behaviour is consistent with the occurrence of a PET mechanism between the unprotonated amines and the chromophoric group. The strongest fluorescence enhancement in water was observed upon complexation of **27c** and **27e** with both Zn^{2+} and Cd^{2+} ions, and was due to the prevention of the above mentioned PET process. With **27e**, complexation with Hg^{2+} resulted in a quenching of the residual fluorescence. Interestingly, the complex **27d**–Cd^{2+} gives rise to a different bandshape and maximum with respect to the free ligand, and this feature can be used to discriminate between Zn^{2+} and Cd^{2+} ions. This behaviour is attributed to the occurrence in this system of a ground state Cd(II) π-complexation. Complete chelation in acetonitrile of Zn^{2+} ions by **28** [44] results in a net 14-fold increase in fluorescence, with saturation occurring after addition of approximately one equivalent. A very interesting selectivity towards Cu^{2+} and Hg^{2+} was found for **29** [45,46]. In water solution at pH 7, this compound displayed in fact chelation-enhanced quenching effects only with these two ions, with overall emission changes of 18-fold ($K_a > 10^6$ M^{-1}) and fourfold ($K_a = 1.8 \times 10^4$ M^{-1}), respectively. As for **27d**–Cd^{2+}, a ground state Hg(II) π-complexation has been proposed in order to explain the high association constant and the strong quenching observed for this complex.



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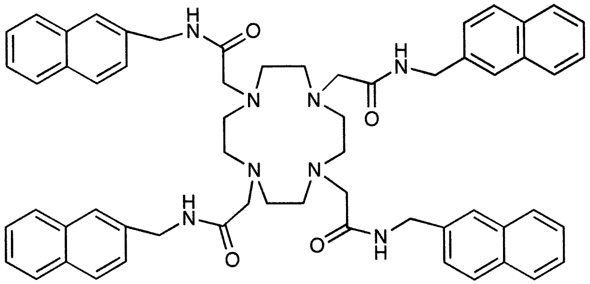

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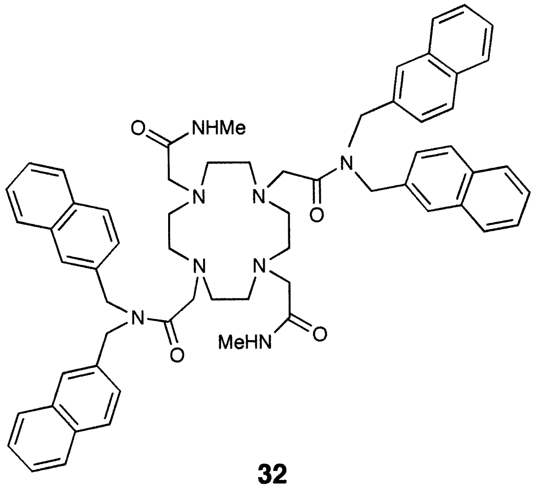
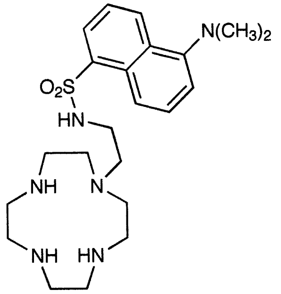


