

Literature Review

Introduction

In recent years, ions of toxic heavy metals, such as lead and mercury, have gained much attention due to their negative effects on the environment and human health. For example, when ingested, these metals have carcinogenic (cancer-causing) effects. They are, as a result, forbidden for use in electronics by the European Union's Restriction of Hazardous Substances (RoHS) (Rasheed, 2017). Additionally, the United States Environmental Protection Agency (EPA) and World Health Organization (WHO) have established allowable limits because of their toxicity in drinking water (Rasheed, 2017). More specifically, the EPA's action level, which is the maximum permissible lead concentration in water, for Pb^{2+} ions in water is 15 micrograms/liter (Environmental Health and Medicine Education, 2017). It is especially important that lead concentration in drinking water does not exceed the EPA specification, so the water is safe for human consumption.

In order to address heavy metal contamination of water, researchers have worked on novel heavy metal detection methods. Numerous methods utilize changes in fluorescence of dyes (such as acid red 94) or quantum dots, which are man-made nanoscale semiconductor crystals (Zhao, Rong, Ma, & Tao, 2013). Upon binding to a target ion, a change in fluorescence occurs relating to the aqueous concentration of contaminant. Using standard aqueous concentrations of ions, to generate a standard curve, unknown contaminant loads can be identified. This is important because it allows one to determine how much lead is

dissolved in the water and whether its concentration is high enough to be concerned about and act to reduce it.

Heavy Metals

Heavy metals are elements in a group of relatively dense and toxic metals such as mercury, lead, copper, and silver. They are considered toxic due to the myriads of negative health effects they have been observed to have on organisms. Consequently, previously common sources of heavy metals such as lead paint and mercury thermometers are no longer used. However, as shown by Figure 1 below, there are still many ways in which people may still encounter heavy metals. While some are part of nature, such as volcanic eruptions and the weathering of rocks, others are the result of human activity (such as pesticides and metal-based batteries). Understanding where heavy metal exposure can come from is important so that if people encounter any of these, they are aware there is a good chance they encountered heavy metals and perform tests to potentially validate such suspicions.

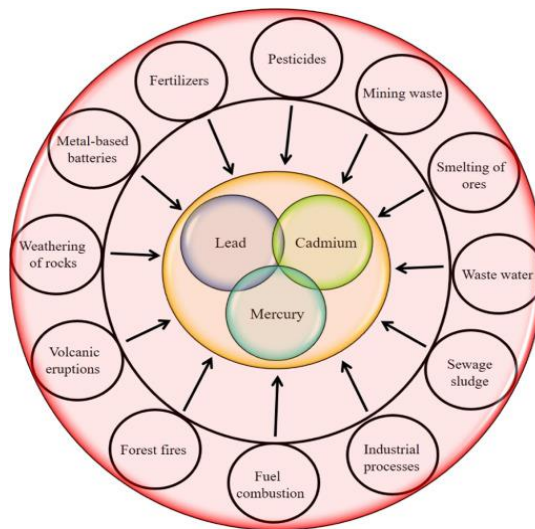


Figure 1: Various sources of lead, cadmium, and mercury. (Rasheed, 2017)

Another notable way lead can reach people, not present in the figure above, is the leaching of lead from the walls of old water pipes into drinking water as underscored by the Flint, MI water crisis in 2014, when the city switched its drinking water supply from Detroit's system to the contaminated Flint River to save money. Contaminated water can quickly spread lead and other toxic metals over a wide area, causing serious environmental and health problems. The insufficient water treatment that followed the Flint, MI drinking water supply switch resulted in a series of major water quality and health issues for Flint residents (Denchak, 2019).

