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New High Throughput Microtiter Plates for Detection of Organophosphorous Pesticides in Environmental Samples

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Abstract

Quenching of lanthanides luminescence of a novel long live Tb(III) - Ethyl 1-Allyl-1,2-Dihydro-4-Hydroxy-2-Oxoquinoline-3-Carboxylate (EADHOC) as a ligand to form a complex with molar ratio 1:3 in solution has been studied in ethanol in presence of organophosphorus pesticides both Di-chlorvos(P1) and paraoxon-Ethyl (P2). The intensity of luminescence of Tb(III) –(EADHOC) decrease as the concentration of pesticides increase. The quenching effect was observed with both pesticides. Direct methods of determination of pesticides under investigated have been developed by using luminescence of Tb(III) –(EADHOC) probe in a solution. The linear range for determination of the selected pesticides P1(Di-chlorovos) and P2(Paraoxon-Ethyl) is1 to 7 and 1 to 10 µM. the detection limits were 1.9×10-7and 9.8×10-7 µM for P1(Di-chlorovos) and P2(Paraoxon-Ethyl) respectively. The Thermodynamic parameters and binding constant (K) of OPs with Tb(III) –(EADHOC) were evaluated. Including the value of enthalpy (ΔH) and entropy(ΔS) changes for Probe interaction with pesticides. As the natural water sample in this study don't contain the above mentioned OPs over the limit detected by the methods, a recovery study was carried out after the addition of the adequate amount of organophosphorus pesticides.

Introduction:

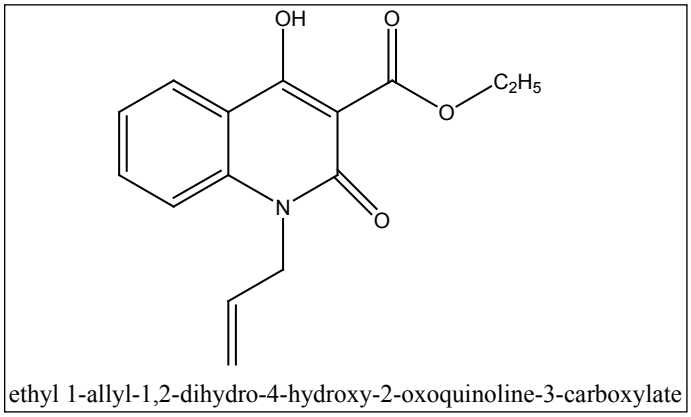
Organophosphorus pesticides (OPs) are used in a wide range in many fields especially in agriculture fields to improve the quality and quaintly of crops, However OPs are synthetic chemical compounds of esters, amides, or thiol derivatives of phosphoric, phosphonic, phosphorothioic or phosphonothioic acids. There are over 100 OPs. According to the chemical structural of organophosphorus pesticides it has a central phosphorus atom, with either double bonded oxygen (P═ O), or a double bonded sulfur atom (P ═S). P═ O pesticide is called an Oxon pesticide, and the P═ S is termed as thion pesticides [1].

Organophosphorus pesticides (OPs) compounds are highly powerful inhibitors of cholinesterase, and Ops have a very highly toxicity effect on human health and bad environmental effect. Organophosphorus pesticides with a Phosphoryl (P=O) function group such as (Di-Chlorvos and Paraoxon-Ethyl) wide spread and frequent commercial use of organophosphate based compound in agricultural lands and other environmental application, So it has resulted in their presence as residues in crops,soil and polutry proudcts and severe risk to the aquatic environment as well as to drinking water quality. Thus, there remains a constant need for the determination of pesticide concentrations in natural water. The European Union (EU) has recently scheduled a monitoring based priority list containing those species of pesticides, which are considered to represent an exceptionally high risk to humans and to the aquatic environment [2].

The design of new sensors with improved analyte sensitive and selective is a great importance in this area. Many analytical techniques was introduced to determination of organophosphorus pesticides (OPs) in water including gas chromatography-mass spectrometry (GC-MS) [3,4,5]. High performance liquid chromatography (HPLC) and mass spectrometric (LCMS) or diode array detection (LC-DAD) [6,7,8,9,10]. A disadvantage of the HPLC, CE and GC analysis in general the analysis takes long time and limited sensitivity, costly and require high purity grade of organic solvents of HPLC[11,12,13]. The lanthanides ions have specific characteristics of its luminescent for the narrow band emission and long life time of the excited state and also it form a stable complex [14,15,16,17,18].