32

The series of compounds **30–32** [47,48] is based on the cyclen receptor and on the naphthalene chromophore. Compound **30** [47] shows the typical fluorescence of the naphthalene, although the intensity is partially quenched in acetonitrile presumably via a PET mechanism involving the nitrogen atoms. In water, partial protonation of two of the four nitrogen atoms prevents PET from occurring, and a more intense fluorescence can be observed. In acetonitrile, addition of zinc or cadmium triflates led to a twofold increase in the intensity of the naphthyl fluorescence, due to the increased oxidation potential of the amines upon metal complexation. Addition of Pb^{2+} ions leads to a fluorescence decrease of ca. 25%; in this case the aforementioned effect is contrasted by the heavy atom effect due to the lead ion. Copper(II) is instead responsible for a 95% fluorescence decrease; a copper to naphthyl PET was reported as the most likely mechanism for such a quenching. The same quenching with copper ions was observed in water, while Pb^{2+} in this solvent caused a fluorescence decrease of ca. 60%. Less than 10% enhancement was observed with zinc and cadmium ions. The fluorescence spectrum of **31** [47] revealed not only the expected monomer emission at 337 nm, but also a broad band, sensitive to solvent polarity, at lower energy that is typical of naphthalene excimers, due to intramolecular interactions. While addition of Zn^{2+} to an acetonitrile solution of **31** did not cause any effect, after addition of Cd^{2+} the monomer emission increased immediately, while the excimer emission decreased quickly. This effect could be due to conformational changes on the receptor moiety that take apart the naphthyl groups precluding excimer formation. Also, the Pb^{2+} ions quenched the excimer fluorescence, in this case without strong effect on the monomer emission. The heavy atom effect has been proposed as responsible for the different behaviour between cadmium and lead ions. Ligand **32** is related to **31** [48] in that it contains four naphthyl groups, but, in this case, they are arranged as two pairs bound to *trans*-related nitrogen atoms of the ring, and, again, it shows an excimer emission. Zn^{2+} and Cd^{2+} caused very weak effects both on the total intensity and on the ratio between monomer and excimer emission, while Pb^{2+}, Ni^{2+}, and Cu^{2+} caused some decrease (36%, 37% and 86%, respectively) of the total emission, with some more effectiveness towards the excimeric one.



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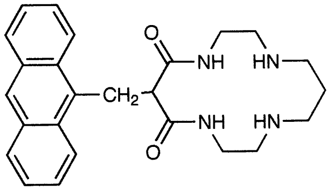
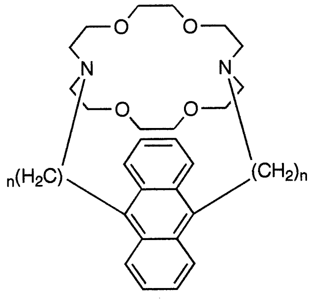


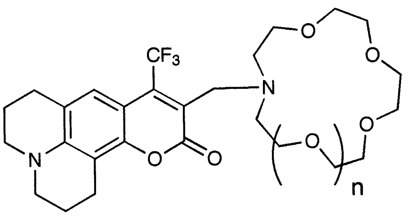
34

A very interesting chemosensor for Zn^{2+} (**33**) was developed by Koike et al. using the cyclen unit [14,49]. The design of this species is due to the high affinity of cyclen towards zinc ions, the strong affinity towards aromatic sulphonamides of the Zn–cyclen complex, and the good luminescence properties of the dansyl chromophore. Since the metal ion complexation requires the deprotonation of the sulphonamide group, the association constant is pH dependent, but is very high at physiological levels (e.g. 7×10^9 M^{-1} at pH 7.0 and 2×10^{12} M^{-1} at pH 7.8). Complexation with zinc ions at physiological pH induces a blue shift of the peak from 582 to 540 nm, and increases the emission intensity by fivefold at 540 nm and tenfold at 490 nm. Some quenching of the luminescence intensity was also observed with Co^{2+}, Pb^{2+}, and Hg^{2+}, while almost complete quenching was observed with Cu^{2+}. This last ion tends to form very stable complexes with **33**, but the use of bovine serum albumin masks its presence.

Chemosensor **34** [22], possessing a dioxocyclam receptor shows a similar behaviour to the open-chain analogue **12**, complexing both Cu^{2+} and Ni^{2+} ions, and exhibiting the same qualitative behaviour. Sensors **34** and **12** differ in the following aspects, which depend on the cyclic or non-cyclic structure of the receptor: (i) titration profiles for **34** for both cations are shifted to lower pH values, due to the thermodynamic macrocyclic effect, and (ii) the separation of Cu^{2+} and Ni^{2+} profiles is narrower for **34**, because the latter ion profits from the macrocyclic effect to a larger extent than the larger Cu^{2+} cation. This effect causes the disadvantage that for **34** the pH interval available for selective titration (in which the two ions can be discriminated) is reduced.

