Fluorescein based Sensing Probes: A Review

Navjot Sandhu[1], Atul Pratap Singh[2*], Jagdeep Kumar[3], Ashish Pratap Singh[4]

[1]Navjot Sandhu-Pursuing Doctorate of Philoshphy in Chemistry at Chandigarh University, Gharuan, Mohali, Punjab, India.

[2]Atul Pratap singh, Associate Professor, Department of Chemistry, Chandigarh University, Gharuan, Mohali, India.

[3]Jagdeep Kumar, Assistant Professor, Department of Chemistry, Chandigarh University, Gharuan, Mohali, India.

[4]Ashish Pratap Singh, Research Associate, Materials Group, Material Processing and Corrosion Engineering Division, Bhabha Atomic Research Center (BARC), Mumbai

navjotsandhu2326@gmail.com, +91-9780447357

atulpiitd@gmail.com

Abstract

There is plethora of fluorescent probes that have been reported in the last decade. Out of these fluorescein based probes have made a class with wide category of probes. In the present review we have tried to sum up various fluorescein based sensing probes developed in the recent years. These probes have wide biological as well as chemical scope.

Keywords:*Fluorescein, Fluorescent probes, sensors*

Introduction

Many efforts have been made for developing sensing technologies to detect the number of chemical species for their utmost importance to the various science branches, mainly including chemical, environmental and biological sciences. In this aspect fluorescence probes are widely encountered, for the monitoring of various environmental pollutants, cellular, sub cellular biochemical processes in the living organisms[1,2]. The fluorescent sensing probes has gained much popularity due to their potential applicability in the numerous fields from biological research to the clinical diagnostics.

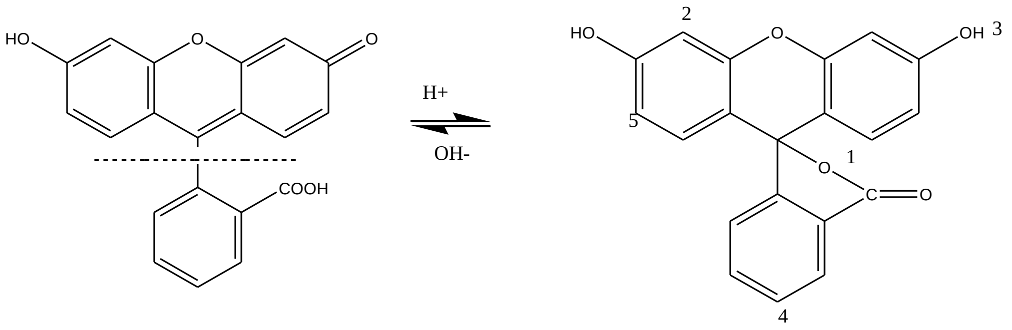
In comparison to the traditional techniques like titrimetry, chromatography, electrochemistry, chemi-luminiscence and flow injection analysis, these probes provide fast responding, highly sensitive, non-sample destructing and on site analysis of specific targets[3,12]. Synthetic organic molecules based fluorophores are more beneficial over available organic molecules, metal nanoclusters, semiconductor quantum dots (QDS) etc. as these can be chemically modifies to control its properties and sensitivity[12].

Spiro lactam structure of Fluorescein makes it an interesting molecule to be used as a fluorescent probe[4,12]. As both the functionalities i.e. nucleophillic (phenol groups) and electrophillic (carboxylate groups) are present in the free acid form of fluorescein, which makes it more available as the good flouregenic building unit for the organic synthesis[5,11]. However, they lack behind in making specific class of analytes due to their poor selectivity because of the presence of oxygenated functional groups all around its molecular structure. Therefore, the modification of these marginal functional groups may allow the enhancement in the specificity and selectivity of this chromophore to make it more widely explored fluorescent probe or dye[5,11]. With this concept in mind, modification of this xanthenes moiety could be envisioned. Fluorescein based derivatives are widely used fluorescence as they generally have good water solubility, give high response to the pH variation, give high-intensity emission peaks, have high molar absorption coefficients. They give good fluorescence quantum yields and have adorable penetration capacity towards cell membrane[6-11]. A plethora of evidences support their ability to detect and quantify metal ions.

