DETECTION OF TRANSITION METAL IONS USING FLUORESCENT SENSORS

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INTRODUCTION

Transition Metal Detection

Transition metal ions can have profound biological effects in animals and the environment even at extremely low concentrations, often less than 1mg/L.[1,2] Therefore, it is important to be able to detect transition metals in low concentrations to assess health risks and for environmental monitoring.[3] The development of such assay methods represents a significant challenge for scientists. The methods to determine transition metal concentrations in drinking water approved by the Environmental Protection Agency (EPA) involve Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma Emission Spectroscopy (ICP-ES), and X-ray fluorescence.[4] While these techniques do an excellent job in accurately detecting low levels of transition metals, they often require extensive sample preparation and expensive instrumentation, and thus are low throughput. The development of other techniques having good specificity and sensitivity, and being inexpensive and fast is of great practical importance.

One method that can be used to detect and quantify transition metal ions is the use of fluorescence organic sensors. This technique has certain advantages, because it does not require extensive sample preparation nor any instrumentation beyond a fluorometer, and it is relatively fast. Intriguingly, specificity for particular metal ions can often be built into this technique. This review will present and evaluate the fluorescent organic sensors that have thus far been developed for transition metals.

Modes of Fluorescent Detection

For transition metal ions to be detected by fluorescence there must be some observable change in the fluorescence emission by the fluorophore. There are three ways in which this can be achieved, (Figure 1) the first being fluorescence enhancement.

This approach involves the use of a fluorophore that has little or no fluorescence in the absence of the target metal ion but, which becomes fluorescent or more fluorescent in the presence of the metal ion. Such an increase in fluorescence efficiency can result from conformational restriction induced upon binding.[5,6] The second approach is fluorescence quenching. This involves the selection of a molecule that has a high fluorescence in the absence of the target metal ion but, greatly reduced or no fluorescence when the target metal ion is present. Many transition metals are known to be fluorescence quenchers,[7] and they can quench fluorescence through energy transfer[8,9] or electron transfer mechanisms.[10] The last

approach involves a shift in the emission maximum of the spectrum depending upon whether the target metal ion is present or not.[11]
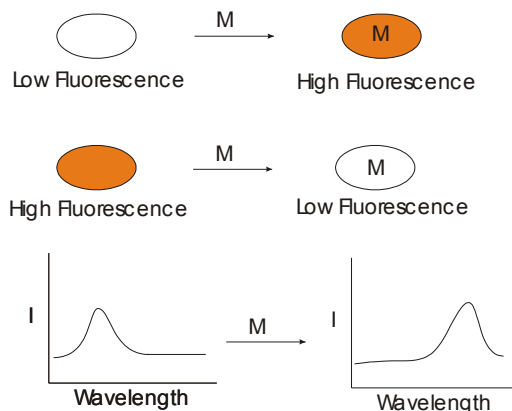

Figure 1: Illustration of Methods of Fluorescence Detection

Evaluation of a sensor

There are several criteria by which to evaluate the quality of an individual sensor; the first is sensitivity. It is important that the sensor be able to detect low concentrations of metal ions. In addition to being sensitive, the detector should have a useful dynamic range, so that it can be used to monitor varying ion concentrations. A good sensor should also be selective, and the best sensors should be selective for one particular transition metal ion and not experience interference from other transition metal ions or from alkali and alkali earth metal ions. Another important factor to consider is the solvents in which the sensor can be used; since many of the applications of these sensors involve an aqueous environment, the best sensors should be able to operate in water.