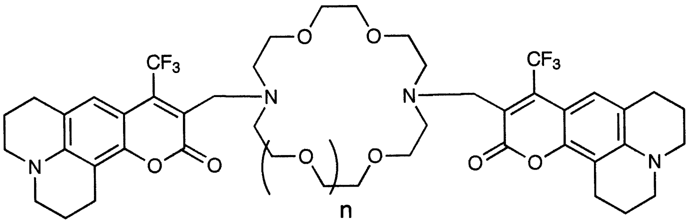


35 (n = 2(a), 3(b))



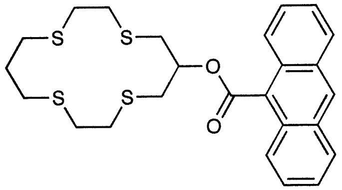
36 (n = 0(a), 1(b))

In low polarity solvents, the fluorescence spectra of **35a** and **35b** [50–52] exhibit a strong exciplex type emission involving the nitrogens and the anthracene ring. In methanol the quantum yield of **35b** dramatically decreases due to the formation, via exciplex intermediates, of non-fluorescent radical ions. In the same solvent, **35a** exhibits a strong, monomer-type emission, since the nitrogen lone pairs, forming hydrogen bonds with solvent molecules, are oriented in this system outside the cavity, thus preventing exciplex formation. Addition of alkali or alkaline earth cations to methanolic solutions of **35** induced a fluorescence increase, with the total disappearance of the exciplex emission. Interestingly, these two species complex Ag^+ quite efficiently ($K_a > 10^9$ M^{-1}). For **35a**, a strong fluorescence quenching has been observed, with evidence for two ground-state complexes: the major one with the silver ion inside the cavity, forming a non-fluorescent charge-transfer complex, and the minor one with the ion residing in the external part of the ring, sufficiently far from the anthracene chromophore, that is in this case able to emit. As far as **35b** is concerned, its fluorescence upon addition of Ag^+ ions is not superimposable to that observed upon addition of alkali metal ions, having a non-structured component peaking at 460 nm, that has been attributed to an exciplex formed by Ag^+ and the anthracene chromophore.



37 (n = 0(a), 1(b))

Complexation studies of **36** and **37** [53,54] were performed in the Valeur's laboratory with alkali, alkaline earth and transition metal ions (Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}). In these compounds the chromophoric group is coumarin 153, whose dipole moment in the excited state differs from that of the ground state of 8 debyes because of the photoinduced charge transfer occurring from the nitrogen atom of the jololidyl ring to the carbonyl group. In these systems the chromophores are linked to azacrowns in such a way that the cations interact directly with the carbonyl group that acts as the electron-withdrawing unit. Therefore, when a cation is coordinated, the excited state is more stabilized than the ground state so that both absorption and luminescence spectra are red-shifted. In addition to the spectral shifts, large changes in fluorescence intensities have been observed upon cation binding. This process reduces the efficiency of PET from the nitrogen of the macrocycle that partially quenches the coumarin chromophore in **36** and **37**. For **36**, fluorescence enhancements were observed with Ni^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}. The stoichiometry of these complexes is usually 1:1, with some exceptions (**36a** can form ML_2 complexes with Zn^{2+}, Cd^{2+}, and Pb^{2+}, **36b** with Pb^{2+}).



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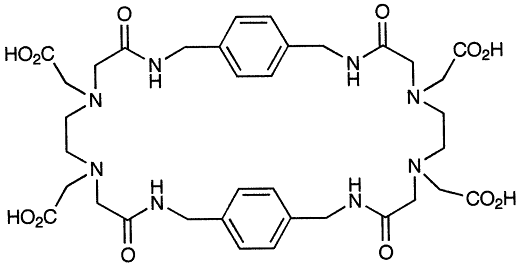
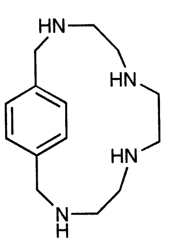


39

The tetrathia-macrocycle **38** [55] was designed and investigated as a chemosensor for Cu^{2+} ions, that are incorporated selectively also in the presence of other 3d metal ions. Copper binding leads to an efficient quenching of the chromophore luminescence centred at 470 nm through PET from the anthracene to the metal ion. Ag^{+} ions are shown to compete in ethanol (but not in acetonitrile) with Cu^{2+} ions, forming a stable complex with 1:2 (metal:ligand) stoichiometry. Complexation with silver ions can not however be signalled, since it does not cause sharp fluorescence changes, due to the higher reduction potential of this ion.

