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Synthesis of A Biodegradable Polymer from Tyrosol, Fluorescein and Caprolactone for Sensing and Removal of Cupric Ions from Pond Water

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ABSTRACT

Anthopogenic actions mostly contribute cupric ions to pond and other water bodies. These ions build rapidly in plants and animals, eventually entering the human food chain and causing public health problems. To address the health risks associated with these ions, a novel polymer molecule has been designed for sensing and removing them. This study entails creating a new polymer using tyrosol, fluorescein and caprolactone. This polymer has a strong selectivity for cupric ions, which causes a dramatic color shift for easy identification. It can detect even trace levels of copper ions in pond water. The hydrophobic properties of this polymer aid in the elimination of cupric ions, resulting in a yellow precipitate that precipitates toxic copper ions. This polymer is biodegradable, hence the process is environmentally beneficial. UV-Vis and EDAX spectra were used to evaluate the polymer's ability to remove ions from water. This invention provides a straightforward method to reduce cupric ion pollution, protect human health and address environmental difficulties.

Keywords: Colourimetry, Biodegradable, Polymer, Caprolactone; Sensing.

INTRODUCTION

Heavy and transition metals are primarily derived from the formation of the Earth's crust, establishing a natural baseline for their presence in the environment. However, the proliferation of human activities has led to a substantial increase in both human exposure and environmental contamination associated with these metals. Key anthropogenic sources of this pollution include industrial activities such as manufacturing processes and waste disposal, along with mining and smelting operations that release significant quantities of heavy metals into the environment. Furthermore, agricultural practices and domestic usage of metal-based products have also contributed to the accumulation of these metals in soil and water systems. Environmental pollution by heavy metals can also occur through various physical and chemical processes[1]. For instance, evaporation of metals into the atmosphere can lead to widespread deposition onto land and water surfaces, while sediment re-suspension can transport metal particles from water bodies into groundwater and soil. Soil erosion can further exacerbate the issue by releasing heavy metal ions into the surrounding environment, and the corrosion of metal structures can lead to leakage of these toxic substances. Additionally, atmospheric deposition through industrial emissions, particularly from processes such as petroleum combustion, coal burning at power plants, metal refining, and wood preservation, contributes significantly to heavy metal pollution in diverse ecological settings[2]. Among the various heavy metals, copper (in the form of cupric ions) is of particular concern due to its widespread industrial applications. It is extensively utilized because of its superior electrical and thermal conductivity, ranking third in terms of usage after iron and aluminium. Despite its essential role as a trace element in biological systems, excessive exposure to copper is linked to a variety of neurodegenerative disorders, including MenkeSs disease, Alzheimer disease, Parkinson disease and Wilson disease[3]. The Environmental Protection Agency (EPA) of USA has made suitable guideline for the permissible concentration of copper ions in drinking water, which is set at 20mM, to mitigate potential health risks associated with copper exposure. The increasing presence of copper as a contaminant in the environment can be attributed to its significant applications in diverse fields such as biotechnology, chemistry, life sciences, and medicine. While copper ions are generally less toxic in comparison to other heavy metals, they can still have detrimental effects on specific organisms even at low concentrations in the submicromolar range[4]. This necessitates the development of effective strategies to detect and remove cupric ions from environmental sources to safeguard ecosystems and human health. In light of these challenges, we have designed a sensor molecule capable of both detecting cupric ions and selectively removing them from water sources. Historically, various analytical techniques for the detection of copper ions have been documented, including graphite furnace-atomic absorption spectrometry, inductively coupled-plasma emission spectrometry, and electrochemical stripping analysis, among others. Despite the high sensitivity offered by these methods, their application is often hindered by factors such as cost, technical complexity, and the requirement for sophisticated instrumentation, limiting their accessibility and practicality for widespread use[5]. In contrast, the development of chemical sensors represents a promising alternative to traditional detection methods. Specifically, colorimetric detection techniques provide a user-friendly and cost-effective means of identifying cupric ions *in situ*. The advantages of this approach include its applicability in fieldwork, the elimination of the need for reference solutions, and the avoidance of bulky equipment and lengthy processing times typically associated with more complex analytical instruments. However, many of the currently reported colorimetric sensors rely on small molecules, which often present limitations regarding sensitivity, real-time detection capabilities, and effective deployment in field conditions. In this context, polymer-based macromolecular sensors emerge as a preferable solution due to their unique detection mechanisms, innovative side chain interactions, and excellent biocompatibility for *in vivo* studies[6]. Moreover, literature reveals that instances of simultaneous sensing and removal of cupric ions using polymer-based sensors are relatively rare. This gap in existing methodologies has motivated us to develop a biodegradable caprolactone-based polymeric macromolecule designed for the selective and sensitive detection of cupric ions, thus addressing both environmental and health concerns associated with copper contamination. The synthesis of compound-1 was done by a simple reaction. Compound-2 was obtained from fluorescein through a condensation reaction. Duff reaction involved a dialdehyde compound and an amine derivative of fluorescein to form compound-3. Following the successful formation of compound-3, this monomer subsequently underwent a ring-opening polymerisation process when it was exposed to caprolactone[7]. This transformation resulted in the production of our final polymer, known as compound-4. In this innovative polymer design, we have integrated a biodegradable polycaprolactone backbone. The introduction of polycaprolactone serves two primary purposes: it enhances the overall biodegradability of the polymer and increases its hydrophobic characteristics. The hydrophobic nature of the polymeric backbone is particularly crucial because it facilitates the effective separation of copper ions (cupric) from aqueous environments. Rigorous testing confirmed that both the synthesized monomer, compound-3, and the resultant polymer, compound-4, displayed excellent limits of detection (LOD) for cupric ions, significantly below the thresholds established by the EPA. This finding underscores the effectiveness of the materials developed in this study. To our knowledge, this represents the first instance of a biodegradable, caprolactone-based polymeric sensor, compound-4, specifically designed for the selective and sensitive detection of cupric ions, while simultaneously functioning as a medium for their removal from water. Such advancements are significant in addressing environmental concerns associated with metal pollutants in aquatic systems[8].

