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Optically selective and electrochemically active chemosensors for Cu (II) ions based on a skeleton of 2-(benzylideneamino)-4,5,6,7-tetrachloro-3′,6′-dihydroxyspiro-[isoindoline-1, 9′-xanthen]-3-one

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A new series of fluorescent chemosensors (**7a-7f**) based on a core skeleton of 2-(benzylideneamino)-4,5,6,7-tetrachloro-3′,6′-dihydroxyspiro-[isoindoline-1,9′-xanthen]-3-one was synthesized and characterized by FT-IR, NMR and mass spectrometric techniques. The sensitivity and selectivity of probes (**7a-7f**) for Cu^{2+} ions were investigated by colorimetric, UV-vis absorption, fluorescence emission spectral studies and electrochemical analysis. These optical probes exhibited higher sensitivity and selectivity towards Cu^{2+} ions in DMSO solution over various other metal cations under consideration. Induced changes were observable by naked-eye. Based on fluorescence titration spectra and Job's-plot, it was found that the complexes formed between probes (**7a-7f**) and Cu^{2+} ions were in 1:1 stoichiometric ratio. The detection limit for chemosensors (**7a-7f**) were calculated to be 3.0834×10^{-7}–3.6425×10^{-7} M.

1. Introduction

Copper is an essential micronutrient that is vital for proper functioning of animals, plants and human bodies. It is the third most abundant transition metal in living systems and also naturally occurs in rocks, soil, water and sediments [1]. The world health organization (WHO) has recommended an adequate and safer level of copper intake to be approximately 1.3 mg/day [2]. Copper plays a crucial role in different biological and regulatory processes such as growth and development of bones, stimulation of immune systems, free radical defense, regulation of nerve impulse, formation and repairing of red blood cells as well as in the metabolism of glucose and cholesterol [3–6]. Likewise, many useful aspects of copper homeostasis are also known at the molecular level. It is an integral part of a variety of cuproenzymes, which are essential for metabolic processes like mitochondrial respiration, cross-linking of collagen, gene expression and synthesis of melanin pigments [7–10]. Furthermore, copper is also involved in ferrous-ferric redox transformation and resulting haemoglobin synthesis. The food sources contribute virtually all of the copper consumed by living organisms. The best dietary sources for copper include seafood, cereals, nuts, legumes, organ meats, fruits and chocolates. Drinking water also comprises about 20–25% of dietary copper. From decades, copper based medications have been suggested for patients suffering from anemia, osteoporosis, and osteoarthritis, for healing of wounds and during bypass surgery [11–13]. Acquired deficiency of copper implicated in the development of colon cancer, blood disorders (myelodysplastic syndrome), diabetes, impaired bones, poor motor coordination, and adult-onset myleoneuropathy. On the other hand, higher levels of copper are also regarded as potentially toxic due to associated health issues; memory loss, Alzheimer's disease, infertility, inflammatory disorders, premenstrual syndrome, anxiety, and stomach upset (diarrhea), Hashimoto's disease and hypothyroidism [14–17]. Accordingly, there is a high-up demand for the development of highly sensitive, selective and facile methods for detection of copper, in both physiological and non-physiological environments to suitably address these health issues, and has now become an active subject of current analytical and biochemical research [18].

Up till now, many technological advancements have been applied for qualitative and quantitative analysis of copper like voltammetry [19,20], ion selective membrane electrode [21–23], atomic absorption spectrometry (AAS) [24] and inductively coupled plasma mass spectroscopy (ICP-MS) [25] but of these, fluorescence based chemosensors provided many advantages [26–28]. Colorimetric and fluorescence techniques are typically of low-cost, highly sensitive and selective having capability of real-time detection of metal ions with high detection limit and naked-eye visualization. To date a variety of fluorescent chemosensors have been designed for Cu^{2+} ion but practical utility of many of them is limited due to complex multistep synthesis procedures and poor selectivity for Cu^{2+} ion as well as interference with Fe^{3+}, Co^{2+}, Hg^{2+} and Ni^{2+} ions [29–32].