The water soluble cyclophane **39** [56] showed a dependence of its fluorescence intensity (λ_{max} at 290 nm) upon changing pH conditions or upon addition of Zn^{2+}, Cu^{2+} or Ni^{2+} ions. The emission intensity increased with decreasing pH, in two steps, centred at pH 3 and ca. 7. This behaviour was attributed to the increased rigidity of the cyclophane upon protonation. The same effect is caused by complexation with zinc ions, that form a 2:1 (metal:ligand) complex, whose fluorescence is pH independent up to pH 2. The fluorescence enhancement factor is 5.0 at pH 6 and 50 at pH 8.6 (due to the pH dependence of the free ligand). Ni^{2+} and Cu^{2+} instead quenched the ligand fluorescence via a PET mechanism. Furthermore, addition of copper ions caused a tenfold increase in the molar absorptivity at 260 nm, since deprotonation of the amide groups resulting from metal ion complexation brings the two phenyl rings closer.



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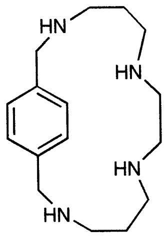
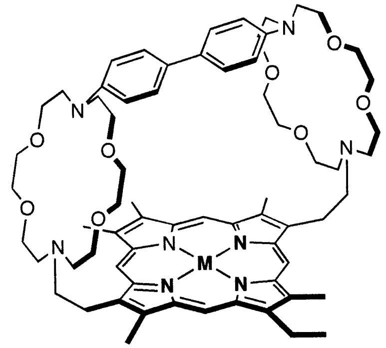


41

Together with the *N*,*N*′-dibenzylated open-chain polyamines analogues **9–11**, Bernardo et al. [24] investigated the polyazacyclophane macrocycles **40–41**. However, the open systems turned out to be more efficient chemosensors in comparison to polyazacyclophanes. These last ones, including a *p*-phenylene spacer, present enough stiffening in the polyamine chain to prevent the simultaneous coordination of both benzylic nitrogens to the same metal ion, leaving one amine able to perform PET even in the complexed state.



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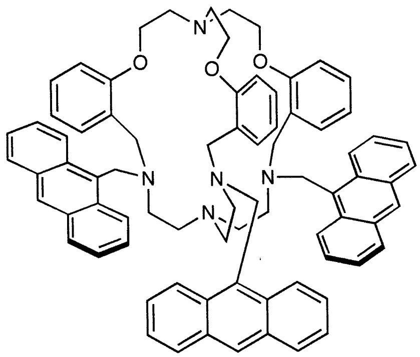

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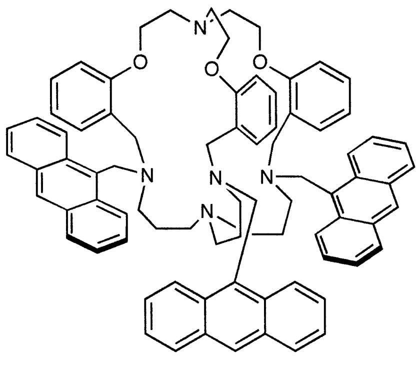

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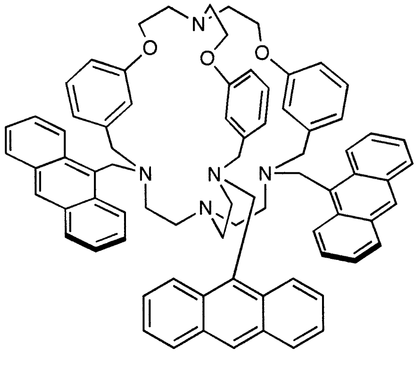
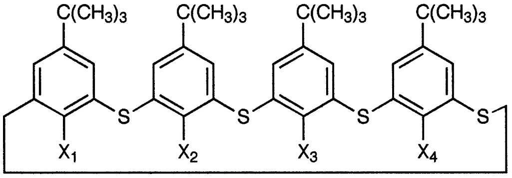
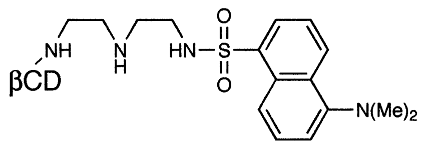


