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Review of the detection of metal ions using fluorescent probes

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Abstract. Metal ions, essential for numerous physiological functions of the human body require accurate monitoring in order to gain precise knowledge of the health status of people. As fluorescent probes advances, the level of metal ions can be measured with high selectivity and sensitivity. Allowing the homeostasis of numerous metal ions to be maintained easily. Fluorescent probes are a hot area of research, providing a prospective tool for the detection of metal ions and to provide critical statistics in maintaining the homeostasis of the human body. This article introduces several fluorescent probes on the detection of both essential and toxic metal ions. It gives a review about the recent development in the detection of metal ions using fluorescent probes. This article explains the mechanism of recognition of each fluorescent probe and summarizes the results obtained from experiments. Outlining the advantages and shortcomings of each fluorescent probe. It includes novel fluorescent probes based on uncommon mechanisms.

Keywords: metal ions, fluorescent probe, detection.

1. Introduction

Fluorescence is displayed as a consequence of the absorption of UV or visible light followed by the emission of light of a longer wavelength. In organic fluorophores, this usually involves delocalized electrons or a conjugated system. The incoming light matches the gap between the pai and pai*. Colors are seen due to the emission of photon of the exact energy separating the excited state at a given configuration [1]. Fluorescence has significant application in the biological system. It could be used in labeling biological molecules, visualization of DNA, cell sorting and the detection of metal ions [1]. Their advantage comes from the difference between excitation and emission wavelength, which allows the easier detection of the wavelengths. Common types of fluorescent probes are small-molecule probes, genetically-encoded probes and hybrid probes.

Metal ions in particular play an important part in regulating the metabolism of the human body. Biological metal ion concentrations, such as Cu (II), Zn (II), Fe (II), should be contained within a certain range. Thus it is significant for researchers and medical workers to be able to sense and quantify the amount of metal ions in organisms. Fluorescent probes are able to perform this function and provide essential data for fluorescent imaging and later analysis. It's also the concern of researchers to be able to generate fluorescent probes that have high selectivity and sensitivity, low detection limit and strong signal [1]. The structure of the probe can be altered to adjust to a better binding affinity and chemical reactivity. In this article, the application of different types of fluorescent

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probes in the detection of metal ions will be introduced, and their advantages and shortcomings will be discussed, as well as pointing into the future of the development of fluorescent probes.

2. Detection of essential metal ions

Essential metal ions including Mn, Co, Na, Mg, K, V, Ni are critical for the metabolism of the human body. These metal ions, essential for performing numerous physiological functions are intake from the environment. The human body has to maintain a homeostasis in terms of the concentration of these ions in order to decrease the risk of any diseases and deficiencies. It is therefore an intriguing area of research to develop florescent probes capable of monitoring minute alterations in the level of these metal ions and revealing their location and function in the human body.

2.1. Cu (I)/Cu (II)

Copper is a heavy metal found in the organism which plays key role in the physiological process. It presents in high concentrations and is closely related to the enzyme catalysis and electron transfer process. It exists in the human body as either Cu (I) or Cu (II) [2]. The shift between these two oxidation states shows their role in oxidation and reduction reactions in the phosphorylation pathway. It participates in the function of cuproenzyme which is important in connective tissue formation, nerve transmission, iron homeostasis and angiogenesis. Copper is essentially involved in many proteins including ceruloplasmin, cytochrome c oxidase, dopamine β-hydroxylase and hephaestin [2]. Also, due to its essential role in participating in redox reactions, free copper could be toxic. Excessive amounts of copper causes numerous neurodegenerative diseases such as the Alzheimer's disease and Menkes disease. Thus, gaining understanding of the concentration and level of copper ions in the human body remains significant. Several fluorescent probes have now gained considerable success in the detection of Cu^{2+} and Cu^{+}.

CTAP-1 is the first developed small molecule-based probe for the detection of labile copper ions, which was developed in 2005. However, this probe is used to detect copper (I), rather than copper (II). This first probe gives the picture that copper ions are largely localized in the Golgi body and mitochondria of the cell. Coppersensor-1 is a later version after CTAP-1. It consists of boron-dipyrromethene (BODIPY) dye platform and a thioether-rich receptor. It presents decreased phototoxicity and increased fluorescence intensity as compared to its former version, CTAP-1 [3].