Ethyl 1-allyl-1, 2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxylate (EADHOC) as shown in Figure. 1 is a water- souble and commercially avaialble has a nitrogen and oxygen atoms that can coordinate to the metal ions. The excited light absorbed by EADHOC is transferred to its triplet state by the intersystem crossing, then intra-moleculary transferred to Tb(III) ion. The sensitized luminescence of widely applied in many fields, such as probes and labels in variety of biological and chemical applications[19,20,21].However the lanthanides complexes widely used to recognize the biological function in the body metabolism [22,23,24,25]. And in a variety of process as enzyme inhibitors [19, 20]. And plant preservation [26].

In the present work, we have studied the interaction of Tb(III)-with Ethyl 1-allyl-1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxylate(EADHOC) with different organophosphorus pesticides (OPs) by using luminescence spectroscopy. We focued our studies on two pesticides Di-chlorovos(P1) and Paraoxon-Ethyl (P2). The structures of the studied pesticides are shown in the Figure. 2. However, this study is considered as a part of our project for development of chemo and biosensors for selective detection of organophsoporus pesticides in enviromental samples [27, 28, 29,30 ,31].



ethyl 1-allyl-1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxylate

Figure 1. Strucutre of EADHOC ligand

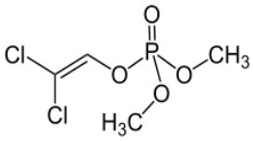
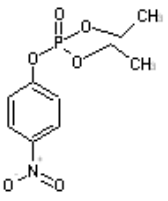
Reagents:

Ethyl1-allyl-1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxylate(EADHOC) and all OPS uesd were obtained from Sigma-Aldrich, St.Louis,Mo.USA. Standard certified pesticides of analytical grade were purchased from Sigma –Aldrich. (Hamburg, Germany) supplied certified reference items. The purity above 95% individual stock solution containing 0.1 µg mL-1 analyte was prepared in high pure ethanol. Stock solutions (1×10-3 M) of OPs were prepared by dilution of the standards using absolute ethanol. This solution was stable; however daily working solutions were prepared by dilution with Absolut Ethanol. All solution were protected against light and humidity with aluminum foil and stored in a refrigerator. Lanthanide metal salt TbCl3.6H2O was from Sigma Chemical co. A 1×10-3 M of standard solution of terbium chloride was prepared by dissolving a high –purity terbium chloride hexa-hydrate (from Merck, Darmstadt, Germany) in ethanol (from Sigma-Aldrish). Stock solutions of Tb (III)-EADHOC were freshly prepared by mixing appreciate amount of TbCl3 and the ligand followed by the stirring for about 30 min. to form Tb (III)-EADHOC. The Tb(III)-EADHOC- Pesticide solution were prepared by transferring of 0.05-1 mL aliquots of the pesticides working standard solution into a 10 mL volumetric flask followed by the addition of the required volume of Tb(III)-EADHOC Solution. The solution were shaking vigorously before irradiation and / or carrying out analytical measurements.

Apparatus:

Allmeasurementswerecarriedoutona Jasco6300spectrofluorimeter equipped with a 150 W Xenon lamp source and quartz cell of 1cm path length. The slit widths of excitation and emission wave length were 5 nm/5nm. All absorption spectra were performed on a perkin-Elmer lambda 20 UV-VIS spectrophotometer equipped with quartz cells. Software programs used for the statistical treatment of the data were stagraphics plus for windows 3.1

Figure 2. Strucutre of studied Pesticides .

Pesticide	IUPAC	Structure
Di-chlorovos (P1)	2,2-dichlorovinyl dimethyl phosphate	
Paraoxon-ethyl (P2)	Diethyl 4-nitrophenyl phosphate	

software package (Statistical Graphics Corporation, USm1994-1997) and Excel software package from Microsoft office 97, version 8.0,1997. Luminescence time-resolved measurements in microtiter plates (MTP) were performed using 96-well flat bottom black microplates. The instrument is equipped with the high Energy xenon flash lamp. The instrumental parameters of the MTP reader were as follows: excitation filter of 320 ± 10 nm, emission filter of 520 ± 10 nm, lag time 50 µs, Integration time 100 µs, 10 flashes per well, time gap between move and flash 100 ms. Luminescence top measurement mode was used and temperature was adjusted to 250C. The gain of the microtiter plate reader was fixed at a value of 1500. The mean fluorescence intensity was calculated as the average of eight independent measurements of each concentration.