45

A very interesting system, **42**, was presented by Gubelmann et al. [57], in which a Zn porphyrin is bound to two crown ethers derivatives forming a macrotetracyclic cryptate. The two receptor moieties are able to complex in $CHCl_3$/MeOH 9:1 Ag^+ ions; in the so formed polymetallic species the silver ions quenches the typical Zn porphyrin fluorescence by a tenfold factor via a PET mechanism, leading to a long-lived charge-separated state. Three heteroditopic cryptands (**43–45**) for transition metal ions have been proposed by Ghosh et al. [58,59]. These systems show the typical structured fluorescence band of the anthracene monomer along with a red-shifted broad structureless band centred at 550 nm. The authors attributed the red-shifted component to an intramolecular exciplex, although a dependence of the ratio of the two intensities on ligand concentration was found [51]. The overall fluorescence yield is anyway much lower for these three receptors ($\Phi_{FT} \leq 0.002$) than that usually observed for anthracene derivatives, since in this case a very efficient PET process also occurs. Addition of many transition metal ions (Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Pb(II)) in THF leads to the formation of 1:1 adducts, in which the monomer fluorescence is increased by more than a 100-fold for all the metal ions, and no exciplex emission could be observed. Among the different receptors, the more rigid **45** (*meta*-phenylene derivative) shows the lower enhancement, while the more flexible **44** (*ortho*-phenylene derivative) gives the highest one. Concerning the different metal ions, Zn(II) induces the highest fluorescence as expected since complexation with this species makes the PET process thermodynamically forbidden, without introducing other quenching mechanisms. The noticeably high enhancement observed with the other metal ions was explained in terms of a communication gap between the metal ion and the anthracene fragments, that would avoid efficient energy- or electron-transfer processes. Experiments were also performed in order to rule out the possibility that the fluorescence enhancement could be due to some artefact, and in particular to protons produced during the addition of metal salts. Silver and mercury ions were instead not able to enhance the fluorescence intensity of the host, probably because they do not enter into the cavity. The selectivity of these hosts among the different metal ions was not discussed.



46: $X_1 = X_3 = OH$, $X_2 = X_4 =$ O-Dansyl
47: $X_1 = OH$, $X_2 = X_3 = X_4 =$ O-Dansyl



48

The use of dansyl-modified thiacalixarens **46** and **47** [60] has recently been proposed for sensing metal ions. Addition of metal ions leads in this case to an increase of the dansyl fluorescence in the case of Cd^{2+}, Cu^{2+}, Zn^{2+}, Cr^{3+}, and Al^{3+}. For the latter ion, association constants of 3500 and 1200 M^{-1} were obtained for **46** and **47**, respectively. A decrease of the fluorescence was instead indicated for Co^{2+} and Ni^{2+}. No mechanism was presented in order to explain the changes of the fluorescence upon complexation. The dansyl moiety was also used in compound **48** [61], in which the chromophore was appended to a β-cyclodextrin via a diethylenetriamino spacer. Compound **48** is also highly luminescent in water, where usually the dansyl chromophore is poorly emissive, since its inclusion in the cyclodextrine cavity provides it with a sufficient, apolar environment. When copper ions are added to a solution of **48**, efficient quenching of the luminescence can be observed. This effect is promoted by the complexation of the ion by a donor group adjacent to the dansyl moiety, with subsequent abstraction of the sulphonamide hydrogen, leading to the observed quenching.

4. Sensors with peptide- and protein-based receptor

Analysis of traces of transition metal ions is particularly important for environmental and biomedical applications but at the same time very difficult because of their extremely low concentrations. Therefore, particularly selective and avid receptor units are required. Protein binding of divalent metal cations can exhibit these characteristics and many researchers are investigating this very attractive field. Imperiali et al. have recently used polypeptide architectures for metal ion recognition and in particular to design fluorescent chemosensors sensitive to nanomolar concentrations of Zn^{2+} [62–65] or Cu^{2+} [66] in water at pH 7. They reported [62] the preparation and characterisation of polypeptides including amino acid building blocks bearing a chromophore (cyanoanthracene), metal coordinating sites (bipyridine derivatives) and a donor unit (L-3,4-dimethoxy-DOPA) able to quench the fluorophore luminescence. This sensor undergoes a metal ion-induced conformational change that produces noticeable differences in the fluorescence properties of the peptide. Different sequences were tried to enhance the aqueous solubility maintaining the structural flexibility. A metal ion-dependent PET luminescence quenching was observed upon titration with Zn^{2+} and/or Co^{2+} in micromolar concentrations in MeOH or aqueous solutions and the system exhibited total reversibility upon addition of 1,10-phenanthroline.

