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Development of Sensitive and Selective Spectrofluorometric Method for the Determination of Heavy Metal Ions

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ABSTRACT

In the current work, Pyranine interaction with different heavy metal ions like Fe^{3+}, Pb^{2+}, Cr^{3+}, Zn^{2+} and Cu^{2+} have been examined in the SDS aqueous solution. All metal ions reduced the luminescence intensity of pyranine, and different levels of quenching were seen. Pyranine quenching was optimized by adjusting the SDS concentration. Three distinct Sodium Dodecyl sulphate (SDS) concentrations 0.100 M, 0.050 M, and 0.01 M were used in the experiments. In 0.100 M SDS, quenching was less substantial, but in 0.010 M SDS, it was more noticeable. Using the Stern-Volmer (S-V) equation, which yielded the Stern-Volmer constant (KSV) value, the quenching of pyranine by heavy metal ions inside the SDS micellar system was successfully interpreted. Pyranine's susceptibility to heavy metal ions is indicated by its KSV value; a large Stern-Volmer constant (KSV) denotes more sensibility, whereas a lower constant denotes decreased sensitivity. The S-V constants were found to be in the following order of Fe^{3+} ion > Pb^{2+} ion > Cr^{3+} ion > Zn^{2+} ion > Cu^{2+} ion. KSV was from 1.021×10^{4} M to 0.669×10^{3} M. The fluorescence quenching of Pyranine using SDS miceller system is an efficient technique for identifying amines in environmental materials

INTRODUCTION

Spectroscopy is the study of light's emission and absorption by materials. They

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primarily process the wavelength of light. Spectroscopy also studies the interactions of particles such as protons, electrons, and ions. Spectroscopy may also be used to investigate the interaction of other particles as a function of their impact energy. Atomic spectroscopy is a powerful analytical method for detecting and measuring components from a sample of great accuracy and sureness [1]. Free gas atoms absorbed electromagnetic energy at wavelengths to provide a detectable signal, which is how Atomic Absorption Spectroscopy (AAS) operates [2].

The interaction of EMR with molecules is studied under the molecular spectroscopy.

Types of molecular spectroscopy are.

- Raman spectroscopy
- Fluorescence spectroscopy
- Phosphorescence spectroscopy
- Infrared Spectroscopy
- Ultraviolet spectroscopy
- Circular Dichroism spectroscopy
- Nuclear Magnetic Resonance spectroscopy
- Microwave spectroscopy
- Vibrational circular Dichroism spectroscopy
- Time resolved spectroscopy

1.1 Fluorescence Spectroscopy

After absorbing UV or visible light, a fluorophore emits light, a phenomenon known as fluorescence. Amphiphilic, hydrophilic, and hydrophobic substances are all possible. Spectroscopy with fluorescence has increased dramatically in recent decade. A sample is excited with light at a certain wavelength, usually from a UV or laser source, and then emits light that is measured to create a signal. This is how fluorescence spectroscopy operates. The indicator contrasts with a reference meeting cleanliness standard. The fluorescence strength spectrum generated by an atom or molecule right after optical stimulation is distinctive to the material, even at very little quantity [3-4]. A light beam is used to excite the sample, causing the emission of lower-energy light that creates a release spectrum that is used to interpret results.

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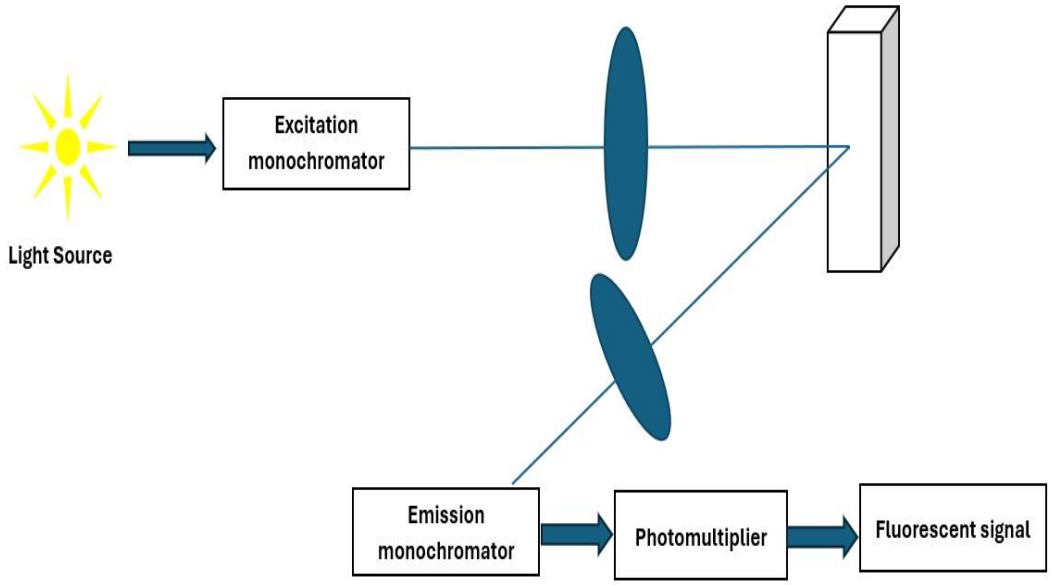

Fig. 1.1: Diagram Of Spectrofluorometer

Figure 1.1 illustrates a simple instrument configuration to determine stable state fluorescence.