On the next page, Figure 2 shows the numerous human health effects of heavy metal exposure. Metal ions tend to disrupt the circulatory system, with ailments such as hypertension and anemia, and the nervous system, with problems such as disrupted memory and reduced IQ. Lead contamination is one of the most serious concerns to human health because even low-level lead exposure can cause several adverse health effects and is especially harmful to children. Lead ions absorbed by the human body can cause huge damage to bones by replacing important elements in our body like Zn^{2+} and Ca^{2+} in hydroxyapatite, the main inorganic constituent of bone (Kamel, El-Nahass, El-Khouly, Fayed, & El-Kemary, 2019). Lead has deleterious effects on the brain and central nervous system, and can lead to decreased intelligence, behavioral problems, and impaired growth. Thus, the careful monitoring of lead in the environment with high sensitivity is of great importance (Zhao, et. al., 2013). Detecting lead in drinking water and other sources can be the first step in avoiding the impact of exposure.

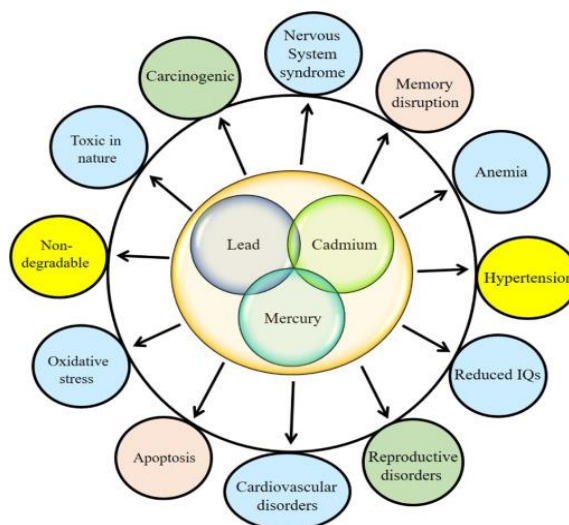


Figure: 2. Major consequences and adverse health effects of lead, cadmium, and mercury. (Rasheed, et al., 2017)

Acid Red 94

Acid red 94 (AR94 also known as rose bengal) is a fluorescent xanthene “acid” dye; acid dyes are those used to dye wool from an acidic bath (Xu & Neckers, 1987). In medicine, it is commonly used in eye drops to stain damaged conjunctival and corneal cells, and it thereby identify damage to the eye. It is also known that AR94 dye is a good probe for monitoring electrostatic interactions and hydrogen bonding interactions and is widely used in photocatalytic reactions because it is inexpensive and easy to prepare (Kamel, et. al., 2019). Its chemical structure is shown in the figure below.

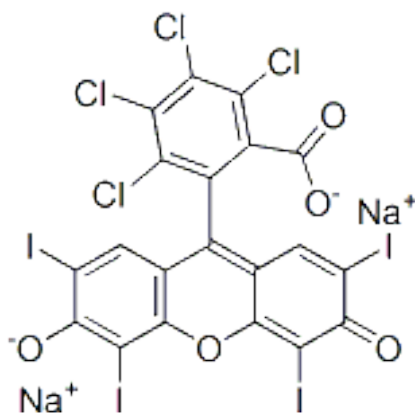


Figure 3: Chemical structure of AR94 (Chemical Book, 2016)

Additionally, AR94 is photodegradable, which means it decomposes, in the presence of light. This means that it should not be exposed to light for too long while in use. However, the fact that the dye is photodegradable is good for disposal purposes. The reaction can even be catalyzed, or sped up, using a catalyst such as ZnO in the presence of ultraviolet (UV) radiation (Kaur & Singhal, 2014).

Fluorescence

Fluorescence is the emission of electromagnetic radiation, usually visible light, caused by the excitation of atoms within a material. The atoms absorb energy to become excited, then return to their ground state and emit the excess energy as light. Often, radiation or particles, such as X-rays or electrons, are used for the initial excitation. The fluorescence ceases as soon as the exciting source is removed since the remission occurs quickly. This is different from what occurs for phosphorescence, which persists as an afterglow (The Editors of Encyclopaedia Britannica, 2017). The process of fluorescence is shown in the figure below.