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Selectivity and sensitivity for copper ions were achieved by introducing various ligands to core fluorophores, such as, rhodamine, fluorescein, pyrene, naphthalimide, coumarin, and BODIPY [29,33–37]. Researchers have also designed efficient and low-cost carbon-dot-based dual emission nanohybrid fluorescent sensors for in-vivo sensing and imaging of copper ions [29,38]. Kyunga et al. reported first example of Boronic acid-linked fluorescent and colorimetric probes for in-vivo imaging of copper ions at physiological pH of 7.4 with high detection limit (2.8×10^3/M) and selectivity [39]. Jun, Eun Jin, et al. reported fluorescein based chemosensor that effectively recognizes copper ions in nanomolar range. Furthermore, they also demonstrated the usefulness of the sensor by monitoring Cu^{2+} ion uptake by copper binding proteins such as transferrin and amyloid precursor protein, and later on, they also utilized this Cu^{2+}-sensor complex for the detection of cyanide ions in aqueous solutions [40].

Interestingly, fluorophores of long-wavelength emissions are often preferred to serve as the reporting group for analyte to avoid the influence of background fluorescence (<400 nm) [41]. Recently, many research groups have reported that the introduction of the rhodamine moiety to construct probes of the “off-on” type is a reliable method because of the well-known spirolactam (fluorescence “off”) to ring-opened amide (fluorescence “on”) equilibrium of rhodamine derivatives [34,42,43].

The past few years have witnessed a large number of reports on conjugated polymer based sensors that have been extensively studied because their absorption, emission, and redox characteristics are highly sensitive to environmental perturbations [44]. Among them, polydiacetylenes (PDAs) have attracted significant attention due to their unique chromatic properties [45]. Researchers have synthesized new azide- and alkyne-functionalized polydiacetylene (PDA-aa) vesicles and applied them as a new method for visual detection of Cu^{2+} ions [46].

Most of the advancements on copper ion sensors are stressed on the design of selective fluorescent probes that can be used in aqueous and polar organic solvents. In contrast, the investigation of sensors on a surface has been rarely studied. However, in terms of practicability, a surface sensor possesses more favorable properties than a fluorescent probe used only in solution phase [47]. So far, intense research interest in surface chemistry endows alternative strategies for making sensors with new or improved functionalities [48]. Zheng, Yujun, et al. have developed Langmuir and Langmuir-Blodgett (LB) films of several peptide lipids to detect copper ions in aqueous sub phase through surface interaction mechanism in the presence of other metal ions [49]. In short, the ever-increasing interest in the design and chemical synthesis of fluorescent probes for Cu^{+2} ions have evoked interests of many scientists.

In order to design simple, reliable and facile sensors for detection of Cu^{2+} ions with improved sensitivity and selectivity, we now report a series of 2-(benzylideneamino)-4, 5, 6,7-tetrachloro-3′,6′-dihydroxyspiro-[isoindoline-1,9′-xanthen]-3-one based probes (**7a**-**7f**) that were synthesized by coupling of 2-amino-4,5,6,7-tetrachloro-3′,6′-dihydroxyspiro-[isoindoline-1,9′-xanthen]-3-one (**5**) with suitably substituted aryl aldehydes (**6a**-**6f**). In the present experimental studies, Cu^{2+} ion is chelated with fluorescent probes (**7a**-**7f**) via nitrogen and oxygen atoms. The chelation effect was studied by UV-vis absorption and emission spectral data. Although, xanthene based derivatives are among the most widely studied fluorescent chemosensors but only few investigations have been reported on their aldehyde conjugates. Herein, we have introduced Schiff base chemosensors (**7a**-**7f**) for Cu^{2+} ions as shown in Scheme 1. Of note, the quenching of fluorescence emission in DMSO is easily observable upon addition of Cu^{2+} ion with a visible color change that can be seen with naked eyes. Cyclic Voltammetry has provided valuable outcomes regarding the electrochemical behavior of theses chemosensors. Accordingly to the results, the probes (**7a**-**7f**) are highly selective for Cu (II) detection as ˝turn-on˝ fluorescent chemosensors.

