1.11: Fluorescence Spectroscopy

Introduction

Atomic fluorescence spectroscopy (AFS) is a method that was invented by Winefordner and Vickers in 1964 as a means to analyze the chemical concentration of a sample. The idea is to excite a sample vapor with the appropriate UV radiation, and by measuring the emitting radiation, the amount of the specific element being measured could be quantified. In its most basic form, AFS consists of a UV light source to excite the sample, a monochromator, a detector and a readout device (figure 1.11.1). Cold vapor atomic fluorescence spectroscopy (CVAFS) uses the same technique as AFS, but the preparation of the sample is adapted specifically to quantify the presence of heavy metals that are volatile, such as mercury, and allows for these elements to be measured at room temperature.

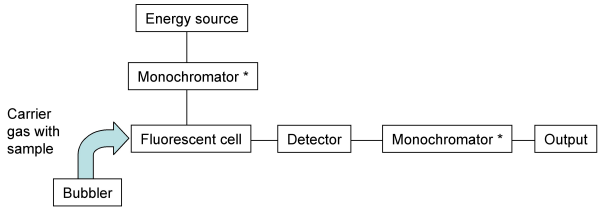

Figure 1.11.1 The basic setup for CVAFS. *The monochromator can be in either position in the scheme.

Theory

The theory behind CVAFS is that as the sample absorbs photons from the radiation source, it will enter an excited state. As the atom falls back into the ground state from its excited vibrational state(s), it will emit a photon, which can then be measured to determine the concentration. In its most basic sense, this process is represented by 1.11.1, where P_F is the power given off as photons from the sample, P_{abs} is the power of the radiation absorbed by the sample, and φ is the proportionality factor of the energy lost due to collisions and interactions between the atoms present, and not due to photon emission.

$$\mathrm{P}_F = \psi \mathrm{P}_{\mathrm{abs}} \tag{1.11.1}$$

Sample Preparation

For CVAFS, the sample must be digested, usually with an acid to break down the compound being tested so that all metal atoms in the sample are accessible to be vaporized. The sample is put into a bubbler, usually with an agent that will convert the element to its gaseous species. An inert gas carrier such as argon is then passed through the bubbler to carry the metal vapors to the fluorescence cell. It is important that the gas carrier is inert, so that the signal will only be absorbed and emitted by the sample in question and not the carrier gas.

Atomic Fluorescence Spectroscopy

Once the sample is loaded into the cell, a collimated (almost parallel) UV light source passes through the sample so that it will fluoresce. A monochromator is often used, either between the light source and the sample, or between the sample and the detector. These two different setups are referred to as excitation or emission spectrum, respectively. In an excitation spectrum, the light source is kept at a constant wavelength via the monochromator, and multiple wavelengths of emitted light are gathered, whereas in the emission spectrum, only the specified wavelength of light emitted from the sample is measured, but the sample is exposed to multiple wavelengths of light from the excitatory source. The fluorescence will be detected by a photomultiplier tube, which is extremely light sensitive, and a photodiode is used to convert the light into voltage or current, which can then in turn be interpreted into the amount of the chemical present.

Detecting Mercury Using Gold Amalgamation and Cold Vapor Atomic Fluorescence Spectroscopy

Introduction

Mercury poisoning can damage the nervous system, kidneys, and also fetal development in pregnant women, so it is important to evaluate the levels of mercury present in our environment. Some of the more common sources of mercury are in the air (from industrial manufacturing, mining, and burning coal), the soil (deposits, waste), water (byproduct of bacteria, waste), and in food (especially seafood). Although regulation for food, water and air mercury content differs, EPA regulation for mercury content in water is the lowest, and it cannot exceed 2 ppb (27 µg/L).

In 1972, J. F. Kopp et al. first published a method to detect minute concentrations of mercury in soil, water, and air using gold amalgamation and cold vapor atomic fluorescence spectroscopy. While atomic absorption can also measure mercury concentrations, it is not as sensitive or selective as cold vapour atomic fluorescence spectroscopy (CVAFS).