MATERIALS AND METHODS

Chemicals

Organic compounds

The following chemical compounds were obtained from the reputable supplier Sigma Aldrich: 4-(2-hydroxyethyl) phenol, Trifluoroacetic acid, Hexamethylene tetra-amine, Fluorescein, Hydrazine, Caprolactone, Tin(II) 2-ethylhexanoate, Dimethyl sulfoxide (recognised as DMSO), Dichloromethane, commonly referred to as DCM, DMSO-d6, and Diethyl ether, abbreviated as Et_2O, in addition to $CDCl_3$. These compounds are essential for various applications in both research and industrial processes due to their unique chemical properties and reactivity profiles.

Inorganic salts

Selected inorganic salts, which include magnesium chloride ($MgCl_2$), iron(II) sulphate ($FeSO_4$), lead(II) nitrate ($PbNO_3$), silver nitrate ($AgNO_3$), nickel(II) chloride ($NiCl_2$), cadmium sulphate ($CdSO_4$), calcium sulphate ($CaSO_4$), and aluminium chloride ($AlCl_3$), was procured from TCI and Sigma Aldrich. These compounds are vital for a wide array of chemical synthesis, analytical procedures, and material formulation, serving as key reagents for numerous experimental methodologies. The sourcing of these chemicals from established suppliers ensures a high level of quality and reliability required for precise scientific work.

Analysis

NMR characterization

The characterization of the samples was conducted using C-13 and Proton-NMR spectroscopy techniques, facilitated by an advanced Bruker 500MHz instruments. The choice of solvents for NMR analysis included dimethyl sulfoxide (DMSO-D6) and deuterated chloroform ($CDCl_3$), both of which are widely used in NMR studies due to their ability to dissolve a variety of compounds while providing a suitable environment for accurate spectral analysis. To ensure precision and consistency in the measurement of chemical shifts, tetramethylsilane (TMS) was employed as an internal standard across all NMR solvents. This approach not only enhances the reliability of the results but also allows for effective comparison of chemical environments within the samples under investigation.

UV-Vis experiments

The ultraviolet-visible (UV-Vis) absorption studies were meticulously done in Perkin-Elmer Lambda-35 UV-Vis spectrometer, which is equipped to provide high-resolution spectral data. During the experiments, a scan rate of 480nm per min was applied, allowing for a thorough examination of the absorption properties of the samples. Each absorption spectrum was acquired individually in a quartz cell with a standard path length of 1cm, ensuring that the optical path was consistent across all measurements. This methodology not only standardizes the experimental conditions but also contributes to the accuracy and reproducibility of the results, thereby enabling an in-depth analysis of the samples' electronic transitions and interactions.

ESI-MS analysis

The mass spectroscopic studies were conducted using a Q-TOF YA263 mass spectrometer, which is recognized for its high-resolution capabilities. The analysis employed negative electro-spray ionization (–ESI) mode, a technique that enhances the ionization of the analyte molecules under investigation, thus facilitating the detection and characterization of the molecular species present in the samples. This advanced instrumentation provides comprehensive information on the molecular weight and structural characteristics of the compounds, contributing significantly to the overall understanding of their chemical behaviour and interactions.

DFT calculation

The theoretical calculations necessary for our study were performed using the Gaussian 09 suite of computational chemistry programs. Within this framework, the geometries of the monomer complex compound-3, as well as its interaction with cupric ions, were optimised. The optimization process employed the local meta-GGA exchange-correlation functional known as M06-L, in conjunction with the 6-31+G basis set, which is widely accepted for the majority of atoms in molecular modeling. Notably, for the copper (Cu) atoms, a double-zeta basis set along with the relativistic effective core potential defined by Hay and Wadt (LANL2DZ) was utilized. This methodological approach ensures a high level of accuracy in simulating the electronic structure and geometrical configurations of the compounds, providing valuable insights into their behaviour at a molecular level[9].