Procedure:

The luminescence spectra and the intensities were monitored at the fixed analytical emission wave length (λem=545 nm) of the complex in 10 ml of ethanol. Luminescence titrations were performed in 1 cm quartz cuvette by successive addition of pesticides (1×10-6 - 1×10-5 M) Tb(III) Chloride and (1.0×10-5 M) of EADHOC. The titration data were analyzed according to modified Stern-Volmer equation to investigate the type of interaction of Tb (III)-Complex with the different pesticides.A1:3 stoichiometry of Tb(III):EADHOC was used in all experiments . The employed pesticides P1 and P2 seem to absorb the excitation light at 360 nm that may affect the luminescence intensity of Tb (III) by filtering effect. The analysis was done by using the decrease of luminescence intensity due to this effect as well as the quenching results from the interaction between the Tb (III)-EADHOC probe and studied pesticides in ethanol.

Results and discussion:

Interaction of EADHOC with Tb(III)

Steady State UV-vis spectroscopy

The absorption spectrum of 3×10-5M EADHOC as the free ligand in ethanol has maximum absorption band at λ1=320nm, λ2= 350nm, the first band could be attributed to π-π* transition while the second one is the characteristic band for n-π* transition as shown in figure 3.Upon addition of 1×10-5M of Tb(III) to the ligand solution, the first band of the ligand is slightly shifted to the lower wavelength (λ=316 nm) with a decrease in the absorbance value, while the second band of the ligand become as a shoulder, hence such behavior is a good configuration of the binding of Tb(III) to the ligand molecule. The behavior of Tb(III)-Complex in ethanol solvent was monitored by UV-VIS Spectroscopy for several times at room temperature . Liberation of ligand wasn't observed under these conditions, so it suggests that the complex is stable under the conditions studies.

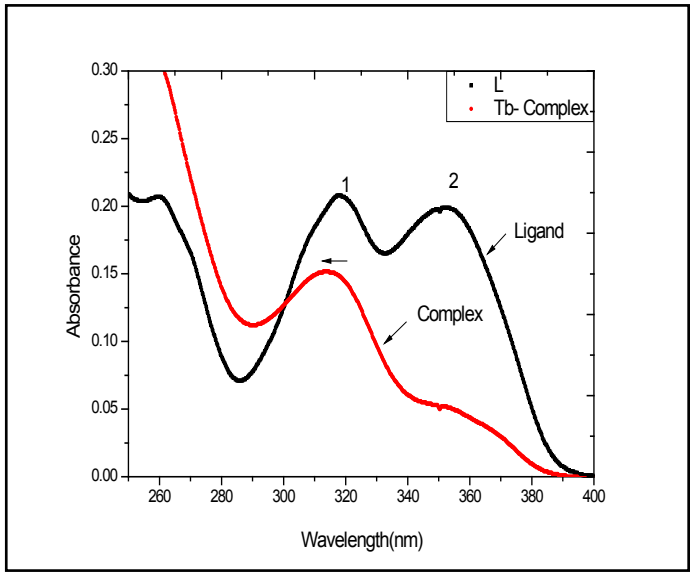


Figure 3. UV Absorption spectra of (3×10-5M) EADHOC (L), and (3×10-5 M) EADHOC + (1×10-5M) TbCl3 in Ethanol at room temperature.

Steady state luminescence spectroscopy:

The luminescence spectrum of the Tb (III) - EADHOC probe investigated at room temperature in ethanol as shown in Figure.4. The best stoichiometry of the formed complex was monitored by fluorescence measurements, where the data reveal that the ligand: metal ratio which give the higher intensity=545 nm is obtained at 3:1 Figure.5 a,b.