Sample Preparation

As is common with all forms of atomic fluorescence spectroscopy (AFS) and atomic absorption spectrometry (AES), the sample must be digested, usually with an acid, to break down the compounds so that all the mercury present can be measured. The sample is put in the bubbler with a reducing agent such as stannous chloride ($SnCl_2$) so that Hg^0 is the only state present in the sample.

Gold Amalgam and CVAFS

Once the mercury is in its elemental form, the argon enters the bubbler through a gold trap, and carries the mercury vapors out of the bubbler to the first gold trap, after first passing through a soda lime (mixture of $Ch(OH)_2$, NaOH, and KOH) trap where any remaining acid or water vapors are caught. After all the mercury from the sample is absorbed by the first gold trap, it is heated to 450 °C, which causes the mercury absorbed onto the gold trap to be carried by the argon gas to the second gold trap. Once the mercury from the sample has been absorbed by the second trap, it is heated to 450 °C, releasing the mercury to be carried by the argon gas into the fluorescence cell, where light at a wavelength of 253.7 nm will be used for mercury samples. The detection limit for mercury using gold amalgamation and CVAFS is around 0.05 ng/L, but the detection limit will vary due to the equipment being used, as well as human error.

Calculating CVAFS concentrations

A standard solution of mercury should be made, and from this dilutions will be used to make at least five different standard solutions. Depending on the detection limit and what is being analyzed, the concentrations in the standard solutions will vary. Note that what other chemicals the standard solutions contain will depend upon how the sample is digested.

Example 1

A 1.00 g/mL Hg (1 ppm) working solution is made, and by dilution, five standards are made from the working solution, at 5.0, 10.0, 25.0, 50.0, and 100.0 ng/L (ppt). If these five standards give peak heights of 10 units, 23 units, 52 units, 110 units, and 207 units, respectively, then 1.11.2 is used to calculate the calibration factor, where CF_x is the calibration factor, A_x is the area of the peak or peak height, and C_x is the concentration in ng/L of the standard, 1.11.3.

$$\mathrm{CF}_x = \mathrm{A}_X/\mathrm{C}_X \tag{1.11.2}$$

$$10/5.0\ \mathrm{ng/L} = 2.00\ \mathrm{units\,L/ng} \tag{1.11.3}$$

The calibration factors for the other four standards are calculated in the same fashion: 2.30, 2.08, 2.20, and 2.07, respectively. The average of the five calibration factors is then taken, 1.11.4.

$$\mathrm{CF}_m = (2.00 + 2.30 + 2.08 + 2.20 + 2.07)/5 = 2.13\ \mathrm{units\,L/ng} \tag{1.11.4}$$

Now to calculate the concentration of mercury in the sample, 1.11.5 is used, where A_s is the area of the peak sample, CF_m is the mean calibration factor, V_{std} is the volume of the standard solution minus the reagents added, and V_{smp} is the volume of the initial sample (total volume minus volume of reagents added). If A_s is measured at 49 units, V_{std} = 0.47 L, and V_{smp} = 0.26 L, then the concentration can be calculated, 1.11.6.

$$[\mathrm{Hg}]\ (\mathrm{ng/L}) = (\mathrm{A}_s/\mathrm{CF}_m)\cdot(\mathrm{V}_{std}/V_{smp}) \tag{1.11.5}$$

$$49\ units/2.13\ units\ L/ng)\cdot(0.47\ L/0.26\ L) = 43.2\ \mathrm{ng/L\ of\ Hg\ present} \tag{1.11.6}$$

Sources of Error

Contamination from the sample collection is one of the biggest sources of error: if the sample is not properly collected or hands/gloves are not clean, this can tamper with the concentration. Also, making sure the glassware and equipment is clean from any sources of contamination.

Furthermore, sample vials that are used to store mercury-containing samples should be made out of borosilicate glass or fluoropolymer, because mercury can leach or absorb other materials, which could cause an inaccurate concentration reading.

The Application of Fluorescence Spectroscopy in the Mercury Ion Detection

Mercury in the Environment

Mercury pollution has become a global problem and seriously endangers human health. Inorganic mercury can be easily released into the environment through a variety of anthropogenic sources, such as the coal mining, solid waste incineration, fossil fuel combustion, and chemical manufacturing. It can also be released through the nonanthropogenic sources in the form of forest fires, volcanic emissions, and oceanic emission.

