Detection of some Heavy Metals Using Rhodamine B Isothiocyanate Fluoresceinated Dye

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Abstract: The spectral behavior of the binary complexes of Rhodamine B Isothiocyanate (RBITC) dye with metal ions in aqueous medium is studied. The observed fluorescence quenching of the dye reveals that the binding constant for RBITC dye-metal ions follow the order $Cu^{2+} > Ni^{2+} > Pb^{2+}$. Also, the spectral changes of the interaction of the studied dye with metal ions in presence of ACES zwitterionic buffer (ternary complexes) is carried out, where the interaction of dye with ACES zwitterionic buffer is accompanied by as light increasing of the peak intensity at $\lambda = 585$ nm and the binding constant for dye-zwitterionic buffer- metal ion systems were determined where the order of binding with metal ions is as follows: $Pb^{2+} > Ni^{2+} > Cu^{2+}$.

Keywords: Metals, Rhodamine B Isothiocyanate dye, Zwitterionic buffer, Binary complex, Ternary complexes.

INTRODUCTION

Rhodamine B is an amphoteric dye, although usually listed as basic as it has an overall positive charge. It is a highly water soluble, basic red dye of the xanthene class (Lide and Milne, 1994). It is found as a reddish violet powder. Rhodamine B can be used to dye silk, cotton, wool, fibers, nylon, acetate fibers, paper, spirit inks and lacquers, soap, wood stains, feathers, leather and distempers on china clay etc. It has also been used as a drug and cosmetic colour in aqueous drug solutions, tablets, capsules, toothpaste, soap, hair waving fluids, bath salts, lipsticks and rouges. It is often used as a tracer dye within water to determine the rate and direction of flow and transport. Therefore, the usage of RBITC as dye sensitizer is expected to reduce the negative impact of dye waste on environment (Jain *et al.*, 2007).

Rhodamine B isothiocyanate (RBITC) was used as the fluorescent dye in this project. The structure of Rh B is very important in that the benzene ring structures provide the electrons capable of excitement and producing fluorescence. Changes to these structures leads to loss of this property known as photobleaching. This may not be a result of molecule breakdown into smaller molecules but just minor changes to the structure (Juan *et al.*, 2008). The structure of Rhodamine B isothiocyanate (RBITC) dye is depicted in Figure (1).
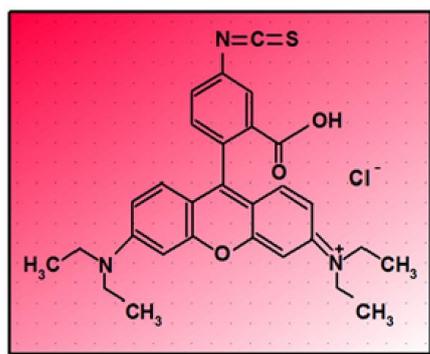
Fig. (1): Structure of Rhodamine B isothiocyanate (RBITC) dye.

Xanthene derivatives especially rhodamine dyes, occupy an important position among different families of dyes, owing to a number of reasons related to their photochemical and photophysical properties. Because of their high fluorescence quantum yield rhodamine dyes have wide spread technszological and scientific applications *e.g.,* single molecule detection (Rigler *et al.*, 1992; Keller *et al.*, 1996), fluorescence labeling (Taylor *et al.*, 1986), dye lasers (Drexhage, 1982; Peterson, 1979; Duarte and Hillmann, 1990), conversion and storage of solar energy (Archer and Ferreira, 1982) etc.

"Heavy metals" is a term which is used to define metals with elemental densities above 5g/cm³ (Morris, 1992). As many other metals they can be found in the earth's shell. Human body also contains small amount of these substances. Mainly they get to organisms via food, drinking water and air. Some heavy metals (e.g. copper, selenium, zinc) are involved in the metabolism of the human body (so called "essential elements") but at very low concentrations (WHO, 1996). If the concentrations of heavy metal in the environment and as a result in a body exceeds certain amount it can be toxic (Duffus, 2002). The reason of heavy metals poisoning is e.g. metals contained water pipes drinking water pollution, or air emissions from factories (Bradl, 2002; He *et al.*, 2005).