In Principle When a fluorophore captures UV or visible light and then releases it at a longer wavelength fluorescence occurs. In Fig.1.2. A Jablonski diagram might help to demonstrate the general ideas. The first step (1) is excitation, in which the molecule absorbs light and transfers it to an electrically excited state, The exciting singlet state, S1, is the result of an electron moving from its initial singlet state, S0. Internal conversion, often known as vibrational relaxation, comes next (2), when the molecule goes from an advanced electronic energy state to a lower energy level S1, without any radiation emission. After 10^{8} s of stimulation, the negative charge particle rebounds to its ground state stability, S0, and emits radiation energy at a wavelength determined by the energy distinction between both states. This clarification is rather oversimplified [5].

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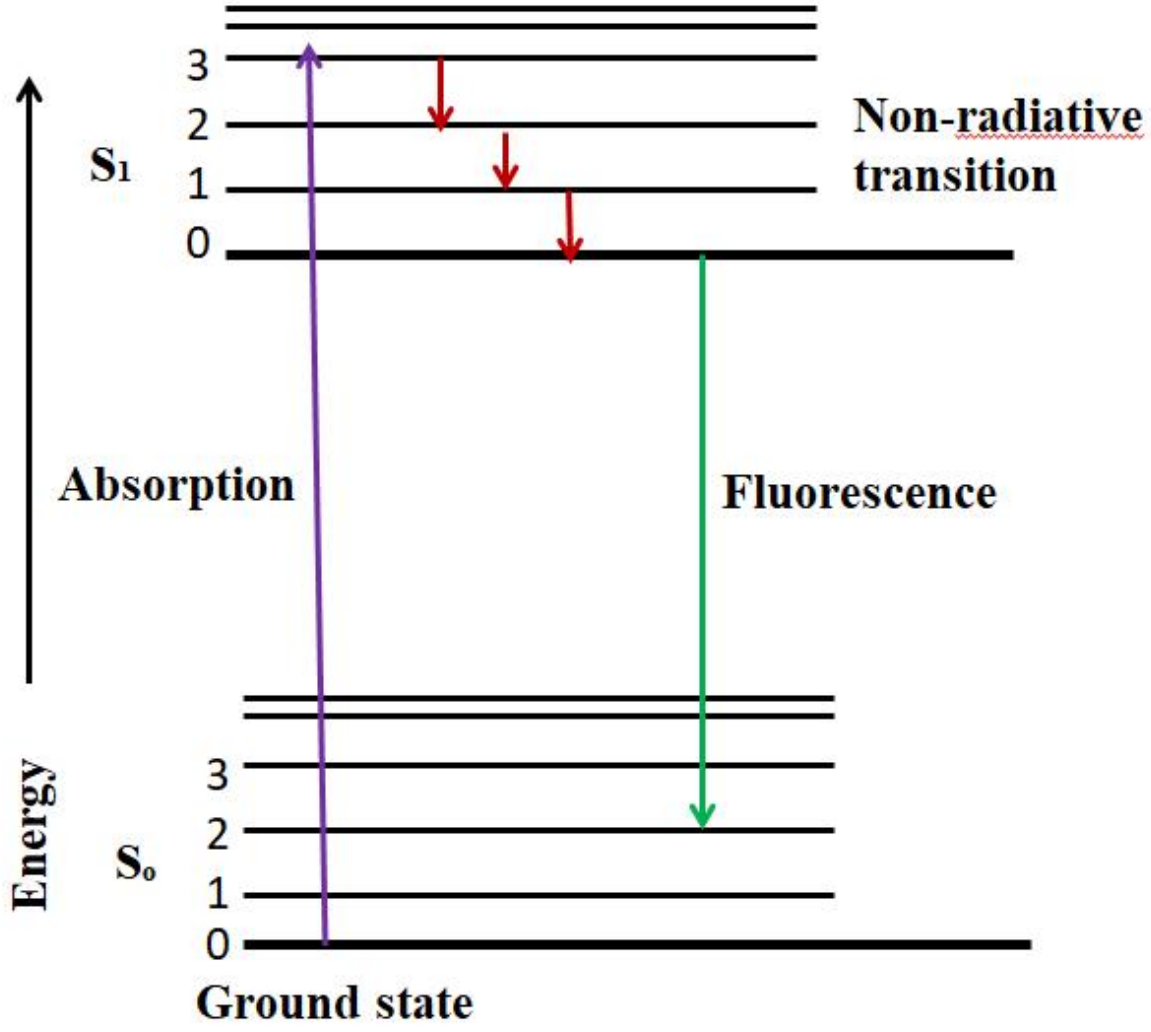

Fig. 1.2: Jablonski Diagram

Both the excitation spectra and emission spectra have in common that both are obtained using fluorescence spectroscopy, which provides useful information about the interactions within the molecule. The excitation spectrum measures the fluorescence the molecule generates while absorbing light, and is usually, but not always, the same as the low-energy absorption spectrum of the molecule, and, in most cases, is independent of the wavelength used to detect the emission [6-7]. The emission spectrum, meanwhile, refers to the distribution of the emitted radiation from the sample at light of specific wavelengths which the sample absorbs. Together, these spectra provide crucial information about the molecular surroundings and the