There are also sensors developed to overcome the existing problem of copper ion sensors, including high phototoxicity, lack of sample penetration and high auto-fluorescence. Single-photon and two-photon excitation microscopy are developed. A naphthalene-based receptor, ACu1 is designed for the detection of copper(I). This is a kind of two-photon excitation microscopy. This receptor can be excited by 2 near-infrared photons. It's advantage is that upon the binding of Cu, a 4-fold increase in intensity is seen. Cao-Cu3 is a single-photon excitation microscopy. This technique uses thio-rich bis(2-((2-(ethylthio) ethyl)- thio) ethyl) amine (BETA) moiety as a high affinity receptor for Cu^{+} [3].

Xanthene hydrazone based fluorescent probe is developed for the detection of copper ions. Xanthese-hydrazone is a form of Schiff base structure, it is electron rich. Many xanthene hydrazone probes are derived from ketones and aldehydes, and they have shown a better biological activity but a lower toxicity to organisms [4]. It's suitable in the application of detection of copper ions due to its great affinity for nitrogenous and oxygenous recognition moiety to copper ions. 27 versions of rhodamine-based fluorescent probes have developed starting from 2011. Probe 1 uses a rhodamine 101 hydrazone. It can be applied to monitor intracellular Cu^{2+} level in living HeLa cells. Maximum absorbance occurs when the molecular fraction is near 50%, a 1:1 stoichiometry is optimal. Later versions of probes have improved both selectivity and sensitivity, gradually employing a turn-on/off mechanism, able to operate with greater stability in various PH environments. Notably, an apparent color change from colorless to pink is observed with the application of probe 16. This probe uses a damantane-modified salicyl- rhodamine B and β-cyclodextrin-modified Fe3O4@SiO2, inducing novel inclusion complex magnetic nanoparticles (SFIC MNPs) colorimetric sensitive for Cu^{2+} [4]. This strategy is super magnetic. Magnetic measurements and collections are easily done in 9 seconds. Its'

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high selectivity is a result of a large number of hydroxyl groups on the surface. Probe 17 is a success in its ability to overcome several biochemical challenges namely spatial resolution in cell imaging, high/low solubility and stability in biological system, and interference from intracellular species. This probe is activated by lysosomal copper ions in a process known as the spiropyran-based proton activation. A new probe 18 shows a linear range detection limitation of 0.11μm. It has 1:1 stoichiometry and works reversibly proven by the pink fluorescence diminishing after the addition of sodium EDTA.

BNQ((E)-2-butyl-6-hydroxy-5-((quinolin-8-ylimino) methyl)-1H-benzo[de]isoquinoline-1,3 (2H)-dione) is a novel fluorescent probe developed for the detection of copper ions based on the mechanism of excited state double proton transfer (ESDPT). It's proved to be highly selective and sensitive in the imaging in HeLa cells. According to experiment, HeLa cells are cultured in 24-well plates, they were first allowed to adhere to for 24 hours and then put with 1μm for 30 inures and 10 μm of Cu^{2+} for a further 30 minutes. Results shows that the optimal PH for the application of BNQ is PH 6.0 to 8.0. Specifically, before the addition of Cu^{2+}, fluorescence intensity is low before PH4. And then florescence intensity rapidly climbs from PH6.0 to PH 8.0. After the addition of Cu^{2+}, beyond PH 9.0, the fluorescence intensity is high. Cu^{2+} reacts completely with BNQ in 2 minutes. The UV-vis absorption study shows that absorption peak between the range 300nm and 500nm increased with increasing Cu^{2+} concentration, accompanying a color change visible to naked eye of bright yellow to colorless. Fluorescence titration shows that fluorescence decreased from 520nm with increasing Cu^{2+} concentration, with color change from green to colorless [5]. Selectivity experiments shows that the addition of metal ions other than copper did not cause a change to the fluorescence spectrum. Indicating a high selectivity toward Cu^{2+}.