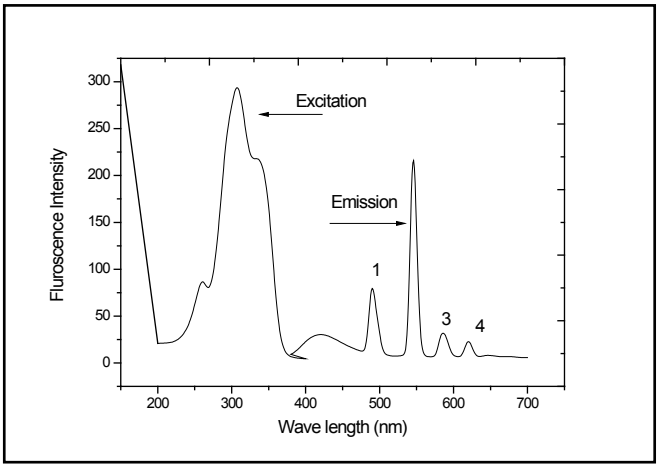


Figure 4. Fluorescence and Excitation spectra for Tb(III)-EADHOC in Ethanol [CTb=1×10^{-5} mol L^{-1} and CEADHOC=3×10^{-5}mol $^{L-1}$].

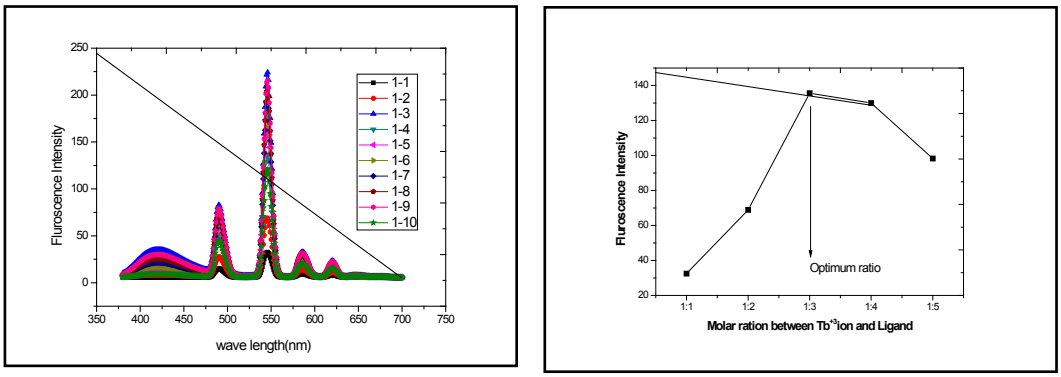


Figure 5. (a) Fluroscence spectra at (λEX: 355 nm) for Tb(III)-Complex with different concentration of EADHOC ligand[CTb(III)=1×10^{-5} mol L^{-1} and CEADHOC =1,2,3,4 and5×10^{-5}] (b) Jop's method.

3.2 Interaction of Tb(III)- EADHOC probe with different pesticides.

3.2.1. Luminescence spectroscopy of Tb (III)-EADHOC Probe with different pesticides.

The effect of the studied two Organophosphorus pesticides (OPs) on the luminescence intensity of Tb(III)-EADHOC probe has been investigated in ethanol solvent, they show quenching effect on intensity of Tb(III)-EADHOC probe as shown in Figure.6

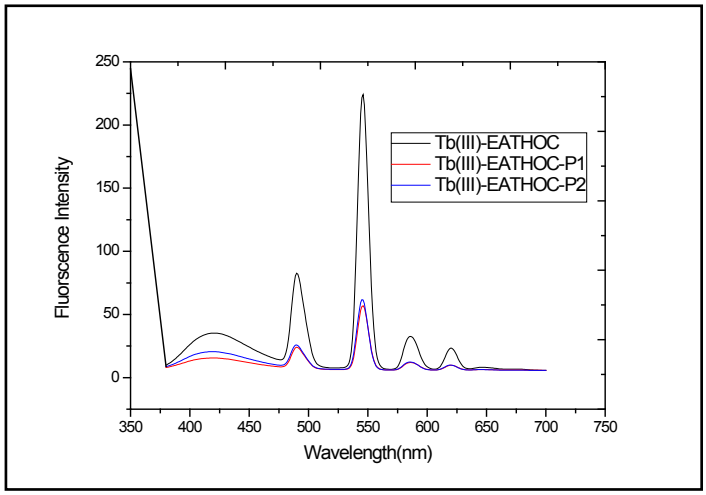


Figure 6. Emission spectra of free Tb(III)-EADHOC and its interaction with Paraoxon-ethyl and Dichlorvos pesticides in ethanol [CTb(III)=1×10^{-5} mol L^{-1} ,CEADHOC= 3×10^{-5} and Cpesticides=1×10^{-6} mol L^{-1}].

