Introduction

Iron is a component of heme and chlorophyll and serves as a micronutrient of plants and animals [1, 2]. Iron is also a part of several enzymes for its function during the biological redox process [3]. Moreover, due to rapid economic growth and industrialization in the past few decades, excessive iron is found in water and thereby the environment [4, 5]. Ferric ion (Fe$^{3+}$) from industrial effluent has the potential to poison animals and plants. Mining industries, wastewater from municipal and manufacturing sectors pose a threat to the environment and humans. Liquid wastes mixed with groundwater; corrosion of pipes was also a concern for iron pollution [6]. Potable water has been transported through pipes made of cast iron for several years, corrosion of such pipes results in soluble iron species contaminating the drinking water system [7]. Accumulation of iron beyond the acceptable level can lead to reactive oxygen and nitrogen molecules, which in turn can influence the peroxidation of lipids in the plasma membrane. Ferric ion waste also originates from iron packaging materials.
and several iron-based products that cause water and soil pollution and thereby harm aquatic organisms [7]. Thus, metal ions can enter the food chain and pose a serious threat to the digestive, nervous, respiratory, and reproductive tracts of human systems.

Among the micronutrients ingested through diet, iron is one among them. Human system need approximately 30 mg of iron per day for erythropoiesis. US Food and Nutrition and World Health Organization recommend 8 mg to 16 mg iron per day for adult men and women respectively [8]. Organic and Inorganic iron are two forms of assimilated iron through food. Inorganic iron exists as Fe²⁺ or Fe³⁺ as an elemental iron. Ascorbic acid present in the GI tract convert Fe³⁺ to Fe²⁺ which is more soluble and absorbed by the enterocytes of the GI tract [9]. Acute ingestion of iron is particularly harmful for children. Life-threatening toxicity is associated with paediatric intake of strong adult additives, such as prenatal vitamins. Also, Iron is present in many over-the-counter (OTC) multivitamins [10]. These days metal nanoparticles are also increasingly used in pharmaceutical, food and cosmetic industries which can also enter both human and environmental system. Nanomedicine can undergo metabolism and distribute in various organs of human systems [11, 12]. Iron associated with copper toxicity is also identified in adults, specifically when the adults are ageing [13]. Metal ion control is thus of considerable significance in living organisms and in environmental processes. Many advanced instrumental methods are commonly used to detect metal ions; however, the fast identification of metal ions requires powerful equipment, such as colorimetric and fluorescent chemosensors.

Over the past few decades, molecular fluorescent probes have been extensively developed for the detection of Fe³⁺ due to its high sensitivity and selectivity. This research summarizes the selective fluorescent probes for Fe³⁺, which demonstrates an increase in fluorescence (turn “ON”) response upon formation of Fe³⁺-probe complex. Fe³⁺ sensing power, processes, and analytical methodologies of recently developed fluorescent probes are discussed.

Such methods are simple, involving low cost, lower detection limit, high selectivity, and sensitivity, and are therefore used to track metal ions in real-time.

**Importance of Fe³⁺ and Its Detection by Rhodamine Chemosensors**

Fe³⁺ is one of the most important transition metal ions in living environments. It plays a crucial part in a multitude of physiological roles such as oxygen absorption, oxygen metabolism, and electron transfer [14, 15]. Deficiency and/or overdose of Fe³⁺ contributes to a wide range of disorders, namely Parkinson’s, Alzheimer’s, Huntington’s etc. [16]. Therefore, in drinking water, the maximum limit of iron was set to be 5.357 μM by the U.S. Environmental Protection Agency (EPA) [17]. Considering its importance in the biological system, many rhodamine based chemosensors were reported for the identification of Fe³⁺ [18-20]. However, researchers are continuing to develop the chemosensors with improved efficiency, sensitivity and selectivity for the recognition of Fe³⁺ ions [21-24]. An attempt also made to develop a Rhodamine B functionalized nanoparticle fluorescent probe and a FRET based composite probe based on UCNPs and Rhodamine Derivative for the detection of Fe³⁺ [25, 26]. In this review, we discussed Rhodamine B and Rhodamine 6G based Chemosensors for the selective detection of ferric ions in ecological and biological samples with their detection limits. Moreover, Rhodamine Chemosensors are also selective in the detection of Cupric (Cu²⁺) ions [27].