SELECTIVE SENSORS

A selective Cd^{2+} sensor

Cadmium, a major pollutant in some areas, is an example of a transition metal that can be selectively detected using fluorescent sensors. The EPA standard for maximum Cd^{2+} concentration in drinking water is 0.005 mg/L or 4.4 x10^{-8}M.[2] Long-term exposure to cadmium above these levels can potentially cause kidney malfunctions.[12,13] Anthryl Tetra Acid (ATA), has recently been reported to be a selective Cd^{2+} sensor.[14] At pH 7, ATA exists in a zwitterionic form, that is fluorescent, having a λ_{max} of emission at 420 nm. Upon addition of divalent metal ions such as cobalt, manganese, and zinc, ATA fluorescence is quenched. The mechanism proposed for this quenching involves a reduction in amine pK_a that occurs when the metal binds to the carboxylate groups. With the pK_a reduced, the amine is no

longer protonated and this results in a quenching of fluorescence. A much different result is observed when Cd^{2+} ions are added to the solution. In this case, fluorescence is not quenched rather, there is a red shift in the spectrum to 435 nm as well as significant broadening. These experiments were performed in an aqueous system at a concentration of 6 µM, and it was not stated whether this sensorsystem would work at lower concentrations such as those closer to the 44 nM limit specified by the EPA for drinking water. The proposed mechanism for the spectral broadening and red shift that occurs upon addition of Cd^{2+} is electrophilic aromatic cadmiation at the 9 position of the molecule. This mechanism is supported by ^{1}H NMR, which shows that there was a decrease and a broadening of the H-9 signal upon addition of Cd^{2+}. Similar instances of electrophilic aromatic cadmiation of anthracene-based fluorophores have also been reported.[15] This cadmiation changes the conformation of the system and converts a π complex to a σ complex (Figure 2).
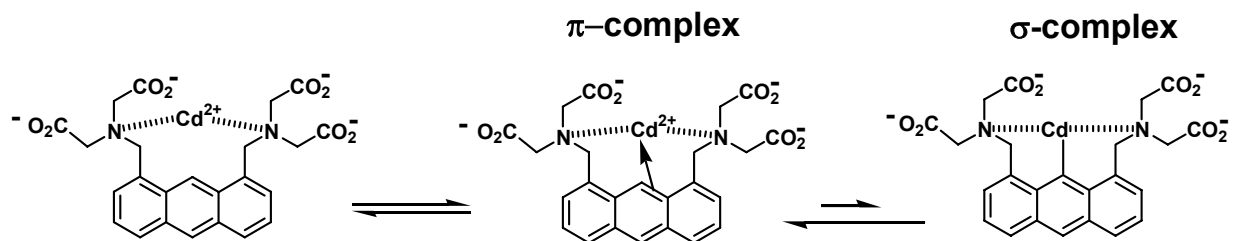

Figure 2. Electrophilic Aromatic Cadmiation

This sensor is very selective for Cd^{2+} ions, and if it proves to be sufficiently sensitive, it may have future medical or environmental applications.

A selective Cu^{2+} sensor

Copper is an essential mineral in the human diet and the Food and Drug Administration (FDA) has recommended a daily copper intake of 2 mg.[13] Although it is an essential mineral, excess copper can have adverse health effects, and the EPA has set the maximum concentration of 1.3mg/L or $2.0x10^{-5}M$ for copper in drinking water.[2] Concentrations above the EPA limit can in the short term cause gastrointestinal problems, and in the long term, kidney damage.[16] Recently, a selective pyrene-based sensor for Cu^{2+} has been developed (Figure 3).[17]
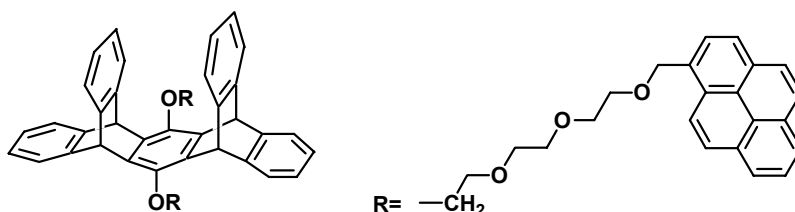