Danger of heavy metals is in their ability for bioaccumulation. Bioaccumulation is a deposition of a chemical substance in a biological organism with an increase of its concentration in the organism every time this substance is emitted to the environment independently from its amount.

Accumulation of compounds in living things is faster than its decomposition and happens at any time they are exposed to the substance (Chang *et al.*, 1996; Wang and Shi, 2001).

Environmental pollution from industry is main source of high amounts of heavy metals in the environment. Food poisoning is a consequence and the easiest way to enter human body (UNEP/GPA, 2004).

The aim of this work is to make different probes to detect heavy metal ions.

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MATERIALS AND METHODS

All materials employed in the present investigation were of A.R grade products from commercial sources (Sigma Aldrich) without further purifications. Rhodamine B Isothiocyanate (RBITC), ACES zwitterionic buffer N-(2-acetamido)-2-aminoethanesulfonic acid and transition metal salts used are (Cu $(NO_3)_2.3H_2O$), (Ni $(NO_3)_2.6H_2O$) and (Pb $(NO_3)_2.3H_2O$).

The spectral behavior of the binary and ternary complexes were measured by A JASCO-FP6300 spectrofluorometer with 1 cm quartz cell was used for the emission and spectral measurements and A Shimadzu-UV Probe Version 2.33 UV-Visible automatic recoding spectrophotometer with 1 cm quartz cell). UV/VIS spectrometer was used to measure absorbance of light wavelengths in particular samples.

Procedures used for the absorbance spectra of RBITC dye in presence of zwitterionic buffers:

1×10^{-3} molL^{-1} of zwitterionic buffers solution + 1×10^{-3} molL^{-1} of RBITC dye solution with Molar concentration ratio (1:1).

Procedures used for the measurements of the binding between RBITC dye and transition metals in aqueous medium (binary complexes):

1×10^{-3} molL^{-1} of RBITC dye solution + 1×10^{-3} molL^{-1} of transition metals solution with Molar concentration ratio (1:1) to 1×10^{-3} molL^{-1} of RBITC dye solution + 1×10^{-2} molL^{-1} of transition metals solution with Molar concentration ratio (1:10).

Procedures used the measurements of the binding between RBITC dye, zwitterionic buffers and transition metals (ternary complexes) in aqueous medium:

(1) 1×10^{-3} molL^{-1} of zwitterionic buffers solution+1×10^{-3} molL^{-1} of RBITC dye solution with Molar concentration ratio (1:1).

(2) 1×10^{-3} molL^{-1} of zwitterionic buffers solution+1×10^{-3} molL^{-1} of RBITC dye solution + 1×10^{-3} molL^{-1} of transition metals with Molar concentration ratio (1:1:1) to 1×10^{-3} molL^{-1} of zwitterionic buffers solution+1×10^{-3} molL^{-1} of RBITC dye solution +1×10^{-2} molL^{-1} of transition metals with Molar concentration ratio (1:1:10).

RESULTS AND DISCUSSION

Visible absorption and emission spectra for (RBITC) dye with various metal ion in aqueous medium:

The visible absorption spectra of 1×10^{-3} molL^{-1} of (RBITC) dye in aqueous medium was carried out. The dye exhibits a maximum absorption at λ_{max}=550nm, with a molar extinction coefficient equals to 0.54×10^3 mol^{-1}L.The interaction of different metal ions with the dye was studied in pure aqueous medium by fluorescence measurements at λ_{em}=585nm as shown in Figure (2 a, b).
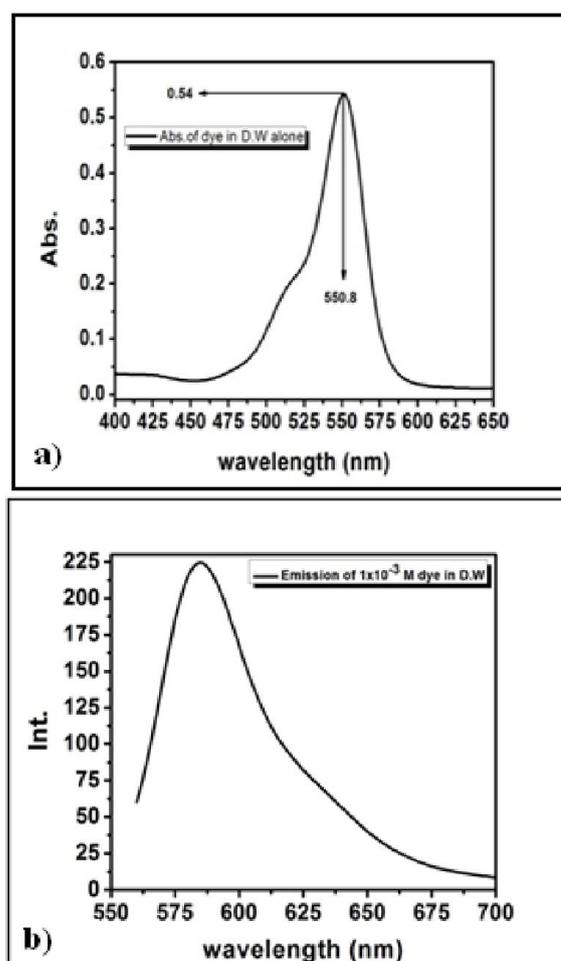
Fig. (2): Absorption and emission spectrum of 1×10^{-3} molL^{-1} RBITC dye in aqueous medium.

The addition of metal ions in the concentration range of 1×10^{-3} to 1×10^{-2} molL^{-1} with RBITC is accompanied by quenching Figures (3-5).
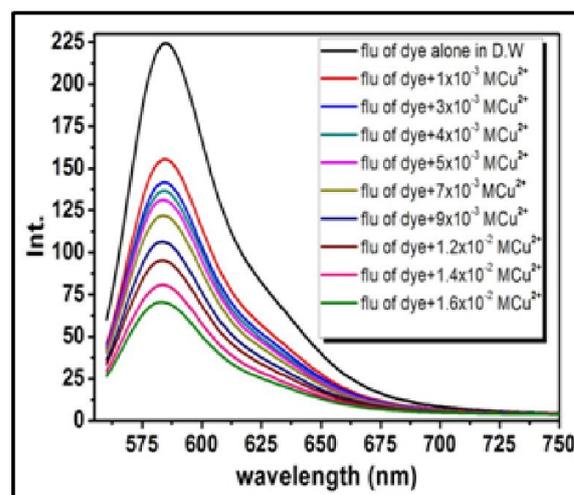
Fig. (3): Fluorescence spectra for the interaction of different concentrations of Cu^{2+} with 1×10^{-3} molL^{-1} of (RBITC) dye in aqueous medium (λ_{ex}= 550 nm, λ_{em}= 585 nm).
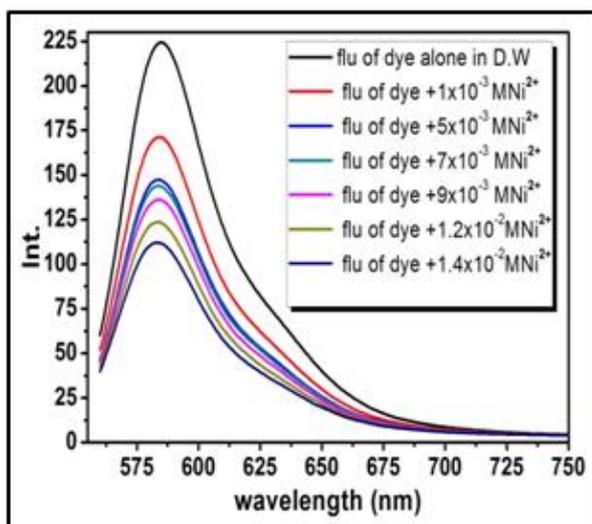
Fig. (4): Fluorescence spectra for the interaction of different concentrations of Ni^{2+} with 1x10^{-3} molL^{-1} of (RBITC) dye in aqueous medium (λ_{ex}= 550 nm, λ_{em}= 585 nm).
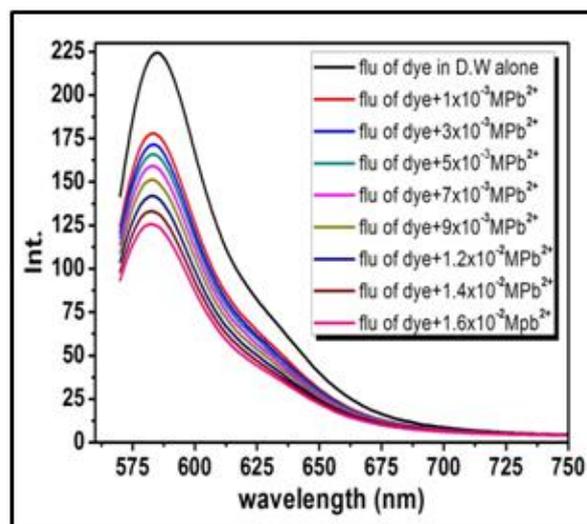
Fig. (5): Fluorescence spectra for the interaction of different concentrations of Pb^{2+} with 1x10^{-3} molL^{-1} of (RBITC) dye in aqueous medium (λ_{ex}= 550 nm, λ_{em}= 585 nm).