2.2. Zn (II)

Zinc is present as Zn^{2+} in mammalian cells. Concentration of zinc ions in cell is in the hundreds of micromolar range. Nearly 1−10 μM is present in the serum or the plasma. On a daily basis, 8 and 11 milligrams of zinc is needed for adult women and men respectively. Zinc has roles in the activity of over 300 proteins It has a modulating effect on neuronal communication, catalyzing certain reactions. Zinc controls the balance of tissues and the integrity of the cell surface membrane, additionally, it prevents cell damaging due to its antioxidant and anti-inflammatory properties. Insufficient uptake of zinc results in hair loss, diarrhea, weight loss and weakening immune system. With excessive zinc in the human body, nausea, vomiting, epigastric pain, lethargy and fatigue would be felt [6]. It is therefore significant to maintain zinc homeostasis.

Small-molecule based fluorescent probe is typical for the detection of Zn^{2+}. The first type of small molecule probes is intensity-based probes. These fluorescent probes works on the principal of photoinduced electron transfer. Without Zn^{2+}, the chelating moiety which is abundant in the number of electrons quenches the flourophore. When Zn^{2+} bind to the fluorophore, the PET between the fluorophore and the chelating moiety is disrupted, causing fluorescence emission. The first developed intensity-based probes used UV-excitable quinoline based fluorophore and sulfonamide chelating group. This technique has achieved some success in the visualization of the axon and the apoptotic activity of cells. However, it exists that the UV-range excitation wavelength obstructs these probes. Photodamage as well as high background fluorescence could be resulted. To overcome this, fluorescein based fluorophore is used. It demonstrates a higher quantum yield and a lower excitation energy. It's more suitable for living cell imaging. Yet these two kinds of intensity-based probe are still affected by changes in PH. Therefore, another probe was developed. On its fluorescein backbone, there is electronegative substitution fluorescein backbone [3]. This probe has a lower pKa. The other type of small-molecule sensor is ratiometric probe. The binding of Zn^{2+} to this probe causes alterations in the excitation wavelength at the emission wavelength or both. The advantage is that the fluorescent images generated are produced at maximum wavelength. However, this technique displays less apparent signal than the intensity based method.

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In 2021 a turn-on unsymmetrical fluorescent probe L based on Schiff is produced which exhibits high selectivity. Fluorescence spectroscopic response of L on different metal ions are conducted in ethanol solution and excitation at 354nm [7]. With the addition of Zn (II), fluorescence is enhanced where emission intensity is observed at 475nm. No responsive changes under the same experimental conditions are observed for other metal ions including lithium, sodium, potassium, silver, magnesium, iron, cobalt, nickel, lead. Fluorescence spectroscopic studies shows that as zinc concentration gradually increases, fluorescence intensity also rises steadily. At 475nm, there's shown to be a high dependence of fluorescence intensity on Zinc ion concentration. The turn-on ratio is observed to increase by over 512 fold when 1 equiv. Of Zn is added. Proposing a 1:1 complexation ion of L-Zn [7]. The detection limit is 9.53 10 mol/L. Probe L, compared with many similar versions, is much more sensitive.