**Chemosensors for Biological Applications**

**Pyridine Type Fluorescent Chemosensors**

Kan along with co-workers reported two new pyridine-type fluorescent chemosensors 1 and 2 for sensing the ferric ion in the aqueous media, further sensing activity was analyzed using fluorescence and UV–Vis spectroscopic techniques [28]. Chemosensors 1 and 2 displayed excellent sensitivity and selectivity towards Fe³⁺ in ethanol/water system (3:1 v/v; 0.5 mM HEPES; pH = 7.33). In addition, cell imaging study performed with living human breast adenocarcinoma (MCF-7) cells, showed that chemosensor 1 and 2 could be used effectively to detect Fe³⁺ in living cells due to their favourable molecular properties. Stoichiometry of 1-Fe³⁺ was determined to be 1:1, whereas 2 showed 1:2 binding stoichiometry towards Fe³⁺.  

**Terephthaloyl Chloride Based Chemosensor**

Liu et. al found an “off–on” colorimetric and fluorescent probe 3 that demonstrated a extremely selective and receptive reaction to Fe³⁺ in the THF–water system (v/v:4/6, pH:7.2) with binding stoichiometry of 1:2 between probe and Fe³⁺ [29]. A linear fluorescence enhancement was observed at 584 nm with the incremental addition of Fe³⁺ to a solution of 3, from 0.1 to 26 μM Fe³⁺ concentration. Other competitive metal ions rarely affected the recognition of Fe³⁺ except for Hg²⁺ and Al³⁺. Probe 3 was used to analyze Fe³⁺ in human blood serum and water samples and cell imaging of Fe³⁺ in living SGC-7901 cells indicates that the probe can have its practical application value in biological and environmental samples.

**Furan-2,5-dicarbaldehyde Based Chemosensor**

Dey and co-workers synthesized a probe 4 from rhodamine hydrazide and furan-2,5-dicarbaldehyde [30]. Probe 4 could detect Fe³⁺ selectively through the “off-
Selective Identification of Ferric Ions...

...mechanism among the other co-existing metal ions in semi-aqueous media. Upon addition of one Equiv. of Fe$^{3+}$ to probe 4, the pH of the solution turned to 3.8 with the formation of a highly fluorescent complex. Further, the fluorescent intensity was decreased gradually up to pH 10 by altering the pH from 2 to 12. Moreover, cell-imaging and cytotoxicity study showed that probe 4 is safe and non-toxic to a wide range of cells.

Benzimidazole Based Chemosensor

Li et. al designed and prepared a sensitive and selective chemosensor 5 based on rhodamine-benzimidazole for sensing of Fe$^{3+}$ in aqueous media over a pH range 5.8-7.4 [31]. After addition of Fe$^{3+}$ to probe 5 in Tris-HCl buffer solution (2.5 mM, pH 7.0), recognizing event was completed in 10 s; this rapid response indicated the usefulness of the probe for real sample analysis. Further, an imaging study using MGC803 cells confirmed that the probe could be used for effective monitoring of Fe$^{3+}$ in living system.

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Chemosensors with Nitric Oxide Donor Atoms

Ali’s group synthesized a novel di-coordinating chemosensor 6 bearing NO donor atoms for selective...
and rapid recognition of Fe$^{3+}$ with co-existing biologically relevant and toxic metal ions [32]. The formation constant of chemosensor 6-Fe$^{3+}$ was computed to be 1.72×10$^4$ M$^{-1}$ with a detection threshold of 0.17 μM. DNA interaction study showed that the interaction of chemosensor 6-Fe$^{3+}$ with DNA takes place through a groove binding mode. Further, negative $\Delta G^0$ and positive $\Delta S^0$ values obtained by the calorimetric technique, indicated that the binding of [chemosensor 6-Fe]$^{2+}$ with DNA is spontaneous. [Chemosensor 6-Fe]$^{2+}$/DNA complex can be used for future photonics and biological applications.