Applying Stern – Volmer equation (Lakowicz, 1999) $\Longrightarrow$ $$\frac{F^0}{F} = 1 + K_{SV}[Q]$$

Where F^0, F are the fluorescence intensity of the free dye and the dye combined with metal ion, the Stern-Volmer constant k$_{sv}$ can be calculated for each metal ion. Table (1) and the data are represented in Figure (6).
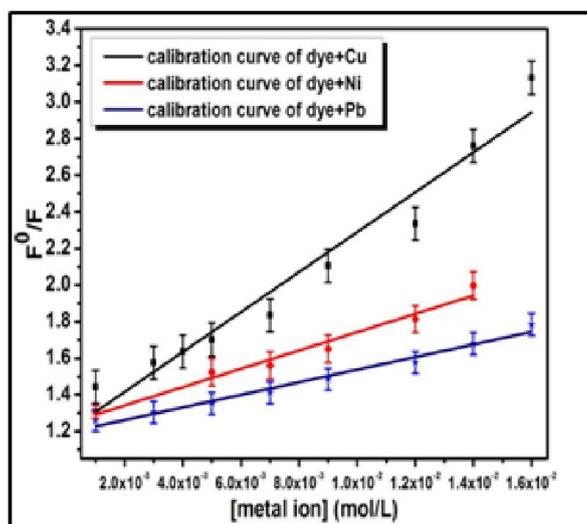
Fig. (6): Calibration plot for metal ions from the fluorescence spectra of its interaction with (RBITC) dye in aqueous medium.

Table (1): Stern-Volmer quenching constant (K**sv**), Limit of detection (LOD), Limit of quantitation (LOQ) for the interaction of metal ions with (RBITC) dye in aqueous medium.

Metal ions	K$_{sv}$ (mol^{-1} L)	R	SD(σ)	LOD (mmolL^{-1})	LOQ (mmolL^{-1})
Cu^{2+}	109.0984	0.95282	0.1112	3.056416	10.188054
Ni^{2+}	50.10541	0.96649	0.0078	0.464022	1.546739
Pb^{2+}	34.42991	0.97766	0.0046	0.403428	1.344761

R: Correlation coefficient**; SD (σ):** Standard deviations

The binding constant of the studied metal the application of Lineweaver- Burk equation on the obtained data Figure (7). The data are collected in Table (2). Lineweaver-Burk equation (Beckford, 2009) is applied in the form:-