Typically, proteins do not show the suitable fluorescence properties to be useful luminescent sensors but this problem can be overcome using an auxiliary fluorophore covalently linked to the peptidyl structures. Imperiali and her co-workers used zinc finger domains [63,64] as selective and sensitive receptors and replaced one of the latter residues with a dansylated β-aminoaniline amino acid derivative to monitor the metal-binding event. Dansyl is an environment-sensitive fluorophore and since the metal binding induces peptide folding, it results in a shielding of the fluorophore from the solvent and in a consequent increase in the fluorescence intensity. The increase of fluorescence relative to the increasing levels of Zn^{2+} in solution is linear between 0.1 and 1.0 μM of the metal ion. The ions Na^{+}, Mg^{2+} and Co^{2+} do not interfere in the analysis of Zn^{2+} even if they are present in much higher concentrations (more then 100 times). The indispensable characteristic of reversibility is also proven by fluorescence intensity measurements before and after the addition of EDTA to a solution of the complexed chemosensor.

The peptide skeleton can be easily modified and this allowed the same group of researchers to investigate [64] several modifications in order to obtain a better oxidative stability and the best luminescence signal. Other different fluorophores have been covalently attached at the peptide structure but dansyl proved to offer the highest sensitivity. The thiolate ligand, formed by cysteine residues, is the weak site towards oxidation but its substitution is not trivial because this would change the binding affinity for Zn^{2+}. After some different attempts, aspartic acid was selected as a replacement for Cys^{6} as this was best able to mimic the steric and charge neutralisation properties of the original binding site. A fluorescent chemosensor with analogous performances [63] but enhanced oxidative stability was obtained in this way.

A further step towards practical application is to support chemosensors on water-solvated resins to build regenerable devices. The size of the fluorosensor is particularly important at this stage: it is impossible to obtain a well-defined sensing device composition using polypeptide structures as big as the one described above. To solve this problem Imperiali et al. [65] have described the synthesis of new residues containing the well-known ligand oxine (8-hydroxyquinoline) and their incorporation in short peptides (seven residues) sensitive to sub-micromolar levels of Zn^{2+}. Particular peptide sequences were designed to promote folding resulting in enhanced metal-binding properties and cysteine was incorporated to favour Zn^{2+} selectivity. A detection limit less than 250 nM was found for Zn^{2+} ions; Fe^{2+} and Cd^{2+} showed minor competition (approximately 10%) while Cu^{2+} is a strong interferent: approximately 40% of binding at equivalent concentrations.

They also studied polypeptide chemosensors selective towards Cu^{2+} [66], using the metal-binding properties of amino terminal motifs found in the serum albumin, which bind both Cu^{2+} and Ni^{2+} with very high affinity. The dansyl fluorophore was appropriately covalently linked and intramolecular quenching signals the metal binding. The fluorescence quenching was linear until the chemosensor was saturated at one equivalent of added metal and further addition of divalent metal produced no further quenching. Cu^{2+} causes a greater change in fluorescence (7% of initial intensity residue) than Ni^{2+} ($\sim$15–35% of initial intensity residue) and the only interfering metal ion seems to be Fe^{2+} ($\sim$10% quenching). The selectivity towards Cu^{2+} can be increased via modification of the peptide backbone and this results in a cross reactivity of Ni^{2+}, Fe^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, Mg^{2+}, Ca^{2+} of less than 4% of the quenching observed. The induced quenching is completely recovered via an addition of an excess of EDTA. Immobilized analogues on solid phase were also prepared.