Sample preparation for UV-Vis studies

The preparation of samples for UV-Vis spectroscopy involved creating stock solutions of compound-3 and compound-4 in dimethyl sulfoxide (DMSO), which were then diluted with water to achieve the desired concentrations for various experimental setups. Additionally, solutions containing metal ions were prepared in water to ensure compatibility for the subsequent analyses. Throughout the UV-Vis spectroscopic investigations, a fixed concentration of compound-3 and compound-4 was maintained, measured in micromolar (μM) units, to establish a controlled environment for the experiments. A systematic UV-Vis spectroscopic titration was performed by incrementally increasing the concentration of cupric ions in the compound-3 solution, which allowed for a detailed assessment of the interaction dynamics between the complex and the metal ions. All real samples were prepared in water to maintain consistency and reliability in the experimental conditions, further ensuring that the results obtained reflect the true nature of the molecular interactions under study.

Synthesis

The synthetic scheme is preparation of a biodegradable polymer from Tyrozol, Fluorescein and Caprolactone. As elaborated below, itt is a four step sequential procedures of synthesizing compounds 1-4. Compound-4 is proposed polymer for sensing and removing cupric ions from pond water[8,10,11].

Synthesis of compound-1

The synthesis of compound-1 was achieved through a straightforward Duff reaction, a well-established method for the introduction of formyl groups into aromatic compounds (Fig. 1). The procedure commenced with the dissolution of 4-(2-Hydroxyethyl) phenol (known as Tyrozol), which weighed 1 g and corresponded to 7.23mmol, in 20mL of Trifluoroacetic acid. Following the complete dissolution of the substrate, 4.05 g of hexamethylenetetramine (HMTA), amounting to 28.89mmol, was carefully added to the reaction mixture. This combination was then subjected to heating at a controlled temperature of 120°C under reflux conditions for a duration of 15 hours. Upon completion of the reaction, as indicated by appropriate monitoring techniques, the reaction mixture was allowed to cool to ambient temperature. Subsequently, the cooled mixture was poured into 200mL of a 2N hydrochloric acid solution to facilitate the acidification process. The resulting acidic mixture was stirred and maintained at a temperature of 80°C for 1 h, which aided in the precipitation of the desired product. Following this stirring period, a yellow solid was obtained from the mixture. This solid was then carefully filtered out of the reaction medium and subjected to thorough washing with 1L of distilled water to remove any impurities and un-reacted starting materials. The purification of the compound was achieved through column chromatographic techniques, which resulted in the isolation of a light yellow solid. The final product was then dried using a vacuum pump to ensure the removal of any residual solvents. The yield of the synthesis process was determined to be 0.63 g, equating to a yield percentage of 63%, an indication of a reasonably efficient reaction. The purity and structural integrity of the synthesized compound-1 were confirmed through the application of proton nuclear magnetic resonance (Proton-NMR) and carbon-13 nuclear magnetic resonance (C-13 NMR) spectroscopic techniques, which provided detailed insights into the molecular structure of the compound.

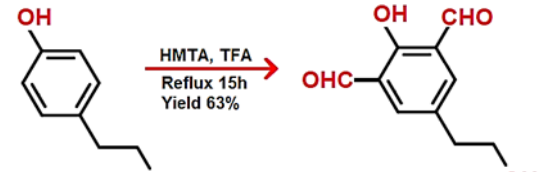

Fig. 1. Synthetic scheme for synthesis of compound-1 from Tyrosol

Synthesis of compound-2

In the synthesis of compound-2, fluorescein was utilized as the starting material (Fig. 2). 1 g of fluorescein anhydride accurately weighed (it is equal to 3.009mmol) This fluorescein was carefully placed into a 250mL round-bottom flask. To facilitate the reaction, 30mL of ethanol was added to the flask, and the contents were stirred using a magnetic bead to ensure thorough mixing and dissolution of the fluorescein. Subsequently, a solution of hydrazine hydride was prepared in ethanol, measuring out 0.9643 g, corresponding to 30.092mmol. This hydrazine hydride solution was then introduced into the reaction vessel containing the fluorescein and ethanol mixture. The reaction was conducted under reflux conditions for a duration of 12 h, allowing for adequate interaction between the reactants. Upon the completion of this reaction period, the next step involved concentrating the resulting reaction mixture. Once concentrated, the mixture was carefully transferred into a 250mL beaker filled with ice-cold water. This step was crucial for the precipitation of the desired product. After allowing sufficient time for the precipitation process to occur, the solid product formed was then filtered from the aqueous phase. The collected precipitate was subjected to multiple washing steps to eliminate any impurities and un-reacted starting materials, thereby enhancing the purity of the final compound. The washed precipitate was then dried under appropriate conditions to yield the final product, which appeared as a yellowish solid. The weight of the purified product obtained was 0.8858 g, indicating a respectable yield of 85%. The characterization of this synthesized compound was carried out using Proton-NMR and C-13 NMR techniques, which provided valuable information regarding its structural and chemical properties, confirming the successful synthesis of compound-2.