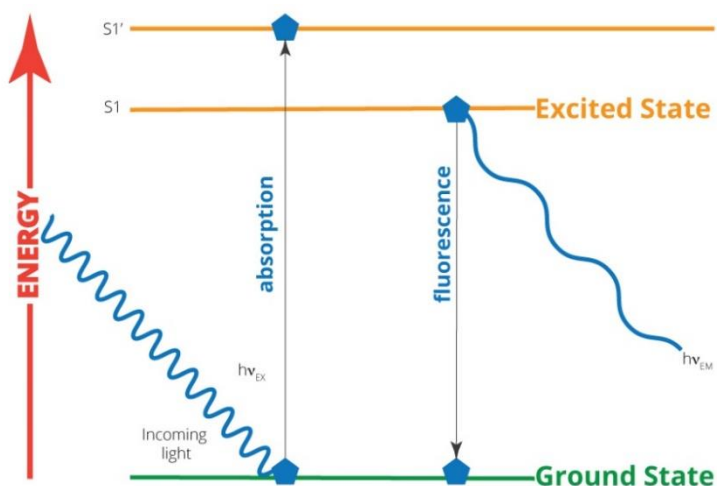


Figure 4: Absorption and fluorescence energy transitions. (Fulghum, L., 2018)

Applications of fluorescence include fluorescent lightbulbs, which are coated on the inside with a powder and contain a gas. Electricity causes the gas to emit ultraviolet radiation, which then stimulates the tube coating to emit light. Another example is the pixels of a television or computer screen fluorescing when electrons from an electron gun strike them. Fabrics appear whiter in sunlight due to the addition of a fluorescing agent with emissions in the blue region of the visible spectrum to detergents. Fluorescence, in the form of fluorescent dyes, is often used to analyze molecules. For instance, X-ray fluorescence is used to analyze minerals (The Editors of Encyclopaedia Britannica, 2017).

Fluorescence can be measured using a machine called a spectrofluorometer. It ranges in size from big enough to fill a lab benchtop (Perkin Elmer spectrofluorometer) to small enough to fit in one's hands (Ocean Optics spectrofluorometer). The spectrofluorometer is connected to a computer that is equipped with an interface-software. The components of a spectrofluorometer are a light source, an excitation monochromator (defined as an optical device that transmits a mechanically selectable narrow band of wavelengths from a wider spectrum of light), a cuvette holder, an emission monochromator and a detector. The light

source emits light at the excitation wavelength, which one predetermines in advance and is absorbed by an analyte in a sample solution. The excitation wavelength is slightly longer than the absorption wavelength of the analyte. Before reaching the sample, the excitation monochromator filters light allowing only a specific wavelength to be absorbed by the analyte and blocking all other wavelengths. As a result, electrons within atoms in the analyte enter a higher energy state. Following excitation, those electrons release the energy at a specific wavelength longer than the excitation wavelength then return to their ground state. The emitted light passes through the emission monochromator positioned orthogonal to the excitation light. The emission monochromator minimizes light scatter and screens the emitted light before it reaches the detector, which measures the intensity of the emitted light and displays the fluorescence emission intensity of the analyte at the chosen wavelength. Alternatively, the spectrofluorometer can be used to display the fluorescence emission intensity of the analyte over a range of wavelength, which is called the fluorescence signature. The fluorescence signature gives information about the identity of the analyte as well as its concentration. The fluorescence value is proportional to the concentration of the analyte in the sample (Genovesi, 2015).

Stern-Volmer Relationship

The fluorescence intensity of the AR94 dye showed that a significant quenching (suppression) of fluorescence was observed with the addition of various amounts of Pb^{2+} metal ions. This is important because it provides a link between AR94 dye fluorescence intensity and Pb^{2+} aqueous concentration. There are two types of quenching mechanism, (1) dynamic (collisional) quenching and (2) static quenching. Dynamic quenching is diffusion

controlled and depends on the viscosity of the solvent. When the excited fluorophore collides with the quencher, the fluorophore returns to the ground state without emission of light. The linear Stern-Volmer equation below describes the dynamic quenching,

$$\frac{I_0}{I} = K_{SV}[Q] + 1 \quad (1)$$

where I_0 is the fluorescence intensity of the fluorophore and I is the fluorescence intensity of the fluorophore in the presence of the quencher, K_{SV} is the Stern-Volmer constant and $[Q]$ is concentration of the quencher.