### Cytocompatible Chemosensors

Chemosensor 7 reported by Jin et. al can be used for the optical recognition of Fe$^{3+}$ over other usually coexisting metal ions in an aqueous system [33]. Adding ions to the solution of chemosensor 7, Fe$^{3+}$ and Hg$^{2+}$ could induce enhanced fluorescence intensity at 582 nm and 587 nm; however, the fluorescence intensity of Hg$^{2+}$ was far weaker than that of Fe$^{3+}$, this would help to distinguish the existence of Fe$^{3+}$ and Hg$^{2+}$. The probe 7 showed good cytocompatibility and low toxicity to HeLa cells and zebrafish, indicating its worth importance in biological aspects.

### Furfuran-Based Chemosensor

A Furfuran based Chemosensor 8 developed and synthesized by Zhou, Tong, et al. can be use effectively for the detection of Fe$^{3+}$ [34]. Chemosensor 8 shows “Turn-On” response in the presence of Fe$^{3+}$ ions and “Turn-Off” response when B$_2$O$_5^{2-}$ added to the solution of 8-Fe$^{3+}$ in EtOH/H$_2$O solution at pH 7.20. The association constant for Fe$^{3+}$ was determined to be 1.33×10$^4$ M$^{-1}$. Further, fluorescence imaging study performed with human cervical carcinoma cells revealed that Chemosensor 8 has low cytotoxicity and good biocompatibility.

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9 can be used as a potential probe for detection of Fe$^{3+}$ in biological samples.

2-morpholinoethyl-1-amine Based Chemosensor

Cuicui Jiang and co-workers synthesized and evaluated two low cytotoxic “OFF-ON” fluorescent probes 10 and 11 which exhibited excellent selectivity towards Fe$^{3+}$ ions [36]. Fluorescence intensity response of 10 and 11 showed excellent linear correlation for Fe$^{3+}$ in the concentration range the range of 0–60 µM. Further, 10 and 11 binds to Fe$^{3+}$ in the 1:1 and 2:1 stoichiometric ratio respectively. The binding of 10 and 11 was reversible to Fe$^{3+}$ in the presence of high concentration of F$^-$ to form Na$_3$[FeF$_6$] complex. Cell viability study performed using HepG2 cells with 10 µM of concentration of both the probes did not show any toxicity, reveals that both the probes could be used for the detection of Fe$^{3+}$ in biological samples.

2,4-di Tertiary Butyl Phenol Based Chemosensor

A Rhodamine-3,4-dihydro-2H-1,3-benzoxazine, sensitive and selective fluorescent probe 12 was synthesized by Habib Ali Molla and co-workers for Fe$^{3+}$ [37]. Upon addition of Fe$^{3+}$, fluorescence intensity of 12 enhanced by 240-fold with binding constant of 1.50×10$^4$ M$^{-1}$. Complex 12-Fe$^{3+}$ revealed reversible binding with tetra-n-butylammonium fluoride in extra as well as intracellular conditions. Bioimaging study performed on HepG2 cells showed that present probe could be used for the recognition of cytoplasmic Fe$^{3+}$ as low as 1 µM concentration.

Thiophene Based Chemosensor

A thiophene based rhodamine derivative 13 synthesized by Wang, Kun-Peng, et al. exhibited high response speed towards Fe$^{3+}$ in ethanol/water system at 7.2 pH [38]. Job’s Plot analysis, $^1$H NMR spectra, mass
spectrum and theoretical calculations confirms the 1:2 stochiometric ratio of Fe$^{3+}$/13. Addition of Fe$^{3+}$ induced intensive absorption band at 560 nm with change in color from colorless to pink. Moreover, among the various background metal ions; Cr$^{3+}$ and Al$^{3+}$ also induced fluorescence signals response, however they were observed about 2 and 3 times lower than that caused by Fe$^{3+}$ indicates selectivity towards Fe$^{3+}$ over the other metal ions. Further confocal fluorescence imaging study revealed that, probe 13 is cell-permeable and could be used to detect Fe$^{3+}$ ions effectively in living cell.