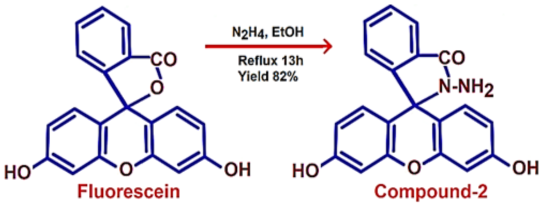

Fig. 2. Synthetic scheme for synthesis of compound-2 from fluorescein

Synthesis of compound-3

The synthesis of compound-3 is achieved using compound-1 and compound-2 (Fig. 3). It was carried out using a 100mL round bottom flask, into which 500mg of compound-1, corresponding to 2.57mmol, was accurately weighed and introduced. Concurrently, 1.78 g of compound-2, equating to 5.14mmol, was added to the flask. Both compounds were then solubilized in ethanol, providing a homogenous reaction medium. The mixture was subjected to heating at a temperature of 85°C, maintained under reflux conditions for a duration of 10 hours. This controlled heating facilitated the required chemical interactions to occur. Upon the completion of the reaction, as determined by appropriate analytical methods, the solvent was systematically evaporated under reduced pressure. A small volume of ethanol, approximately 1-2mL, was retained for further processing. This residual solvent was subsequently added to water in a meticulous drop-wise manner to induce precipitation of the desired product. The resulting precipitate was collected and filtered efficiently, followed by a drying step conducted under vacuum to obtain the final product in a yield of 0.4 g, which constitutes an 80% yield based on the starting materials. The characterization of the synthesized compound was meticulously performed using Proton-NMR and C-13 NMR techniques, providing insights into the molecular structure and confirming the successful synthesis of compound-2.

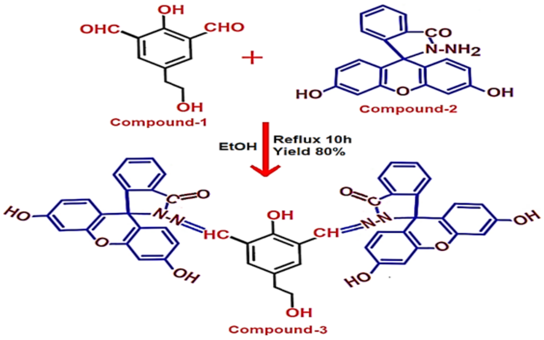

Fig. 3. Synthetic scheme for synthesis of compound-3 from compounds-1 & 2

Synthesis of compound-4

The synthesis of compound-4 polymer is achieved from compound-3 (Fig. 4). The process commenced with the transfer of the previously synthesized compound-3 (100mg, 0.117mmol) to a Schlenk tube. To this, 321.89mg of caprolactone, amounting to 2.808mmol, was added, along with a magnetic bead to assist in the mixing process. The system was then supplemented with 5mL of dry toluene to facilitate the dissolution of the reaction components, ensuring a well-mixed reaction environment. Following this, tin octanoate (4mg, 0.0117mmol) was introduced to the mixture, serving as a catalyst for the polymerisation process. The reaction was stirred continuously for a period of 20 h while maintained at a temperature of 110°C under a nitrogen atmosphere to prevent moisture interference. Upon completion of the heating period, the reaction mixture was allowed to cool. The polymerisation product was then precipitated by a careful drop-wise addition of the reaction mixture into a cold ether solution, which promotes the formation of solid polymer while minimizing solubility. The precipitated product was filtered to remove any un-reacted materials and excess solvent, and was subsequently dried under vacuum conditions to yield a purified polymer. Yield is 90%. The extent of polymer formation and molecular characteristics were confirmed through extensive Proton-NMR spectroscopy. Additionally, the synthesis resulted in the production of three distinct polymers by varying the ratio of caprolactone, thus enabling an investigation into the effects of composition on the polymer's properties. The average molecular weight of the synthesized polymers was determined through Proton-NMR analysis and gel permeation chromatography.

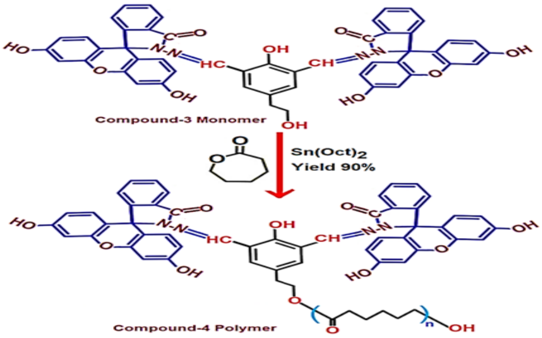

Fig. 4. Synthetic scheme for synthesis of compound-4 from compound-3

RESULTS AND DISCUSSION

Photophysical Studies of Monomer (compound-3)