Mercury can be easily transported into the atmosphere as the form of the mercury vapor. The atmospheric deposition of mercury ions leads to the accumulation on plants, in topsoil, in water, and in underwater sediments. Some prokaryotes living in the sediments can convert the inorganic mercury into methylmercury, which can enter food chain and finally is ingested by human.

Mercury seriously endangers people's health. One example is that many people died due to exposure to methylmercury through seafood consumption in Minamata, Japan. Exposure in the organic mercury causes a serious of neurological problems, such as prenatal brain damage, cognitive and motion disorders, vision and hearing loss, and even death. Moreover, inorganic mercury also targets the renal epithelial cells of the kidney, which results in tubular necrosis and proteinuria.

The crisis of mercury in the environment and biological system compel people to carry out related work to confront the challenge. To design and implement new mercury detection tools will ultimately aid these endeavors. Therefore, in this paper, we will mainly introduce fluorescence molecular sensor, which is becoming more and more important in mercury detection due to its easy use, low cost and high efficiency.

Introduction of Fluorescence Molecular Sensors

Fluorescence molecular sensor, one type of fluorescence molecular probe, can be fast, reversible response in the recognition process. There are four factors, selectivity, sensitivity, in-situ detection, and real time, that are generally used to evaluate the performance of the sensor. In this paper, four fundamental principles for design fluorescence molecular sensors are introduced.

Photoinduced Electron Transfer (PET)

Photoinduced electron transfer is the most popular principle in the design of fluorescence molecular sensors. The characteristic structure of PET sensors includes three parts as shown in Figure 1.11.2

- The fluorophore absorbs the light and emits fluorescence signal.
- The receptor selectively interacts with the guest.
- A spacer connects the fluorophore and receptor together to form an integral system and successfully, effectively transfers the recognition information from receptor to fluorophore.

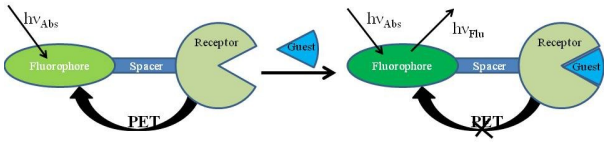

Figure 1.11.2 The general view of the principle of PET fluorescence molecular sensor.

In the PET sensors, photoinduced electron transfer makes the transfer of recognition information to fluorescence signal between receptor and fluorophore come true. Figure 1.11.2 shows the detailed process of how PET works in the fluorescence molecular sensor. The receptor could provide the electron to the vacated electoral orbital of the excited fluorophore. The excited electron in the fluorophore could not come back the original orbital, resulting in the quenching of fluorescence emission. The coordination of receptor and guest decreased the electron donor ability of receptor reduced or even disrupted the PET process, then leading to the enhancement of intensity of fluorescence emission. Therefore, the sensors had weak or no fluorescence emission before the coordination. However, the intensity of fluorescence emission would increase rapidly after the coordination of receptor and gust.

Intramolecular Charge Transfer (ICT)

Intramolecular charge transfer (ICT) is also named photoinduced charge transfer. The characteristic structure of ICT sensors includes only the fluorophore and recognition group, but no spacer. The recognition group directly binds to the fluorophore. The electron withdrawing or electron donating substituents on the recognition group plays an important role in the recognition. When

the recognition happens, the coordination between the recognition group and guest affects the electron density in the fluorophore, resulting in the change of fluorescence emission in the form of blue shift or red shift.

Excimer

When the two fluorophores are in the proper distance, an intermolecular excimer can be formed between the excited state and ground state. The fluorescence emission of the excimer is different with the monomer and mainly in the form of new, broad, strong, and long wavelength emission without fine structures. The proper distance determines the formation of excimer, therefore modulation of the distance between the two fluorophores becomes crucial in the design of the sensors based on this mechanism. The fluorophores have long lifetime in the singlet state to be easily forming the excimers. They are often used in such sensors.