Figure 3. Structure of the Cu^{2+} selective Sensor

This sensor system is based upon the difference in the emission spectra of the pyrene monomer vs. excimer emission: λ_{max} monomer = 375 nm, and excimer = 475 nm. With this sensor Ca^{2+} is selective over other alkali and alkali earth metals, decreasing excimer formation while increasing monomer formation. Cd^{2+} is selective among nine other transition metal ions and it has a similar effect on the spectrum of the sensor. In the presence of Cu^{2+}, by contrast, there is an enhancement of excimer formation along with a substantial, 35 nm blue shift in the emission spectrum, from 475 nm to 440 nm. This is unusual, because Cu^{2+} is typically considered a quenching metal,[18] and the change is particularly significant because, not only is excimer fluorescence enhanced, there is also a shift in the wavelength.

These studies were conducted in dichloromethane at a concentration of 1×10^{-5}M. In acetonitrile no fluorescence changes were caused by either Ca^{2+} or Cd^{2+}; however, the excimer shift and enhancement associated with Cu^{2+} was still retained. The difference in fluorescence spectra for Ca^{2+} and Cd^{2+} compared to that of Cu^{2+} suggests that two different conformations of this sensor are possible, depending upon the metal ion that is present. Size of the ion was discounted as a factor, since the radius of Cu^{2+} was not unique to the group of metal ions studied. To fully understand this sensor system, one must look at the nature and cause of the two very different (440 nm and 475 nm) excimer emission signals (Figure 4), which are caused by two different types of pyrene excimers.[19,20] The sandwich-type structure is thought to produce the 475 nm emission, whereas the partially overlapping dimer form gives the 440 nm emission. Typically, the system is dynamic, and the two states can interconvert, both in the ground state and the excited state (Figure 4).
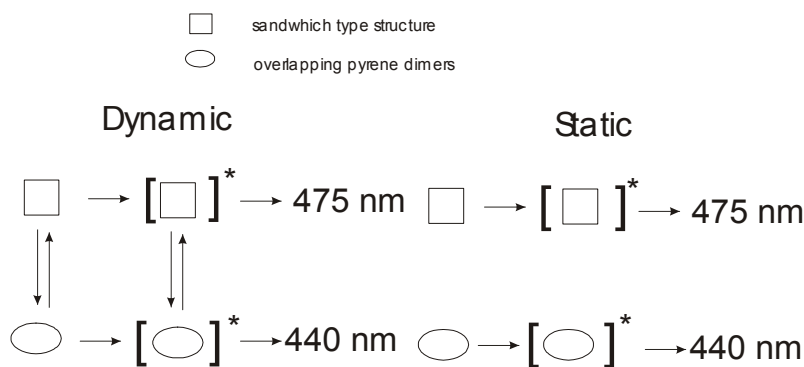

Figure 4. Difference Between Static and Dynamic Pyrene Excimers

In this dynamic system, the equilibrium favors the sandwich state, and the 440 nm emission from the overlapping state is not seen. When Cu^{2+} is added, the system becomes static and there is no longer interconversion between the two excimers. The Cu^{2+} not only locks the system into a static state, but it also stabilizes the overlapping structure with an emission at 475 nm.

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A selective Pb^{2+} sensor

Lead is one of the more serious environmental concerns and health threats. Lead toxicity effects the kidneys, reproductive system, and nervous systems.[21] The FDA limit for lead in drinking water is 0.015mg/L or 7.2×10^{-8}M.[2] Recently, a selective based sensor for Pb^{2+} ions in acetonitrile solutions has been developed (Figure 5).[22]
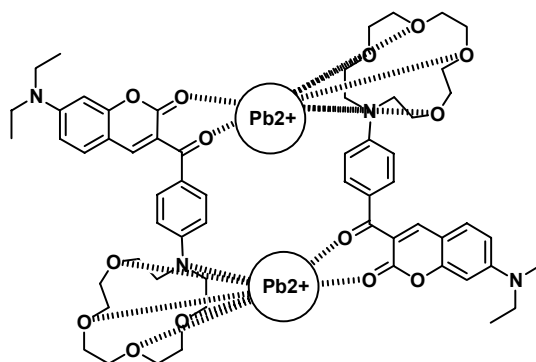