**Chemosensor Consisting Thiocarbonyl Unit**

Gao, Zhigang, et al. reported a rhodamine B-based fluorescent probe 14 consist of thiocarbonyl moiety for Fe$^{3+}$ [39] pH stability study showed the stability of probe 14 over the pH range of 5.0-11.0 with little background fluorescence. With addition of Fe$^{3+}$ to a solution of 14, a naked eye color change observed from colorless to pink with significant fluorescence enhancement at 582 nm. The binding constant of 14 with Fe$^{3+}$ was determined to be 2.75×10$^4$ M$^{-1}$ with 1:1 stoichiometry. Further, live cell imaging study in the Hella cells revealed that probe 14 is biocompatible in nature and could be used as ana effective tool for rapid detection of Fe$^{3+}$ in the Biosystem.

**Pyrrole-2-carbaldehyde Based Chemosensor**

A pyrrole-based Rhodamine-B derivative 15 synthesized by X. Bao and co-workers could specifically recognize Fe$^{3+}$ ions in a MeOH/H$_2$O (3:2, v/v, pH 7.10, HEPES buffer, 0.1 mM) system by forming 1:1 complex with Fe$^{3+}$ [40]. With addition of 5 equiv. of Fe$^{3+}$ to a solution of 15, a more than 100-fold fluorescence enhancement was observed. 15-Fe$^{3+}$ complex was found to be dissociable in the presence of sulfide ions among many other, indicates that 15-Fe$^{3+}$ complex could be

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an efficient sensor for S²⁻ ions. Applicability of probe was examined on cultured normal human liver cells (L-02) and study showed that probe 15 might be a cell permeable and non-toxic to the cell.

### Chemosensors for Ecological Applications

#### Glyoxal Based Chemosensor

Wang et al. reported probe 16 based on Rhodamine 6G that exhibited a strong response to Fe³⁺ in CH₃CN/Tris-HCl buffer (9:1, v:v, pH 7.00) [41]. Upon interaction of Fe³⁺ with probe 16, the colorless solution turned to pink. Further, Job’s plot analysis revealed 2:1 binding stoichiometry for 16-Fe³⁺ with an association constant of 1.4×10¹⁰ M⁻². The interaction between probe 16 and Fe³⁺ was chemically reversible to 200 μM of AcO⁻. As a practical applicability probe was used for the determination of Fe³⁺ concentrations in real water samples (Drinking water and distilled water) with a good recovery from 93.60% to 97.70%.

#### 2-Naphthylamine Based Chemosensors

Han, Xiang, et al. reported a Rhodamine B lactum based double schiff’s base fluorescent probe 17 for the recognition of Fe³⁺ [42]. Upon addition of Fe³⁺, the probe 17 solution turns from colorless to pink and showed strong absorption enhancement in a wide pH range of 5.54 to 10.12. The binding constant of 17 with Fe³⁺ was determined to be 0.25×10⁴ M⁻¹ suggesting that the probe has well binding affinity towards Fe³⁺. Further, intensity of 17-Fe³⁺ complex was completely quenched in the presence of EDTA solution, indicates reversibility of probe 17. The recovery of Fe³⁺ from 88.5% to 112.8% in Weihe River water sample showed that, probe 17 has great potential in real sample analysis.

#### Hybrid PVOH Film-Based Sensor

A Rhodamine based reusable Hybrid polymeric (PVOH) chemosensor 18 synthesized using sol-gel

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<td><img src="image" alt="Structure 18" /></td>
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technique by A. Pipattanawarothai and T. Trakulsujaritchok gives high selectivity towards the detection of Fe$^{3+}$ in aqueous system [43]. Out of three chemosensor films PV-3TE-S10, PV-3TE-S15 and PV-3TE-S20; PV-3TE-S15 showed the most distinct sensitivity towards Fe$^{3+}$ with shorter time to reach optimum absorption intensity. Sensor film turned to be prominently pink in color upon interaction with Fe$^{3+}$, moreover original color of sensor film regained after washing with 0.1 M ethylenediamine enabling repeated use in the detection. Applicability and reusability of sensor film was proven using tap water and commercial drinking water without significant reduction in its response.