$$\frac{1}{F^0 - F} = \frac{1}{F^0 k_a [Q]} + \frac{1}{F^0}$$

Where ka is the binding constant of RBITC with the quencher (metal ions) in molL-1. The data reveal that, the binding constants of the metal ions with RBITC dye following the order {Cu2+ > Ni 2+ > Pb2+}.
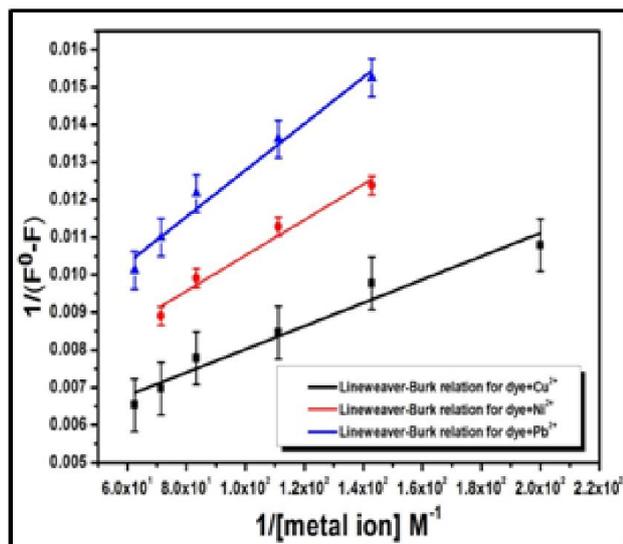
Fig. (7): Line weaver-Burk equation plot for the interaction of different concentrations of metal ions with (RBITC) dye in aqueous medium.

Table (2): Binding constant (K_a) of metal ions with (RBITC) dye in aqueous medium.

Metal ions	$K_a(mol^{-1}L)$	R	SD (σ)
Cu^{2+}	284.8935	0.95821	2.56 x 10^{-6}
Ni^{2+}	122.4529	0.98674	1.84 x 10^{-7}
Pb^{2+}	106.4065	0.98973	3.44 x 10^{-7}

R: Correlation coefficient; **SD (σ):** Standard deviations

Visible absorption and emission spectra for (RBITC) dye with various metal ions in the presence of ACES zwitterionic buffer:

The zwitterionic amino acid buffer solutions recommended by Good *et al.* (1966) and Ferguson *et al.* (1980) have been studied in the authors' laboratory for the purpose of pH measurement control in the physiological range of pH. Concerning the current investigation, the authors' goal is to provide pH values for the ACES, which is depicted by the following structure:-
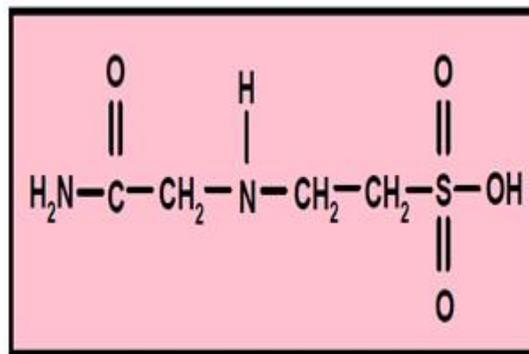
Fig. (8): Structure of ACES Buffer.

The zwitterionic buffer compound ACES may form complexes with cations such as Mg^{+2} and Ca^{+2}. At a high NaCl concentration, the complex formation is mini-mized.

ACES is a zwitterionic buffer used in biochemistry and molecular biology research. It is one of the Good buffers developed in the 1960's to provide buffers in the pH range of 6.15-8.35 for wide applicability to biochemical studies.

The potential inhibition of ACES and other Good buffers in γ-aminobutyric acid receptor binding to rat brain synaptic membranes has been investigated (Tunnicliff and Smith, 1981). The survival of a strain of Listeria monocytogenes Scott A tolerant to high hydrostatic pressure has been studied in ACES buffer (Karatzas and Bennik, 2002).

ACES was used recently to assist calorimetric determination of the affinity of Cu (II) to Alzheimer's Disease's A β peptide and some other bioligands (Grossoehme *et al.*, 2010; Sacco *et al.*, 2012).

The absorption and emission spectra for 1x10^{-3} molL^{-1} of (RBITC) dye, and the interaction with 1x10^{-3} molL^{-1} of ACES buffer, where a considerable decrease in the absorbance value is observed with as light shift in the maximum absorptivity, also, a slight increase of the peak intensity at λ= 585 nm is obtained when the dye is interacted with the zwitterionic buffer molecule, which could be assigned to the formation of associates between the two molecules Figure (9 a, b).

