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Direct quantification of a trace amount of iron (III) by using room-temperature fluorescence excitation-emission matrix spectroscopy

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***Abstract*---**In this research, the organ fluorescent reagent was used. 4 (4-sulphophenylazo) pyrogallol Prepared by the method of forming diazonium salt in an acidic medium by coupling the compound sulfadiazine with the compound pyrogallol, which is a solid substance with a brown color and a melting point of 167–165 C. The reagent (4-SPAP) was determined by fluorescence spectrometry, and the excitation and emission spectra were determined at room temperature by using maximum wavelength emission of 320 nm and excitation of 276 nm. With its high sensitivity, simplicity, and low cost compared to other analytical techniques, this technique was used to estimate and reagent some metal ions. This technique is widely employed for the selective reagent of metal ions. It was determined that the detector's limit of detection (LOD) and limit of quantification (LOQ) are both 110.1031 ppb (equal to 367.0103 ppb).

***Keywords*---**fluorescence spectrometer, azo fluorescent reagent, iron ions III**.**

Introduction

Azo compounds are a very important class of chemical compounds that have received interest in scientific research (1,2). They are very important reagents in separating and determining the trace concentrations of different metal ions (3). Organic reagents are compounds with high molecular weights, most of which are poorly soluble in water because they have covalent bonds (4). One of its most important advantages is its high sensitivity and the bright colors of its complexes when they are in harmony with the elements of the periodic table, and it exploited

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the solubility of its complexes in organic solvents in analytical chemistry to perform extraction processes [5,6]. It was also used in the field of quantitative and qualitative estimation of the trace concentrations of many metal ions in different models and using various techniques [7,8]. Fluorescence spectroscopy was used to estimate and detect some metal ions with its high sensitivity, simplicity, and low cost compared to other analytical techniques, and this technique was used to study the selectivity and sensitivity of the reagent 4 (4-Sulpho phenyl azo) pyrogallol (4-SPAP) prepared under study. This technique is widely used for the selective detection of metal ions. Fluorescence spectroscopy is a type of electromagnetic spectroscopy that analyzes the fluorescence of a sample. In the fields of research chemistry, biochemistry, and medical analysis of organic compounds, the spectrometer is also used to identify many organic compounds and aromatic molecules that are common and effective in medicines. New [9] fluorescent dyes have been synthesized with a lot of effort to ensure that no two molecules have the same fluorescence spectra. This is the principle that makes fluorometry a highly specific analytical technique. These photonic processes involve transitions between electronic and vibrational states. For polyatomic fluorescent molecules (fluorophores) [10, 11, 12], which are the components found in molecules that cause fluorescence, [13] fluorophores play a major role in fluorescence spectroscopy.

Methods

4 (4-Sulphophenylazo) pyrogallol synthesis

The reagent (4-SPAP) was prepared according to the standard azo method, where 0.01 mol (2.27 g) of sulfadiazine was dissolved in a mixture consisting of 10 ml of 37% HCL and 80 ml of distilled water, and the mixture was cooled to a temperature of (5-0 C̊). Then a solution of 0.01 mol of sodium nitrite dissolved in 20 ml of distilled water was added to it, and then a solution of diazonium chloride was added to a solution of (1.26 g) of pyrogallol dissolved in a mixture of 30 ml of ethanol plus 100 ml of sodium hydroxide NaOH prepared by dissolving (4 g) in 100 ml of distilled water with continuous stirring. It was observed that the solution turned orange when they left the mixture for 24 hours, so it formed a precipitate [14,15,16] brown in color and had a melting point of 167–165 C.

Study of the excitation-emission spectrum of the organic reagent (4-SPAP)

The excitation and emission spectra of the organic reagent (4-SPAP) were calculated as 0.001 mg with a concentration of 100 ppm was dissolved in the ethanol solvent, which is the mother solution. The dissolved compound was used to calculate the excitation and emission spectra at the longest wavelength.

Study of the excitation-emission spectrum of a reagent with Fe (III) iron ions

The excitation and emission spectra of the organic reagent (4-SPAP) were calculated with iron ions (Fe(III) at their PH where they are mixed in molar ratios 2:1 (metal: reagent).

Concentration effect study
When employing varied concentrations, it's crucial to investigate the influence of c oncentration on the absorption and emission spectra attributes of the (4-SPAP) reagent and the iron complex.

Study the effect of the solvent: The absorption and fluorescence spectra of organic reagent solutions (4-SPAP) were investigated in various solvents to determine the influence of solvent polarity on the displacement of the reagent's maximum emission and absorption spectra. At room temperature, 0.001 mg of the reagent was dissolved in polar and non-polar solvents at a concentration of 100 ppm.