Design Mechanism of Fluoresein Probes

In fluorescein two moieties: Xanthene ring and benzoic acid moiety, orthogonal to each other, are available for the modification by organic synthesis. Both the moieties possess unique characteristics. Strategies based on the rational designing on the five available modification sites (fig. 1) of the two moieties allows us to design a plethora of fluorescein probes.

Xanthene Ring (6-hydroxy-3H-xanthen-3-one)



(Benzoic Acid moiety)

Fig. Fluorescein's pH changes and the sites available for modification.

The number of fluorescein based probes have been developed in recent years, that recognize various metal ions like Fe^{3+}, Fe^{2+} Cu^{2+}, Zn^{2+}, Hg^{2+} and Mg^{2+}. It has been well proved that, these fluorescein based fluorescent probes generally exist in a colorless and non-fluorescent spiro-cyclic form. But, when any specific metal ion is added, the ring-opening of the fluorescein moiety occurs upon complexation of these probes with these metal ions, which results in the color and fluorescence.[13a]. In this review, we have tried to sum up the various Fluorescein based fluorescent probes, fabricated in the last few years. Many efforts have been made in last few years by many reviewers to summarize the various fluorescein based probes that are used for metal ion detection and in some sensing in biological system. We have listed some of them over here. Formica et al[13b] explored number of chemosensors that has been reported out of which they widely encountered fluorescent probes based on fluorescein which are successfully used for the detection of heavy metal ions. Carol et al [13c] has tried to analyse the various available fluorescent probes for the detection of Zn^{2+}. Davis[13d] has reviewed the various newly modulated probes of fluorescein with the modern techniques and their applicability in metal detection and biological field. Aron et al[13e] has gone through the various fluorescein probes for detection of iron in biological system. Li et al[13f] checked out various crown ethers bearing fluoresceinprobes for detection of various analytes. Yan et al [13g] has clubbed up various fluorescein based probes that are used for the detection of ROS/RNS or metal ions and highlighted the properties of synthesized probes. Goldberg et al[13h], in his review focussed on the available sensors for mobile zinc ions and in the review he has widely checked out the applicability of fluorescein based sensors for zinc detection.

Carrying forward we have summarized the ongoing research in this field in the present review paper

Various fluorescein based Probes

Li et al synthesized novel chromone-fluorescein conjugate i.e. 6-Hydroxy-3-formylchromone fluorescein hydrazine and proved it as Mg^{2+} turn on fluorescent sensor. The probe showed high selectivity and sensitivity for Mg2+ in comparison to other metal ions, and showed good enhancement in fluorescence at 504 nm in the presence of Mg2+, which was marked to be the ring opening process of the fluorescein fluorophore in probe 1 because of complexation of the probe with Mg2+. Furthermore, the authors claimed that the “turn on” response of this probe towards Mg2+ was almost completed in 3 min, it indicates that the designed probe could be used to sense Mg2+ for real-time detection.[14]