The interaction of 1x10^{-3}molL^{-1} of the studied metal ions (Cu^{2+}, Ni^{2+}, Pb^{2+}) with 1x10^{-3} molL^{-1} of (RBITC) dye in the presence of1x10^{-3} molL^{-1} of ACES buffer is studied by monitoring the change in fluorescence at λ=585 nm, where quenching effect is observed in the case of all the concerned metal ions Figures (10, 11 and 12).

Fig. (11): Fluorescence spectra for the interaction of different concentrations of Pb^{2+} with $1x10^{-3}$ molL^{-1} of (RBITC) dye (in the presence of $1x10^{-3}$ molL^{-1} zwitterionic buffer ACES) in aqueous medium.
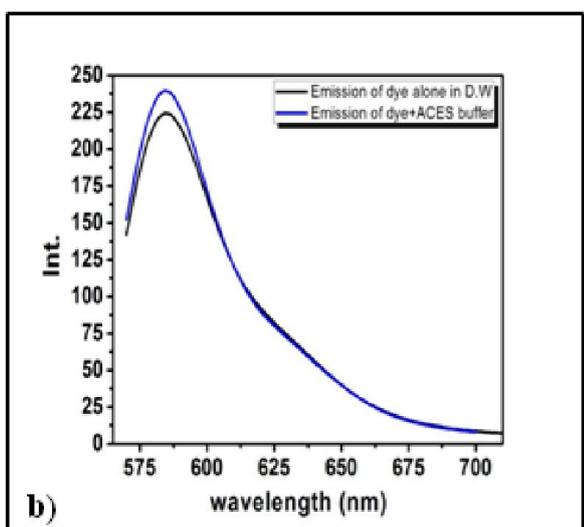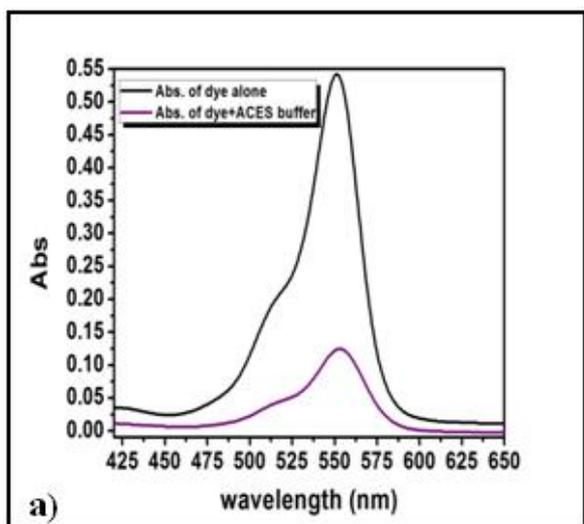
Fig. (9): Absorption and emission spectra of $1x10^{-3}$ molL^{-1} RBITC dye and $1x10^{-3}$ molL^{-1} of ACES buffer.
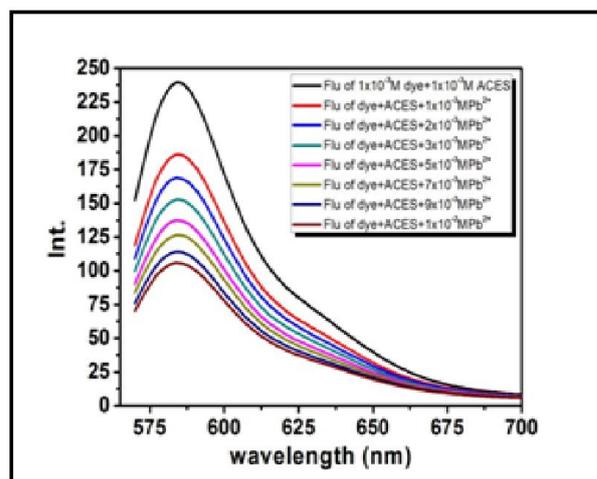
Fig. (12): Fluorescence spectra for the interaction of different concentrations of Ni^{2+} with $1x10^{-3}$ molL^{-1} of (RBITC) dye (in the presence of $1x10^{-3}$ molL^{-1} zwitterionic buffer ACES) in aqueous medium.