K.Okuda et al developed a reaction based fluorescent probe for the detection of Zn^{2+}, which is a unconventional approach. Zinc (II) do not have quenching properties as that observed in copper (II) and other transitional metal ions [8]. A turn-on fluorescent probe is thus more easily realized in fluorescent probes targeting Zinc (II). More conventionally, recognition-based fluorescent probes are developed for zinc detection and imaging. However, problems exist with these kinds of probes. In terms of detection in living cells, they have significantly lower sensitivity. The metal-ligand complex formation for recognition based is reversible, the dissociation of the analyte metal ions from the Logan's moiety of a probe and capture by competing intracellular chelations hinders FL readout [8]. Additionally, trivial changes in zinc ion concentrations are hard to sense. Reaction based fluorescent probes, on the other hand, with their metal ligand complex formation being irreversible, has the advantage of minimizing interference from other metal ions and addresses these problems of recognition based probes. Although they have a distinct disadvantage of having slower response to metal ions. K.Okuda et al. And his group developed reaction-based probes for zinc. Zinc which acts as a Lewis acid, can increase the electrophilicity of carbonyls and decreasing pKa of water [8]. The probe they developed is Dpa-LBC. This probe consists of an antibiotic Cepheus core, a zinc-ligand moiety (DPA), and a FL-quenched umbelliferone [8]. It works on the principal of a zinc-catalyses hydrolysis reaction activated by β-lactamase after a complex is formed with DPA [8]. Zinc-bound water attacks -lactamase, resulting in hydrolysis of amide bond. Electron-transfer then releases the dye which demonstrates fluorescence. This is a cyclical process, the remaining skeleton of the probe releases zinc ions, which could then react with several probes, strengthening the FL signal. This probe is investigated to have the ability to detect Zn even during a 40-fold change in FL intensity. Zinc reacts with 2.8 DPA-SoxLC per hour [8]. The detection limit is lower than other versions. With the prescience of GSH, Zn at low concentrations could be detected intracellularly (at 1 um).

2.3. Fe (II)/Fe (III)

Iron in the human body occurs in two oxidation states, Fe (II) (in acidic conditions) and Fe (III) (in alkaline conditions). Iron serves an important role in the hemoglobin transport of oxygen. Apart from that, it is also involved in the phosphorylation reactions of body cells since it is found in numerous oxidative phosphorylation proteins. However, free iron ions are also toxic when remained free in cell plasma due to its ability to generate hydroxyl radical from hydrogen peroxide. Excessive amount of iron in the human body causes cellular dysfunction and damage, hemochromatosis and bronze diabetes. Likewise, a insufficient amount of iron ions results in decreased intestinal absorption, disruption of hemoglobin transport of oxygen and mutations in the iron response elements of iron regulated mRNAs [2]. Here, the detection of both Fe (II) and Fe (III) will be given.

Fe (II) are easily oxidized to Fe (III) in the human body in aerobic aqueous environment, yet the most common iron ion in the human body is still Fe (II). It has been challenging to develop fluorescent probes that have high selectivity toward Fe (II) [9].

Early probes for Fe^{2+} are pyrene-TEMPO and DanSQ. TEMPO is used for the sensing of Fe in the aqueous environment. Upon Fe^{2+} binding, TEMPO is reduced and fluorescence is restored. This displays high selectivity toward Fe (II). However, it must be implemented under acidic conditions and

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it could also be activated by other radicals, making it unsuitable to be put in use in intact biological systems. The DanSQ, in contrary, demonstrates poorer selectivity of Fe (II) and is only soluble in acetonitrile and 10% H_2O [9]. But has a 25 fold increase in fluorescence.

For better living cell application of the probes, several others are introduced. The first one is the N-oxide reduction-based fluorescent probe. The investigation of this type of probe has started in 2013. N-oxide is able to prevent the tertiary amines from being deprotected during hydrogenation catalyzed by Pd and Zn/HCl [9]. In the probe developed. The N-oxide makes the nitrogen atoms in the rhodamine fluorophore less electron-donating [9]. Fluorescence is induced when the N-oxide is transformed to tertiary amine through Fe^{2+} binding (deoxygenation). This probe is demonstrated to have high selectivity toward Fe^{2+}. It is also applicable in sensing alterations in Fe^{2+} intracellular level. Results obtained from experiments shows that fluorescence intensity grows by 30 fold within 1 hour upon binding of only 20μm of Fe and a high selectivity toward Fe (II).

The second probe is the cyclization of Schiff based fluorescent probe. This probe has its fluorescence quenched by the C=N bond isomerization. When Fe^{2+} binds, the 2 hydroxyl groups are converted into benzoxazole ring, displaying fluorescence [9]. In investigating the selectivity of this probe, fluorescence intensity is measured. Only with the addition of Fe^{2+} are obvious enhancement shown in the fluorescence intensity. With other metals, including Fe^{3+}, Cu^{+}, Mg^{2+}, Hg^{2+}, no obvious enhancement are shown. Suggesting a high selectivity. The results of electron paramagnetic resonance (EPR) demonstrates that this probe displays nitroxide radical-type triplet. In the addition of human fibreblast cells, weak emission signal are observed. While upon addition of Fe^{2+}, obvious signal are observed, suggesting it can be used in the detection of intracellular Fe^{2+} level [9].