Following the successful synthesis of monomer (compound-3), attention was directed towards conducting an in-depth exploration of the photophysical properties of this sensor. Characterization commenced with an assessment of its absorption characteristics, revealing a prominent absorption maximum located at approximately 372nm within a colourless solution prepared in a dimethyl sulfoxide (DMSO) and phosphate-buffered saline (PBS) mixture, specifically in a volumetric ratio of 8:2 and adjusted to a physiological pH of 7.4. The absorption spectrum exhibited significant alterations upon the introduction of cupric ions into the medium. Notably, a new absorption peak emerged at 454nm, coinciding with a discernible decline in the intensity of the peak at 372nm. This variation in the UV–Vis spectra, coupled with a visible transformation of the solution's colour from colourless to yellow, suggests a strong interaction between the cupric ions and the monomer sensor molecule, indicative of their binding affinity. In sensor applications, the detection capability constitutes a critical parameter that determines the overall effectiveness of the system. To evaluate the responsiveness of monomer to the presence of cupric ions, the temporal behaviour of the sensor within the DMSO/PBS (8:2v/v, pH 7.4) medium was investigated. The results indicated that the sensor monomer molecule exhibited an immediate response to cupric ion introduction, achieving a noticeable colour change that transitioned from colourless to yellow within a remarkably short timeframe of one minute. Concurrently, the appearance of a new absorption maximum at a wavelength of 454nm confirmed the rapid interaction between the sensor and the metal ions. To further ascertain the stability of the resulting colour change in the solution, we conducted a prolonged observation period spanning two days. This assessment concluded that the yellow hue of the solution remained stable throughout this duration, providing compelling evidence of the robust binding interaction between the cupric ions and monomer in the solution. Such stability is a critical aspect of sensor performance, ensuring reliable detection over time. To examine the selectivity properties of monomer, comprehensive UV–Vis spectroscopic analysis was conducted to observe the interactions of monomer with various metal ions. In line with our expectations, a distinct new absorption peak was identified at 454nm exclusively in the presence of cupric ions. In contrast, no similar spectral modifications were observed when other metal ions were introduced. Additionally, a noticeable change in colour was documented, transitioning from colourless to yellow solely in the presence of cupric ions, indicating a lack of interaction with the other metal ions tested, as depicted in Fig. 5. These findings corroborate that the monomer sensor molecule is capable of selectively detecting cupric ions within a timeframe of one minute, accompanied by a significant change in colour from colourless to yellow and marked spectral alterations.

Fig. 5. Selectivity of compouind-3 (100 μM) for cupric ions compared to other metal ions

The promising results obtained regarding the time sensitivity and selective detection capabilities of monomer prompted us to conduct a more detailed investigation into its responsive behaviour towards cupric ions. To this end, we executed UV–Vis titration experiments, systematically adding cupric ions in increasing concentrations ranging from 0 to 100μM. As anticipated, with the incremental addition of cupric ions, the absorption maximum near 372nm exhibited a decrease, while the absorption maximum at approximately 454nm displayed a corresponding increase in a ratiometric fashion, ultimately reaching a plateau following the addition of 50μM of cupric ions. Notable linearity was observed, with an R^2 value of 0.98522, reflecting a consistent rise in absorption intensity at 454nm corresponding to varying concentrations of cupric ions within the range of 0-30μM. This linearity enabled the determination of the limit of detection (LOD) for cupric ions, yielding a value of 29nM, which is impressively lower than the threshold established by the World Health Organization (WHO). The results discussed affirm the exceptional selectivity and sensitivity of monomer towards cupric ions. In light of these findings, we were motivated to investigate the effects of pH on the performance of monomer. To achieve this, UV–Vis spectroscopic studies were conducted to assess monomer's behaviour across various pH levels, both in the absence and presence of cupric ions. The pH-dependent observations revealed that the spectral characteristics of monomer remained unchanged when diverse pH levels were applied in the absence of cupric ions. Conversely, in the presence of cupric ions, there was a gradual increase in absorption intensity at 454nm with rising pH values, peaking at pH 6, after which it approached a state of saturation. These findings from the pH-dependent investigations suggest that monomer demonstrates effective performance within the physiological pH range, reinforcing its potential utility in practical applications.

Determination of the Binding Constant and Stoichiometry

The investigation into the binding interactions between the monomer sensor molecule and cupric ions has unveiled a significant transformation in the solution's colour, progressing from a clear, colourless state to a distinctly yellow hue. This transition occurs rapidly and demonstrates a high degree of selectivity and sensitivity towards the target ions. To elucidate the specific binding stoichiometry of this interaction, we employed Job's plot methodology alongside UV–Vis spectroscopic techniques. The absorption measurements were meticulously conducted while systematically varying the mole fraction of cupric ions in relation to the monomer sensor (Fig. 6). The analysis of the resulting plot indicated a binding stoichiometry of 1:1 between monomer and cupric ions, implying that each molecule of the sensor interacts with one cupric ion. Furthermore, the association constant of this interaction through a series of titration experiments yielded a value of 7.25×10^5M. This calculation was facilitated by applying the Benesi–Hildebrand equation (Figure 7).