The Stern-Volmer quenching constants were calculated and collected in Figure (13), Table (3).

Fig. (10): Fluorescence spectra for the interaction of different concentrations of Cu^{2+} with $1x10^{-3}$ molL^{-1} of (RBITC) dye (in the presence of $1x10^{-3}$ molL^{-1} zwitterionic buffer ACES) in aqueous medium.
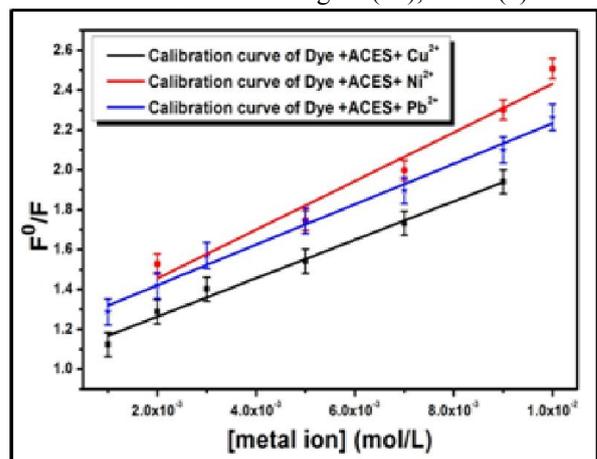
Fig. (13): Calibration plot for metal ions from the fluorescence spectra of its interaction with (RBITC) dye in aqueous medium containing ACES zwitterionic buffer.

Table (3): Stern-Volmer quenching constant (K_{sv}), Limit of detection (LOD), Limit of quantitation (LOQ) for the interaction of metal ions with (RBITC) dye in aqueous medium containing ACES zwitterionic buffer.

Metal ions	K_{sv} (mol^{-1} L)	R	SD(σ)	LOD (mmol L^{-1})	LOQ (mmol L^{-1})
Cu^{2+}	96.38899	0.98726	0.9277	2.887363	9.624543
Ni^{2+}	122.064	0.95499	0.02145	0.527182	1.757275
Pb^{2+}	101.528	0.98971	0.00654	0.193247	0.644157

R: Correlation coefficient**; SD (σ):** Standard deviations.

The binding constant values for the metal ions with the two ligands (dye and ACES) were calculated by using Lineweaver-Burk equation Figures (14), and the data are collected in Table (4), the binding follows the order: {$Pb^{2+} > Ni^{2+} > Cu^{2+}$}**.**
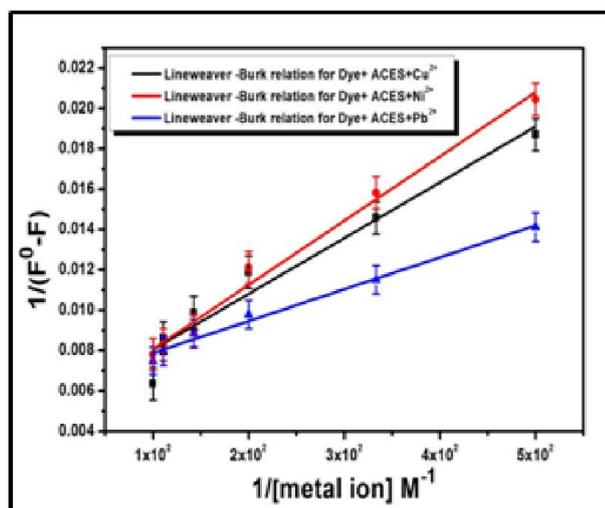
Fig. (14): Lineweaver-Burk equation plot for the interaction of different concentrations of metal ions with (RBITC) dye in aqueous medium containing **ACES** zwitterionic buffer.

Table (4): Binding constant (K_a) of metal ions with (RBITC) dye in aqueous medium containing zwitterionic buffer (**ACES**).

Metal ions	K_a(mol^{-1} L)	R	SD(σ)
Cu^{2+}	125.1478	0.98406	9.3214 X 10^{-6}
Ni^{2+}	152.5468	0.99083	2.8797 X 10^{-8}
Pb^{2+}	573.2905	0.97153	2.2944 X 10^{-6}

R: Correlation coefficient**; SD (σ):** Standard deviations.

