Topics in Fluorescence Spectroscopy

Volume 9
Advanced Concepts in Fluorescence Sensing
Part A: Small Molecule Sensing

Topics in Fluorescence Spectroscopy

Edited by JOSEPH R. LAKOWICZ and CHRIS D. GEDDES

Volume 1: Techniques
Volume 2: Principles
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Part A: Small Molecule Sensing
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Edited by

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Springer

The Library of Congress cataloged the first volume of this title as follows:

Topics in fluorescence spectroscopy/edited by Chris D. Geddes and Joseph R. Lakowicz.
p. cm.
Includes bibliographical references and index.
Contents: v. 1. Techniques
1. Fluorescence spectroscopy. I. Geddes, Chris D. II. Lakowicz, Joseph R.

QD96.F56T66 1991 91-32671
543'.0858—dc20 CIP

ISSN: 1574-1036
ISBN 0-387-23334-2 Printed on acid-free paper

Printed in the United States of America

9 8 7 6 5 4 3 2 1 SPIN 11319535

springeronline.com

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PREFACE

Over the last decade fluorescence has become the dominant tool in biotechnology and medical imaging. These exciting advances have been underpinned by the advances in time-resolved techniques and instrumentation, probe design, chemical / biochemical sensing, coupled with our furthered knowledge in biology.

Ten years ago Volume 4 of the Topics in Fluorescence Spectroscopy series outlined the emerging trends in time resolved fluorescence in analytical and clinical chemistry. These emerging applications of fluorescence were the result of continued advances in both laser and computer technology and a drive to develop red/near-infrared fluorophores. Based on the advancements in these technologies, it was envisaged that small portable devices would find future common place in a doctor's office or for home health care.

Today, these past emerging trends in fluorescence sensing are now widely used as either standard practices in clinical assessment or commercialized health care products. Miniature lasers in the form of laser diodes and even light emitting diodes are widely used in applications of time-resolved fluorescence. Computer clock-speed is now not considered a hurdle in data analysis. Even our choice of fluorophores has changed dramatically in the last decade, the traditional fluorophore finding continued competition by fluorescent proteins and semi-conductor quantum dots, to name but just a few.

This volume "Advanced Concepts in Fluorescence Sensing: Small Molecule Sensing" aims to summarize the current state of the art in fluorescence sensing. For this reason we have invited chapters, encompassing a board range of fluorescence sensing techniques. Chapters in this volume deal with small molecule sensors, such as for anions, cations and CO2.

While many of the changes in recent fluorescence have been well received, its continued growth in the world has created a challenge in trying to archive and document its use. Subsequently Chris D. Geddes has now become co-series editor of the Topics in Fluorescence Spectroscopy series. We have also recently launched the Reviews in Fluorescence series, which co-edited also by Dr's Geddes and Lakowicz and published annually, is meant to directly compliment the Topics in Fluorescence Spectroscopy series, with small chapters summarizing the yearly progress in fluorescence.

Finally we would like to thank all the authors for their excellent contributions, Mary Rosenfeld for administrative support and Kadir Aslan for help in typesetting the volume.

Chris D. Geddes
Joseph R. Lakowicz
Baltimore,Maryland, US.
August 2004

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PROBES AND SENSORS FOR CATIONS

Luca Prodi,* Marco Montalti, Nelsi Zaccheroni, and Luisa Stella Dolci

1.1. INTRODUCTION

1.1.1. The Need of Chemical Sensors

Sensors of any kind are nowadays substantially ubiquitous with the aim of improving the quality of our lives in any technologically advanced application. Following a definition given by IUPAC, a sensor is a system that, stimulated by any form of energy, reacts changing its own state and thus one or more of its characteristics. Among the different kinds of sensors, chemical sensors, i.e., those sensors that transform chemical information (ranging from the concentration of a specific sample component to total composition analysis) into an analytically useful signal, are of particular importance.[1] They have, in fact, already found a wide application in many fields, such as environmental monitoring, process control, food and beverage analysis, medical diagnosis, and, lately, toxic gases and explosives detection. It is evident that all these fields are of great importance from a social and an economical point of view. The development of chemical sensors seems thus predestined to revolutionise the potentialities of chemical analysis. Up to now the convenience of characterising from a chemical point of view an environment was strongly conditioned by many practical factors, mostly related to time and cost, and in many cases these two variables, in the final balance, could make this kind of analysis unsuitable or even useless.[2] Classical methodologies require collection, transportation, eventual pretreating of the sample, and, in many cases, expensive instrumentation manageable only by trained personnel. Chemical sensory devices have been conceived to bypass these restrictions and cover a large field of applications where conventional strategies result to be, even when feasible, inadequate. Chemical sensors, however, are valuable not only since they are cheap and user-friendly analytical tools; they indeed offer more than this: if properly designed they allow monitoring analyte concentrations in real-time and real-space.[3-5]

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1.1.2. The Power of Luminescence Spectroscopy

Among the different chemical sensors, 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 sub millisecond temporal resolution. 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. The versatility of fluorescence-based chemosensors originates also from the wide number of parameters that can be tuned in order to optimize the convenient signal. Even very complex analytical problems can be indeed overwhelmed by controlling the excitation and emission wavelengths, the time window of signal collection, and the polarization of the excitation beam or of the emitted light. In most cases luminescence intensity changes represent the most direct detectable response to target recognition; more recently, however, also other properties such excited state lifetime and fluorescence anisotropy have been preferred as detectable parameters, since they are less affected by the environmental and experimental conditions.[5]

1.1.3. Sensory Devices and Chemosensors: the Role of Chemists

It is evident from what discussed above that the design of efficient chemical sensory devices requires a multidisciplinary approach: it is in fact essential that chemists, biologists, physicists and engineers collaborate in order to obtain the desired system. The main role of chemists in this multidisciplinary team consists on the design and development of the interface among the matrix to be analyzed and the device itself. Chemists are therefore responsible of the part of the device that interacts at the molecular level with the analyte, that is responsible for the affinity and selectivity of the whole device, and that, in many cases, is also responsible of the signal transduction process, determining, as a consequence, the sensitivity of the system.