Another group of researchers followed the same kind of strategy to obtain a good chemosensor for Zn^{2+}. Berg et al. reported [67] the study of a new ratioable fluorescent probe that exhibits as a receptor unit the zinc finger consensus peptide (CP) that binds zinc tightly and selectively with consequent changes in flexibility and structure as already reported [68]. They modified CP covalently linking two fluorescent moieties: fluorescein as the donor and lissamine as the acceptor. When the peptide is unfolded the dyes are relatively far apart and they almost do not interact. Zn^{2+} ions induce the folding taking the fluorophores closer and consequently an appreciable intramolecular energy transfer can be observed. The fluorescence spectrum presents the two maxima corresponding to the fluorescein and lissamine emissions and the intensity changes differently upon addition of zinc yielding a ratioable chemosensor. This is also an excellent candidate for in vivo studies since the emission in the visible region is not covered by other cellular components.

Thompson et al. are actually working in the field of optical sensors for Zn^{2+} in water using the specific ion recognition ability of the enzyme human carbonic anhydrase II [69–73]. Contrary to what has been described so far, the fluorophore that allows the monitoring of the target species is not covalently attached to the skeleton of the enzyme but is diffusing in solution. Dansylamide is able to bind the Zn^{2+} only when it is linked to the active site, in this condition its fluorescence is enhanced and shifted towards higher energies, so that the concentration of Zn^{2+} ions is proportional to the ratio of fluorescence intensity at two wavelengths. Mn^{2+} and Co^{2+} give minor interference. One problem is reversibility since the off rate of Zn^{2+} is too slow even for very low data rate applications. The application of this system to a fibre optic needs some further investigation since the membrane that should permit the exchange of the Zn^{2+} ions between the sample and the fibre optic is also permeable to the dansylamide molecules.

Changes in fluorescence are very useful to monitor metal ions concentration but sometimes optical effects and other sources of error invalidate the measurements. Methods based on fluorescence lifetime [70] measurements are more precise but the instrumentation is not a routine one.

Thompson and co-workers introduced fluorescence anisotropy as a new approach for determination of metal ions in solution [71–73]. This is a well-known technique that measures the difference in fluorescence intensity observed through polarisers parallel and perpendicular to the polarisation of the excitation light. The changes in fluorescence anisotropy can be caused by changes in rotational motion, molecular size or fluorescence lifetime. In a first attempt [71] they synthesized some *para*-substituted benzenesulfonamide which is able to bind Zn^{2+} ions when it is complexed by the enzyme. The change in mobility and molecular size of the fluorophore results in an appreciable fluorescence anisotropy able to signal nanomolar concentrations of Zn^{2+} even if non-negligible enzyme-inhibitor affinity makes the data interpretation quite difficult. Thus, they synthesized [72] a new aryl sulphonamide ligand which shows a large difference in affinity between the apoprotein (CAII) and holoprotein (CAII–Zn^{2+}). The new fluorescent ligand presents a very low anisotropy when free in aqueous solution but it becomes high when it is bind to the Zn^{2+} in the holoprotein. This system allows the determination of Zn^{2+} in solution in the picomolar range concentration. The authors suggest the use of this new fluorescent ligand in the same situations for wavelength ratiometric and lifetime-based determinations.

A further development of this work [73] presents some CAII variants obtained by replacing one residue near the binding site of the enzyme with a cysteine covalently substituted with derivatives of benzoxadiazole sulphonamide. This kind of approach opens many possibilities for applications since this system can, for example, be attached on a surface of an optical fibre. Two different variants are discussed, the first one with a high affinity for Cu^{2+} (0.3 pM) and Co^{2+} (100 nM), but $Zn^{2+}$and Cd^{2+} ions can interfere, and the second one for Zn^{2+} (80 pM), both studied in water at pH 7. The transition metal ion concentrations are monitored both by variations in fluorescence intensities or by fluorescence anisotropy, this last being particularly important (30% of increase) in the enzyme binding the Zn^{2+}. The results demonstrate that free transition metal can be quantitatively determined at trace levels.

5. Conclusions

In this review, we have reported a large variety of fluorescence chemosensors for transition metal ions. This clearly indicates that the chemistry community devotes strong attention to the development of systems able to monitor the concentrations of these analytes in real-time and real-space. This interest can be easily understood, since they represent an environmental concern and, at the same time, some of them are present as essential elements in biological systems. We believe however that for many transition metal ions good selectivities and sensitivities have still to be reached in order to address practical needs. A wide horizon is therefore open to further investigation and developments. In other words, as stated by Czarnik [74], there is still a great demand for more and more efficient chemosensors.

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