3.3. Luminescence quenching and calibration curve for organophosphorus pesticides.

- The effect of different pesticides concentration on the intensity of luminescence of Tb(III) complex exhibited a pronounced change in the emission intensity in ethanol solvent as appear in Figure.6. At experimental conditions. The decrease of the luminescence intensity of Tb(III) –EADHOC after adding pesticides may be attributed to a combined effect including both photo- absorption by the solute and the possible interaction with the pesticides by additional coordination with Tb(III)-EADHOC which may take place at (P=O) functional group. The luminescence intensity decrease due to its chelation to the receptor unit of the sensor and formation of a non-luminescent complex and therefore the net luminescent is quenched. However the quenching process can be classified into two types according to their mechanism, static and dynamic quenching. Both of two types distinguished by their different dependences on the temperature and excited life time. Dynamic quenching is diffusion controlled because the quencher must be diffuse to the fluorophore during the lifetime of the excited state. So high temperature mean more diffusion coefficient. However the bimolecular quenching constant are expected to increase with temperature while if (Ksv) decrease with increase temperature, the quenching process is static rather than dynamic [32, 33,34]. Statics quenching implies either existence of a sphere of effective quenching where dynamics or collisional quenching involve the collision followed by the formation of a transient complex between an excited-state fluorophore and a ground state quencher. The effect of temperature on the stern-volmer quenching constant (KSV) was examined for the two pesticides as calculated from the obtained data Figure7, 8. The data reveal that the mechanism of quenching for Dichlorovos is static, on other hand dynamic or collisional quenching is that for Paraoxon-Ethyl.

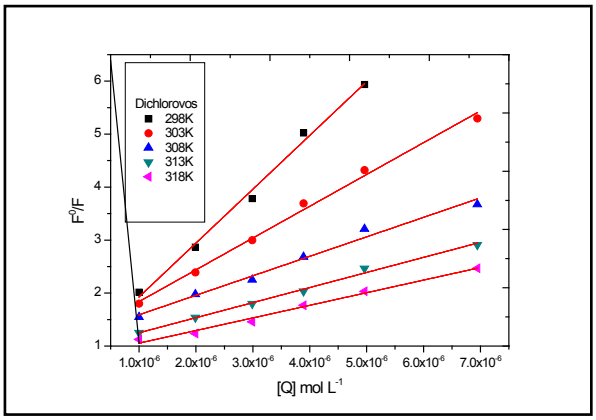


Figure 7. Steren-Volmer calibration plot for detection of Paraoxon-Ethyl using Tb(III)-EADHOC Complex in Ethanol at different temperature.

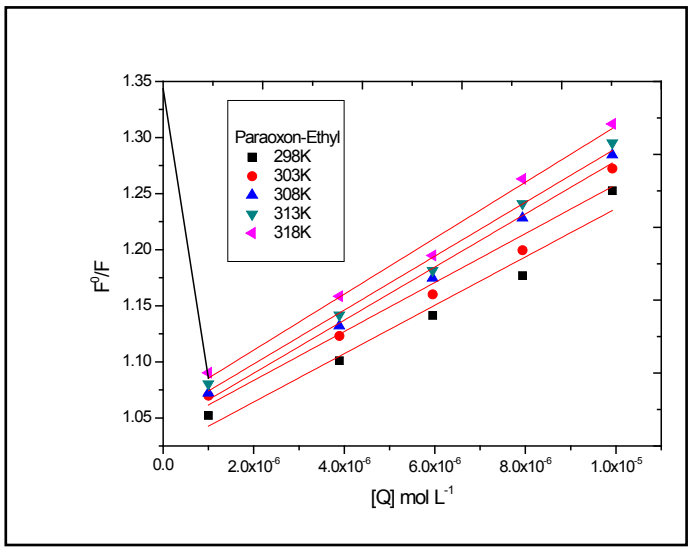


Figure 8. Steren-Volmer calibration plot for detection of Paraoxon-Ethyl using Tb(III)-EADHOC Complex in Ethanol at different temperature.

Under optimum conditions, the calibration graphs are desired by equation: Y=a+bX (where Y=F0/F, a= intercept, b=slope and x =concentration of pesticides in M). The limit of detection (LOD) calculated for Di-chlorvos and paraoxon-ethyl pesticides in ethanol are 1.9 and 9.8 µ M respectively and limit of quantitation (LOQ) for Di-chlorvos and paraoxon-ethyl pesticides are 6.5 and 1.3 µ M respectively.