In comparison to the binding constant of metal ions with the RBITC dye in a pure aqueous medium, Cu^{2+}exhibit low binding constant in the presence of zwitterionic buffer ACES, which may be attributed to the strong binding of Cu^{2+} with ACES (Taha *et al.*, 2007; Azab *et al.*, 2000; Pope *et al.*, 1980; Sigel and Martin, 1982) as shown in Figure (15).
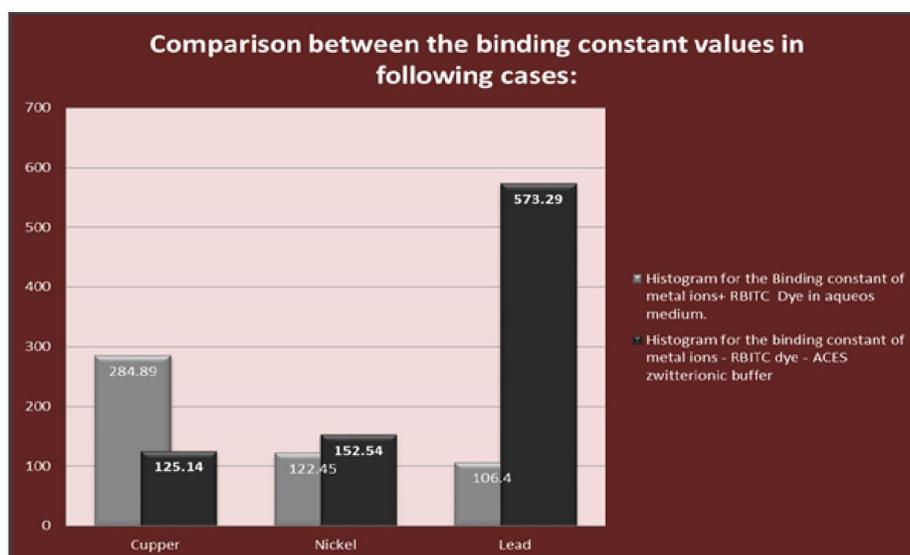
Fig. (15): Histogram for the Binding constant of metal ions – RBITC dye in both pure aqueous medium and in presence of **ACES** zwitterionic buffer.

Hence the net result gives lower value of binding with the dye. In contrary, the other metal ions acquire high binding constants values in ACES buffer than with the dye only. The promising result is the great increase in the binding constant of Pb^{2+} with the dye in presence of ACES zwitterionc buffer.

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الكشف عن بعض أيونات المعادن الثقيلة باستخدام الصبغة الفلورسينية (الرودأمين أيزوثيوسيانات)

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لقد تم دراسة السلوك الطيفي لصبغة الرودامين أيزوثيوسيانات في وجود بعض من أيونات المعادن الثقيلة (تكوين متراكب ثنائي) في الوسط المائي وقد لوحظ أنه يحدث انخفاض واضح في شدة الانبعاث للصبغة وتم حساب قيم ثوابت الارتباط ووجد أنها تتبع الترتيب التالي النحاس > النيكل > الرصاص. وأيضا تم دراسة طيف الامتصاص الإنبعاثي لصبغة الرودأمين ب أيزوثيوسيانات مع أيونات العناصر تحت الدراسة في وجود أيون الزفيتر المنظم (آسس) (تكوين متراكب ثلاثي) وقد أوضحت النتائج أنه عند تفاعل أيونات العناصر مع الصبغة في وجود أيون الزفيتر المنظم يحدث انخفاض واضح في شدة الانبعاث للصبغة عند طول موجي ٥٨٥ نانومتر ويعتمد معدل هذا الانخفاض على نوع أيون الزفيتر المستخدم في القياس مما يؤكد تكون متراكب ثلاثي   (أيون الفلز-الصبغة-أيون الزفيتر) وقد تم حساب قيم ثوابت الارتباط ووجد أنها تتبع الترتيب التالي: الرصاص > النيكل > النحاس**.**