Fluorescence Resonance Energy Transfer (FRET)

FRET is a popular principle in the design of the fluorescence molecular sensor. In one system, there are two different fluorophores, in which one acts as a donor of excited state energy to the receptor of the other. As shown in Figure 1.11.2, the receptor accepts the energy from the excited state of the donor and gives the fluorescence emission, while the donor will return back to the electronic ground state. There are three factors affecting the performance of FRET. They are the distance between the donor and the acceptor, the proper orientation between the donor emission dipole moment and acceptor absorption moment, and the extent of spectral overlap between the donor emission and acceptor absorption spectrum (Figure 1.11.3).

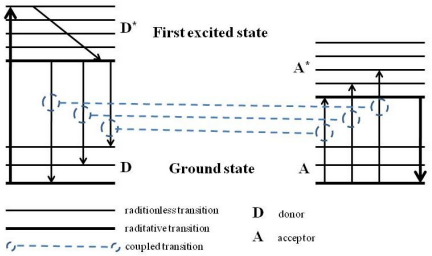

Figure 1.11.2 A schematic fluorescence resonance energy transfer system.

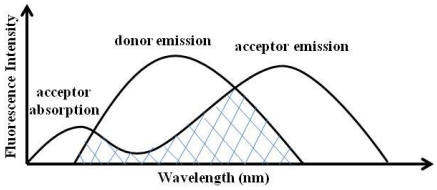

Figure 1.11.3 Diagram showing the spectral overlap for fluorescence resonance energy transfer system.

Introduction of Fluorescence Spectroscopy

Fluorescence

Fluorescence is a process involving the emission of light from any substance in the excited states. Generally speaking, fluorescence is the emission of electromagnetic radiation (light) by the substance absorbed the different wavelength radiation. Its absorption and emission is illustrated in the Jablonski diagram (Figure 1.11.4), a fluorophore is excited to higher electronic and vibrational state from ground state after excitation. The excited molecules can relax to lower vibrational state due to the vibrational relaxation and, then further retune to the ground state in the form of fluorescence emission.

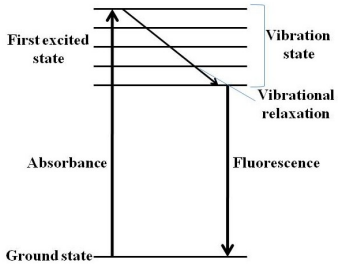

Figure 1.11.4 Jablonski diagram of fluorescence.

Instrumentation

Most spectrofluorometers can record both excitation and emission spectra. They mainly consists of four parts: light sources, monochromators, optical filters and detector (Figure 1.11.5).

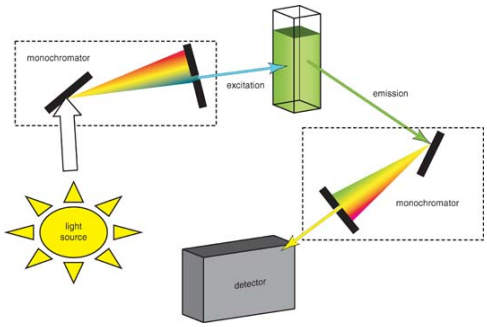

Figure 1.11.5 Schematic representation of a fluorescence spectrometer.

Light Sources

Light sources that can emit wavelength of light over the ultraviolet and the visible range can provide the excitation energy. There are different light sources, including arc and incandescent xenon lamps, high-pressure mercury (Hg) lamps, Xe-Hg arc lamps, low pressure Hg and Hg-Ar lamps, pulsed xenon lamps, quartz-tungsten halogen (QTH) lamps, LED light sources, etc. The proper light source is chosen based on the application.

Monochromators

Prisms and diffraction gratings are two mainly used types of monocharomators, which help to get the experimentally needed chromatic light with a wavelength range of 10 nm. Typically, the monocharomators are evaluated based on dispersion, efficiency, stray light level and resolution.

Optical Filters

Optical filters are used in addition to monochromators in order to further purifying the light. There are two kinds of optical filters. The first one is the colored filter, which is the most traditional filter and is also divided into two catagories: monochromatic filter and long-pass filter. The other one is thin film filter that is the supplement for the former one in the application and being gradually instead of colored filter.