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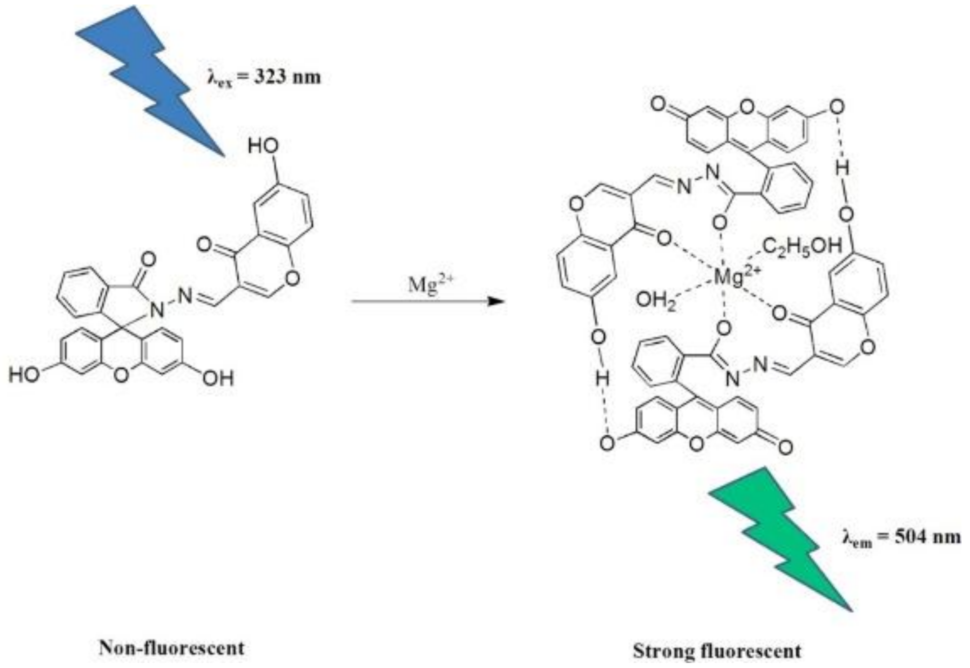
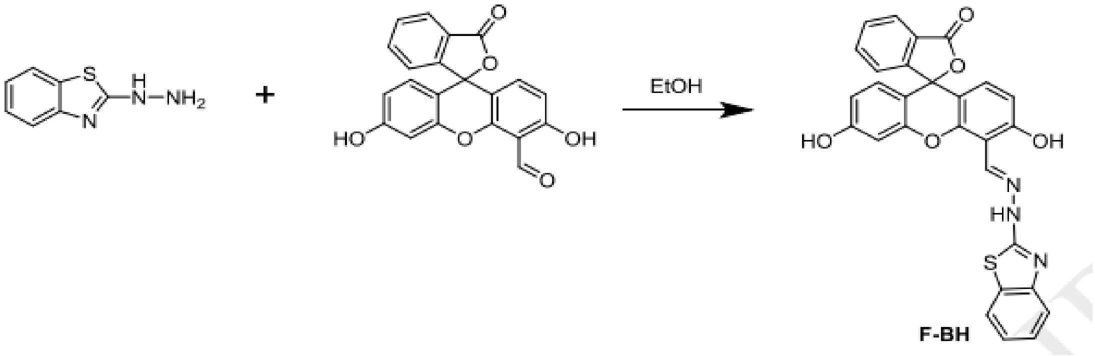
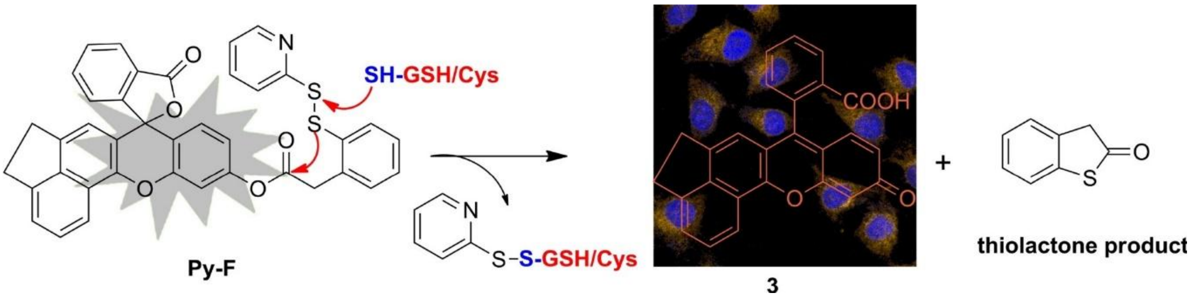


