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ROBUST DETECTION OF Hg^{2+} IONS AND VALIDATION USING NEWLY PREPARED THERMOSENSITIVE POLYMERIC GEL

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ABSTRACT

The current report describes the fabrication of a polymer based on dithioacetalization of quinoline-2,4-dicarbaldehyde and characterized using spectroscopic techniques. The synthesized polymer showed the ability to form a temperature-dependent polymeric gel in a few solvents. The effect of varying solvent and temperature conditions was investigated for the polymeric gel. The synthesized polymer was used as a sensor to detect Hg^{2+} ions in various samples. In the presence of Hg^{2+} ions, the polymer acts as a turn-on fluorescence sensor, and the detection was achieved at 416 nm. The ability to sense the Hg^{2+} ions was investigated by analyzing different samples from different sources at a nanomolar scale. The effect of altering the concentration of Hg^{2+} ions on the durability of a polymeric gel and the sensing ability of the polymer was examined. The developed method for sensing Hg^{2+} ions was validated, and the limit of detection and quantification was calculated. The DFT calculations were performed to develop the lowest energy structures and HOMO-LUMO orbitals of the polymer.

Keywords: Dithioacetalization, Quinoline, Polymeric Gel, Fluoresce Sensing, Metal Ions.

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INTRODUCTION

The identification of hazardous metal ions from environmental and biological samples is very important and demanding [1]. Several studies have been conducted on metal ion detection in aqueous samples. These studies employed macromolecule or small molecule-based chemosensors to identify metal ions.[2–5] Due to the chemosensor's unique range of fluorescence emission with a specific metal ion, fluorescence detection techniques are regarded as the best among them.[2,3] The fluorescence sensor based on polymers has yet to be thoroughly investigated for sensing applications.

The polymer-based sensors, due to the repetition of desired monomer units, will advance the sensing application more than single-molecular chemosensors. Nowadays, polymers are widely used substances or materials in a wide range of applications, such as petrochemicals, drug delivery, water filtration, solar cells, lithium-ion batteries, cosmetics, food, household materials, self-healing materials, electronic and magnetic materials, and luminescent materials.[6–18] The utility of the polymer for different applications depends on the types of monomer units used in synthesis.

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The present report on the synthesis of a quinoline-based polymer describes the formation of the thermo-responsive polymeric gel and the polymer's application in the selective detection of mercury ions, selectively, in aqueous samples. Quinolines are aromatic compounds that naturally arise as fused rings that belong to a heterocyclic class. Quinoline and its derivatives are widely used in numerous industrial and medical applications.[2-5] Apart from that, the substituted quinoline also received the most excellent attention among the chromophores employed to create luminous materials due to its unique coordination and photophysical properties.[19-22] For example, tris(hydroxyl-8-quinoline)aluminium(III) is one of the most significant metal complexes for OLED applications that have been investigated so far. Quinoline's remarkable optical and chelating properties are the reason for this.[23-25] The quinolines exhibit exceptional luminescence and fluorescence properties in the presence of the fused ring aromatic structure. Thus, there has been a lot of interest in the development of fluorescent and luminous materials. The heteroatom (nitrogen atom) in quinoline acts as an electron donor group. It shows high binding capacity with various analytes when used as a chemosensor, such as styryl-substituted 8-hydroxyquinoline derivatives.[2,3,26] Chemosensors based on quinoline derivatives have been used to detect a range of metal cations,[2-5,27] including zinc, iron, copper, calcium, magnesium, and others, in various samples from chemical, environmental, clinical, and biological samples.[3-5] This report describes the condensation polymerisation of quinoline with thiols, forming a new linear polymer and its application in the selective sensing of mercury ions. The quinoline-2,4-dicarboxylic acid was first converted to quinoline-2,4-dicarbaldehyde and then performed a reaction with Pentaerythritol-tetrakis(3-mercaptopropionate) under a dithioacetalization reaction, and a linear polymer was obtained. The synthesized polymer turned into a soft gel in the presence of suitable solvents and showed sensitivity toward different temperature conditions. The stability of the prepared polymeric gel was studied by varying temperature conditions and mixing different metal ions. Using the prepared polymer, a selective turn-on fluorescence detection method for the Hg^{2+} ions was investigated and validated. The detection sensitivity for Hg^{2+} ions of the developed method was examined by adding other metal ions. Additionally, the stable structure, HOMO-LUMO, and energy gap optimizations were carried out by DFT simulations.