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presence of quenching. One of the important factors of fluorescent behavior is the addition of surfactants. The surfactant molecules have both hydrophilic ("water-loving") and a hydrophobic ("water-fearing") section, surfactants molecules can change the solubility and the environment of fluorescent species because they form micelles. Surfactants, based on the charge on their head groups, can be anionic (soaps, SDS, $RCOO^-$, and alkyl sulphates), or cationic (with a positively charged hydrophilic head) [8-9]. By altering the microenvironment's polarity and charge distribution, surfactants can enhance the fluorescence quenching effects via dynamic and static mechanisms. Any process which causes a reduction in emission intensity is termed fluorescence quenching. This is the result of an excited-state reaction, molecular rearrangement, energy transfer, collisional quenching, or the formation of a ground-state complex. Proximity is a requirement for both static and dynamic quenching and is especially important in the study of many metal ions of biological and environmental importance, which due to their redox activity and complexity, serve as quenchers. Metals including lead, Chromium, copper, zinc and iron, are most researched in this regard. One of the prominent examples in the domain is lead and its interaction with fluorescent compounds. Chronic exposure to lead (Pb^{2+}) is harmful, and it is an environmental contaminant as well. On the other hand, as with other chemicals compounds, chromium (Cr^{3+}) exists mostly within its trivalent state [10]. Trivalent chromium, though needed by the human body in small doses and often taken in the form of chromium picolinate to boost glucose metabolism and suggested chromium in the trivalent form picolinate form, on the other hand, can often tend to be harmful. Another chemical copper is also potentially harmful as it exists in both cupric (cupric and cuprous state Cu^{2+} and Cu^{+}) and can lead to the formation of the reactive oxygen species (ROS) by virtue of it as well as generates and also in the other form (Cu) and leads to the unstable cycle (catalytic cycle) and shines a light to the over stressed and fluorescence [11]. One of the chemical constituents, which is zinc (Zn^{2+}), on the other hand, is needed in minute quantities by both plants and animals and even the fluorescence with biomolecules as well as and automatically aligned to irradiation to bio coordination. In a like manner, iron (Fe), which is essential for the sustenance of all living forms is in most of the (II) and (III) oxidation state also as primarily exists in the physiological forms Are in

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oxidation. While the soluble state is Oxide for (Fe^{3+}) and (Fe^{2+}) and (Fe^{3+}) and above which leads to self in the homo polymer and both oxidation states can be in effective process of the quench able system [12-13].

The relationships that exist between the metal ions and surfactants and fluorescence has been a recent interest in investigating the relationships on a molecular level. The equilibrium condition by which the equilibrium system of the complex formed by the surfactant is satisfied in the (General interaction of fluorophores with cationic surfactants as complex to fluorophores bound) and the surfactant with the metal ions where the metal ions influence the surfactant microenvironment [14].

2. MATERIALS AND METHODS

2.1 Chemicals

All the chemicals used were analytical grade.

i. Pyranine (which has a purity of 98%), (Sigma-Aldrich, Steinem Germany),

ii. Nitric acid was used (which has a purity of 65%, riedel-dehaen GMbH).

iii. SDS (ASC reagent, which has a purity of 99.9%). (Steinem Germany, Sigma-Aldrich).

2.2. Metals

i. Chromium ion ii. Copper ion iii. Zinc ion iv. Iron Ion v. Lead ion (II)

2.3. Stock Solutions Preparation

2.4 Pyranine standard solution preparation

Pyranine standard solution (0.010 mol L^{-1}) was made by dissolving 0.13109g in 25mL of ethanol.

2.5. mixture solution preparation

Nitric acid, Pyranine, and SDS are present in the combination. SDS and nitric acid are dissolved in distill water, and pyranine is diluted in ethanol of analytical quality in a 25 mL, and we use stock solutions for preparation of the following mixtures.

i. Nitric acid

ii. Pyranine

iii. SDS

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2.6. Preparing two more mixtures

Solutions X & Y

We split the 50 mL mixture into 2 falcoon tubes, each holding 25 mL of the mixture. We call these two falcon tubes, X and Y. In the falcon tube, we introduce several types of heavy metals in microliters. These two combinations will be frequently prepared. Only heavy metals were modified in this study for each analysis and for the quenching analysis.

Falcon tube "X" contains a blend of Falcon tube "X" has the following elements, which are part of this combination.

HNO_3: 0.24 mol L^{-1}

Pyranine: 0.010 mol L^{-1}

SDS: 0.010, 0.050 and 0.10 mol L^{-1}

The mixture's entire volume is 25ml, which we finish with distal water.

Falcon tube "Y" has a combination of

HNO_3: 0.24mol L^{-1}

Pyranine: 0.01mol L^{-1}

SDS: 0.01, 0.05 and 0.1mol L^{-1}

2.7. Methods of Experimentation

The mixtures "X" and "Y" are collected in several volumes that are contained in falcon tubes X and Y, and all data and information were derived from these mixtures, which are shown diagrammatically in the figure below. 3.1