A very fruitful approach recently followed by chemists for the design of new efficient chemical sensors is based on the principles of supramolecular chemistry.[3-9] This approach implies indeed to start the design of a sensory device from the molecular level, where the space resolution achievable is the lowest possible. In addition to this, the knowledge reached so far in the field of supramolecular chemistry and molecular recognition can suggest the right choice to obtain the desired affinity and selectivity towards the target analytes, and an efficient signal transduction mechanism. The molecular or, more often, supramolecular entities that carry out these functions are conventionally referred to as chemosensors, mostly to distinguish them from chemical sensors, that usually are intended as macroscopic devices.[10-13] It is noteworthy, however, that also chemosensors and probes respond to the definition of sensor given by IUPAC. In fact, chemosensors designed following the supramolecular approach are chemical species 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 properties.

Because of the two different processes occurring during analyte detection, i.e., molecular recognition and signal transduction, luminescent chemosensors can usually be schematised as the assembly of three possible different components (Figure 1.1): a receptor (responsible for the selective analyte binding), an active unity (whose properties should change upon complexation) and, eventually, a spacer that controls the geometry of the system and tunes the electronic interaction between the two former moieties.

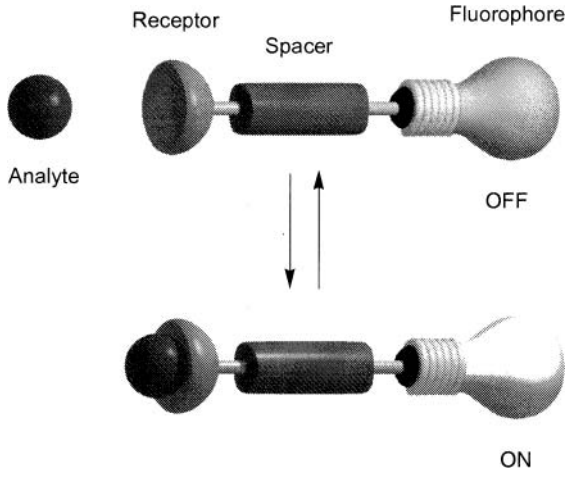


Figure 1.1. Schematic representation of a luminescent chemosensor whose signal transduction mechanism is a photoinduced electron transfer process.

This is the most common approach, that includes also the large family of PET (photoinduced-electron-transfer) based systems. In this case, the fluorophore is electronically coupled to a quencher unit constituting together the electron donor-acceptor pair involved in the PET process through which the luminescent excited state thermally deactivates. The donor, which can be in the simplest case an aminic nitrogen, is usually an integrating part of the receptor and plays an active role in coordinating the analyte, strongly decreasing its electron donating properties upon complexation. As a result the interaction with the analyte can destabilise the charge separated state to such an extent that the PET process cannot compete anymore with the radiative deactivation of the fluorophore. It is worth noticing that this mechanism operates by modifying the electronic properties of the donor moiety but usually does not alter the geometry of the donor acceptor pair.

Another possible arrangement includes all those systems where alterations of the fluorescence properties arise from direct interaction between the fluorophoric moiety and the target species.

In a third representative model of chemosensor, the reconfiguration of the system imposed by complexation plays, on the contrary, a fundamental role. In this case, the structure presents two active units involved in excimer formation or in energy or electron

transfer processes between them, (Figure 1.2). Complexation of the analyte, tuning the relative distance of these two moieties, modulates the efficiency of the intervening inter-component process.

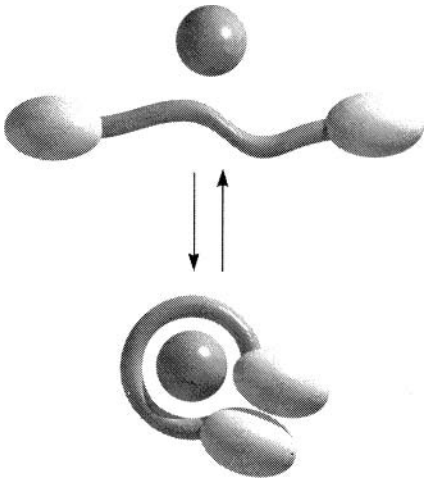


Figure 1.2. Schematic representation of a luminescent chemosensor whose signal transduction mechanism is based on conformational changes of its structure.

As already discussed, luminescent chemosensors can and do already 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.[3-9] In biochemistry, clinical and medical sciences, and cell biology, freely mobile sensor molecules are employed extensively in microscopy allowing to draw maps of the concentration of a given target analyte in a sample in real time.[14]

For many other applications in analytical chemistry and environmental sciences, however, only the immobilization of luminescent chemosensors to yield insoluble sensitive materials and the consequent development of a sensory device can allow continuous measurement of analyte concentrations. The characteristics of the holding up material and the methods of immobilisation are in this context crucial for the efficiency of the sensory devices, and they are again a special task for chemists. The support, in general, must be transparent to the wavelengths of the light absorbed and emitted by the chemosensor and, on the other hand, the active unit must be immobilised at such densities that a facile observation of changes in absorption and/or emission is possible. Solutions are many. One possibility is to immobilise effective chemosensors directly on glass surfaces (Pyrex or quartz).[15,16] This procedure leads however to the formation of a single layer of active units and the output signal could be too weak in the case of luminophores having low emission quantum yield. In order to obtain a larger density of chromophoric units and a stronger signal, the immobilization can be performed grafting on the glass

surface a polymer branched with chemosensor molecules. Choosing polymers of different molecular weight can control the amount of chemosensor immobilized on the glass, as well as the thickness of the film. In general, immobilization does not result in large changes in affinity or selectivity of the receptors.[17] The development of chemical sensors with optical transduction can take advantage of the development of optical fibres.[11,12,18] This technology has already extended the possibility of performing remote real-time measurements with optical sensors by monitoring changes in the absorption or luminescence spectra of fluorescent compounds immobilised on a 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.