EXPERIMENTAL

Materials and Methods

Quinoline-2,4-dicarboxylic acid, Pentaerythritol-tetrakis(3-mercaptopropionate), tris(trimethylsilyl)silane, *fac*-Ir(ppy)$_3$, K_2HPO_4, and NMR solvents were ordered from Sigma-Aldrich. The water samples were collected from nearby locations. Avra Chemicals (India) supplied this investigation's solvent and other reagents.

Instruments and Equipment

FT-IR spectrophotometer (Agilent), UV-2450 spectrophotometer (Shimadzu), Fluorescence spectrometer (Agilent), HR-MS (Bruker HD), NMR instrument (Jeol 500 MHz), and CHNS-elemental analyser were used to characterize synthesis products.

Synthesis of Quinoline-2,4-dicarbadlehyde

The *fac*-Ir(ppy)$_3$ catalyst was used to convert carboxylic groups of the quinoline-2,4-dicarboxylic acid to aldehydes, and quinoline-2,4-dicarbaldehyde was obtained.[28] The crude product was purified using column chromatography, and a pale yellowish-brown solid (yield 89%) was obtained. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 10.03 (s, 1H), 9.94 (s, 1H), 8.51 – 8.46 (m, 1H), 8.37 (s, 1H), 8.26 (dd, J = 7.5, 1.6 Hz, 1H), 7.87 (td, J = 7.2, 1.3 Hz, 1H), 7.67 (ddd, J = 8.4, 6.9, 1.3 Hz, 1H).

SiO_2-$SOCl_2$ Catalyst

15 g silica gel in 30 mL dry CH_2Cl_2 was treated with 15 g $SOCl_2$ was added dropwise into the solution containing 30 mL dry CH_2Cl_2 and 15 g silica gel, and allowed to stir for one hour. The release of the SO_2 and HCl indirectly confirms the formation of the SiO_2-$SOCl_2$ catalyst. After the reaction, the solvent and remaining $SOCl_2$ were removed under a vacuum, and the dried product was directly used as a catalyst for the polymerisation reaction.[29]

Polymerisation (Dithioacetalization)

0.6 g SiO_2-$SOCl_2$ and Quinoline-2,4-dicarbaldehyde (1.5 mmol) were added to a 20 mL solution of dithiol (Pentaerythritol-tetrakis(3-mercaptopropionate); 2.2 mmol) in dry toluene. The resulting mixture was stirred overnight at 65 °C.[29] The reaction was monitored by TLC, and after the reaction, the product was filtered off and loaded onto a small column. Then, it was washed thoroughly with CH_2Cl_2 to remove unreacted substrates and isolate the desired product. The CH_2Cl_2 was then removed under reduced pressure, and the desired product was obtained (yield 78%; Fig.-1). The polymer synthesis was confirmed using NMR spectroscopy (Fig.-2A). The average molecular weight of the synthesized polymer was obtained using gel permeation chromatography (a styrene-based standard was used), and the obtained average molecular weight was 4892 g/mol.

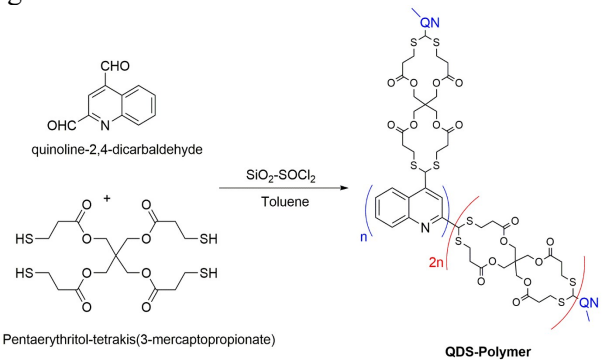

Fig.-1: Synthesis of Quinoline-Based QDS-Polymer From Quinoline-2,4-dicarbaldehyde and Pentaerythritol-tetrakis(3-mercaptopropionate) using SiO_2-$SOCl_2$ Catalyst

Preparation of Gel

The QDS-polymer and dioxane were mixed in a glass vial at a 1:30 molar ratio. A non-sticky, light yellowish gel was formed. Similarly, polymer gel was prepared using other solvents (benzene, diethyl ether, and THF) and a combination of the solvents (dioxane and THF). The gel prepared in dioxane is shown in Fig.-2B.