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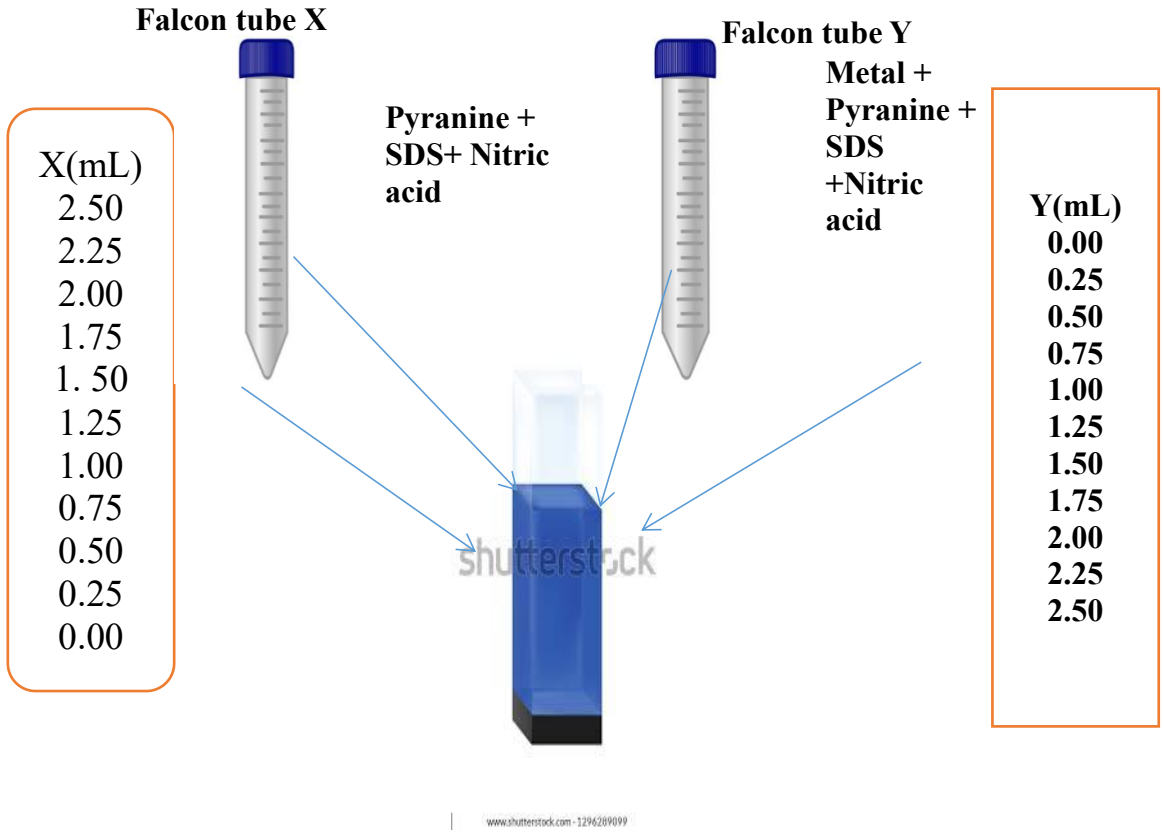

Fig: 1.3 Mixture Preparation Of Pyranine, Sds And Metal.

Amount of mixture in a cuvette = X+Y=2.5 mL

Various quantities in milliliters are obtained from mixtures X and Y in these two falcon tubes.

2.8. Pathway for Analysis

The analytical technique makes use of both the X and Y mixtures. The cuvette has a total volume of 3ml, and we use 2.50ml of the combination X and Y. A cuvette is used to extract various volumes from the mixture X. It will vary from 2.500 to 0.00 ml, and the various types of quantities that are extracted from mixture Y in a cuvette will range from 0.00 to 2.500 ml. We added 0.250 ml of mixture Y to a cuvette and decreased the volume of mixture X from 2.500 to 2.250 ml for the second reading. For the first reading, we took 2.500 ml of mixture from mixture X and placed it in the cuvette for the second reading, we reduced the volume of combination X from 2.500 to 2.250 ml and added 0.250 ml of mixture Y to a cuvette. We took 2.500 ml of mixture from combination X and put it in the cuvette for the

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initial reading. The volume of the cuvette is full of both mixes X and Y, and the total volume collected in the cuvette was 2.50 ml in each reading. The heavy metal ions in the mixture were responsible for the quenching of pyranine. Consequently, it was found that numerous heavy metal ions were quenched.

2.9. Pyranine Fluorescence Quenching By Heavy Metal Ions

The stock solution combination will be created and divided into two falcon tube mixtures, X and Y, in the same manner as described before, the stock solution includes nitric acid, pyranine, and SDS. The combination X will include simply the stock solution, whereas the mixture Y will contain the heavy metal ions that we utilized as a quencher. This reduces the fluorescence intensity of the mixture X containing the Pyranine probe. The Pyranine probe interacts in distinct ways with various kinds of heavy metal ions, leading to different types of quenching.

RESULTS AND DISCUSSION

3.1. Fluorescence Quenching of Pyranine by Ferric Ion (Fe^{3+})

Pyranine fluorescence behaviors were investigated in aqueous SDS and in the presence of Fe^{3+}. Pyranine fluorescence was quenched by Fe^{3+}. The study was performed in three distinct concentrations of SDS as 0.010, 0.050, and 0.10 mol L^{-1}. So, the interaction between Fe^{3+} and pyranine was studied to find the optimum SDS concentration for maximum quenching of pyranine. Pyranine (probe) quenching was highest in lower concentrations of 0.010 mol L^{-1} SDS and lowest at higher concentrations of 0.10 mol/L. The quenching was present in this sequence 0.01 > 0.05 > 0.10 mol L^{-1} SDS, respectively. Because of dilution of pyronine the conc of SdS go upward which leads to the reduction of intensity of fluorescence. Micelles are produced in greater quantities as the quantity of SDS rises, and they struggle for quenchers' attention. Pyranine's fluorescence quenching with Fe^{3+} ion was evaluated using the Stern-Volmer equation (SV) (Eq-1)

$$X_0/X = 1 + KSV\,[\text{Metal Ions}] \ldots. \text{Eq-1.0}$$

Wherever X_0 represents the fluorescence intensity of Pyranine probe when metal ions are absent and X represents the fluorescence intensity of the Pyranine probe if metal ions are present. So *K*SV stands for the Stern-Volmer constant, and [Metal Ions] represents the heavy metal ions' concentration in molar range. These plots were produced by using the Stern-Volmer (SV) equation to the fluorescence