3.4. Determination of binding parameters of Tb (III)-EADHOC-complex with Di-chlorovos.

Quenching of luminescence intensity of Tb(III)-EADHOC in presence of different concentrations of Dichlorovos can be analyzed to obtain various binding parameters according to the following equation [35-36].

Log [(□-F)/F]=Log K+n Log [Q]

Where K and n are the binding constant and the number of binding sites respectively .the plot of log F0-F/F versus Log [Q] gave a straight as shown in Figure.9.

Slope of such curve equal to n while the intercept equal to Log K. The values of n approximately equal to 2 indicating that there is two binding sites in Tb(III)-EADHOC for the selected pesticide P1 as in Table.1.

3.5. Determination of thermodynamics parameters for the interaction of Tb(III)-EADHOC-with Di-Chlorovos pesticide.

The interaction of Tb(III)-EADHOC with Di-chlorovos may proceed through hydrophobic interaction force either van der waales or hydrogen bond[37]. Thermodynamics parameters can be calculated using Van't Hoff equation

LnK=-ΔH/RT+ΔS/R

Where R is the gas constant, T is the experimental temperature, and K is the binding constant at the corresponding temperature

.ΔH and ΔS are enthalpy and entropy change of reaction

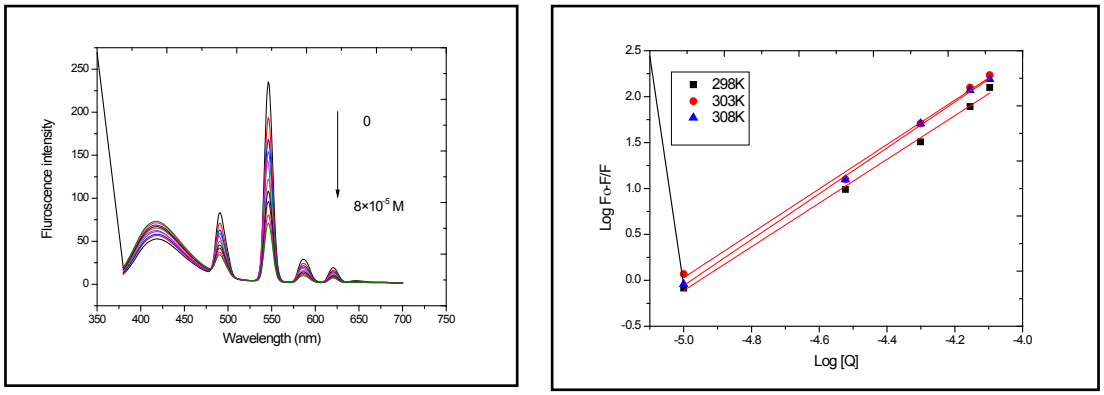


Figure 9. Effect of Concentration of Di-Chlorvos pesticide on the emission spectra of Tb(III) -EADHOC in Ethanol at 25ºC(λEX = 355 nm) .

Table 1. Binding parameters of Tb(III)-EADHOC with Di-chlorovos (P1) pesticide.

Pesticides	Temperature(K)	K(×10⁴M⁻¹)	n	r	SD (σ)
P1	298	6.1	2.3	0.996	0.03
	303	1.2	2.41	0.995	0.07
	308	2.6	2.5	0.999	0.03

SD (σ) is a standard deviation.

Pesticides	Temperature	K_D	R	ΔG^o	ΔHo	ΔSo
	(k)	($Lmol^{-1}$)		(KJ/mol)	(KJ/mol	(J/mol K)
Di-chlorovos (P1)						
	298	**9.78×10^5**	**0.9057**	**-35.09**		
	303	**8.17×10^5**	**0.9752**	**-35.2**		
	308	**7.41×10^5**	**0.9377**	**-35.5**	**-21.6**	**44.9**
	313	**6.81×10^5**	**0.9585**	**-35.9**		
	318	**6.1×10^5**	**0.9970**	**-36.01**		

Table 2. Binding Constant (KD) and thermodynamics parameters for the interaction of Tb-Complex with the Di-chlorovos.