Detector

An InGaAs array is the standard detector used in many spectrofluorometers. It can provide rapid and robust spectral characterization in the near-IR.

Applications

PET Fluorescence Sensor

As a PET sensor 2-{5-[(2-{[*bis*-(2-ethylsulfanyl-ethyl)-amino]-methyl}-phenylamino)-methyl]-2-chloro-6-hydroxy-3-oxo-3H-xanthen-9-yl}-benzoic acid (MS1) (Figure 1.11.6) shows good selectivity for mercury ions in buffer solution (pH = 7, 50 mM PIPES, 100 mM KCl). From Figure 1.11.7, it is clear that, upon the increase of the concentration of Hg^{2+} ions, the coordination between the sensor and Hg^{2+} ions disrupted the PET process, leading to the increase of the intensity of fluorescence emission with slight red shift to 528 nm. Sensor MS1 also showed good selectivity for Hg^{2+} ions over other cations of interest as shown in the right bars in Figure 1.11.8; moreover, it had good resistance to the interference from other cations when detected Hg^{2+} ions in the mixture solution excluding Cu^{2+} ions as shown in the dark bars in the Figure 1.11.8.

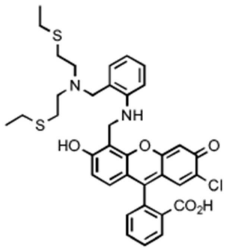

Figure 1.11.6 Structure of the PET fluorescence sensor 2-{5-[(2-{[*bis*-(2-ethylsulfanyl-ethyl)-amino]-methyl}-phenylamino)-methyl]-2-chloro-6-hydroxy-3-oxo-3H-xanthen-9-yl}-benzoic acid.

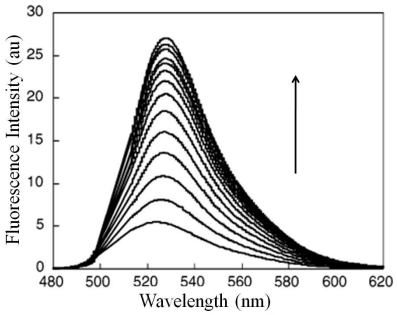

Figure 1.11.7 Fluorescence spectra of sensor MS1 (1 μM) upon addition of Hg^{2+} (0 - 3 μM) in buffer solution (pH = 7, 50 mM PIPES, 100 mM KCl) with an excitation of 500 nm.

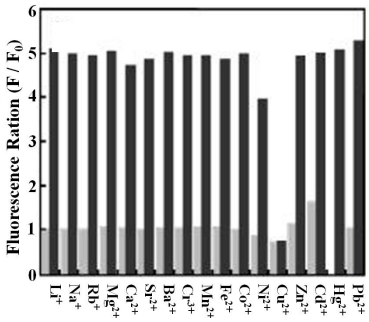

Figure 1.11.8 The selectivity of MS1 for Hg^{2+} ions in the presence of other cations of interest. The light bars represent the emission of MS1 in the presence of 67 equiv of the interested cations. The dark bars represent the change in integrated emission that occurs upon subsequent addition of 67 equiv of Hg^{2+} to the mixed solution.

ICT Fluorescence Sensor

2,2',2",2"'-(3-(benzo[d]thiazol-2-yl)-2-oxo-2-H-chromene-6,7-diyl) *bis*(azanetriyl)tetrakis(N-(2-hydroxyethyl)acetamide) (RMS) (Figure 1.11.9) has been shown to be an ICT fluorescence sensor. From Figure 1.11.10, it is clear that, with the gradual increase of the concentration of Hg^{2+} ions, fluorescence emission spectra revealed a significant blue shift, which was about 100-nm emission band shift from 567 to 475 nm in the presence of 40 equiv of Hg^{2+} ions. The fluorescence change came from the coexistence of two electron-rich aniline nitrogen atoms in the electron-donating receptor moiety, which prevented Hg^{2+} ions ejection from them simultaneously in the excited ICT fluorophore. Sensor RMS also showed good selectivity over other cations of interest. As shown in Figure 1.11.11, it is easy to find that only Hg^{2+} ions can modulate the fluorescence of RMS in a neutral buffered water solution.