Very recently, we also proposed the modification of silica nanoparticles as a possible way for immobilizing luminescent chemosensors.[19,20] The results obtained in this direction will be discussed at the end of this chapter.

As outlined so far, however, the development of new chemosensors and probes is the first (and, for some applications, the only one) essential step for the design of efficient sensors, and the main goal of chemists working in this field. For this reason, this chapter will mainly deal with the results and perspectives in tailoring chemosensors.

1.1.4. Chemosensors and Probes for Metal Ions

Among the different target analytes, a special interest is devoted to develop chemosensors for metal ions. Detecting cations is, in fact, of great interest for many applications. For example, sodium, potassium, magnesium, and calcium, are involved in biological processes such as transmission of nerve impulses, muscle contraction, and regulation of cell activity. In medicine, it is also important to control the serum levels of lithium in patients under treatment for manic depression, and potassium in the case of high blood pressure.

As far as the transition metals are concerned, they can represent an environmental concern when present in uncontrolled amounts, while some of them, such as zinc, copper, and cobalt are present in biological systems in trace amounts as essential elements.[21]

As we discussed in the previous section, the final goal of a luminescent chemosensor is to convert a chemical signal, represented by the concentration of the target analyte into a luminescence related signal (change in the intensity, spectral distribution, temporal decay etc.), which can be quantitatively interpreted.

This condition implies the ability of the chemosensor to recognise the analyte through a specific interaction, leading to the formation of a complex whose fluorescence properties can be easily distinguished from those of the free ligand. As a consequence, a chemosensor must be able to perform two main different functions: the metal ion binding and the signalling of this event. The simplest approach to this problem consists in using fluorescent moieties that are at the same time metal ion receptors. Classical fluorogenic ligands or fluorescent indicators (such as for example hydroxyquinoline derivatives) belong to this class of compounds. The field of applicability and the characteristics of these species are mostly well established.[22] A major limitation of this molecular approach to cation sensing resides in its lack of versatility. The recognition and the transduction

mechanism cannot be modulated independently since adjustments in both these directions require modification of the same moiety. On the other hand these properties are often difficult to be tuned and it could become impossible to induce in the ligand peculiar characteristics such as a high selectivity. The need to operate individually on the recognition and the signalling process suggested, as discussed above, to confine them to separated parts of the whole structure following a supramolecular tactic, as depicted in figures 1.1-1.2. In this way a huge library of photophysically inactive well characterised receptors become available to be integrated into luminescent sensor structures. Historically this process was of course conditioned by the pioneering work of Pedersen, Cram and Lehn who started a systematic research on the synthesis of abiotic metal ions receptors. From then onwards, hundreds of possible chemosensors for metal ions were listed.[3-9,21,23-35]

This chapter will not be an exhaustive review of the systems published so far; rather, it will try do describe, using mainly examples from our laboratory, the possible approaches to the development of luminescent chemosensor for 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 containing dendrimer-, peptide-, or protein-based receptors, and one, as discussed above, concerning nanoparticles, since we believe that these paragraphs allow a better comprehension of the state-of-the-art in the field of sensors and probes for metal ions.

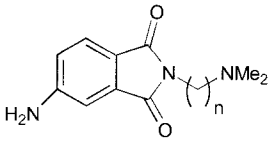
1.2. CHEMOSENSORS WITH ACYCLIC RECEPTOR

As outlined above, in principle chemosensors for metal ions can be designed in a very simple and direct way by interconnecting a proper luminophore with an anchoring function, typically an amino group, though an alkyl chain.

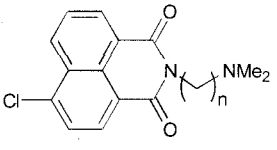
For example, Ramachandaram et al.[36,37] and Mitchell et al.[38] have exploited this possibility for transition metal ion detection. The fluorescence of these systems is quenched by the occurrence of a photoinduced electron transfer (PET) process between the lone pair of the nitrogen and the appended chromophore. A significant enhancement of the fluorescence of **1a**, **1b**,[36] **2a**, and **2b**[37] (> 30 times for **1a**, >100 for **2a**) can be observed in acetonitrile solutions upon complexation of several transition metal ions. It is interesting noticing that some species that are usually reported to quench luminescence, such as Cr^{3+}, Fe^{3+}, Co^{2+}, Ni^{2-} and Cu^{2+} in this particular case induce an increase of the luminescence intensity. Such an atypical effect, according to the authors, is the result of the involvement of the nitrogen lone pair electrons in the coordination of the metal, which makes the PET quenching process energetically inaccessible. This effect prevails on the quenching effect due to the metal ion. The proposed mechanism is supported by the observation that the greatest enhancement effect has been obtained with the Zn^{2+} ions, which, having a d^{10} electronic configuration, do not usually introduce new electron- or energy- transfer processes for the deactivation of the excited state.

The absence of a specific receptor in the above-described systems is a major drawback of their simple design and is responsible for the lack of marked selectivity experienced by these molecular systems.

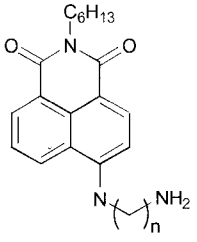
This limitation has been partially overwhelmed in the case of compounds **3a** and **3b**. Their fluorescence in 2-propanol solution (10 μM) is affected[38] by sub-ppm levels of Cu^{2+}, and only by much higher concentrations (10-100 ppm) of Ni^{2+} and Mn^{2+} while it is totally unaltered by the presence of other metal ions such as Zn^{2+}, Al^{3+}, and Ca^{2+}. A three-fold luminescence enhancement upon metal complexation has been observed in the case of ligand **3b**. It is to note that da Silva et al.[39] have described a very similar compound as a PET based pH sensor.



