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RESEARCH ARTICLE

Fluorescence sensing of dichlorvos pesticide by the luminescent Tb(III)-3-ally-salicylohydrazide probe

Ibrahim Ahmed Ibrahim | Abbas Mamdoh Abbas | Hatem Medhat Darwish

Chemistry Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

Correspondence
Hatem Medhat Darwish, Chemistry Department, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt.
Email: hatem.mdhat@gmail.com, chem.hatem85@gmail.com

Abstract

A fluorescent probe was developed and characterized, it consisted of terbium(III) with 3-ally-salicylohydrazide in ethanol, in which the 1:2 [Tb^{3+}:S_1] molar ratio was the best stoichiometric ratio for the probe. The ligand 3-ally-salicylohydrazide (S_1) was synthesized, then was confirmed by IR, CHN, LC–MS and ^{1}H NMR. The sensitivity of the probe's fluorescence spectra towards the presence of eight organophosphorus pesticides in ethanolic solution was studied, in which the probe showed marked sensitivity towards dichlorvos pesticide. A tangible enhancement of the probe's fluorescence intensity was observed as a consequence of the gradual addition of dichlorvos pesticide. The calculated limit of detection (LOD) was 1.183 μM and limit of quantitation (LOQ) was 3.94 μM. Further characterization of the nature of forces acting in the interaction of the probe with dichlorvos was performed by calculation of binding constants at different temperatures according to the Benesi − Hildebrand equation, and the thermodynamic parameters ΔH, ΔS and ΔG. In order to assess the analytical applicability of the method, the influence of various potentially interfering anion and cations that naturally occur in water and soil were calculated.

KEYWORDS

dichlorvos, fluorescent probe, salen derivative, salicylohydrazide, terbium

1 | INTRODUCTION

Organophosphorous pesticides are the most widely used group of insecticides, worth nearly 40% of market sales.[1] OPs are very strong acetylcholinesterase (AChE) inhibitors and transport in environment by volatilization, leaching and runoff.[2] This work focused on dichlorvos, which is an organophosphorus insecticide with fumigant action used for controlling mosquitos, flies, aphids and spider mites on fruits and vegetables.[3] Dichlorvos is found in commercial products such as Atgard, Chlorvinphos, Cyanophos, DDVP and Nogos.[4] It has a high acute toxicity, in rats, the oral LD_{50} is 25 to 80 mg/kg, while the dermal LD_{50} is 75 to 900 mg/kg. It is classified by the World Health Organisation (WHO) as a possibly carcinogen to humans (Group 2B).[5] The dichlorvos conventional measuring analytical technique in environmental samples is by gas chromatography using electrolytic conductivity or microcoulometric detector[6], which is expensive, complicated and requires operator training. Despite the fact that these techniques are very accurate, sampling and sample preservation before proesssing may interrupt the results in addition to the complicated extraction procedure. There is a new approach in analytical chemistry based on luminescent materials that are sensitive to various parameters of physical and chemical origin. Taking advantage of the lanthanides ions absorption or emission properties, these can be either simply detected or modulated by a process depending on the concentration of the analyte, itself reversibly binding to the lanthanide probe. The hypersensitive transitions of lanthanides, for instance in terbium (III) the transition $^5D_4 \rightarrow {}^7F_5$ between 535 and 555 nm, are good reporters in view of their sensitivity to minute changes in the Ln(III) environment.[7]

In the present work, we report the characterization of a sensitive innovated luminescent probe for dichlorvos pesticide detection based on the Tb(III)-3-allyl-salicylohydrazide probe (Figure 1). For future studies, the probe ligand incorporated with an allyl group as a polymerizable group is investigated for the development of a molecularly imprinted probe.

Abbreviations used: HPLC, high pressure liquid chromatography; LOD, limit of detection; LOQ, limit of quantitation; MIP, molecularly imprinted polymer; QY, quantum yield.