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quenching of pyranine. Figure 4.1 displays the Stern-Volmer (SV) plots for the quenching of fluorescent pyranine by the Fe^{3+} ion in 0.01 mol L^{-1}, 0.05 mol L^{-1} and 0.10 mol L^{-1} SDS, while Table 4.1 displays the Stern-Volmer constant (KSV)

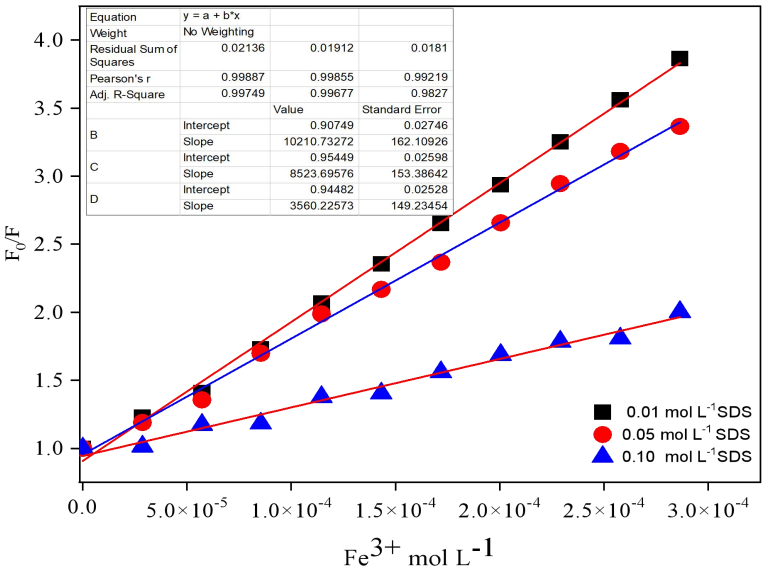

***Fig. 1.4.*:** Plots of Stern-Volmer (SV) for Pyranine fluorescence reduce by the Ferric (Fe^{3+}) ion in different SDS concentration.

Table 1.1. Stern-Volmer constant on behalf of Fe^{3+} ion-induced suppression of fluorescence of pyranine (1.00 x10^{-4} mol L^{-1}) at various concentrations.

S.NO.	SDS mol L^{-1}	K_{SV} mol L^{-1}
1.0	0.01	1.021×10^4
2.0	0.05	8.524×10^3
3.0	0.10	3.560×10^3

For lower SDS concentrations, the Stern-Volmer constant was larger, while for higher SDS concentrations, it was lower. Stern-Volmer constants show how sensitive the procedure is to the metal ions under study [15]. Higher Stern-Volmer constantly higher sensitivity and vice versa. Stern-Volmer constants in 0.010, 0.050 besides 0.10 mole/liter SDS were noted in the sequence of $1.021 \times 10^4 > 8.524 \times 10^3 > 3.560 \times 10^3$ mol L^{-1}.

3.2. Fluorescence Quenching of Pyranine by Pb^{2+}

Pyranine interaction with Pb^{2+} was investigated in 0.01, 0.05, and 0.10mol L^{-1} SDS. Although the degree of quenching varied across the studied three different SDS

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concentrations studied, quenching was observed in all the three different SDS concentrations studied. To determine the ideal SDS concentration for the greater quenching of pyranine by the Pb^{2} ion, the SDS concentrations was optimized. The Pb^{2+} ion quenched pyranine more in 0.01mol L^{-1} of the surfactant, intermediate in 0.050 mole /liter SDS, lowest in 0.100 mol L^{-1} SDS. The Stern-Volmer (eq 1.0) equation was used to treat the drop in pyranine fluorescence intensity caused by the Pb^{2+} ion. The Stern-Volmer plots and Stern-Volmer constants were obtained by applying the equation to the quenching data. Figure 4.2 shows the Stern-Volmer plots and table 4.2 displays the Stern-Volmer constants.

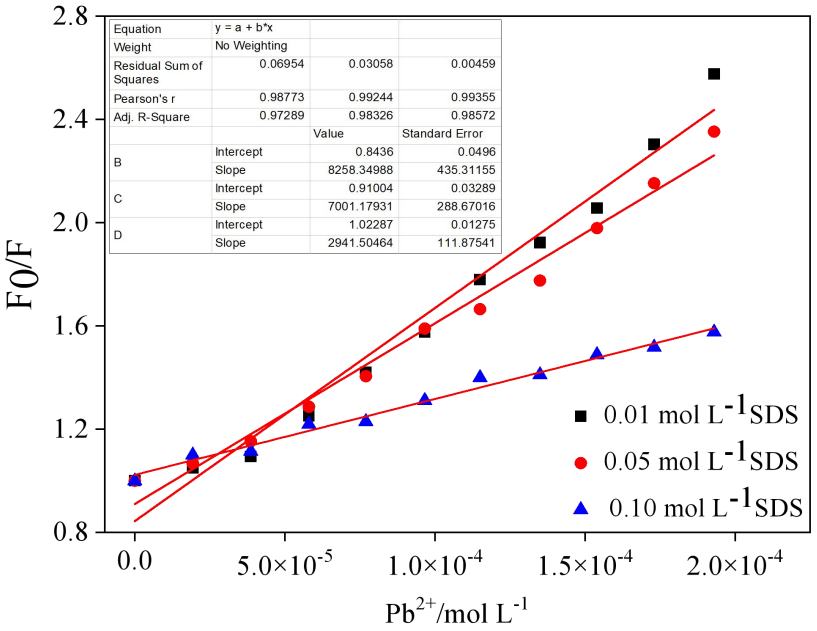

Fig 1.5: SV graph for pyranine reduction by Pb^{2+} ion in selected concentration of SDS.