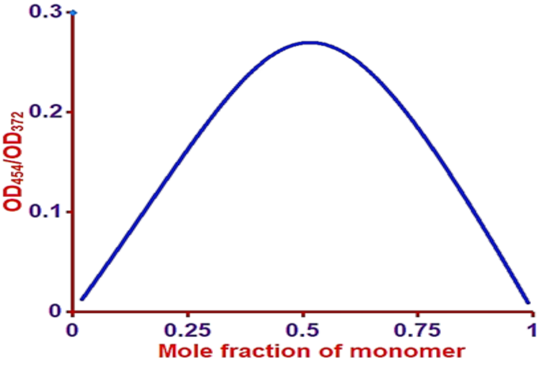

Fig. 6. Job's plot showing 1:1 binding of compound-3 monomer to cupric ions

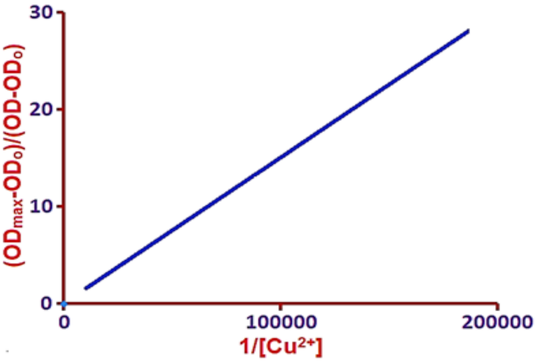

Fig. 7. Plot of Benesi-Hindebrand equation to get association constant of polymer to cupric ion

Photophysical Behavior of Polymer (compound-4)

Building on the insights from the preceding studies, it has become apparent that the sensor molecule monomer (compound-3) possesses the capability to detect cupric ions with remarkable selectivity and sensitivity. However, a limitation was identified in that the monomer was unable to effectively separate cupric ions from the solution, which necessitated a more advanced approach. To address this challenge, a biodegradable polymer based on caprolactone, denoted as polymer (compound-4), employing the ring-opening polymerisation technique. This polymer was designed not only for the detection of cupric ions but also for their subsequent separation from solutions. In pursuit of this objective, three distinct polymer variants were fabricated, designated as polymer-1, polymer-2, and polymer-3, each characterised by differing caprolactone ratios and average molecular weights, and polydispersity index (PDI), as shown in Table 1.

Table 1: Average molecular weight comparison table of polymer

Polymer	Mn by NMR	Mn by GPC	Caprolactoneunit targeted	Caprolactoneunit incorporated	PDI
Polymer-1	1200	2000	4	3	1.08
Polymer-2	1500	2400	6	6	1.02
Polymer-3	3590	4200	25	24	1.05

For the photophysical studies, the focus was primarily on polymer-3 to assess its sensing properties. Initial investigations involved time-dependent studies to evaluate the response of polymer-3 upon the addition of cupric ions. Remarkably, polymer-3 exhibited a rapid detection of cupric ions, with a visible colour change observable within one minute, transitioning from colourless to yellow at a wavelength of 446nm. This swift response paralleled that of monomer and provided a promising indication for further sensitivity assessments. Based on these encouraging findings, UV–Vis titration experiments to quantitatively ascertain the sensitivity of polymer-3 in the presence of varying concentrations of cupric ions were done. During the titration, cupric ion concentrations were incrementally increased from 0 to 100μM. The results revealed a progressive decrease in the absorption maxima at 371nm concomitant with a gradual increase in the absorption maxima at 446nm, ultimately achieving a plateau following the introduction of 60μM of cupric ions. Fig. 8 shows absorption peaks for polymer and cupric ions absorbed on polymer. Furthermore, a significant linear relationship was observed with an R^2 value of 0.99213, exemplifying the correlation between the advancement of absorption intensity at 446nm and the escalating concentrations of cupric ions within the range of 0 to 50μM. Through analysis of this linearity, we established that the limit of detection for cupric ions using polymer-3 is 0.3μM, a value that is substantially lower than the permissible limits set forth by the World Health Organization (WHO). This capability underscores the efficacy of the polymer sensor in both detecting and separating cupric ions, positioning it as a valuable tool in environmental monitoring and public health applications.

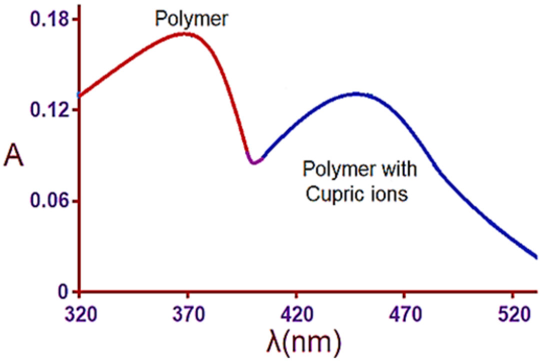

Fig. 8. UV-Vis spectra for separate polymer solution and polymer bonded with cupric ion