Reaction of QDS-polymer with Mercury Ions

The stranded solution of 20 mM Phosphate-buffered saline (PBS) was prepared as reported in the literature.[30] The solution of 1 µM QDS-Polymer in CH_3CN was mixed with aqueous Hg^{2+} solution (1 to 10 equivalents) and 20 mM PBS buffer (7.5 pH). The prepared solutions were incubated for 45 min before fluorescence detection studies. The different organic solvents, pH of buffer, and concentration ratio of solutions of QDS-polymer and Hg^{2+} ions for fluorescence detection study were investigated.

RESULTS AND DISCUSSION

Synthesis of Aldehyde from Acids

In the presence of a photoredox catalyst [fac-Ir(ppy)$_3$], the carboxylic groups of quinoline-2,4-dicarboxylic acid underwent a direct conversion to aldehydes, which was succeeded by a reductive-oxidation process..[28] K_2HPO_4 and tris(trimethylsilyl)silane were used as base and hydrogen sources, respectively, in the reaction. During the synthesis, 89% of quinoline-2,4-dicarbadlehyde was produced using the single electron transfer (SET) mechanism. The acetonitrile (CH_3CN; polar aprotic solvent) was found suitable for the synthesis of quinoline-2,4-dicarbadlehyde. The nitrogen of quinoline and the solvent didn't interfere with the SET mechanism during the reaction. NMR and HRMS confirmed the synthesis of the compound.

Synthesis of Polymer

The polymerisation process involved the dithioacetalization reaction between pentaerythritol-tetrakis(3-mercaptopropionate) and quinoline-2,4-dicarbaldehyde, with the aid of SiO_2-$SOCl_2$ catalyst (Fig.-1).[29] The SiO_2-$SOCl_2$ catalyst was prepared by the addition of $SOCl_2$ to the silica gel solution under dry conditions. The $SOCl_2$ vigorously reacts with silica gel and activates its surface by replacing one oxygen atom with a chlorine atom. The release of SO_2 gas is direct evidence of the formation of the SiO_2-$SOCl_2$ catalyst.[29] The aldehyde groups of quinoline-2,4-dicarbaldehyde adsorbed on the surface of the SiO_2-$SOCl_2$ catalyst, and the aldehydic oxygen atom was substituted with two thiol groups [Pentaerythritol-tetrakis(3-

mercaptopropionate)] in a dithioacetalization substitution reaction, and a linear 3D polymer (QDS-polymer) was obtained. The synthesis conditions were examined by altering the solvents, catalyst, and base and adjusting the reaction time and other factors. A low yield of the product was obtained when silica gel was used in the procedure without being modified to SiO_2-$SOCl_2$. The dry toluene as a solvent in the reaction yielded 78% conversion of the quinoline-2,4-dicarbaldehyde and Pentaerythritol-tetrakis(3-mercaptopropionate) to linear 3D QDS-polymer, but dry solvents such as benzene, acetone, diethyl ether, dichloromethane, acetonitrile, and THF cause low yield (18-52%) of polymerisation. The suitable temperature condition for the reaction was investigated by varying the temperature from 10 to 110 °C. Heating the reaction mixture at 65 °C in dry toluene was optimal for the reaction and yielded the highest. The hydrogen peaks from the thiol and aldehyde groups disappeared in the NMR spectrum, indicating that the polymer had been synthesized.