Li et al[15] reported a simple fluorescent probe **F-BH** in which they introduced $C=N$ group which acted as a recognition site for hypochlorous acid in the fluorescent probe molecule. They synthesized the probe by using 2-benzothiazole hydrazone and formylated fluorescein. The probe **F-BH** solution possessed very fluorescence intensity, however when ClO^- ion was added to probe solution its intensity get largely enhanced. The selectivity and sensitivity experiments proved that the probe is highly selective towards hypochlorous acid over other ROSs/RNSs and anions with a low detection limit (17.5 nM).



Hou[16] with his co-workers prepared a new probe based on fluorescein (**Py-F**) which was an optical as well as fluorometric sensing tool in case of biological thiols. The probe was fitted on with disulfide group, which showed good selectivity for glutathione (GSH) as well as cysteine (Cys) in comparison to homocysteine (Hcy) with the detection limit of 0.12 µM and 0.13 µM, respectively and sensitivity too. The recognition mechanism was explained by DFT calculations, and was confirmed by mass spectroscopic measurements. Biological studies revealed that the probe has good permeability towards cell membrane so can be used to detect these thiols in the biological system too.



Xu and his co-workers[17] designed a new probe for detection of hydrazine. The probe was based on ICT and PET mechanism. Phthalimide and acetyl ester groups acted as site for recognition. When hydrazine was added, it resulted into a turn-on fluorescence at 525 nm and also the color change was observed, from dark to yellow. The probe had good detection limit 0.057 µM and was able to successfully detect hydrazine selectively and bio-imaging was done too.

PET (photo-induced electron transfer) in the layman terms is the transfer of an excited electron from donor to acceptor. Due to PET process separation of charge occurs, *i.e.*, in excited state redox reaction occurs. When a molecule gets excited by a photon, higher energy orbital can be occupied by an electron in a ground state orbital. Excitement of electron leaves a hole or vacancy in the ground state which an electron donor can fill. It corresponds to donation of an electron to acceptor by leaving electron in an orbital with higher energy. So, this way, molecule which is photo-excited can act as reducing agent or oxidising agent in good manner.

In the fig below, the simple design of fluorescent sensors has been shown. A fluorescent sensor is made up of three components Fluorophore, spacer and receptor. Transfer of electron occurs from receptor to fluorophore, due to excitation of fluorophore part of the chemo-sensor in its off state. Energy of fluorophore should be sufficient such that it can match with fluorophore’s reduction and receptor’s oxidation potential. The condition was stated by Weller first time in 1968[18]. However, when analyte reaches receptor site the PET process from receptor to fluorophore stops and results in turning on or fluorescence.

Fig. 1 The 'fluorophore–spacer–receptor' format of fluorescent PET sensors.

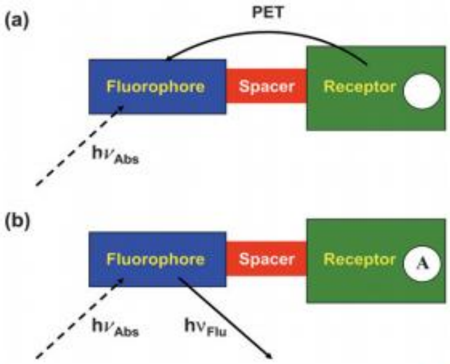


Fig. 2 (a) An electron transfer from the analyte-free receptor to the photo-excited fluorophore creates the 'off' state of the sensor. (b) The electron transfer from the analyte-bound receptor is blocked resulting in the 'on' state of the sensor.