Figure 5. Structure and Binding of Ketoaminocoumarin Based Sensor

This ketoaminocourmarin sensor operates through a fluorescence enhancement mechanism: The fluorophore is not fluorescent until binding of an appropriate metal ion occurs. This sensor is highly selective for Pb^{2+} ions. A variety of univalent metal ions do not cause significant fluorescence emission changes. In competition experiments with other divalent transition metal ions none show fluorescence enhancements that are comparable to Pb^{2+}. The high specificity that this sensor system shows for Pb^{2+} is hypothesized to result from carbonyl lariat formation, and evidence for this can be found in both the NMR and IR spectra. Upon addition of Pb^{2+} ions to a solution of this sensor there was a downfield shift in the ^{13}C NMR carbonyl signals, indicating involvement of the carbonyl in Pb^{2+} binding. There was also a significant decrease in the carbonyl absorption band in the IR spectra. The large Hill coefficient and sigmodial binding curves suggests that a 2:2 rather than a 1:1 complex is formed (Figure 5). The fluorescence enhancement can be understood through an increase in conformational rigidity upon Pb^{2+} binding. This sensor is useful at concentrations down to $\sim 5 \times 10^{-6}$M in acetonitrile solutions, but when tested in aqueous acetonitrile solutions the fluorescent enhancement was diminished.

A Tunable Ni^{2+}/Cu^{2+} Sensor

Although nickel is one of the more benign transition metals, environmentally and medically, large amounts of nickel can cause health problems. The EPA limit for nickel in drinking water is 0.04mg/L or 8×10^{-7}M[1], and concentrations above this can cause liver and kidney damage.[23] A bishydroxamate Calix[4]arene receptor that has tunable specificity for Cu^{2+} and Ni^{2+} ions has recently been developed (Figure 6).[24,25]
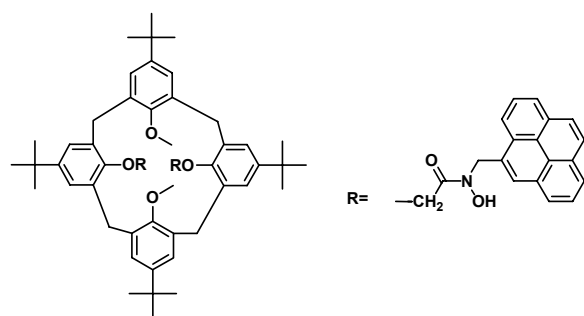
Figure 6. Structure of the Bishydroxamate Sensor

Bishydroxamates have been previously shown to have specific binding for certain transition metal ions.[26,5] This sensor is also based on the monomer/excimer spectral properties of pyrene, but involves quenching of fluorescence. Other divalent metal ions did not have any appreciable effects on the observed fluorescence. The rationale for fluorescence quenching in this system is the existence of low-lying excited states on the metal once it is chelated. These bishydroxamate ligands are known to chelate Ni^{2+} and Cu^{2+}.[27] The pH dependence of the specificity is governed by the relative stability of the ligand to the two different metals. The stability is greater for Cu^{2+} than it is for Ni^{2+} since Cu^{2+} is d^9 and Ni^{2+} is d^{10}.

FUTURE ADVANCES

It has been shown that transition metal ions can be detected using fluorescent organic sensors. Some of these sensors show remarkable specificity for a single transition metal ion, at low concentrations, and in many different solvents. Further advancements in this field could result in sensors having even lower detection limits as well as sensors that can operate effectively in aqueous systems. In addition, the use of combinatorial chemistry may facilitate the development of sensors for a wider variety of metal ions.

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