The third probe is the nitro ice reduction-based fluorescent probe. It has a rhodamine fluorophore and nitroxide radical for Fe (II) binding. During the binding of iron ions, the nitroxide radical is reduced, converting to diamagnetic hydroxylamine [10]. It has high selectivity, suitable for monitoring Fe (II) in environments with interference. Results shows that fluorescence intensity increased by 24-fold in the addition of labile Fe^{2+}.

RhB-EDA is another probe used in the detection of Fe (III) based on rhodamine B. This is based on near-infrared fluorescent dyes. Many of these dyes have low quantum yield and poor photostability, but Rhodamine (Rh) dyes display high quantum yield, good light stability and low photobleaching degree. The probe is synthesized by incorporating an EDA molecule to RhB through an ideation reaction [10]. Upon recognition of Fe (III) and binding, it undergoes amide spiral ring opening in the formation of the coordination structure, showing fluorescence. The structure of RhB-EDA could be proved by both ultraviolet spectrum and fluorescence spectrum. Results from ultraviolet spectrum shows that with RhB, an ultraviolet peak could be observed between 500-600 nm, the same position has signals absent for RhB-EDA. Analysis of the fluorescence spectrum shows RhB has an excitation wavelength at 560nm and an emission peak at 590, where RhB-EDA shows no peak. This shows an internal change in the structure of RhB after the addition of EDA, proving a successful synthesis of the compound. In the experiment of mixing diluted RhB-EDA (1×10^{-2}mol/L diluted in $1x10^{-4}$mol/L absolute ethyl alcohol) to numerous metal ions shows the solution displays a color change from pink to colorless upon addition of Fe (III) [10]. While the addition of other ions displays no color change (except Al^{3+} which shows little color change). Consistent results could be obtained in the experiment with optical instruments. Only when there is the addition of Fe^{3+}, are their absorbance seen near 560nm wavelength. These shows the high selectivity of the probe. The stoichiometry of binding of the probe with Fe^{3+} is investigated through a series of experiments. Ultraviolet spectrophotometer was used to measure absorbance at difference concentrations of Fe^{3+}, which ultimately displays the highest absorption when the molar fraction is 0.5. Showing a 1:1 stoichiometric ratio in the formation of the complex between the probe and Fe (III). In investigating how changes in PH values impacts the fluorescence intensity, UV absorption and fluorescence spectra are analyzed. Both of these two parameters gradually decrease as PH decreases from 3.92 to 3.36. Above PH3.92 no signals in the fluorescence spectra are seen. Under sunlight, when PH is below 3.92, there is a color change from

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colorless to pink. These changes can be explained by a ring opening mechanism of the spirolactam and the proton action of amine functional groups under acidic conditions.

3. Detection of toxic ions

Toxicity from non-essential metals are extremely detrimental to the human body when above a certain limit. Examples of them are lead, cadmium, mercury and arsenic. Their homeostasis should be especially carefully monitored, reducing the risk of any biological dysfunction and even death ultimately. These metals are not essential to the human body. Environmental non-essential metal exposure are usually the way in which they get involved in biological metabolism. Leading to severe chronic symptoms.

3.1. Hg (II)

Hg ion is highly toxic to organisms as well as to the environment. Mercury poisoning comes in 3 types: elemental mercury, inorganic mercury and organic mercury [11]. Symptoms of mercury poisoning are caused by the body's immune system trying to eliminate the mercury in body parts, including heart, nervous system and kidney. Symptoms of mercury poisoning includes trouble breathing, urine color changes, diarrhea, and even memory loss [11]. Pregnant women, if exposed to mercury, could cause brain damage to the developing fetus. Long-term exposure of mercury causes death. Thus it is important to carefully monitor its concentration inside organisms.