1a n = 2
1b n = 3



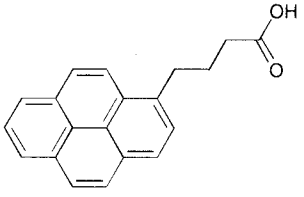
2a; n = 2
2b; n= 3



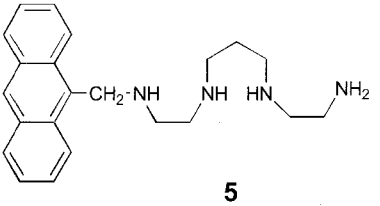
3a; n = 2
3b; n = 3

Carboxylate can also be employed as coordinative groups for metal ions recognition. **4** is a very simple and commercially available chemical species; nevertheless we proposed it as fluorescent chemosensor for metal ions[40] because of the unique ability of pyrene to give strongly luminescent dimers and exciplexes and of the co-ordinating abilities of the carboxylate group. Addition of earth metal ions to acetonitrile solutions of **4** causes in fact strong changes in the absorption and fluorescence spectra (Figures 1.3-1.4).

The results obtained were interpreted assuming that a complex with 1:2 (metal:ligand) stoichiometry was formed, in which the two pyrene units, lying very close to each other, could interact even in the ground state forming a pyrene dimer. Using this methodology, a detection limit of 4 ppb was obtained in acetonitrile for Ca^{2+} ions. Complexation with other ions, such as Sr^{2+}, Ba^{2+}, and Zn^{2+} induced very similar photophysical changes, although with lower association constants. A quenching of the fluorescence was instead observed with Cu^{2+} ions.



4



5

A more specific receptor unit is however required for working in aqueous medium, a typical condition for practical application, because of the strong solvation energy experienced by ionic species in the presence of water molecules.

With this aim in mind, Fabbrizzi et al.[41] synthesized compound **5**, having an anthracene chromophore and a polyamine chain as receptor unit. In aqueous media the metal binding properties of a ligand containing basic centres is strongly affected by the pH conditions. For this reason these authors have first examined the effect of pH on the fluorescence of **5** in acetonitrile/water solutions. In particular, they observed a fluorescence decrease at pH values above 4 where the deprotonation of the polyaminic chain starts enabling the free nitrogen lone pairs to take part to PET process towards the anthracene fragment causing its quenching. The pH dependence of the fluorescence intensity is affected by the presence of Cu^{2+}, Ni^{2+}, and Zn^{2+}. For the two former ions, a decrease of the intensity is observed at lower pH (< 3 for copper ions), indicating the formation of complexes where the deactivation of the anthracene excited state occurs via an energy transfer process from the chromophore to metal centred states. In the case of Zn^{2+}, complex formation is revealed by an almost complete recovering of the chromophore luminescence, which, after an initial quenching on increasing pH, gradually raises in the interval between 3.7 and 6. In the first part of the intensity *vs* pH curve, the Zn^{2+} 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, the PET is not anymore thermodynamically allowed. It is worth to note that in this system, as for every ligand which contains basic atoms, complexation competes with protonation. Hence metal binding induces a shift of the apparent pK_a of the receptor which is as higher as stronger is the interaction with the metal ion. Consequently, the observed behaviour is consistent with a larger stability of the Cu^{2+} and Ni^{2+} complexes with respect to the Zn^{2+} one.

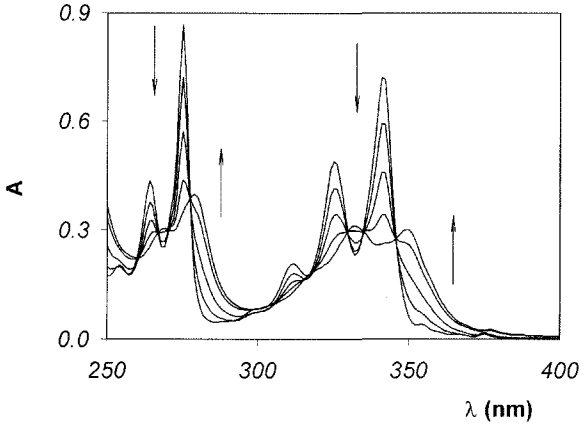


Figure 1.3. Absorption spectra of a 1.4 x 10^{-5} M acetonitrile solution of 4 and upon addition of 0.12, 0.25, 0.38, and 0.51 molar equivalents of $Ca(ClO_4)_2$.

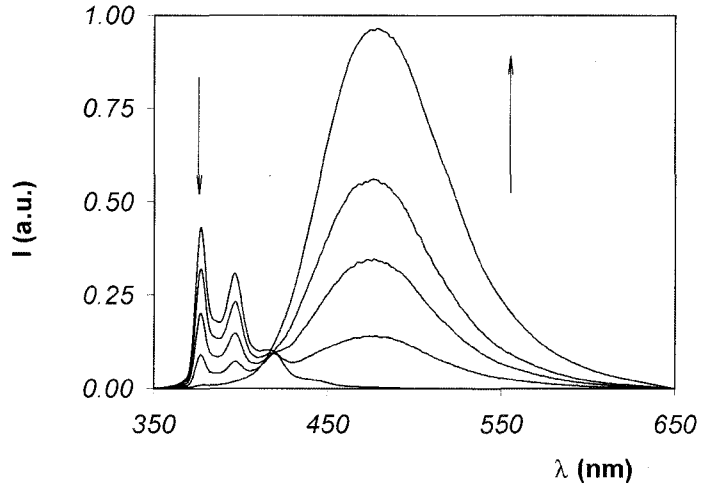
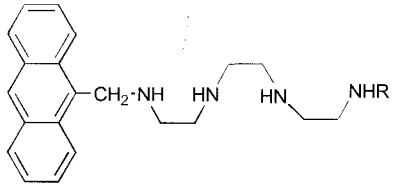
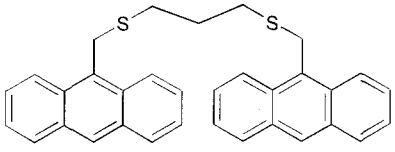


Figure 1.4. Fluorescence spectra (λ_{exc} = 330 nm) of a 1.4 x 10^{-5} M acetonitrile solution of 4 and upon addition of 0.12, 0.25, 0.38, and 0.51 molar equivalents of $Ca(ClO_4)_2$.