Polymeric Gel and Thermal Stability

In the presence of non-polar and polar aprotic solvents, the synthesized polymer formed soft gels. The dioxane forms a more stable gel than other solvents like diethyl ether, THF, benzene, etc.[31] The high polarity of the solvent makes the polymer soluble in solvents. The gel formation was carried out using dioxane, and its durability with temperature was investigated. The polymer begins to swell when it comes into contact with dioxane, and when the concentration rises, a non-sticky gel forms. The concentration of the polymer and dioxane was varied to 1:25, 1:50, 1:75, 1:100, 1:125, and 1:150 molar ratios. It was found that a 50:1 solvent (dioxane)-to-polymer ratio was suited efficiently to produce a polymeric gel. Similarly, benzene, diethyl ether, and THF solvents were used to prepare the polymeric gels. The higher concentration of the solvent leads to dissolving the gel and forming a polymer solution. Polar solvents, due to their high polarity, were unable to form a polymeric gel. The mixed solvents such as dioxane and THF or acetonitrile (1:1) were also tested in preparation of the polymer base gel for fluorescence studies. Compared to gels made with other solvents, the polymer-based gel created in dioxane was more durable but was sensitive to high temperatures.[31,32] Thus, the effect of heating the gel at different temperatures was investigated. Synthesized gel shows high stability at room temperature. However, increasing the temperature above 48-58 °C leads to the deformation of the gel into a liquid solution. The effect of heating on the gel is shown in Fig.-2B.

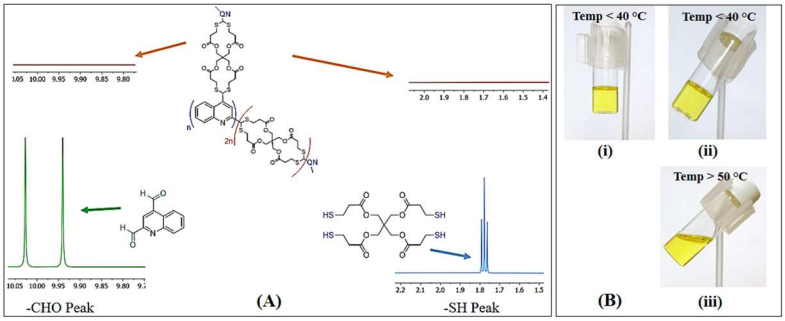

Fig.-2: (A). The Confirmation of the Synthesis of QDS-polymer using ^{1}H-NMR. (B). Effect of the Temperature on the Prepared QDS-Polymer-Based Gel in Dioxane. (i) and (ii) show the Gel form at Low Temperature, while (iii) shows the Solution Phase at High Temperature

Shape of the Polymer and Mechanism of the Metal Ions Detection

The Gaussian program was used to develop the homo-lumo orbitals and 3D structure of a fragment of the polymer (Fig.-3).[33,34] The linear polymer forms a helical structure by overlapping polymeric chains and creates random voids due to the presence of aromatic stacking moieties (quinolines). The voids in the polymer allow the insertion of metal ions in the empty spaces, thus binding strongly with polar groups. Due to a high number of polar atoms, the metal cations easily interact with the QDS-polymer via ionic interactions. In the case of the Hg^{2+} ion, it has an excellent affinity with sulphur atoms and thus interacts strongly with the dithioacetal group and breaks it down.[30] The optimised Homo-Lumo orbitals of the fragment of the polymer (Fig.-3) show that the sulphur group has higher electron density (homo-orbitals), and due to this, cation (Hg^{2+} ions) shows stronger binding with the dithioacetal group because of the opposite charge. The de-acetalization of QDS-polymer with Hg^{2+} ions forms quinoline-2,4-dicarbaldehyde,

which is a highly fluorescent compound. Therefore, this polymer is used for fluorophoric detection of the Hg^{2+} ion.