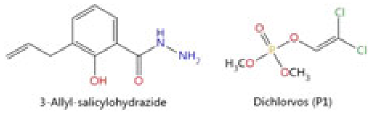

FIGURE 1 Chemical structure of 3-ally-salicylohydrazide and dichlorvos

2 | EXPERIMENTAL

2.1 | Materials

Terbium chloride hexahydrate ($TbCl_3.6H_2O$) of analytical grade was purchased from Sigma-Aldrich. The stock solution was prepared in ethanol. Solvents were purchased from Sigma-Aldrich and Fisher chemicals of high pressure liquid chromatography (HPLC) grade. The water used was bi-distilled in the laboratory.

Material used in organic synthesis and salts used in work were of analytical grade and used without further purification including: heavy metals (nitrate of Ni^{2+}, $Cd^{2+}$and Pb^{2+}), alkali metals (chlorides of Ca^{2+}, Na^+ and K^+ also NH_4^+), anions (sodium salt of CO_3^{2-}, NO_3^{2-}, $H_2PO_4^-$, HPO_4^{2-}, Br^-, and I^-). Pesticides used in this work included dichlorvos (P1), malathion (P2), Crotoxyphos (P3), chlorpyrifos (P4), Paraoxon (P5), Profenofos (P6), Endosulfan (P7) and Heptachlor (P8). All pesticides were of analytical standard brought from Sigma-Aldrich.

2.2 | Chemical and physical measurements

^{1}H–NMR was performed on Bruker Ascend 850 MHz. Infrared spectra were obtained on a 4100 JASCO Japan FT-IR. Fluorescence spectra were performed on a Jasco FP-6300 spectrofluorometer with a 1.0 cm path using q Hellma quartz cell type 111-QS with a 150 W xenon lamp for excitation. Absorption spectra (UV–VIS) were determined using a Shimadzu UV-1800, Double Beam photometric system, and 1.0 cm path length cell. Elemental analysis (CHN) was performed on a Elementer Vario EL. Mass spectra were recorded on a LTQ XL linear ion trap mass spectrometer.

2.3 | Ligand preparation

Synthesis of 3-Ally-salicylohydrazide (S_1): was performed via three steps, Figure 2 shows the scheme of preparation of the concerned ligand S_1:

1. A mixture of methyl 2-hydroxybenzoate (0.1 mol), allyl bromide (0.15 mol) and anhydrous potassium carbonate (0.2 mol) was refluxed in acetone for 8 h, then the mixture was filtered off, and washed with acetone. The product 'methyl 2-(allyloxy) benzoate' was obtained by concentration of the filtrate in a vacuum.
2. Synthesis of methyl-3-allyl-2-hydroxybenzoate based on Claisen rearrangements.[8] Methyl 2-(allyloxy) benzoate was refluxed for 24 h. The reaction mixture was cooled in an ice-bath and the mixture was extracted with ethyl acetate and washed with 0.2 M NaOH solution. The basic solution was acidified with diluted HCl. The methyl-3-allyl-2-hydroxybenzoate was obtained by concentrating the filtrate in a vacuum.
3. Methyl 3-allyl-2-hydroxybenzoate (0.2 mol) and hydrazine hydrate (0.3 mol) were refluxed in ethanol (100 mL) for 4 h. The hydrazide was crystallized, filtered off and washed with ethanol. Further purification was carried out by recrystallization from ethanol, yielding a pale butter yellow fine powder of S_1. Melting point = 162–164°C. Elemental Analysis: calculated: (C, 62.49; H, 6.29; N, 14.57) found (C, 62.39; H, 6.24; N, 14.48). MS (ESI, positive mode): m/z = 193.2 $[M + H]^+$, calculated mass for $C_{10}H_{12}N_2O_2$ 192.22. IR (cm^{-1} KBr) ν(O − H) phenolic 3445 cm^{-1}, ν(N − H) at 3319 cm^{-1}, ν(C = O) 1638 cm^{-1}, δ(N − H) amide II band 1584 cm^{-1}. Combination of δ(O − H) deformation and ν(C − O) stretching vibration 1385–1352 cm^{-1}, ν(C − N) 1103 cm^{-1}. ^{1}H NMR (850 MHz, DMSO-d_6) δ ppm 3.34 (2H, *s*, h8), 4.65 (2H, *s*, NH_2), 5.02–5.07 (2H, *m*, h10), 5.97 (1H, *ddt*, h9), 6.79 (1H, *m*, h4), 7.24–7.27 (1H, *m*, h3), 7.67 (1H, *dd*, h5), 10.15–10.24 (1H, *m*, NH), 13.08–13.23 (1H, *m*, OH) (Figure S1 and S4).