6 R = H
7 R = CH_2An

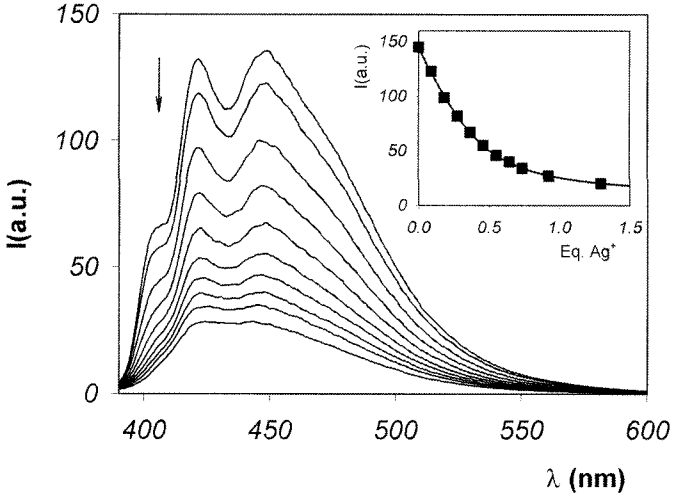


8

A similar molecule, **6**, synthesized by Sclafani et al.[42], shows with Zn^{2+} in pure water a comparable behaviour, although in this solvent the chelation enhanced fluorescence is lower (about 6-fold). From than onward, many other systems possessing an anthracene chromophore and a linear polyamminic chain were synthesized and studied in detail.[43-45]

In all the examples reported up to this point complexation always resulted in a modulation of the luminescence intensity. This parameter unfortunately may be influenced by factors independent from the target species concentration such as environmental or instrumental effects. As a consequence, the signal measured may be not

correctly correlated only to the metal complex concentration causing an error in the analyte determination. This possibility of misreading may be minimised when a different fluorescence signal coming from the uncomplexed ligand can be detected and used as reference. This situation has been achieved in the case of **7**,[42] where two anthracene fragments are present in the same ligand unit. In the fluorescence spectrum of the free molecule there is evidence of 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. A similar effect was also observed[46] with the molecule **8** consisting of two anthryl groups connected by a –SCH2CH2CH2S– spacer, a species that was designed pursuing the aim of the formation of thia-anthracene receptors. The absorption spectrum of **8** (in dichloromethane:methanol, 8:2, v/v) presents the typical pattern of the anthracene moieties, indicating that almost no interaction between the two units takes place in the ground state. On the contrary, the fluorescence spectrum differs greatly from that of simple anthracene (see Figure 1.5), revealing the presence of a



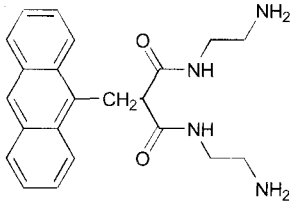
broader band superimposed on the typical structured emission of the isolated fluorophore.

Figure 1.5. Changes in the fluorescence spectra of a solution of 8 in dichloromethane:methanol, (8:2, v/v) upon addition of silver cations.

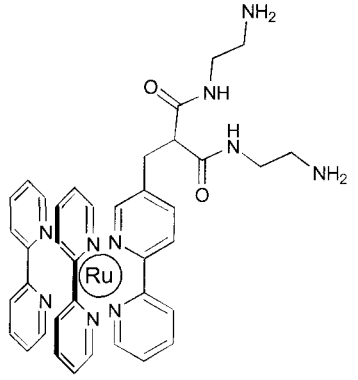
In addition, a double exponential model was needed to interpolate correctly the experimental data, giving two distinct lifetime values. It is worth to note that the above-described photophysical properties are not altered by changing the concentration in the range between 1 x 10^{-6} and 1 x 10^{-4} M. This observation clearly indicates that an intramolecular process is responsible for the double luminescence. In addition, the shape of the luminescence band clearly depends on the polarity of the solvent; in particular, the

contribution of the unstructured component in $MeOH/CH_2Cl_2$ mixtures increases for higher methanol fractions, i.e., increasing the polarity of the mixture itself. Finally, no differences were observed between excitation spectra recorded at different emission wavelengths, all being superimposable to the absorption spectrum. The appearance of the broader, red shifted fluorescence band can thus clearly be attributed to the formation of an intramolecular excimer between the two anthracene moieties of **8**.[47] In the presence of silver ions, the above-described fluorescence spectrum of **8** undergoes remarkable changes. Titration experiments showed that a gradual increase in the concentration of silver ions causes a progressive decrease in the structured luminescence band, while the other, broader band ends by prevailing (Figure 1.5). It is worth noting that a weakening of the luminescence occurs even when only the lower-energy section of the spectrum, namely, over 500 nm, is examined, indicating that the disappearance of the structured band comes together with a decrease in intensity of the other band. Also the absorption spectrum undergoes significant changes upon complexation: a slight red shift of the lower-energy bands takes place together with a broadening of the same band. The coordination of the metal ion imposes a much more rigid structure to the ligand, and in such a geometry the two anthracene moieties are much closer than in the free ligand. This makes their interaction much easier in both the ground and the excited state. The quenching of the fluorescence of the low energy component can be attributed to an electron transfer process involving the silver ions, that is thermodynamically possible. Moreover, other possible interpretations such as the occurrence of an energy transfer process from the anthracene to the metal-centered excited states must be ruled out, since the d^{10} silver ions do not have a low-energy metal-centered state.

A deeper investigation indicated that the association process takes place in two steps, and when a solution of silver ions is added to **8**, there is the formation, first, of $[Ag^+.\mathbf{8}_2]$ and, then, of $[Ag^+.\mathbf{8}]$. The association constants for the two consecutive equilibria can be estimated to be $K_1 = 2 \times 10^5\ M^{-1}$ and $K_2 = 8 \times 10^4\ M^{-1}$. A large number of metal ions usually show a good affinity toward sulfur-containing compounds. Titration experiments with Hg^{2+}, Cd^{2-}, Cu^{2+}, Ni^{2-}, Zn^{2+}, and Co^{2+} however evidenced that **8** does not complex these species, indicating its good selectivity.