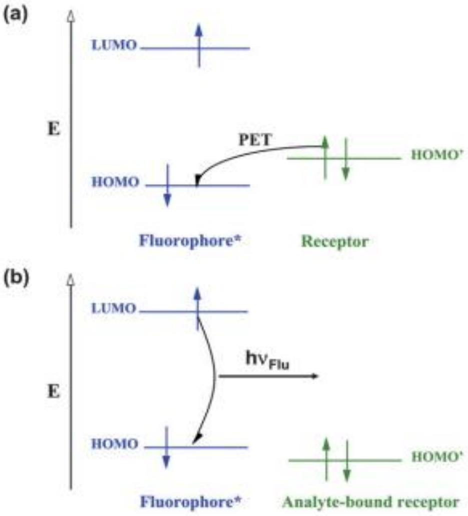
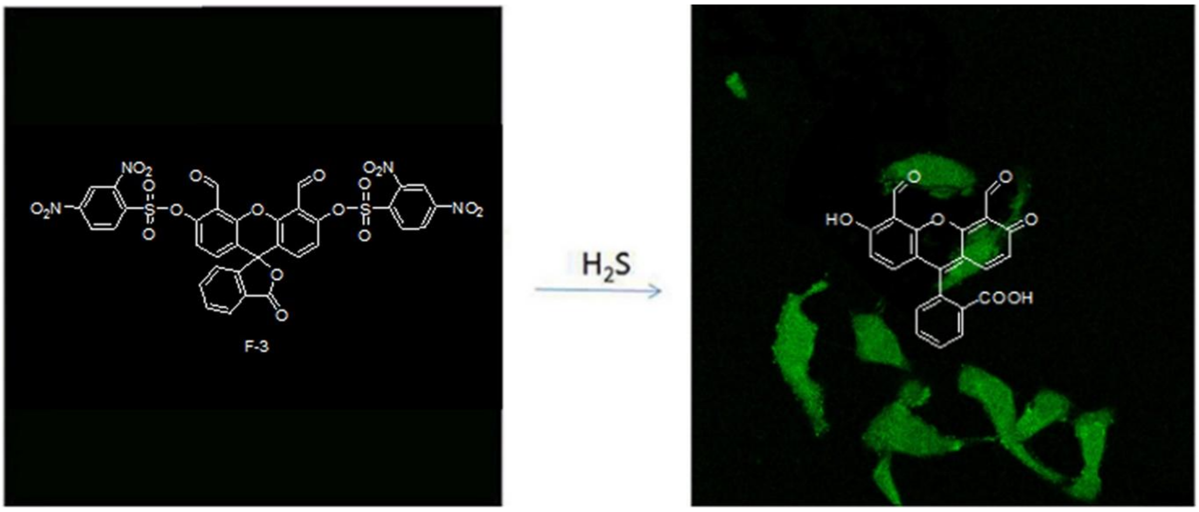


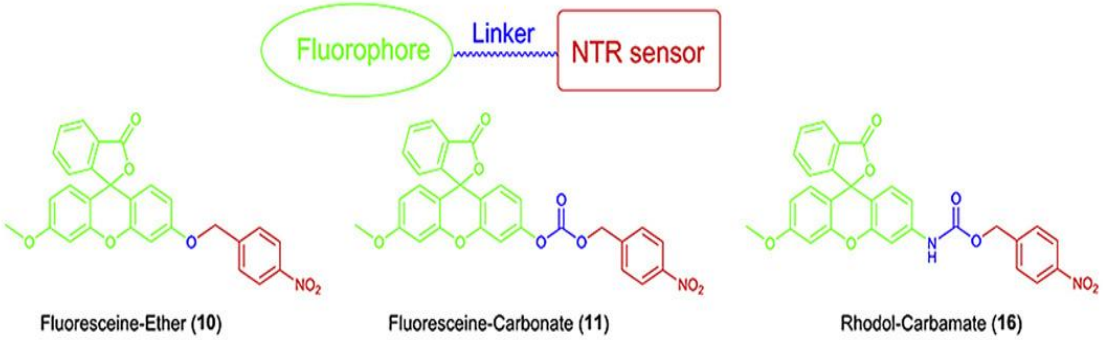
Fig. 3 Molecular orbital energy diagrams which show the relative energetic dispositions of the frontier orbitals of the fluorophore and the receptor in (a) the analyte-free situation and (b) the analyte-bound situation.