5-nitro-2-thiphenecarboxaldehyde Based Chemosensor

Geetha Balakrishna R. and group demonstrated a robust reversible biocompatible Rhodamine 6G based probe 19 for the recognition of Fe$^{3+}$ ions [44]. The detection limit of Fe$^{3+}$ was determined to be 8.2 nM. The effectiveness of the probe towards the recognition of Fe$^{3+}$ was proved in human blood samples and the obtained results were also validated by AAS and ICP-OES techniques. Moreover, the probe 19 was found to be highly effective for the recognition of Fe$^{3+}$ ions in real samples like Iron syrup, Iron tablets, industrial effluents and tap water as well. Obtained results of these samples with AAS technique were found to be comparable to that of obtained using probe. Confocal laser scanning microscopy study with NIH 3T3 cells revealed that probe 19 could be used effectively for the detection of Fe$^{3+}$ in live cell.

Sulfonate Substituted Chemosensor

Y. Yang, Z. Guo, J. Ye et al. designed and synthesized two rhodamine hydrophilic fluorescent probes 20 and 21 bearing sulfonate groups exhibited selective response to Fe$^{3+}$ [45]. Both the probes were demonstrated an apparent fluorescence enhancement with exponential

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linear relationship from 0 to 16 µM concentration of Fe$^{3+}$ \[R_1^2 = 0.9942 \text{ (for 20-Fe}^{3+})\], \[R_2^2 = 0.9967 \text{ (for 21-Fe}^{3+})\]. Both the probes were displayed a satisfactory recovery and accuracy when applied to the real water sample analysis (tap water and river water) for the detection of Fe$^{3+}$. Fluorescence imaging study performed in lampfish confirmed that probe 21 more likely to distribute into abdomen through blood circulation and digestive process than probe 20 implies that probe 21 could be effective tool for the detection of Fe$^{3+}$ in living organism.

**Amino Acid Derivative Chemosensor**

Among the five different kinds of Rhodamine B/ amino acid based fluorescent probes (RhB-Gly, RhB-Ala, RhB-Try, RhB-Cys, and RhB-His) synthesized by H. Li, Z. Liu and R. Jia, RhB-His probe 22 displayed remarkably sensing performance towards Fe$^{3+}$[46]. After the addition of Fe$^{3+}$ about 202 folds fluorescence enhancement observed at 584 nm. This fluorescence enhancement was linear in the concentration range of 0 to 20 µmol/L for Fe$^{3+}$ with a detection limit of 0.25 µmol/L which is remarkably lower as compared to other four probes. Further application of probe in real sample analysis (tap water and river water) exhibited good recovery ranges from 92.7% to 104.0%. Moreover, probe 22 was successfully applied to the determination of Fe$^{3+}$ in tannic acid iron ink.

**Cuminaldehyde Based Chemosensor**

A fluorescent reversible probe 23 developed by Pattan Sirajuddin Nayab and group displayed 48-fold fluorescence enhancement for the Fe$^{3+}$ with naked eye detection [47]. Probe exhibits greater sensitivity and selectivity towards Fe$^{3+}$ with low LOD (5.05 µM) and high association constant ($3.42 \times 10^4$ M$^{-1}$) values over other biologically important metals. Since probe found to be insensitive to the pH between 5-8 it can be served as a potential sensor for the biological application under

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<td>Fe$^{3+}$: 0.91×10$^{-4}$ M, Cu$^{2+}$: 1.04×10$^{-4}$ M</td>
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physiological conditions. Applicability of probe 23 was proven for the determination of Fe$^{3+}$ in drinking, distilled and tap water samples with a good recovery value ranges from 101.3% to 106.6% with a good RSD limit within 5% for three determinations of each sample.