9



10

Amidic groups are usually poorly effective in metal ion coordination. Their binding tendency can be anyway widely enforced when they are inserted in a receptor structure where other anchoring centres co-operatively take part to the complexation. Fabbrizzi et al. have recently shown[48-50] 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 (**9**[48,49]) or $Ru(bpy)_3^{2+}$ (**10**[50]). 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 that profit of a large ligand field stabilisation, in these cases only Ni^{2+} and Cu^{2+}. This condition makes ligands of this kind extremely selective, and the different ligand field stabilisation, higher for Cu^{2+} with respect to Ni^{2+}, allows also to distinguish between these two cations. One major drawback of **9**, however, is the need of organic/aqueous solvent mixtures as the working media, while **10** can be dissolved in water with concentrations ranging up to 10^{-2} M.

In particular, the complexation properties of **10** towards transition metal cations have been examined by comparing the changes of its fluorescence intensity (I_f) and lifetime (τ) as a function of pH, in presence or absence of transition metal cations. When no metal cations are added to solutions containing **10**, I_f remains constant in the $2 < pH < 12$ range, as it has already been observed for related systems. In this pH range, in fact, the luminescence quantum yield (0.030) and lifetime (440 ns) of **10** in aerated water solutions are very similar to those observed for the parent $Ru(bpy)_3^{2+}$ chromophore in the same conditions, indicating that the dioxo-2,3,2-tet fragment does not substantially perturb the excited state properties of the Ru core. On the other hand, when Ni^{2+} or Cu^{2+} (as their perchlorate or chloride salts) are added in 1:1 molar ratio with respect to system **10**, the I_f *vs* pH plot shows a typical sigmoidal profile (figure 1.6), which indicates that binding of the metal ion by the dioxo-2,3,2-tet fragment takes place in the narrow pH range of the steeply descending portion of the sigmoid.

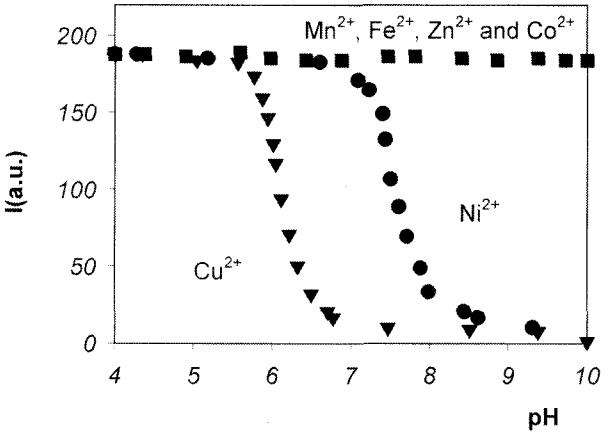


Figure 1.6. Luminescence intensity vs. pH for solutions containing ligand 10 and a metal cation in 1:1 stoichiometry. The metal species referring to each curve is indicated in plot.

Thus, from figure 1.6 it can be said that complexation by **10** ($1x10^{-5}$ M) begins at pH 5.8 and it is complete at pH 6.8 for Cu^{2+}, while for Ni^{2+} it begins at pH 7.5, being complete at pH 8.5. In the descending I_f *vs* pH portion it can also be observed the appearence in the excited state decay profile of a second component with a much shorter lifetime (11 and 15 ns for Ni^{2+} and Cu^{2-}, respectively), clearly indicating an intramolecular quenching process; this component becomes the only one present at higher pH values.

From flash photolysis experiments, no evidence of the presence of Ru^{I} species could be detected, the only transient absorbing species being due to the excited state of the Ru^{II} chromophore. Furthermore, steady state experiments performed at 77 K showed that the quenching process was very fast also in frozen medium at low temperature. All these findings suggest that the energy transfer (ET) mechanism is the most likely explanation for the luminescence quenching of **10**.

The selectivity of this system is proven by the negligible fluorescence intensity chenges in the I_f *vs* pH plots in the presence of other metal centres (1:1 molar ratio), such as Mn^{2+}, Fe^{2+}, Zn^{2-} and Co^{2+}, indicating that no complexation takes place at the dioxo-2,3,2-tet fragment with these ions.

In addition, working on a solution of **10**, buffered at pH = 7.0, no variation in I_f is observed by addition of up to 2 equiv of Ni^{2+} (or other divalent first row transition metal cations), while subsequent addition of Cu^{2+} causes the expected fluorescence quenching, demonstrating that selectivity of Cu^{2+} on Ni^{2+} can be obtained by choosing the correct pH value (see figure 1.7). Finally, solutions buffered at pH 7.0 and containing system **10** at concentrations as low as 10^{-7} M revealed an easily detectable variation of I_f on addition of 1 equiv of Cu^{2+}, indicating that **10** is a suitable sensor for copper cations under analytically relevant conditions.

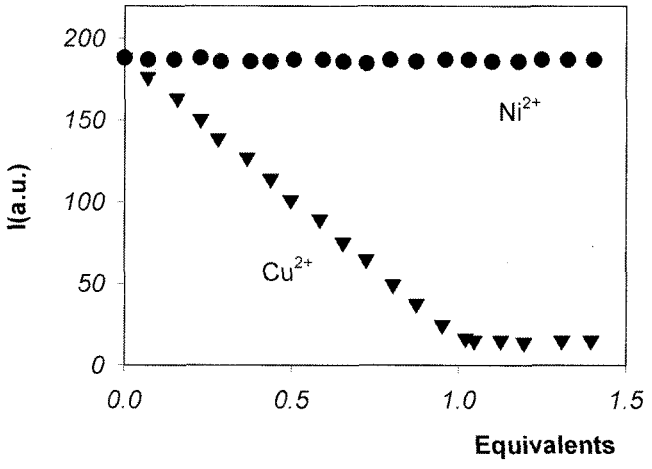
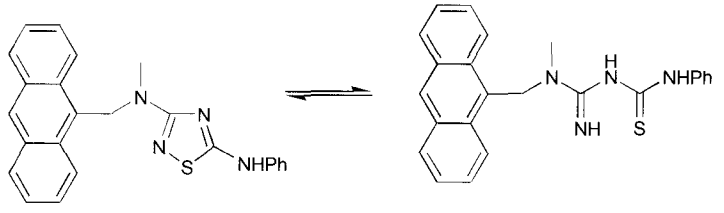


Figure 1.7. Luminescence intensity vs. equivalents of added Ni^{2+} and Cu^{2-} for ligand 10, in a solution buffered at pH 7.0.