The method's sensitivity to the Pb^{2+} ion is demonstrated by the KSV. KSV were lower at higher concentrations of 0.100 mol L^{-1} SDS, medium for 0.05 mole/liter SDS, and higher for 0.010 mol L^{-1} SDS. The technique for Pb^{2+} ion is more sensitive at 0.01mol L^{-1} SDS than at 0.050 mole/liter SDS and 0.100 mol L^{-1} SDS, according toward the higher KSV mean.

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Table. 1.2. The KSV for Pb^{2+} ion induced fluorescence decreasing of pyranine (1.0×10^{-4} mol L^{-1}) .

Serial No.	SDS mol L^{-1}	K_{SV} mol L^{-1}
1	0.01	8.258×10^3
2	0.05	7.001×10^3
3	0.10	4.942×10^3

KSV of 8.25834988×10^3 was conclude for Pyranine quenching with Pb^{2+} ion in 0.01 mole/liter SDS whereas K_{SV} in 0.05 mol L^{-1} SDS was 7.00117931×10^3 and KSV in 0.100 mol L^{-1} SDS was 4.9415048×10^3 [16].

3.4. Pyranine Fluorescence Quenching by Cr^{3+}

The interaction of Cr^{3+}, another heavy metal ion, with pyranine was investigated in 0.01mol L^{-1}, 0.050 mol L^{-1} and 0.100 mol L^{-1} SDS. Although the degree of intensity reduction varied depending on the SDS content, quenching was observed in these concentrations. To determine the ideal SDS concentration for increased pyranine quenching by chromic ions, three distinct SDS concentrations were used for pyranine quenching by Cr^{3+} ions. When the Cr^{3+} ion is added, the fluorescence intensity of pyranine decreases. This is explained by dynamic quenching, which occurs when the quencher and pyranine probe collide in an excited state. Whereas SV equation was utilized to handle the reduction in intensity of pyranine fluorescence by Cr^{3+} ion. The stern-Volmer plots and stern-Volmer constants were produced by applying the equation to the quenching data. Figure 4.3 displays stern-Volmer plots, and table 4.3 displays stern-Volmer constants.

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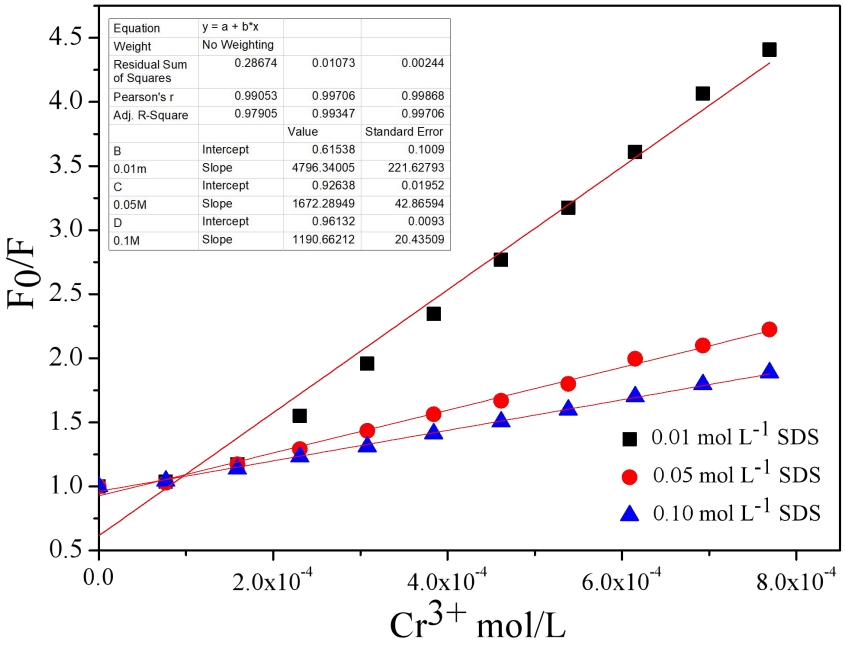

Fig. 1.6: Quenching by Cr^{3+} ion in selected concentration.

Table. 1.3 Stern-Volmer constant as a function of SDS concentration for the quenching of fluorescence of pyranine ($1.0 10^{-4}$ mol L^{-1}) by the Cr^{3+} ion.