Study of the Sensing Mechanism

The binding mechanism of cupric ions with the monomer sensor molecule has been thoroughly investigated using Job's plot alongside mass spectroscopic techniques, providing a comprehensive understanding of the interaction between these entities. Through the Job's plot experiment, we established a binding stoichiometry of 1:1 between the monomer sensor and the cupric ions. The design of the sensor incorporates two spirolactam rings, each containing fluorescein units, and the presence of a hydroxyl (–OH) group within the phenyl ring creates an optimal binding cavity for the interaction with the copper ion. The significance of nitrogen (N) and oxygen (O) atoms in this binding process cannot be understated, as their inherent electronegativity contributes to their ability to effectively engage with cupric ions. Specifically, the nitrogen atoms associated with the carbon-nitrogen (C-N) groups and the oxygen atoms from the carbon-oxygen (C-O) groups, in addition to the –OH groups, are believed to play a pivotal role in the formation of a charge transfer complex with the cupric ion (Fig. 9). This interaction results in a notable change in colour to yellow, which serves as a visual indicator of the binding event. The selectivity of monomer towards copper ions can be attributed to the ideal structural configuration and appropriate cavity size that facilitate this specific interaction. To validate the predicted binding mechanism, mass spectrometry analyses of monomer in the presence of cupric ions was done which confirmed the anticipated 1:1 stoichiometry. The mass spectrometry data revealed a distinct peak at 913.17, which corresponds precisely to the molecular weight of the complex formed between monomer and cupric, thereby substantiating the conclusion of a 1:1 binding ratio. For monomer alone, the peak was at 815.25, revealing 851.230+63.546≈913.169 due to the formation cupric ion-polymer charge transfer complex.

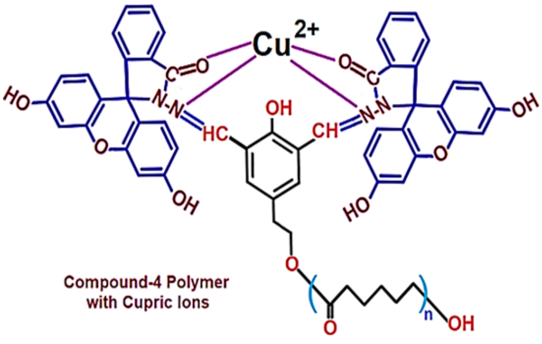

Fig. 9. Absorption of cupric ions in polymer forming stable charge transfer complex

DFT Calculation

To further substantiate our findings on the sensing mechanism, density functional theory (DFT) calculations were performed on the monomeric unit of monomer and its complex with cupric ions. The initial step involved the optimisation of the structures for both the monomer sensor and its resultant complex upon binding with cupric ions. Following this optimisation, calculation of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) was done for both monomer and the cupric complex. The energy levels of these orbitals were also determined, revealing that the energy gap existing between the HOMO, and the LUMO of the monomer sensor without the copper ion is 3.14eV. However, upon the formation of the complex with one cupric ion, this energy gap diminishes significantly to 1.57eV. This reduction indicates a more stabilised electronic structure resulting from the strong complex formation. The DFT calculations corroborate the experimental results, reinforcing the conclusion that a single cupric ion binds to one monomer molecule in a 1:1 ratio, thereby validating our proposed sensing mechanism.

Real Sample Analysis

To test the performance of polymer in real situation, the compounds-3&4 were tested in pond water samples[11]. The impressive performance exhibited by monomer and polymer during the selectivity and sensitivity evaluations prompted an investigation into the practical application of polymer-3 for the identification of cupric ions within real-world systems. To initiate this assessment, a UV–Visible titration experiment was conducted employing poymer-3 at a concentration of 50μM in pure water. During this process, the concentration of cupric ions was systematically varied from 5 to 45μM. The resulting data allowed for the generation of a linear plot depicting the ratio of optical density at 446nm to that at 371nm (OD446/OD371) against the concentration of cupric ions. This plot demonstrated excellent linearity with a corresponding R^2 value of 0.99671. This linear relationship serves as a critical calibration curve, enabling the quantitative determination of unknown cupric ion concentrations present in various real samples. To evaluate the efficacy of polymer-3 in practical applications, pond water samples were collected from the nearby ponds. These samples were then spiked with varying concentrations of cupric ions, alongside the addition of polymer-3 at the same concentration of 50μM. Following the UV–Vis spectroscopic analysis, distinct absorption maxima were observed, yielding different OD446/OD371 values. These values were then correlated with the previously established calibration curve to ascertain the actual concentration of cupric ions within the tested solutions. Subsequently, the percentage recovery of cupric ions was calculated using the calibration curve. Remarkably, the recovery percentage in all tested scenarios exceeded 95%, as detailed in Table 2. Such high recovery rates indicate the potential applicability of polymer-3 for the sensitive and selective detection of cupric ions in real environmental samples.