A case study can easily be done by using H^+ ion as an analyte, where an ion increases receptor's oxidizing potential by electrostatically attracting the electron, which makes un-favorable thermodynamic conditions for PET[19,20,21]. This can also be explained using Molecular orbital energy diagram (Fig. 3). We found that PET process takes place if the receptor's oxidation potential is less than fluorophore. However, opposite condition in case of sensor's on-state.

Lv[22] with his team successfully synthesized three fluorescein based tools. The probes were incorporated with dinitrobenzene sulfonate groups on hydroxy positions of fluorescein. Three probes were formed by incorporating this group on two hydroxyl positions in fluorescein, mono-aldehyde and bis-aldehyde of fluorescein. The author found that all the three probes responded to H_2S. However, response of bis-aldehyde fluorescein based derivative was much faster than that of fluorescein derivative. It is due to the reason that ortho-effect of aldehyde, leads to rapid nucleophilic addition of H_2S to aldehyde of the fluorescein and thiolysis of dinitrophenyl ether intramolecularly.



Hypoxia which is a major characterstic of tumor and hinderance in chemotherapy, can be easily biomarked by the nitroreductase which reduces the nitro group of fluorophore or drug to amine and leading to 1,6- reductive elimination for free drug release or free fluorophore. More[23] with his research team synthesized probes incorporated with nitrobenzyl moiety that respond to nitroreductase. Fluorescein ether and fluorescein carbonate based tools were among the synthesized probe.