An other family (**11-14**) of effective and selective chemosensors for transition metal ions were developed[51-51] using as receptor units the 1,2,4-thiadiazole derivative and its reduced form, the corresponding iminoyl thiourea. For **11**, a PET process from the alkylated nitrogen atom to the excited state of the chromophore is the mechanism responsible for the fluorescence quenching of the appended anthracene. Complexation with Hg^{2+} leads to a tremendous increase in the fluorescence (44-fold), while modest increases were observed upon addition of Cu^{2+} and Pb^{2+} (2-fold and 7.7-fold, respectively). The observed fluorescence increase was attributed to a generic chelation effect, rather than to a suppression of the above mentioned PET process, since addition of protons did not lead to any intensity enhancement. All of the other cations tested yielded no significant changes in emission intensity. In the case of **12**, instead, a 6-fold enhancement of the fluorescence was observed upon addition of Cd^{2+}, and, to a minor extent (3.6-fold) upon addition of Zn^{2+}. For compounds **11** and **12** the detection limit in acetonitrile, for Hg^{2+} and Cd^{2+}, respectively, is in the range of 10 μmol. **13**, closely related to **11**, displays a strong modulation of its fluorescence behaviour selectively upon addition of Cu^{2+}. In this case, the strongest fluorescence enhancement is observed for a concentration ratio 1:2 (metal:ligand). The increase of the emission intensity is time dependent, varying additionally with the concentration of the chemosensor. Immediately after addition of 0.5 eq. of Cu^{2+}, a 4-fold luminescence enhancement is obtained, and an approximately constant signal (a 46-fold intensity increase) is reached after 6 h. **14** functions instead as a chemodosimeter for Hg^{2+}, since this ion promotes the desulfurization of the iminoylthiourea leading to the corresponding urea. This process leads to a red shift and to an increase in the naphthalene emission.

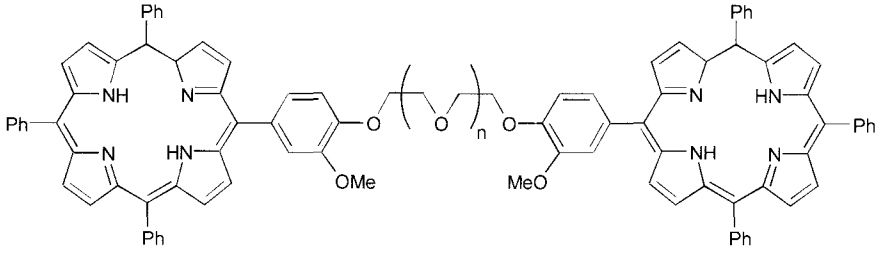


11 **12**

13 **14**

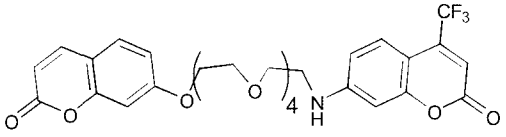
Many groups have instead choose to use derivatized polyoxyethylene chains as receptor units.[53-57] **15a-c**[55] are an example of chemosensors of this family. ^{1}H NMR, UV-

visible and fluorescence studies reveal that the receptors bind to alkali and alkaline-earth metal ions to give a supramolecular complex in which the ion is nestled within the oligooxaethylene framework. The formation of the complexes induces a change in the geometry of the system toward a conformation in which the two tetrapyrrolic macrocycles tend to face together. This causes a blue shift and broadening of their Soret bands. The fluorescence quantum yield is almost completely unaffected by complexation; the lowering of the absorbance causes however a decrease also in the fluorescence intensity at low concentration. In particular, the changes observed allow the complexation process to be monitored at sub-micromolar concentrations. The association constants in chloroform/acetonitrile (1/1) lie within the range 25 - 1 x 10^5 M^{-1} depending on either the nature of the diporphyrinic receptors or the interacting metal ions.



15a; n = 2
15b; n = 3
15c; n = 4

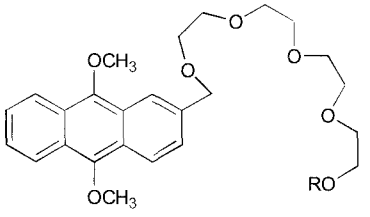
In alternative to the use of the modulation of π–π interactions as signal transduction mechanism, Valeur et al.[56] tried to change the energy-transfer efficiency between a donor and an acceptor (two different coumarins) appended at the end of an ethoxy chain (**16**), taking advantage of the changes induced in the donor-acceptor distances by metal ion complexation. Pb^{2+} is indeed complexed by **16** in acetonitrile, causing a decrease of the average distance of the two chromophores and, consequently, leading to some changes in the luminescence spectrum, due to an increase of the energy transfer rate constant among the two emitting units.



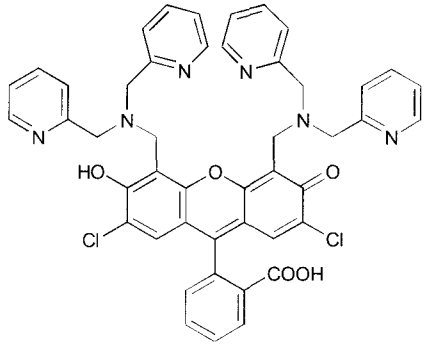
16

As a matter of fact, however, to have a complete switch off/on of an energy transfer process using flexible chain is not an easy task, as proved also by other systems.[55] A

more efficient approach could be to modulate a photoinduced electron transfer reaction via conformational rearrangement induced by the binding process. This mechanism presents a much stronger dependence on the distance, if compared with Förster-type energy transfer processes, and this possibly allows to design more efficient switching systems. Following this strategy, the luminescence of the anthracene is quenched in the series **17a-f**[57] by the electron acceptor linked at the end of the ethoxy chain with an efficiency that depends on the free energy associated at the electron transfer process in agreement with the Marcus law.