Zhu et al. developed a rhodamine spirolactams based indicator for mercury ion. Rhodamine is chosen as the fluorophore, sulfur-based functional unit is added due to its high affinity for Hg^{2+}. This indicator do not show fluorescence and color in the absence of mercury ion. When mercury ions are present, the probe shows a yellowish red fluorescence and the solution turns from colorless to pink. This involves ring opening in the structure. This technique has the advantage of being able to apply in living cells. This is because of its high emission quantum fields, large molar extinction coefficients, low energy absorption and emission wavelength. The fluorescence intensity of the probe is studied to explore the probe's operation. Fluorescence intensity is measured at various concentrations of Hg^{2+} solution ranging from $8.0x10^{-8}$ to $1.0x\ 10^{-5}$ mol/L. The detection limit is $3.0x10^{-8}$, showing a higher sensitivity than previously developed probes for mercury ions. The result from UV-vis is consistent with the result from fluorescence intensity. The stoichiometry of the addition of Hg^{2+} to the probe is determine to be 1:1 as shown by the molar fraction of Hg^{2+} at maximum absorbance. In studying the effect of PH on the detection of Hg ion of the probe, fluorescence intensity is measured. The result indicates that upon addition of mercury ions, strong fluorescence is exhibited in the PH range 4.50-8.50. It demonstrates a high selectivity toward Hg^{2+}. The fluorescence intensity is measured with addition of many metal ion solutions including Ca^{2+}, Fe^{3+}, Al^{3+}, Cd^{2+}, Zn^{2+}, Ag^{+}, Mg^{2+}, Pb^{2+}, Ba^{2+}, and Cr^{3+}. With the addition of Hg, an evident increase in fluorescence intensity is shown and no effect is resulted from the addition of other metal ions. This formation of the complex is reversible with this probe. This was determined by adding EDTA solution. After adding Hg^{2+}, the solution turns pink. With further addition, the solution turns back to colorless The response time is 60s, this was reported to be shorter than previously discovered probes [12].

Probe L, another probe synthesized for the detection of Hg^{2+}, acting as a colorimetric probe. It has high selectivity as indicated by the experiment of measuring the fluorescence response with the addition of numerous metal ions. Results show that at 251nm and 315nm, weak absorbance are shown, only with the addition of Hg^{2+} solution are the absorbance at these two peaks enhanced significantly. The solution color changed from colorless to pink. The data from florescence spectroscopy confirmed the conclusion. Competition experiment is carried out for the L-Hg^{2+} complex, and its unaffected by other metal ions. Fluorescence titration shows when the concentration of Hg is increased to $1.3x10^{-5}$, the fluorescence emission is increased by 55 fold [13]. A 1:1 stoichiometry is determined for the formation of the L-Hg^{2+} complex. It operates at an optimal PH range of 7.0 to 8.0. This probe is also reversible as shown with the addition of EDTA solution and could be used for practical applications.

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Overall, it shows high selectivity and low detection limits. Having prospective application in living cell bioimaging.

3.2. Pb (II)

Lead can come from various routes including the inhalation of lead on painting, endogenous exposure and ingestion. Pb (II) is very toxic to the human body. Pb2+ enters living cells easily by taking the pathway of other essential metal ions, it then mimic or antagonize the physiological effects of divalent metals [3]. The excessive amount of lead ions causes neurological, cardiovascular, reproductive and developmental effects [14]. Currently, the detection lead ions is on the detection of aqueous Pb2+.