Static quenching occurs when the fluorophore forms a nonfluorescent complex with the quencher. The ratio of I_0/I vs. $[Q]$ results in an upward curvature described by a modified Stern-Volmer exponential function shown below,

$$\frac{I_0}{I} = Ae^{K_{SV}[Q]} \quad (2)$$

A plot of $\ln(I_0/I)$ vs $[Q]$ yields a linear plot with a slope of K_{SV} .

If both dynamic and static quenching are observed for the same fluorophore, the modified Stern-Volmer plot is a quadratic function as shown below,

$$\frac{I_0}{I} = K_D K_S [Q]^2 + (K_D + K_S)[Q] + 1 \quad (3)$$

where K_D is the dynamic quenching constant and K_S is the static quenching constant.

A plot of $I_0/I - 1/[Q]$ vs. $[Q]$ yields a straight line with a slope of $K_D K_S$ and y-intercept of $(K_D + K_S)$.

The quenching follows the Stern–Volmer relation ($I_0/I = 1 + K_{SV}[Q]$), where I_0 and I are the emission intensities of AR94 in the absence and presence of a quencher of concentration $[Q]$, respectively, and K_{SV} is the Stern–Volmer quenching constant. The magnitude of the K_{SV} scales with the strength of the association of the fluorophore (AR94) with the quencher $[Pb^{2+}]$. The Stern–Volmer plots are linear for low concentrations but show an upward

curvature for higher concentrations, confirming the static quenching (resulting from the formation of a nonfluorescent fluorophore-quencher ground state complex) as shown in Figure 5C below.

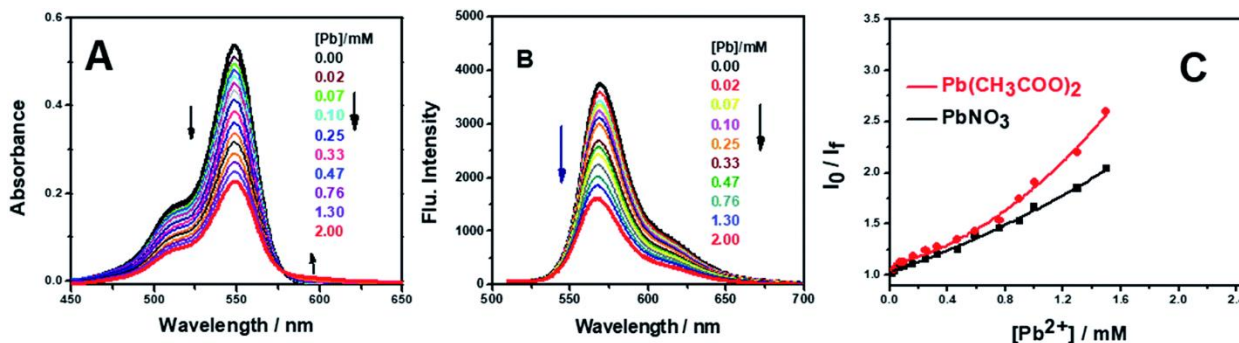


Figure 5: (A) UV-visible absorption spectra and (B) emission spectra of AR94 in the absence and presence of Pb²⁺ metal ions in water. (C) Stern–Volmer plots for the fluorescence quenching of the singlet AR94 in the presence of lead metal nitrate and lead acetate in water (Kamel, et. al., 2019).