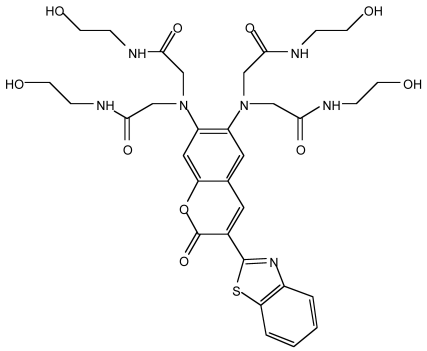

Figure 1.11.9 Structure of the ICT fluorescence sensor 2,2',2",2'"-(3-(benzo[d]thiazol-2-yl)-2-oxo-2-H-chromene-6,7-diyl)*bis*(azanetriyl) tetrakis(N-(2-hydroxyethyl)acetamide) (RMS).

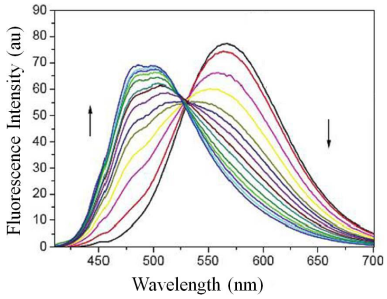

Figure 1.11.10 Fluorescence spectra of RMS (5 μM) upon addition of Hg^{2+} (0 μM to 200 μM) in 0.05 M phosphate-buffered water solution (pH 7.5) with an excitation of 390 nm.

Figure 1.11.11 Fluorescence response of 10 μM RMS in the presence of 20 equiv of different cations of interest at the same condition: control (0), Cd^{2+} (1), Hg^{2+} (2), Fe^{3+} (3), Zn^{2+} (4), Ag^{+} (5), Co^{2+} (6), Cu^{2+} (7), Ni^{2+} (8), and Pb^{2+} (9).

Excimer Fluorescence Sensor

The (NE,N'E)-2,2'-(ethane-1,2-diyl-*bis*(oxy))*bis*(N-(pyren-4-ylmethylene)aniline) (BA) (Figure 1.11.12is the excimer fluorescence sensor. As shown in Figure 1.11.13, when BA existed without mercury ions in the mixture of HEPES-CH_3CN (80:20, v/v, pH 7.2), it only had the weak monomer fluorescence emission. Upon the increase of the concentration of mercury ions in the solution of BA, a strong excimer fluorescence emission at 462 nm appeared and increased with the change of the concentration of mercury ions. From Figure 1.11.14, it is clear that BA showed good selectivity for mercury ions. Moreover, it had good resistance to the interference when detecting mercury ions in the mixture solution.

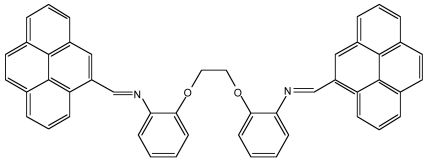

Figure 1.11.12 Structure of the excimer fluorescence sensor (NE,N'E)-2,2'-(ethane-1,2-diyl-*bis*(oxy))*bis*(N-(pyren-4-ylmethylene) aniline) (BA).

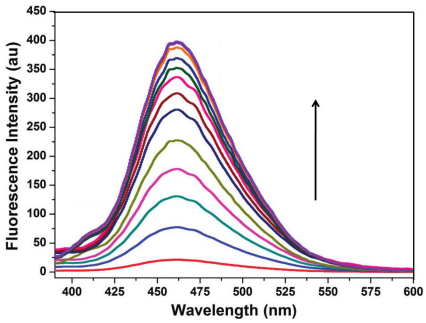

Figure 1.11.13 Fluorescence spectra of BA (1 μM) upon addition of Hg^{2+} (0 μM to 10 μM) in the mixture of HEPES-CH_3CN (80:20, v/v, pH 7.2) with an excitation of 365 nm.