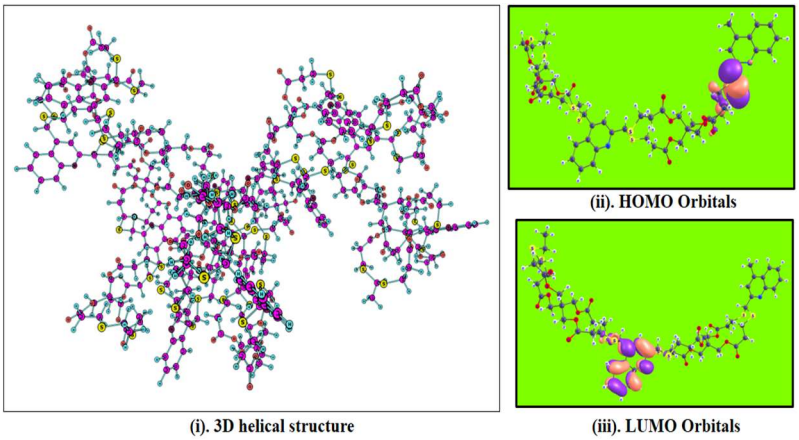

Fig.-3: The 3D Helical Structure (i) and Optimized HOMO and LUMO Orbitals (ii and iii) of the Fragment of the Prepared QDS-Polymer Developed using Gaussian Program

Fluorescence Sensing of Hg^{2+}

In this report, a turn-on fluorescence method for the sensing of Hg^{2+} has been described. The synthesized polymer shows excellent affinity to bind with Hg^{2+} ions in the presence of the sulphur atoms of the dithioacetal group. As a result, the dithioacetal group breaks and the aldehyde groups are obtained.[30] The polymer exhibits a poor emission in the fluorescence region because the dithioacetal group on the quinoline molecule quenches the polymer's fluorescence emission properties.[30] Due to the strong binding of the mercury with the dithioacetal group, the synthesised polymer began to degrade into quinoline-2,4-dicarbaldehyde when it was mixed with a solution of Hg^{2+} ions, and it displayed intense fluorescence emission in the fluorescence region. Consequently, the polymer functions as a turn-on fluorescence chemosensor for Hg^{2+} ions. Due to the QDS-polymer containing adequate binding sites, we wished to study the chemosensing ability of Hg^{2+} metal ions in the presence and absence of other metal ions such as Ag^{+}, Al^{3+}, Na^{+}, Cr^{3+}, Ni^{2+}, Pb^{2+}, Fe^{3+}, Cd^{2+}, Co^{2+}, $Zn^{2+,}$ and Cu^{2+}. Mercury ions are recognised to be toxic environmental contaminants that can be harmful to health even in small amounts.[35] When individual solutions of Ag^{+}, Al^{3+}, Na^{+}, Cr^{3+}, Ni^{2+}, Pb^{2+}, Fe^{3+}, Cd^{2+}, Co^{2+}, $Zn^{2+,}$ and Cu^{2+} ions were mixed with the polymer solution, there was no notable change in the fluorescence spectrum, except for Hg^{2+}. The polymer solution showed intense peak fluorescence spectra at 416 nm when treated with Hg^{2+} ions solution (Figure 4(i) displays the typical fluorescence spectra of QDS-polymer). The QDS solution in CH_3CN with PBS buffer containing 0.25% DMSO produced excellent results for fluorophoric detection of the Hg^{2+} ions. Increasing the equivalent ratio (0 to 10 equivalents) of the Hg^{2+} ions solution concerning the 1.0 µM QDS-polymer solution gave a more intense fluorescence spectrum. Increasing the heating time of the Hg^{2+} ions and QDS-polymer solution reduces the need for high equivalents of the Hg^{2+} ions. The 30 equivalent concentrations of the Hg^{2+} ions solution produced the highest fluorescence emission with 1.0 µM QDS-polymer solution [Fig.-4(i)]. The dethioacetalization reaction proceeds faster in the presence of the PBS buffer and DMSO.[30] The detection limit (LOD) of the prepared chemosensors (polymer) for Hg^{2+} ions was estimated. The IUPAC guidelines define a standard relationship (i.e., LOD = (3.3 x standard deviation)/slope) to determine the LOD values. Also, ten blank samples were taken and measured to determine the relative standard deviation in the current study. The calculated LOD value of **polymer** was 9.02 nM, towards Hg^{2+}, Fig.-4(ii). The binding constant of the chemosensor towards Hg^{2+} was found to be 3.96×10^{5} M^{-1}, using the Benesi-Hildebrand method. To evaluate the excellent selectivity of QDS-polymer towards Hg^{2+} ions, we conducted comparative experiments with various cations (Ag^{+}, Al^{3+}, Na^{+}, Cr^{3+}, Ni^{2+}, Pb^{2+}, Fe^{3+}, Cd^{2+}, Hg^{2+}, Zn^{2+}, and Cu^{2+}) present under similar experimental circumstances. These studies demonstrated that the capacity of QDS to detect Hg^{2+} was not considerably hindered by other metal cations [see Fig.-4(iii)]. As a result, QDS is an incredibly selective fluorescence chemosensor for Hg^{2+} identification.