Stern Volmer plots provide a link one can use to relate something easy to directly measure, fluorescence, and something often more difficult to measure, the concentration of harmful water contaminants. In this case, the Stern-Volmer plot in Figure 5C above shows the fluorescence (I_f) of each AR94 solution in the presence of increasing concentration of lead ions, after the first one, decreases in relation to the fluorescence of only AR94 (I_0) (causing I_0/I_f to increase) as lead nitrate concentration increases. The decrease in fluorescence (due to higher quenching of AR94 by lead nitrate) in a predictable way that can be modeled by a linear equation relating it to lead nitrate concentration. The quenching of AR94 in the presence of lead acetate was found to be higher than that of AR94/lead nitrate,

which may be explained by the high pK_a (a measure of the strength of an acid) and high protonation energy of acetate anions compared to nitrate anions. It is most likely that the high selectivity of acid red 94 towards Pb^{2+} metal ions may be related to their appropriate ionic radii (115 and 119 pm for Ag and Pb, respectively) (Kamel, et. al., 2019). In addition, the formation of the complex between AR94 and Pb^{2+} has been confirmed via Fourier Transform Infrared Spectroscopy (FTIR) analysis. As seen from Figure 6 below, a new band centered at 716 cm^{-1} has been observed that can be attributed to Pb–O bond vibrations and confirms the formation of AR94– Pb^{2+} complexes.

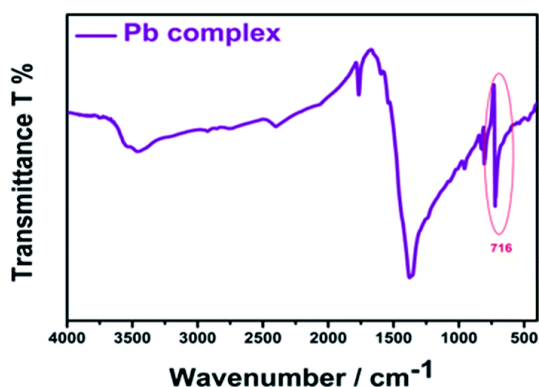


Figure 6: FTIR spectra of the AR94–Pb complex. (Kamel, et. al., 2019)

Stern-Volmer plots help show evidence of interactions, such as complexation, between two or more molecules or atoms as the concentration of one is fixed while the concentration of the other is either increased or decreased. The trend shown could possibly only be valid for the range of the concentration used to generate the plot. It is important to note that although Stern-Volmer plots are often reported in literature as linear plots, it is possible for them to either be nonlinear or be linear but conceal a nonlinear pattern or trend (Pan, B. Xing, Liu, G. Xing, & Tao, 2007) that becomes apparent at higher concentrations. This can give further clue about and prompt further investigation into how the two or more chemicals of interest interact with each other.

Conclusion

As previously mentioned, there is a demand for rapid, efficient, and inexpensive means for the detection of heavy metals in the environment, especially in drinking water. Fluorescent probes show promise for fulfilling this demand. The main two types currently under investigation are quantum dots and fluorescent dyes. They each have their own advantages and disadvantages worthy of further research and investigation (Zhao, et. al., 2013). For example, while quantum dots are valued for not photodegrading like organic dyes do, they could have a potentially negative longer lasting environmental impact. It is not ideal to further harm the environment when the goal is to detect a contaminant that could already be harming the environment. In regard to fluorescent dyes in particular, from the notable spectral changes and fast color changes of AR94 with the addition of heavy metals, such as silver (Shahamirifard, Ghaedi, & Hajati, 2018) and lead, it can be concluded that AR94 can potentially become a sensitive and selective sensor for both the qualitative and quantitative detection of specific heavy metal ions and furthermore could be used a visual indicator (Kamel, et. al., 2019).

The spectral changes can be analyzed using a Stern-Volmer plot to determine an estimate for heavy metal concentration in water. The entire process is fast since the spectral changes take seconds for a spectrofluorometer to measure and graph. Substituting fluorescence intensities, often in the form of a ratio with the fluorescence intensity of a control solution, called a blank, with no analyte, into a Stern-Volmer plot equation can quickly yield a reasonably accurate heavy metal concentration estimate.