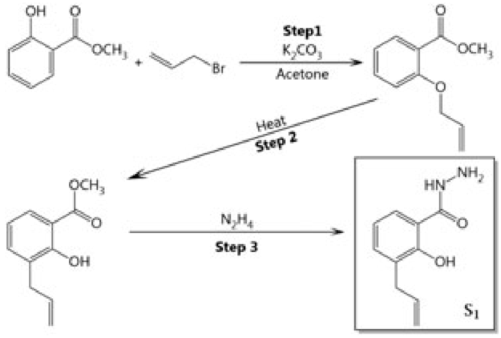

FIGURE 2 Method of preparation of the organic ligand L_1

2.4 | Methods

Preparation of complex samples and testing samples was achieved using stock solutions of materials and by adding a precise volume of these to the solvent, then mixing. The desired measurement was taken at room temperature (22–24°C) or using a water bath to raise sample temperature. Fluorescence measurements were carried out in a 1-cm quartz cell and excitation wavelengths were selected based on excitation and absorption spectra.

2.5 | Determination of quantum yield

The quantum yield (QY) of Tb(III)-S_1 [1:2] was determined in ethanol at a concentration of 1 μM. The QY was calculated with 3-(2-benzothiazolyl)-7 diethylamino-coumarin (Coumarin-6) in ethanol (QY = 0.78) at a concentration of 1 μM as the reference. Using a diluted solution to maintain the absorbance values below 0.1 (at excitation wavelength) and to avoid inner filter effects, the QY was calculated using: [9]

$$Q_X = Q_R \frac{A_R \times I_X \times n_X^2}{A_X \times I_R \times n_R^2} \qquad (1)$$

where Q_R is the QY of the reference, A_R and A_X are absorbances of the reference (R) and Tb(III)-B_1 (X) at the excitation wavelength (350 nm), I_R and I_X are the integrated areas under the corrected emission spectra of the reference and Tb(III)-S_1 and n_R and n_x are the refractive indices of the solutions.

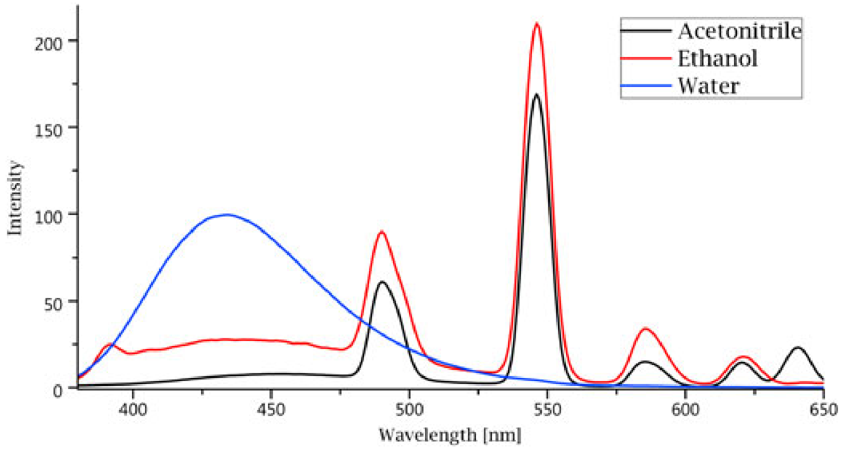

FIGURE 3 Fluorescence spectra of 1:1 Tb(III)-S_1 [20 μM] in different solvents λ_{ex} = 350 nm