In 2023 Xiao et al. developed a probe based on quantum dots with low cytotoxicity. The probe is CIZS-QDs. Quantum dots are semiconductor nano-crystals which have strong quantum confinement effect and unique optoelectronic properties [15]. Due to their high photoluminescence efficiency and their large stokes shift, it is a great source for synthesizing fluorescent probes [15]. Quaternary structure of quantum dots are mostly investigated and used in bioimaging and sensors because of their great optical properties. This probe is synthesized by a single hydrothermal reaction [15]. Optimum conditions of the reaction conditions are investigated by measuring fluorescence intensity. At a reaction time of 3.0 hours, reaction temperature of 120 ℃, PH value of 9.0, and a molar ratio of precursors: Cu:In:Zn:S = 1:10:70:70, fluorescence intensity is the maximum [15]. UV-vis absorption is implemented to observe the complex formations. The investigation suggests that with CIZS-QDs mixed with 8 different metal ion, all 8 shows absorbance. Among these, Pb^{2+} displays the most noticeable decrease in absorbance, showing a slight red shift, which demonstrates a high sensitivity in the sensing of Pb (II) with the formation of Pb-CIZ QDs complex [15]. The peak pattern obtained from XRD analysis also shows consistent results as the UV-vis. Fluorescence detection of Pb (II) is determined by measuring the PL intensities. This experiment is conducted with numerous metal ions. With metal ion concentrations increased to 10mM, Pb shows the highest quenching effect to the CIZ-QDs without extra modifications. The fluorescence lifetime is analyzed using a fluorometer, and is determined to be 2.7μs. With lifetime slightly reduced with addition of Pb (II) ions. This probe has low toxicity and high sensitivity, but has relatively lower selectivity.

A TCT (4-tert-Butylcatechol-Based Triazole) is synthesized for the detection of both Pb^{2+} and Hg^{2+}. It is synthesized through archetypal click reaction (CuAAC) [16]. This material is ideal because it has a high selectivity, a low response time as well as a strong biological activity. They bind to metals through ion-dipole interaction [16]. In proving its sensitivity for Pb and Hg, the Uv-vis analysis is carried out. 1nM concentrations of numerous metal ions (Cr (III), Mn (II), Co (II), Ni (II), Cu (II), Zn (II), Cd (II), Hg (II), Pb (II), Na (I), Mg (II)) are tested with TCT. Only with Pb^{2+} and Hg^{2+} are absorption spectrum fluctuations demonstrated. In investigating the response of the probe for Hg^{2+} and Pb^{2+}, UV-vis analysis is explored. The titration of TCT with 15 equiv. of 1nM of Pb^{2+} shows that with increasing concentrations of Pb^{2+} solution, there is an intense hyperchromic shift and a blue shift of 22nm. Proving the binding of Pb^{2+} to the probe. In investigating the selectivity of the probe, the competitive metal ion titration is carried out with 0.4 mM probe solution and different metal ions of the same concentration. Hg^{2+} identification is unaffected by any other metal ions. The effect of factors including time and temperature are also studied. The effect of time is investigated for 1 hour, absorption changes in TCT-Pb complex is investigated. With the pass of time, absorption intensity of TCT-Pb complex gradually decreases over time. Additionally, instantaneous absorption and fluorescence are observed. As for temperature, the absorption intensity of TCT-Pb gradually decreases with the decrease in temperature. This could be rooted to the time dependence of the complex.

4. Conclusion

In summary, fluorescent imaging and has been a significant area of research for researchers globally. fluroescent probes provide accurate data for the analysis of the metal ion concentrations of the human body, providing substantial biologically important data. Fluorescent probes are ideal for numerous detections due to their high specificity. Various mechanisms are adopted for different fluorescent

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probes each with their own advantages. For example, recognition based probes are common while reaction-based probes shows higher novelty. Both of them have high potential of further investigations.

This article introduces several fluorescent probes used in the detection of both essential ions and non-essential toxic ions. Many fluorophores are discussed, namely rhodamine, quinine, etc. Newer fluorophores such as quantum dots and carbon dots are under exploration. Different fluorophores are currently under research to increase fluorescence intensity and display a shorter response time in recognizing metal ions. In the future, fluorescent probes are targeting to be more adaptive in more complex environment, suitable for a wider range of temperatures and PH.

References

[1] Fu Y, Finney NS. Small-molecule fluorescent probes and their design. RSC Adv. 2018 Aug 14;8(51):29051-29061.