Serial No	SDS mol L^{-1}	KSV mol L^{-1}
1.0	0.01	4.796×10^3
2.0	0.05	1.672×10^3
3.0	0.10	1.191×10^3

Pyranine quenching by Cr^{3+} ion in 0.01mol L^{-1} SDS has a KSV of 4.79634005×10^3, whereas KSV in 0.05mol L^{-1} SDS was 1.67228949×10^3 and for 0.10mol L^{-1} was 1.19066212×10^3. The method's sensitivity to the Cr^{3+} ion is shown by the Stern-Volmer constant. *K*SV was lower at greater concentrations of 0.10 mol L^{-1} SDS, medium for 0.050 mol L^{-1} SDS, besides higher for 0.01 mol L^{-1} SDS. The Cr^{3+} ion technique is more sensitive at 0.01mol L^{-1} SDS than at 0.050 mol L^{-1} SDS and 0.100 mol L^{-1} SDS when the KSV mean is higher. Sensitivity increases with increasing KSV and conversely [17].

3.5. Fluorescence Qing of Pyranine Via Zinc (II) Ion

The interaction of zinc (II) ions was investigated in 0.01mol L^{-1} SDS. Considering that the same molecule has both hydrophilic and hydrophobic component, SDS aids in increasing the solubility of the Zn^{2+} ion and the Pyranine probe. SDS condenses

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the pyranine, creates micelles, and boosts its fluorescence. Fluorescence intensity of pyranine quenched by zinc (II) ions. As the concentration of Zn^{2+} ions increases in all three of these concentrations 0.010 mol L^{-1} SDS, 0.050 mol L^{-1} SDS, besides 0.10 0 mol L^{-1} SDS the intensity of pyranine fluorescence decreases.

It is evident that these three 0.010 mol L^{-1} SDS, 0.050 mol L^{-1} SDS, then 0.100 mol L^{-1} SDS have different levels of pyranine quenching. Since the spectra showed no changes, the quenching was dynamic. The S-V equation was utilized to analyze the outcomes of pyranine quenching by Zn^{2+} ions. This led to the KSV and Stern-Volmer plots. KSV is provided in table 4.4, and Stern-Volmer graphs are displayed in Fig. 4.4.

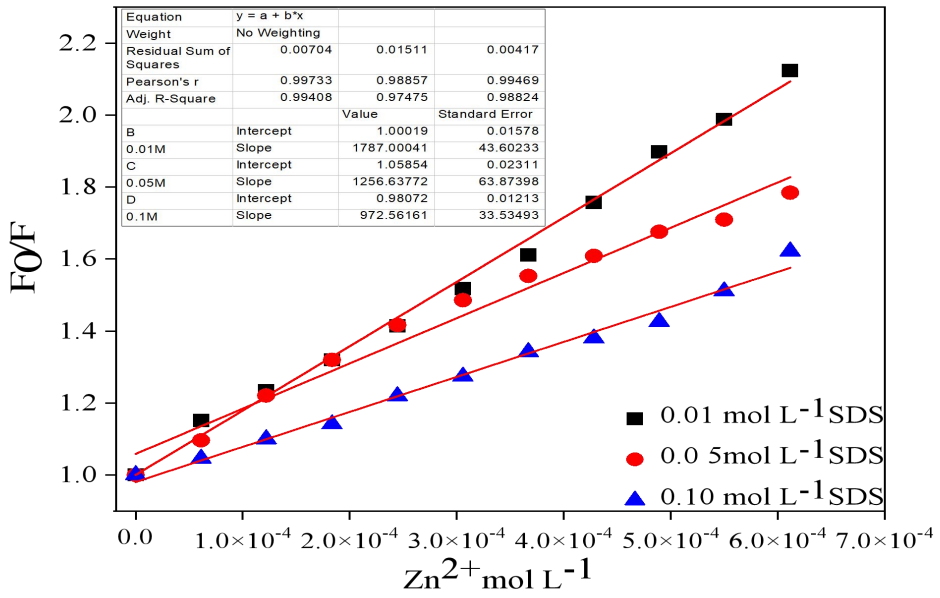

Fig 1.7: F-Quenching by Zn^{2+} ion in selected concentration of SDS.

Table. 1.4. KSV (Stern-Volmer constants) on behalf of minimizing the fluorescence of pyranine (01.010^{-4} mol $L^{-1)}$ via Zn^{2+} at selected concentrations of SDS.

Serial No.	**SDS** mol L^{-1}	**KSV**mol L^{-1}
1.0	0.01	1.787×10^3
2.0	0.05	1.256×10^3
3.0	0.10	9.726×10^2

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KSV was determined to be 1.787×10^{3}, 1.25×10^{3}, and 9.726×10^{2} via the fluorescence quenching Pyranine probe by Zn^{2+} ion in 0.01mol, 0.05 Mol, and 0.10 mol L^{-1}, respectively. Therefore, at lower SDS concentrations, the sensitivity for the Zn^{2+} ion will be greater [18].

3.6. Fluorescence Quenching of Pyranine by Cupric Ion (Cu^{2+})

Pyranine interaction with Cu^{2+} was studied in selected SDS concentration. Different SDS concentrations were used in the study to find the optimum SDS concentration for maximum quenching. Pyranine fluorescence was quenched by the heavy metal ion Cu^{2+} in a micellar concentration of SDS, an anionic surfactant.

The pyranine probe was quenched more readily at lower concentrations (0.010 mol L^{-1}) and less on higher concentrations (0.100 mol L^{-1}) of surfactant. The stern-Volmer equation, which generated S-V plots and KSV, was applied to the quenching of the pyranine probe in three SDS concentrations. S-V plots are presented in Figure 4.5, but constants are specified in Table 4.5.