Table 2: Real environmental water sample analysis

Samples	Spiked [Cupric Ions] (μM)	Obtained [Cupric Ions] (μM)	% of recovery
Pond Water-1	0	0	100
Pond Water-2	15	14.12 ± 0.184	95.36
Pond Water-3	25	24.04 ± 0.239	97.08
Pond Water-4	35	34.87 ± 0.455	98.32
Pond Water-5	45	44.01 ± 0.325	98.52

Separation of the Cupric Ions from the Water Samples

The favorable results accumulated thus far suggest that polymer-3 possesses substantial capabilities for the selective and sensitive detection of cupric ions, characterised by a notably low limit of detection (LOD). This prompted a further investigation into the polymer's ability to separate cupric ions from aqueous solutions. To explore this, ring-opening polymerization of monomer was performed in the presence of caprolactone, utilising tin(II) 2-ethylhexanoate as a catalyst. The underlying hypothesis was that the introduction of increased hydrophobicity into the monomer structure through polymerization would enhance the mechanism for binding and separating cupric ions from the water medium. To examine this hypothesis, three distinct sets of polymers were synthesised, each with varying ratios of caprolactone to adjust the overall hydrophobicity of the resulting polymer. Following polymer synthesis, solvent-dependent experiments were conducted for each polymer by varying the ratio of DMSO and water in the presence of cupric ions. Notably, the experiment revealed a trend; as the percentage of water increased, the absorption maxima at 446nm decreased significantly. At a 9:1 ratio of water to DMSO, the observed absorption maxima at 446nm were markedly reduced, indicating a minimal presence of the cupric ions, coinciding with the formation of yellow precipitates. Additionally, it was observed that increasing the caprolactone units within the polymer correlated with a further decrease in the absorption maxima at 446nm, indicating enhanced precipitation from the solution. This observation underlines the successful strategy of utilising polymer chemistry to facilitate the selective separation of cupric ions from aqueous environments. Through a comprehensive solvent-dependent investigation of three distinct polymers, findings indicated that polymer-3 exhibited negligible absorption maxima at 446nm coupled with a pronounced tendency to form precipitate in environments containing cupric ions when tested within a 9:1 mixture of water and dimethyl sulfoxide (DMSO). This distinct behaviour rendered polymer-3 a favourable candidate for conducting further separation studies focused on cupric ion removal from aqueous solutions. To effectively extract copper from water, a controlled concentration of copper solution was prepared, after which polymer-3 was introduced into the system. The mixture was subjected to stirring for a duration of two hours to ensure adequate interaction between the polymer and the copper ions. Following this period of agitation, the resulting product was subjected to a separation process where the yellow precipitate formed during the reaction was isolated from the colourless filtrate. In order to ascertain the presence of cupric ions within the isolated precipitate, two independent ultraviolet-visible (UV–Vis) spectroscopic analyses were performed. The precipitate was dissolved in DMSO for examination alongside the colourless filtrate. The results indicated a significant differentiation between the two: the filtrate demonstrated no observable absorption maxima at 446nm, confirming its lack of cupric ions. Conversely, the precipitate exhibited a distinct absorption peak at 446nm, thereby substantiating the presence of cupric ions and directly correlating this absorption feature with their existence. To further characterise the composition of the precipitate, energy dispersive analysis of X-ray (EDAX) was conducted, yielding additional insights into the elemental presence within the precipitate. This analysis corroborated the previous findings, affirming the presence of cupric ions in the precipitate. Consequently, the combined evidence obtained from the UV–Vis absorption study and the EDAX analysis unequivocally demonstrates that the precipitate contains cupric ions. Therefore, it can be conclusively stated that polymer-3 possesses the capability to effectively separate copper ions from aqueous environments.

CONCLUSION

The successful synthesis of the fluorescein-based monomer, designated as compound-3 monomer, alongside the caprolactone-based biodegradable polymer, known as compound-4 polymer, was accomplished through the process of ring-opening polymerization. The synthesized compound-3 has demonstrated its efficacy as a highly selective and sensitive agent for the ultrafast detection of cupric ions. Notably, the detection is characterized by a discernible change in the colour of the solution, transitioning from colourless to a distinct yellow hue. The lowest limit of detection achieved for cupric ions using compound-3 monomer is an impressive 29nM, underscoring the high sensitivity of this detection method. Further investigations into the interaction between compound-3 monomer and cupric ions were conducted through mass titration experiments and Job's plot analysis. These results conclusively indicated that compund-3 monomer binds to cupric ions in a 1:1 stoichiometric ratio. This finding was further corroborated by density functional theory calculations, which provided a theoretical framework supporting the experimental observations. In terms of the polymeric material, compound-4 polymer not only possesses the capability of detecting cupric ions but also excels in the separation of these ions from aqueous solutions. The introduction of caprolactone units into the polymer structure significantly contributes to its functionality. This structural modification enhances the hydrophobic character of the polymer, which, in turn, facilitates the binding of cupric ions present in the solution. As a result, the polymer undergoes a phase separation, precipitating out of the solution upon binding with the copper ions. To further validate the practical applicability of compound-4 polymer, real-world testing was performed to assess its performance in detecting cupric ions within actual environmental samples. The outcomes of these experiments indicated that compound-4 polymer is highly effective in identifying cupric ions under real conditions, showcasing its potential utility in environmental monitoring. To the best of our knowledge, this study represents a pioneering effort in developing a biodegradable caprolactone-based polymeric system that not only facilitates the detection of cupric ions but also enables their effective separation from aqueous environments, marking a significant advancement in the field of polymer chemistry and environmental science.

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Conflict of interests

There is no conflict of interests in this work.

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