Determination of metallic elements by the reagent (4-SPAP) in the sample: After researching the fluorescence spectrum of SPAP-4 reagent, which possesses a fluorescence characteristic, it was effectively used in fluorescence spectrometry to determine numerous metallic elements with low concentrations.

Calibration Curve Research: A series of standard solutions of known concentrations are prepared and their fluorescence intensity is measured at wavelength λ max, and then a standard curve is drawn for these solutions between concentration and fluorescence intensity, as the relationship is linear and conforms to the Beer-Lambert law.

Results and Discussion

The excitation-emission spectrum of the organic (4-SPAP) reagent

The excitation and emission spectra of the organic reagent (4-SPAP) were calculated as 0.001mg (100 ppm) was dissolved in the ethanol solvent, which is the mother solution**.** The dissolved compound was used to determine the excitation spectrum and the emission spectrum at the greatest wavelengths (emission = 320nm, excitation = 276nm), and the measurements were made at room temperature. Different concentrations (100 ppb, 80 ppb, 60 ppb, 40 ppb, 20 ppb) were prepared by diluting a certain volume of the prepared solution at a concentration of 100 ppm with ethanol. Then the excitation-emission spectrum of the organic reagent (4-SPAP) was found. As shown in Figure (1) at a concentration of 80 ppb and Figure (2) with different concentrations.

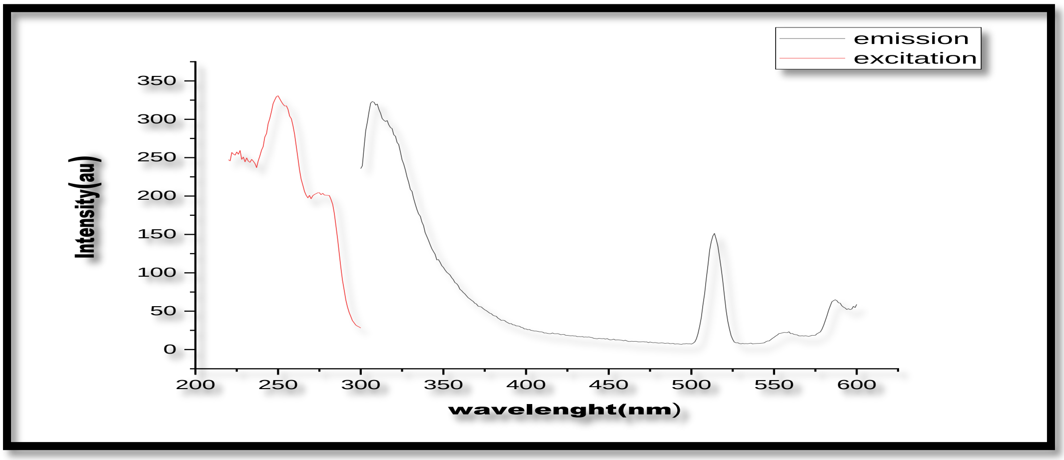

Figure (1) shows the peaks of the emission and absorption spectrum of the organic reagent (4-SPAP) at a concentration of 80 ppb.

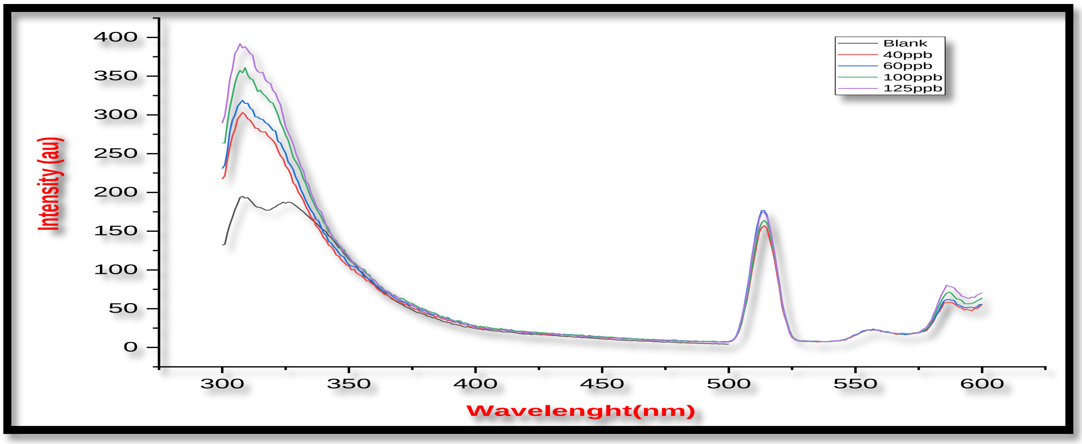

Figure (2) shows the emission spectrum peaks of the organic reagent (4-SPAP) at different concentrations