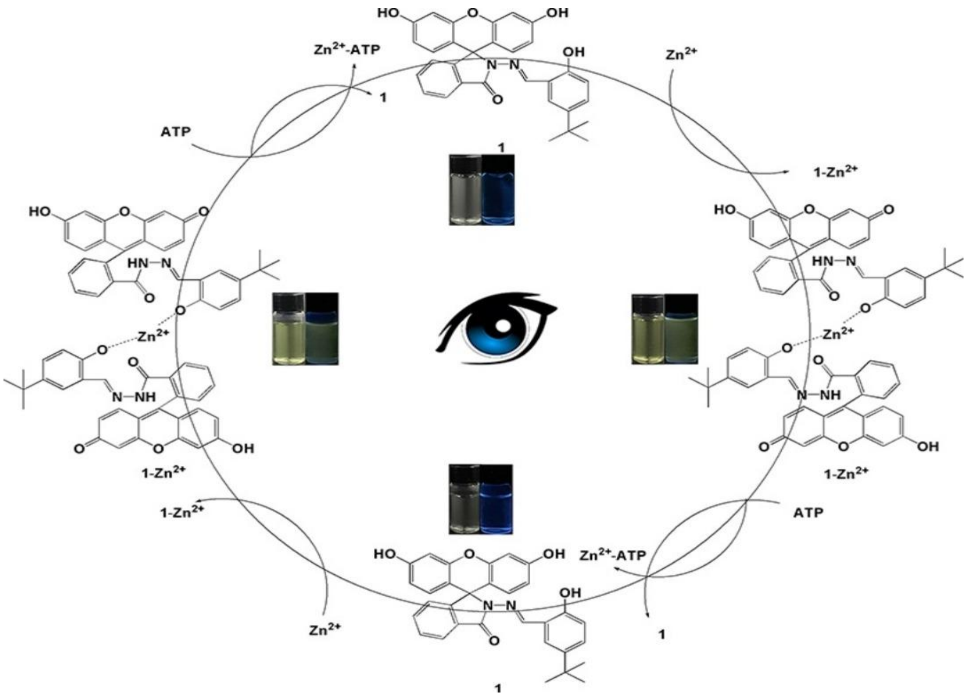


Nitroreductase-responsive fluorescent probes

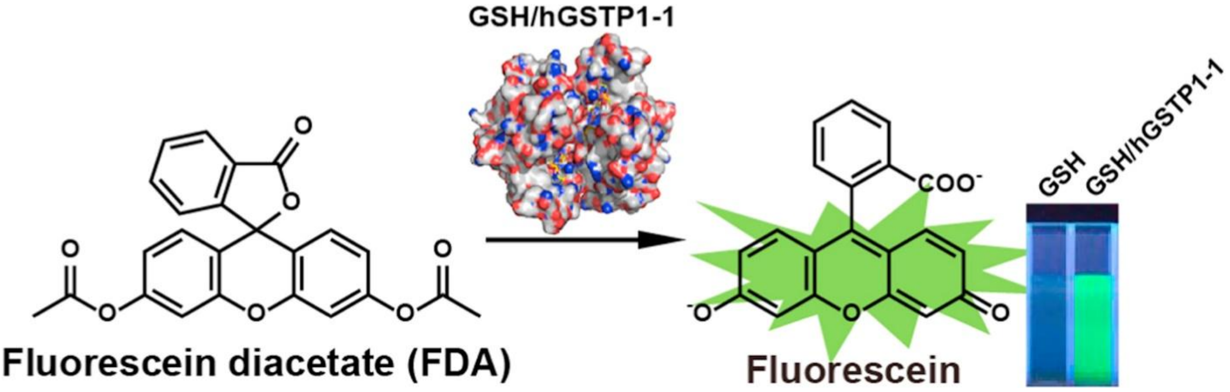
These probes have been extensively used for the detection of heavy metal ions and reactive oxygen species. Heavy metals are the components that have low density[24] , and as they are non-biodegradable they have ill effects on human health[25,26,27,28].Due to their accumulation in biosphere they enter in food chain and finally in living beings resulting in ill effects on human health [29,30] . Heavy metal pollution is of high concern, not only among the community with scientific background, but also general population, who is aware of the disadvantages associated with these heavy metal ions are highly concerned. Even that is the fact that some heavy metal ions are important for living systems, but they are very toxic and hence capable of causing serious environmental and health problems[31-36]. Some heavy metal ions, such as Fe(III), Zn(II), Cu(II), Co(II), Mn(II), and Mo(VI), are essential for the maintenance of human metabolism. However, high concentrations of these ions can lead to many adverse health effects[31,32,37-50]. However, Hg(II), Cd(II), Pb(II), and As(III) are the heavy metal ions that do not have any benefits even in low concentration. Accumulation of these over time in the bodies of humans and animals can lead to severe side effects[32]. In Ganga levels of the toxic heavy metal ions metals is much higher than the US EPA permissible limits for acquatic organisms[51]. Pb pollution was found in ground water resources of Tamil and Pondicherry[52]. Life line of Delhi, i.e. Yamuna river is found to be most polluted river of the country and Heavy metals are found to be one of the dangerous contaminants[53]. Even as reported by The Hindu in Feburary 2012, studies at TERI revealed that vegetables that are grown with Yamuna water are found to contain heavy metals[54]. The rivers of Punjab and ground water are reported to be contaminated by the pollutants like cancer causing Ur, As, Hg and other heavy metal ions in The Hindu in July 2012[55]. Many methods, like atomic absorption spectroscopy, inductively coupled plasma atomic emission spectrometry, electrochemical sensoring, and the use of piezoelectric quartz crystals are known to detect heavy metal ions[56-59], however these methods are becoming less important due to their high time consumption, more cost and laborious procedures. So, analytical techniques based on fluorescence detection are becoming much popular as fluorescence measurements are usually not much costly, easily performable and sensitive [56,60,61-64]. Furthermore, the photo physical properties of a fluorophore can be easily moulded[65]. As clean water resources are the need of an hour which leads to quick research in the field of fluorescent probes to detect heavy metal ions. These heavy metal ions can be detected by measuring the change in fluorescence, color or luminescence of rationally designed fluorescent probes.