### Bifunctional Chemosensor

Wang, Lei, et al. designed, synthesized and characterized a dual chemosensor 24 for the concurrent detection of Fe$^{3+}$ and Cu$^{2+}$ among various background metal ions [48]. With addition of Fe$^{3+}$ and Cu$^{2+}$ to a solution of 24, new absorption bands were appeared at 552 nm and 545 nm for 24-Fe$^{3+}$ and 24-Cu$^{2+}$ complexes respectively in UV-Visible spectroscopy, whereas other metal ions did not exhibit formation of new band. In the presence of Fe$^{3+}$, a solution of 24 turns to pink colored, while it turns to red purple in the presence of Cu$^{2+}$. Interestingly in fluorescence spectroscopy, 24-Fe$^{3+}$ showed fluorescence emission band at 582 nm, however, 24-Cu$^{2+}$ not responded to fluorescence spectra because of quenching of fluorescence due to paramagnetic nature of Cu$^{2+}$. Further, as practical application-soaked bar paper in 10$^{-3}$ M solution of 24 was dried and dipped in various concentrations of Fe$^{3+}$ and Cu$^{2+}$ ranging from 0 M to 10$^{-3}$ M, where color of solution changed from white to red in 10$^{-3}$ M Fe$^{3+}$ solution, whereas it turns to purple in 10$^{-3}$ M Cu$^{2+}$ solution indicates that probe could be used quick visual test strip for Fe$^{3+}$ and Cu$^{2+}$ in environmental samples.

### Arylpropenone Azo Dye Appended Rhodamine Based Dual Chemosensor

Rhodamine based Arylpropenone Azo dye chemosensor 25 developed by A. Sağırli and E. Bozkurt could be competently used for the detection of Fe$^{3+}$ as well as for Cu$^{2+}$ among the other metal ions [49]. A shoulder at 342 nm appeared in absorption band and fluorescence peak at 589 nm for the 25-Fe$^{3+}$ complex. Job’s plot analysis showed that the stoichiometry ratio for the 25-Fe$^{3+}$ complex was determined to be 1:2 and it showed reversibility with EDTA. Response time of probe for both the ions was determined to be as short as 0.5 minutes indicates that probe 25 could be used for the faster detection of Fe$^{3+}$ and Cu$^{2+}$ in environmental samples.

### Chemosensor for Logic Gate Application

Diarylethene Derivative

Xu et al. synthesized a new diarylethene derivative 26 bearing rhodamine 6G by Schiff base condensation probe 26 co-ordinates with Fe$^{3+}$ in 1:1 stoichiometry with a color transition to distinct pink from a colorless solution [50]. When 10.0 Equiv. of Fe$^{3+}$ was added to probe 26 fluorescence intensity was enhanced by 168-fold. The co-ordination of probe 26 with Fe$^{3+}$ was restored upon the addition of 10.0 equivalent of EDTA solution to 26-Fe$^{3+}$. Probe demonstrated an obvious acidic chromism to TFA and could function as a reversible fluorescence photo switch in response to UV/vis light irradiation, therefore a logic circuit was constructed with the combinational stimuli of UV/vis light and Fe$^{3+}$/EDTA as inputs and the fluorescent emission intensity at 585 nm as the output.

### Conclusions

Ferric ion-based pollutants arise from various environmental and biological sources. Excess ferric ion in the human system [51] can pose a threat to human health. Therefore, it is important to identify and detect ferric ion using a simple, rapid and reliable method. Rhodamine chemosensors are demonstrated as a reliable analytical method to detect the ferric ions. Notably the detection limits of Fe$^{3+}$ were found to be comparable for the biological and ecological applications. For biological applications LOD values for Fe$^{3+}$ were determined to be in the range of 2.74 µM to 11.6 nM and that of for environmental applications LOD values were calculated
to be in the range of 5.05 μM to 4.63 nM. Probe used in logic gate application demonstrated a detection limit of 65 nM for Fe

3+. Data revealed that, most of the interaction ratios between the probe and Fe

3+ were 1:1. Chemosensors are also cost-effective platform for detection of ions [52]. Structures of chemosensors [53, 54] can also be modified to enhance its sensitivity and selectivity.

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

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