3.6 Interference of other relevant metal ions

To study the effect of the interference of other ions present in real sample a systematic study proceed on samples containing 1×10^{-5} M P1 Di-chlorovos and P2 Paraoxon-Ethyl by Tb(III)-EADHOC at different concentration levels depending on their respective solubilizes as shown in table 3,4.

Table 3. Recovery and tolerance of Di-chlorvos (10µmol/L) using Tb(III) –EADHOC complex in the presence of different interfering species.

Interfering species	F°	Concentration of an interfering species		Tolerance
		1 µM	2µM	
Co^{+2}	54.4836	53.8354	53.3911	3µM (0.174µg/ml) Q
Cd^{+2}	112.044	111.435	102.849	3µM (0.337µg/ml) Q
Cu^{+2}	56.8326	56.551	52.8315	3µM (0.19µg/ml) Q
Hg^{+2}	62.6095	59.2308	56.0091	2µM (0.4µg/ml) Q
K^+	54.8205	53.5068	48.0075	2µM (0.078Mg/ml) Q
Na_2CO_3	55.499	54.3903	52.6241	4µM (0.424µg/ml) Q
Na_2HPO_4	55.2254	52.7138	48.6147	2µM (0.28µg/ml) Q
NaCl	54.762	51.0818	48.735	4µM (0.23µg/ml) Q
Na_2So_4	55.894	55.476	50.7206	2µM (0.28µg/ml) Q
NH_4Cl	48.5206	45.5185	43.9488	3µM (0.160µg/ml) Q
Ni^{+2}	110.094	103.468	100.975	3µM (0.17µg/ml) Q
Pb^{+2}	84.5201	82.9189	74.9271	3µM (0.62µg/ml) Q

Pesticides	samples	Regression equation	r^2	added (µM)	Found (µM)	Recovery (%)
Paraoxon-ethyl (P2)	Tap Water	F_0/F= 0.65+0.95[P]	0.98142	2	1.8	90
				4	4	100
				6	6.3	105
				8	7.6	95
				10	9	90
	Mineral Water	F0/F =0.842+0.116[P]	0.96245	2	2.4	120
				4	2.7	67
				6	7.2	120
				8	8.4	105
				10	9.9	99
	Sediment	F0/F =0.949+0.0366[P]	0.93243	2	2	100
				4	3.2	80
				6	5.7	95
				8	7.9	98
				10	13	130
Dichlorvos(P1)	Tap Water	F0/F= 0.95+0.085[P]	0.98921	2	2.5	125
				4	4	100
				6	6	100
				8	8	100
				10	14	140
	Mineral Water	F0/F =0.0938+0.047[P]	0.95575	2	2.4	120
				4	2.7	67
				6	7.2	120
				8	8.5	106
				10	9	90
	Sediment	F0/F =0.845+0.239[P]	0.93437	2	1.7	85
				4	4.8	120
				6	5.2	86
				8	8.8	110
				10	9.9	99

Table.5 Recovery of organophosphorus pesticides in different type of natural water sample

The proposed methods was applied to determination of organophosphorus pesticides P1(Di-chlorvos) and P2(Paraoxon-Ethyl) in different types of water(tap water, mineral water and sediment) as water in this study didn't contain the studied pesticides OPs a recovery was carried out after addition of the adequate amount of pesticides under investigation. The analysis performed after the addition of P1 (Di-chlorvos) and P2 (Paraoxon-Ethyl) in water samples as the result obtain as shown in table 5.

5. Conclusion

These papers describe the application of Tb (III)-EADHOC luminescence quenching for sensing organophosphorus pesticides in ethanol solvent. The luminescence changes observed on adding of P1(Di-chlorovos) and P2 (Paraoxon-Ethyl) may use for detection of this analytes .However this method has many advantages as it cheap, rapid direct for determination of the pesticides The linear range for determination of the selected pesticides P1(Di-chlorovos) and P2(Paraoxon-Ethyl) is 7and 1 µM. the detection limits were 1.9 and 9.8 µM for P1(Di-chlorovos) and P2(Paraoxon-Ethyl)

respectively. Also this method was applied to determination of organophosphorus pesticides P1(Di-chlorovos) and P2(Paraoxon-Ethyl) in different types of natural water sample(tap water, mineral water and sediment) as water in this study didn't contain the studied pesticides OPs a recovery was carried out after addition of the adequate amount of pesticides under investigation.

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