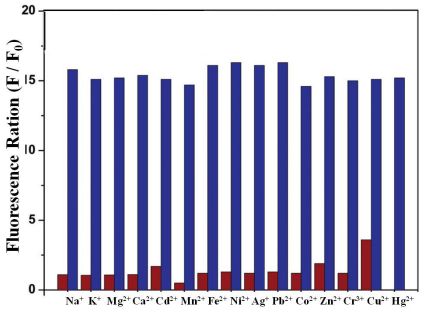

Figure 1.11.14 Fluorescence response of BA (1 μM) with 10 equiv of other cations of interest in the same condition. Bars represent the final (F) over the initial (F_0) integrated emission. The red bars represent the addition of the competing metal ion to a 1 μM solution of BA. The blue bars represent the change of the emission that occurs upon the subsequent addition of 10 μM Hg^{2+} to the above solution.

FRET Fluorescence Sensor

The calix[4]arene derivative bearing two pyrene and rhodamine fluorophores (CPR) (Figure 1.11.15) is a characteristic FRET fluorescence sensor. Fluorescence titration experiment of CPR (10.0 μM) with Hg^{2+} ions was carried out in $CHCl_3/CH_3CN$ (50:50, v/v) with an excitation of 343 nm. As shown in Figure 1.11.16, upon gradual increase the concentration of Hg^{2+} ions in the solution of CPR, the increased fluorescence emission of the ring-opened rhodamine at 576 nm was observed with a concomitantly declining excimer emission of pyrene at 470 nm. Moreover, an isosbestic point centered at 550 nm appeared. This change in the fluorescence emission demonstrated that an energy from the pyrene excimer transferred to rhodamine, resulting from the trigger of Hg^{2+} ions. Figure 1.11.17showed that CPR had good resistance to other cations of interest when detected Hg^{2+} ions, though Pb^{2+} ions had little interference in this process.

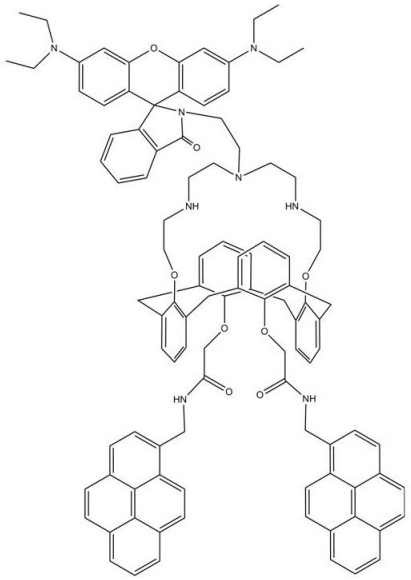

Figure 1.11.15 Structure of the FRET fluorescence sensor calix[4]arene derivative (CPR) bearing two pyrene and rhodamine fluorophores.

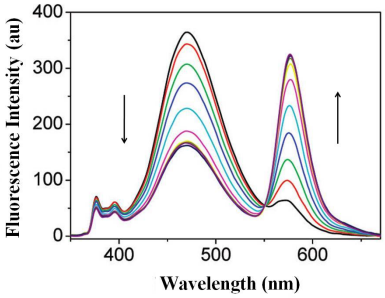

Figure 1.11.16 Fluorescence spectra of CPR (10.0 μM) in $CHCl_3/CH_3CN$ (50:50, v/v) upon addition of different concentrations of $Hg(ClO_4)_2$ (0 μM to 30 μM).

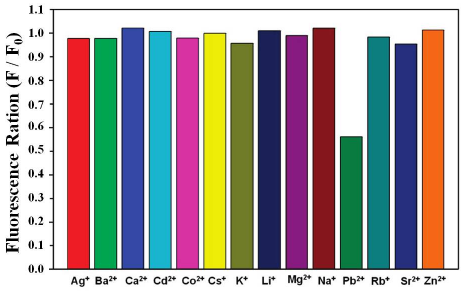

Figure 1.11.17 Competition experiment of 10.0 μM CPR at 576 nm with 10 equiv of other cations of interest in the presence of Hg^{2+} (3 equiv) in the same condition. F_0 and F denote the fluorescence intensity of CPR and Hg^{2+} ions and the interested metal ions in the presence of CPR and Hg^{2+} ions.