[2] Iron and Copper Homeostasis.2023. https://themedicalbiochemistrypage.org/iron-and-copper-homeostasis/

[3] Carter KP, Young AM, Palmer AE. Fluorescent sensors for measuring metal ions in living systems. Chem Rev. 2014 Apr 23;114(8):4564-601.

[4] Ma, Siyue, Wang, Yaqi, She, Mengyao, Wang, Shen, Yang, Zheng, Liu, Ping, Zhang, sShengyong and Li, Jianli. "Design strategies and progress on xanthene-based fluorescent probe for metal ions" Reviews in Analytical Chemistry, vol. 36, no. 2, 2017, pp. 20160024.

[5] Wang X, Li Z, Nie J, Wu L, Chen W, Qi S, Xu H, Du J, Shan Y, Yang Q. A novel hydrophilic fluorescent probe for Cu2+ detection and imaging in HeLa cells. RSC Adv. 2021 Mar 10;11(17):10264-10271.

[6] Role of zinc in the human body.2022.https://steptohealth.com/the-role-of-zinc-in-the-human-body/

[7] Shen LY, Chen XL, Yang XJ, Xu H, Huang YL, Zhang X, Redshaw C, Zhang QL. A Highly Selective Turn-On Fluorescent Probe for the Detection of Zinc. Molecules. 2021 Jun 23;26(13):3825.

[8] Okuda K, Takashima I, Takagi A. Advances in reaction-based synthetic fluorescent probes for studying the role of zinc and copper ions in living systems. J Clin Biochem Nutr. 2023 Jan;72(1):1-12.

[9] Y. Sun, P. Sun and W. Guo, Fluorescent probes for iron, heme, and related enzymes, Coordination Chemistry Reviews, https://doi. org/10.1016/j.ccr.2020.213645.

[10] Qin Z, Su W, Liu P, Ma J, Zhang Y, Jiao T. Facile Preparation of a Rhodamine B Derivative-Based Fluorescent Probe for Visual Detection of Iron Ions. ACS Omega. 2021 Sep 17;6(38):25040-25048.

[11] Mercury poisoning.2022.https://my.clevelandclinic.org/health/diseases/23420-mercury-poisonin-g

[12] Zhu N, Xu J, Ma Q, Geng Y, Li L, Liu S, Liu S, Wang G. Rhodamine-Based Fluorescent Probe for Highly Selective Determination of Hg2. ACS Omega. 2022 Aug 10;7(33):29236-29245.

[13] Li B, Tian F, Hua Y. A turn-on fluorescent probe with high selectivity for Hg2+ and its applications in living cells. RSC Adv. 2022 Aug 1;12(33):21129-21134.

[14] Lead toxicity.2023. www.atsdr.cdc.gov/csem/leadtoxicity/physiological_effects.html

[15] Han XL, Yu F, Lei JW, Zhu JH, Fu HY, Hu JC and Yang X-L (2022) Pb2+ Responsive Cu-In-Zn-S Quantum Dots With Low Cytotoxicity. Front. Chem. 10:821392.

[16] Singh G, George N, Singh R, Singh G, Kaur JD, Kaur G, Singh H, Singh J. CuAAC-Derived Selective Fluorescent Probe as a Recognition Agent for Pb (II) and Hg (II): DFT and Docking Studies. ACS Omega. 2022 Oct 20;7(43):39159-39168.

[17] Hu, XL., Gan, HQ., Meng, FD. et al. Fluorescent probes and functional materials for biomedical applications. Front. Chem. Sci. Eng. 16, 1425–1437 (2022).

DOI: 10.54254/2753-8818/22/20230954

[18] He Y, Du E, Zhou X, Zhou J, He Y, Ye Y, Wang J, Tang B, Wang X. Wet-spinning of fluorescent fibers based on gold nanoclusters-loaded alginate for sensing of heavy metal ions and anti-counterfeiting. Spectrochim Acta A Mol Biomol Spectrosc. 2020 Apr 5; 230:118031.
[19] Li L, Wang J, Xu S, Li C, Dong B. Recent Progress in Fluorescent Probes For Metal Ion Detection. Front Chem. 2022 Apr 13; 10: 875241.