Jiao[66] with his team developed fluorescein based sensor for the recognition of Cu(II) ions. The sensor was incorporated with 5- aminoisopthallic acid methyl ester which quenched the fluorescence on addition of Cu(II) ions to the probe. The biological study of the probe was done too.

A reversible probe for Zn^{2+} and ATP in living cells was synthesized by Jin[67] team. The sensing mechanism was based on the spiro-lactam structure of fluorescein and proceed via complexation of zinc ion. The probe was used for in-vivo studies.

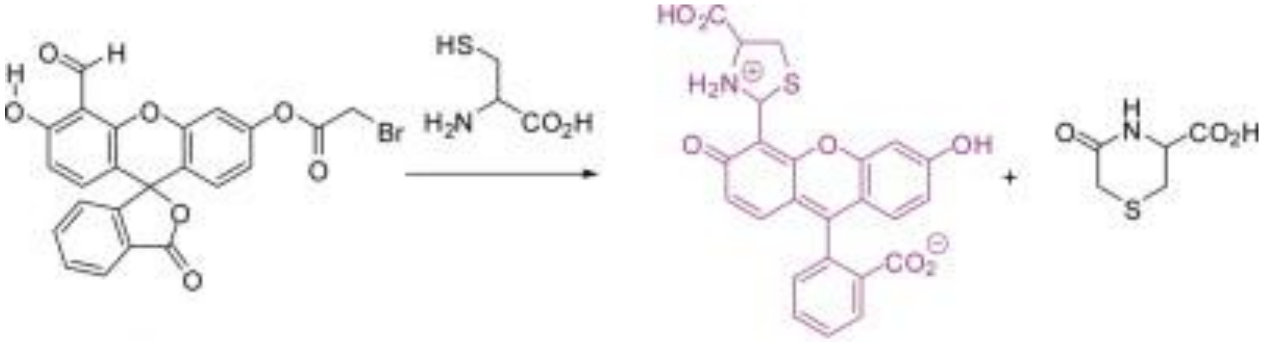


Fujikawa[68] et al reported diacetate and mono acetate derivative of fluorescein as a tool for the detection of human glutathione S-transferase. The probe gets fluorescently activated due to the esterolytic activity of this human glutathione S- transferase.



Wang[69] and his co-workers synthesized a probe showing the applicability of these fluorescent sensors in food chemistry. They developed dinitrobenzene sulfonate probe for the detection of cysteine in milk and water samples. Here, cysteine cleaves the sulfonate bond rendering the more fluorescent molecule. Hou[70] et al synthesized 2-hydrazino-pyridine incorporated fluoresceind based derivative for the dual sensing of Al^{3+} and Cu^{2+}. The probe showed turn on response towards these two with different fluorescence and also different binding modes in different media with good detection limit.

Lee[71] et al synthesized bromo acetyl bromide based three probes with fluorescein, fluorescein mono aldehyde and fluorescein dialdehyde which act as turn on probes for cysteine over other bio-thiols.



Conclusions

Since, the study of the fluorometeric probes became a growing area, fluorescein based probes has become a wide area of interest for the researchers. This review addresses the literature covering various fluorescein based probes and has highlighted the properties of some of the probes. In general, a wide range of fluorescein based have been developed and are proven to be good sensing systems in terms of wavelength, non-toxicity, selectivity, and sensitivity. These fluorescein based probes have good applicability in aq. media, and have good scope in bioimaging of the heavy metal ions. Even the research in this field has been going on since past many years but still it is a growing area. We believe that our present review will help for the future designing and fabrication of new fluorescein based probes.

Acknowledgement

This research was supported by the grant from SERB-DST, India (Project: YSS/2015/001237). We thank SERB-DST (India) for providing Research Assistant Fellowship to Ms. Navjot Sandhu to carry out her research work smoothly.

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