17a; R = benzoyl
17b; R = 2,4-dichlorobenzoyl
17c; R = 2-Chloro-5nitrobenzoyl
17d; R = 4-Nitrobenzoyl
17e; R = 3,5-Dinitrobenzoyl
17f; R = 4-Chloro-3,5dinitrobenzoyl

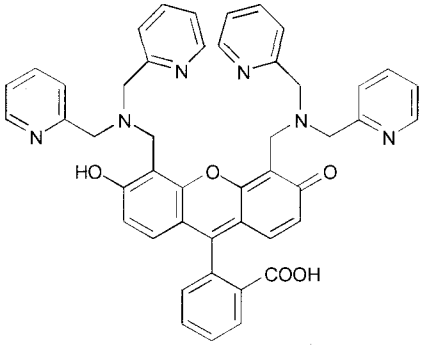


18

In this context, it is worth to remind the excellent work of Roger Tsien,[58-62] a work that, started from the late 70', makes him the first pioneer in the field of chemosensors. He presented a number of very interesting examples of chemosensors; as a recent example, we will cite here two systems (**18** and **19**) based on a fluoresceine platform for the determination of intracellular concentrations of zinc ions. Both these chemosensors have excitation and emission wavelengths in the 500 nm region, association constants for $Zn^{2+} > 10^9$ M^{-1}, quantum yields around 0.9, and cell permeability, making them well suited for intracellular applications. It has been observed a 3- to 5-fold enhancement upon zinc complexation, due to the inhibition of a PET process. As stated by the authors, the primary shortcomings of these sensors are their sensitivity to protons and their relatively modest fluorescence enhancement upon binding of the zinc cations, but they represent a very interesting approach for the development of new zinc sensors suitable for the neurosciences studies, that is indubitably a very promising field.

Tripodal ligands are also often used as receptor moieties. A very interesting example is that proposed by Castagnetto and Canary,[63] who synthesised the chiroptically enhanced fluorescent sensor **20**. 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 6-fold, respectively. In this case, the complexation changes the nature of the lowest excited state from a $n\pi^*$ (that usually tends to give intersystem crossing processes, and thus phosphorescence) to $\pi\pi^*$ state (that

usually gives a more intense fluorescence). Complexation with Fe^{2+} and Cu^{2+} did not result in a fluorescence increase. Complementary information can come 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 fluorescence and ECCD properties of the complexes can lead to the identification of the metal. In fact this 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 findings stress the principle that both isotropic and anisotropic detection may be used to maximise the information given by a single sensor molecule.



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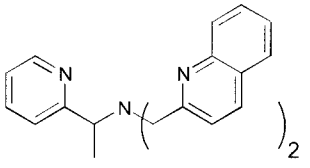

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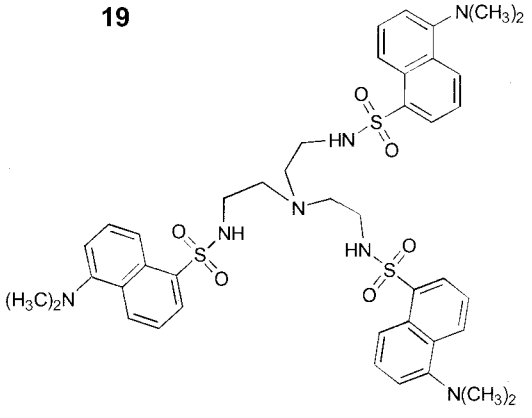
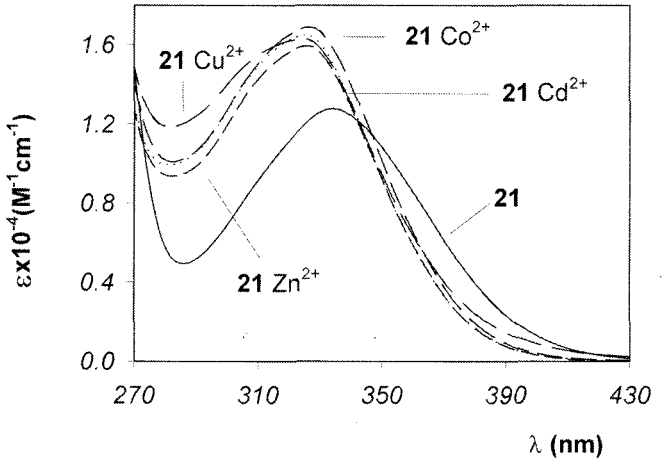


21

Addition to each terminal amine nitrogen atom of *tris*(2-aminoethyl)amine of a dansyl group leads to **21**.[64] The dansyl chromophore is well known to show intense and

large luminescence bands in the 400-600 nm region. These bands are very sensitive to the polarity of the solvent and have considerable charge-transfer character, caused by mixing of the ^1L_a and ^1L_b states of naphthalene with a charge-transfer state arising from the promotion of a lone-pair electron on the amino group into an antibonding orbital of the naphthalene ring. The fluorescence of **21** does not depend on the pH conditions in the 3-11 pH range. However, strong changes on its absorption and luminescence properties were indeed observed upon addition of Cu^{2+}, Co^{2+}, Zn^{2+}, and Cd^{2+} at pH 9.5 (see figures



1.8 and 1.9).

Figure 1.8. Absorption spectra of 21 (9.2 x 10^{-5} M) and of its complexes with Cu^{2+}, Co^{2+}, Zn^{2+}, and Cd^{2+} in acetonitrile/water (1:1, v/v) solution at pH 9.5.