Study the effect of the solvent

The absorption and fluorescence spectra of organic reagent solutions (4-SPAP) have been studied in different solvents. We find that the energy of the peak of the absorption spectrum (abs) and the fluorescence spectrum (flu) differ from one solvent to another. (0.001 mg) was dissolved at a concentration of 100 ppm of the reagent in polar and non-polar solvents and at laboratory temperature for all used solvents. It has been observed that the intensity of fluorescence intensity increases in polar solvents and decreases in non-polar solvents as shown in Table (1). Figure (3) shows the emission spectrum of the detector (4-SPAP) in different solvents.

Table (1): The effect of solvent on reagent 4 (4-SPAP) at a concentration of 100 ppm in different solvents using fluorescence spectrometry.

protic solvents				aprotic solvents			
Solvent	λmax			Solvent	λmax		
	Excitation	Emission	Intensity		Excitation	Emission	Intensity
Ethanol	254	308	603.094	DMSO	289	330	161.468
Methanol	250	306	356.018	THF	289	313	89.320
2-propanol	299	318	770.998	Acetone	280	296	200.976
Ethylene glycol	252	309	137.237				

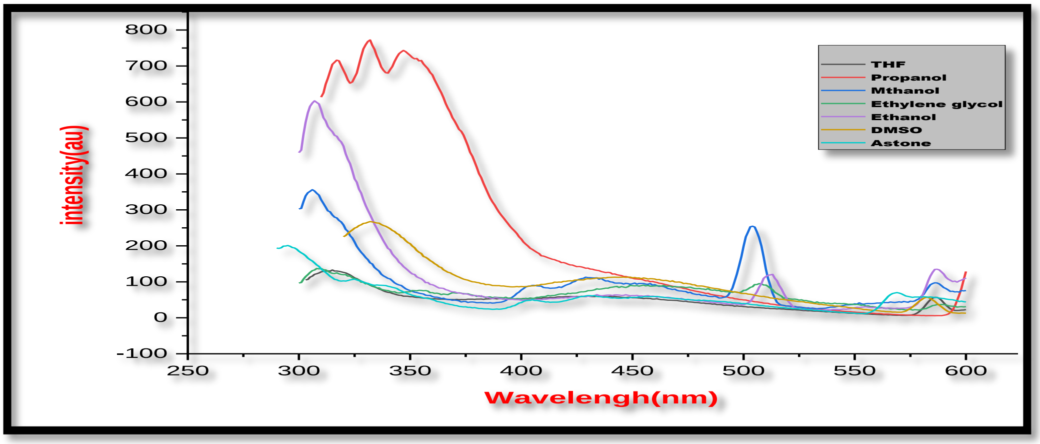

Figure (3) shows the emission spectrum peaks of the organic reagent (4-SPAP) in different solvents.

Iron Fe(III) ion determination

Iron is in the eighth group of the periodic table and belongs to the d sub-level elements. It belongs to the transition metal group chemically. The organic reagent solution, 4-SPAP, dissolved in ethyl alcohol, was mixed in molar ratios of 2:1 with the metal's ionic solution at PH = 9. (metal: reagent). At room temperature, at maximum emission wavelengths of 310nm and excitation wavelengths of 249nm, the excitation and emission spectra of varied concentrations were measured. As concentration decreases, the intensity of the fluorescence spectra peaks increases, as illustrated in Figure 4. When the fluorescence detector is coordinated with the laser, the spectrum of the fluorescence detector shifts.