2. Experimental

2.1. Materials

All the reagents and materials used for analysis were of 99% purity. Tetrachlorophthalic anhydride was obtained from Sigma-Aldrich. Calcium oxide and zinc chloride were supplied by local suppliers. 4-Aminoobenzaldehyde, 4-Bromobenzaldehyde, 4-Methoxybenzaldehyde, 3-Nitrobenzaldehyde, 2, 4-Dichlorobenzaldehyde, 3-Hydroxybenzaldehyde and hydrazine were purchased from Merck. Solvents such as methanol, ethanol, petroleum ether, ethyl acetate, DMF and DMSO were common laboratory grade reagents. Ethanol was used after distillation at 78 °C with activated calcium oxide. Progress of the reaction was monitored by thin layer chromatography (TLC), using pre-coated silica gel aluminum plates with layer thickness 0.2 mm, HF_{254}, Riedel-de Haën purchased from Merck. Whatman Filter Paper 40 was used for filtration of the synthesized derivatives. For preparative thin layer chromatography glass plates (20×20 cm) coated with silica gel (HF_{254}, Fluka) of 0.5 mm layer thickness were used. Pre-coated silica gel plates (0.25 mm, Aldrich) were used for purification of the compounds under analysis.

2.2. Methods

^1H NMR spectra were recorded on 300 MHz Bruker NMR spectrometer in acetone-d_6. Splitting patterns were as follows: s (singlet), d (doublet), t (triplet), and br (broad). Chemical shifts were given in δ (ppm) scale. ^{13}C NMR spectrum were recorded at 75 MHz NMR spectrometer. FT-IR spectra were recorded on a Bio-Rad-Excalibur Series Model No. FTS 300 MHz spectrophotometer in the 4000–400 cm^{-1} region. Mass spectra (EI, 70 eV) was recorded using Agilent technologies (GC–MS) instrument. Elemental analysis was carried out on an Elemental Vario EL analyzer. Melting points of the synthesized derivatives were determined by using digital Gallenkamp (SANYO, Moriguchi, Japan) melting point apparatus model (MP-D BM 3.5) and were uncorrected. Ultraviolet-visible (UV-vis) absorption spectra were recorded on double beam Perkin-Elmer Lambda 900 UV-vis-NIR spectrophotometer with quartz cell of 1 cm optical path length at 25 °C. Fluorescence spectra were generated on Hitachi RF-5301 spectrophotometer. Cyclic Voltammetric studies were accomplished by Electrochemical Analyzer CH1830C and resulting data was used for finding redox potentials and LUMO and HOMO energy levels, following the reported procedures [25–28].

2.3. Synthesis of Xanthene Derivatives (7a-7f)

Synthesis of xanthene based derivatives (**7a**-**7f**) was accomplished in three steps as depicted in Scheme 1.

2.3.1.1. Synthesis of 4, 5, 6, 7-Tetrachloro-3′, 6′-Dihydroxy-3H-Spiro-[Isobenzofuran-1, 9′-Xanthen]-3-One (3). Tetrachlorophthalic anhydride (**1**) was first ground to a fine powder by using pestle and mortar. In a 250 ml, two-necked round bottom flask tetrachlorophthalic anhydride (**1**) (0.286 g, 1 mmol) and resorcinol (**2**) (0.22 g, 2 mmol) were heated under reflux conditions at 180 °C in the presence of catalytic amounts of $ZnCl_2$ as Lewis acid catalyst. After 2 h, a yellowish brown colored compound was obtained. The resulting compound 4, 5, 6, 7-tetra- chloro-3′, 6′-dihydroxy-3H-spiro-[isobenzofuran-1, 9′-xanthen]-3-one (**3**) was purified by recrystallization from methanol and then dried under vacuum filtration.

2.3.1.2. Synthesis of 2-(Amino)-4, 5, 6, 7-Tetrachloro-3′, 6′-Dihydroxyspiro-[Isoindoline-1, 9′-Xanthen]-3-One (5). In **step-2** of the synthetic Scheme 1, the compound, 4, 5, 6, 7-tetrachloro-3′, 6′-dihydroxy-3H-spiro-[isobenzofuran-1, 9′-xanthen]-3-one (**3**) (0.470 g, 1 mmol) was dissolved in hot ethanol (10 ml) and heated under reflux