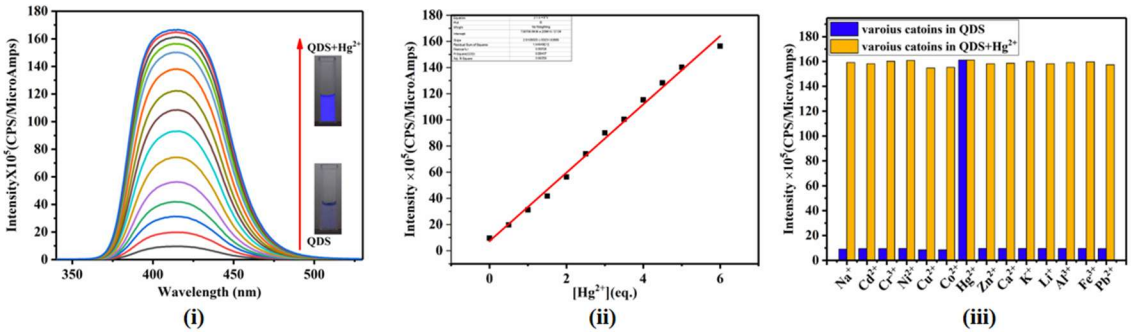

Fig.-4: (i) Fluorescence Spectra of QDS-Polymer (1 µM) upon Incremental Addition of Hg^{2+} Concentration (0 to 10 Equivalents). (ii) The Limit of Detection of QDS-polymer with Hg^{2+}. (iii) Fluorescence Response of QDS upon Addition of different Metal Ions (blue bars), followed by Addition of Hg^{2+} (yellow bars)

Analysis of River Water

To do this analysis, we prepared two sample solutions of river water, A1 and A2. Sample A1 contained only a riverwater sample that was boiled for five to ten minutes. Sample A2 was prepared purposefully by adding a known amount of Hg^{2+} ions to river water. Similar analysis conditions were applied for both river water samples to detect Hg^{2+} ions, as described earlier in this report. Both samples were tested separately with QDS-polymer solution (1.0 µM), and similar results were obtained, as provided in Figure 4(1). The addition of sample A1 to this solution (QDS-polymer in CH_3CN + PBS+ DMSO) did not appreciably change the fluorescence intensity. However, once sample A2 containing Hg^{2+} ions was added, the intensity of the QDS-polymer solution fluorescence was enhanced (turn-on). In river water, the detection limit for QDS-polymer (as a chemosensor) towards Hg^{2+} was calculated to be 9.68 nM, which was found to be the same as calculated for a standard Hg^{2+} solution.

CONCLUSION

This report has proposed and synthesized Quinoline-2,4-dicarbaldehyde-dithioacetal-based linear polymer. The ability of the polymer to form the soft gel and its Thermo-responsive behaviour was investigated by varying temperature and solvent conditions. The synthesized polymer showed excellent fluorescence sensitivity for Hg^{2+} ions and detected them very efficiently in the nanomolar concentration range. These polymeric chemosensors can be employed in bioimaging living cells and in chemical analysis to find Hg^{2+} ion residues in river water and other waste. In conclusion, for the first time, a unique class of well-defined quinoline-dithioacetal moieties containing polymeric chemosensors was developed for tunable sensing of Hg^{2+} ions. Besides the chemosensor uses in this work, earlier studies suggest these compounds hold great potential as electron-transport and electroluminescent materials. Analyzing similar chemosensors' characteristics can lead to their development with considerable promise for the aforementioned uses.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-6: Clean Water and Sanitation*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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