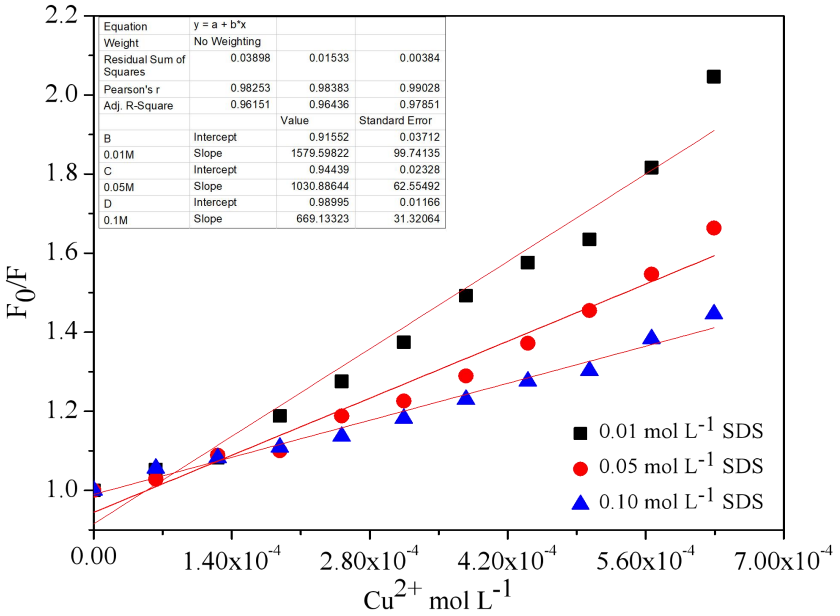

Fig. 1.8: Stern-Volmer S-V plots and pyranine fluorescence minimized through Cu^{2+} ions, in water based SDS were employed.

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Table. 1.5 SV constants for the Cu^{2+} ion's ability to quench pyranine fluorescence at varying SDS concentrations.

Serial No	SDS mol L^{-1}	KSV mol L^{-1}
1.0	0.01	01.579 $\times 10^3$
2.0	0.05	01.031 $\times 10^3$
3.0	0.10	0.669$\times 10^3$

In order of 1.579 $\times$ 10^3 mol L^{-1} 1.031 $\times$ 10^3 mol L^{-1}, then 0.669$\times 10^3$ mol L^{-1}, respectively, KSV on behalf of Cu^{2+} ions in 0.01 mol L^{-1}, 0.05 mol L^{-1}, besides 0.10 mol L^{-1} were detected. Sensitivity, detection, and quantification limit were all greater at lower SDS concentrations because KSV was higher there.

3.7. Fluorescence quenching of Pyranine by Fe^{3+}, Pb^{2+}, Cr^{3+}, Zn^{2+}, Cu^{2+}

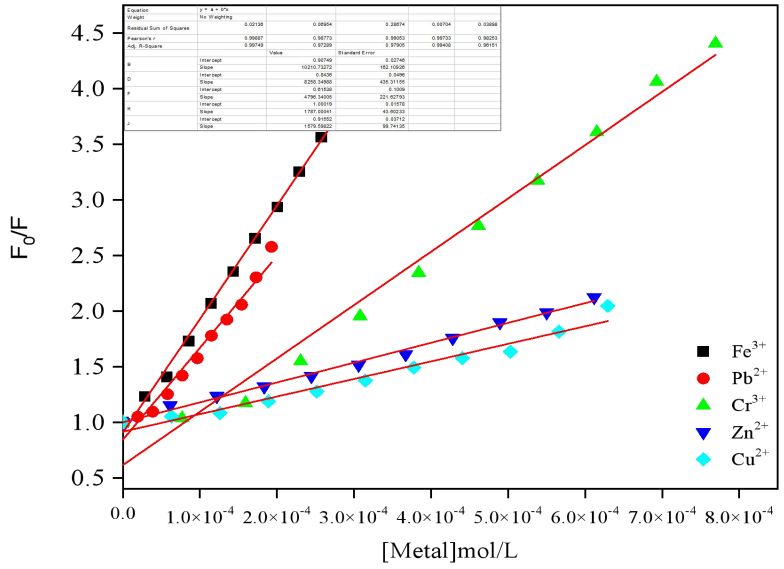

Fig. 1.9: Stern-Volmer plots and pyranine fluorescence quenching by Fe^{3+}, Pb^{+2}, Cr^{3+}, Zn^{2+}, Cu^{2+} ions in aqueous SDS were employed.

CONCLUSION

Heavy metal ion detection in biological samples was successfully discovered by using the process of fluorescence quenching of Pyranine probe by heavy metal ions in water based SDS. SDS improved the probe's solubility and local concentration. The Pyranine fluorescence intensity was muted by all five heavy metal ions, and the order of their sensitivity was Fe^{3+} > Pb^{2+} > Cr^{3+} > Zn^{2+} > Cu^{2+}. The information on the method's sensitivity was supplied by the Stern-Volmer constants. Even a little

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number of heavy metal ions can significantly reduce fluorescence intensity. The constants of Volmer were observed in the order $Fe^{3+} > Pb^{2+} > Cr^{3+} > Zn^{2+} > Cu^{2+}$, which shows the sensitivity of the method for the above metal ions. The Sensitive nature of metals ions depends on the constants, higher its values greater will be the reactivity. The method developed has high sensitivity for Fe^{3+} while smaller sensitivity was found for Cu^{2+}. The heavy metal ion identification technique is a highly sensitive and repeatable methodology that is helpful for identifying heavy metal ions in biological samples.

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