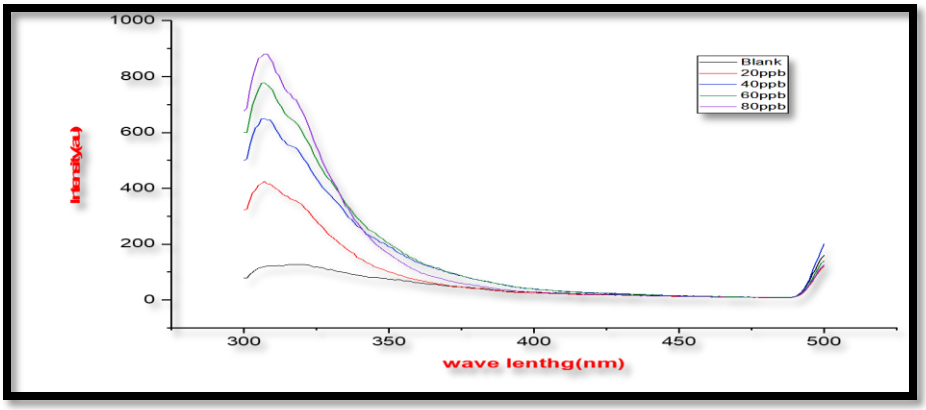

Figure (4) shows the emission spectrum peaks of the organic reagent (4-SPAP) with Fe(III) ions

Determination of iron ion concentration using the calibration curve

The emission intensity of a detector was determined when it was coordinated with the iron ion (III) at the greatest wavelength in the fluorescence device, as shown in the previous step, after which standard solutions were prepared with different concentrations ranging from (20-80**).** The emission intensity of the concentration of the iron complex solution of unknown concentration was found to be equal to 776.025 and it was projected onto the tooth axis that represents the concentration and was equal to (65 ppb), where we note the linear relationship between intensity and concentration, and the value of the coefficient of determination R2 (correlation coefficient) is equal to (0.9519), as shown in Figure (5):

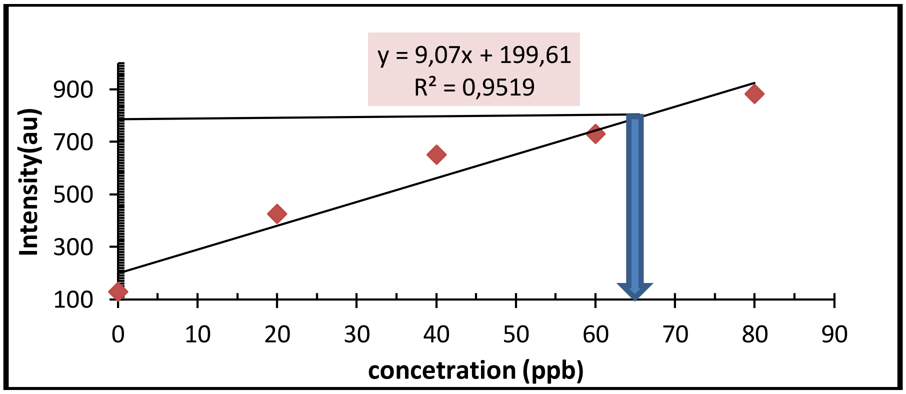

Figure (5): The calibration curve of the iron complex shows the linear relationship between intensity and concentration

Detection limits and quantitative limits in fluorescence spectroscopy:

The accuracy of this analytical method was determined by using the fluorescence technique to determine the low concentrations of iron ions. It was found that the relationship between intensity and focus is linear, and this is subject to Beer's law. where the relative percentage deviation (%RSD) and measurement deviation (S.D) were calculated, as well as the calculation of the limit of detection (LOD), the calculation of the quantitative limit (LOQ), and the correlation coefficient (R2 for low concentration solutions (ppb), as shown in Table (2). This means that fluorescence technology can be used to estimate low concentrations of [10] metal ions.

Table (2): Estimating the accuracy and validity of the spectroscopic method using fluorescence spectroscopy

parameters	Fe(III) ppb	4-SPAP ppb
RSD%	52.27208	8.869945
LOD	97.23859	110.1031
LOQ	324.1286	367.0103
S.D	293.9847	17.73761
Slope	9.07	0.4833
$\bar{x}$	562.4124	199.9743
R^2	0.9519	0.9096

Conclusion

The fluorescence of the organic detector was studied, and the emission and absorption spectra were determined at the greatest wavelength: emission = 320nm, excitation = 276nm. The measurements were made at room temperature using a Spectro fluorophotometer RF-5301pc. As well as studying the effect of solvents on the reagent in polar and non-polar solvents and at laboratory temperature, it was noted that the intensity of the fluorescence intensity of a reagent increases in polar solvents and decreases in non-polar solvents. Where the fluorescent reagent was used to estimate the iron ions and find the unknown concentration in the sample using the calibration curve, we notice that the intensity of the peaks of the fluorescence spectrum increases with the decrease in the concentration of the solutions. The relationship between intensity and concentration is linear, in compliance with Beer-Lambert's law, and the value of the coefficient of determination R^2 (correlation coefficient) is equal to 0.9519. This means that the fluorescence technique can be used to estimate the small concentrations of metal ions.

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