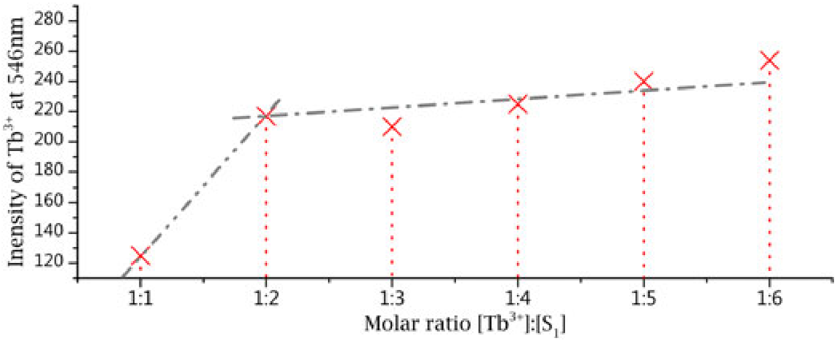

FIGURE 4 Molar ratio study of $[Tb^{3+}]:[S_1]$ versus intensity of Tb^{3+} band at 546 nm in ethanol

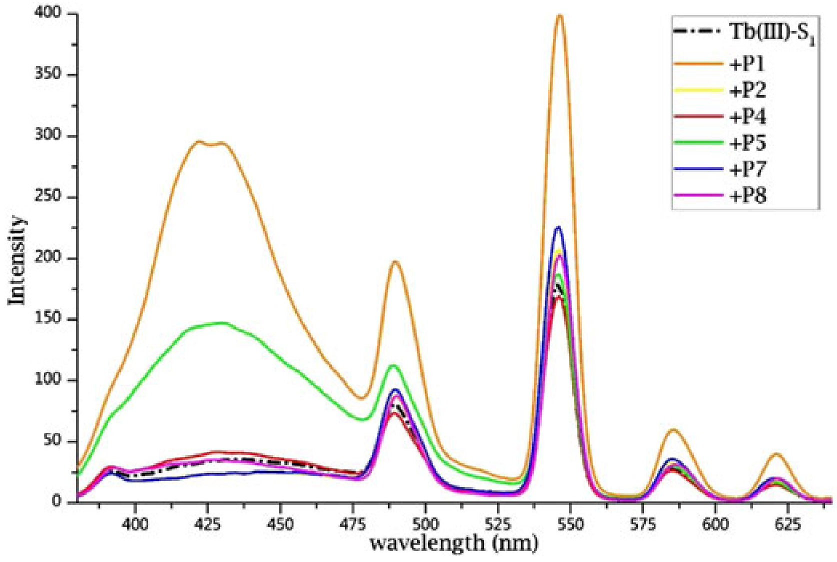

FIGURE 5 Fluorescence spectra for the interaction of [10 μM] Tb(III)-S_1 (1:2) complex with [10 μM] of different pesticides; dichlorvos (P1), malathion (P2), chlorpyrifos (P4), paraoxon (P5), profenofos (P6), endosulfan (P7) and heptachlor (P8) in ethanol, excitation at 350 nm with high sensitivity

3 | RESULTS AND DISCUSSION

Absorption spectra of 3-allyl-salicylohydrazide (S_1) (Figure S2 and S3) indicated the presence of three absorption bands at 207, 244 and 307 nm, the first one may be attributed to the $\pi \rightarrow \pi^*$ transition, while other two bands could be assigned to the $n \rightarrow \pi^*$ transition. Upon complex formation of Tb(III)-S_1 the absorption spectra showed a shift in bands of free S_1, This result was indicative of complex formation. The molar absorptivity for Tb(III)-S_1 [1 μM] $\varepsilon = 7.5 \times 10^3$ L mol^{-1} cm^{-1} in ethanol. While the brightness of the probe (ε.Qy) is 113 L mol^{-1} cm^{-1} in ethanol.

Fluorescence spectra of Tb(III)-S_1 in different solvents is depicted in Figure 3. All characteristic Tb^{3+} bands appeared at 490, 546, 585 and 621 and corresponded to Tb^{3+} f − f transitions $^5D_4 \rightarrow {}^7F_6$, $^5D_4 \rightarrow {}^7F_5$, $^5D_4 \rightarrow {}^7F_4$ and $^5D_4 \rightarrow {}^7F_3$ respectively. These were observed in acetonitrile and ethanol, while in water no bands appeared at all. Water is a strong quencher to Tb^{3+} emission due to the high-frequency vibrational modes of O − H oscillators (νOH = 3300–3500 cm^{-1}, νNH = 3100–3300 cm^{-1}).[10] The stoichiometry for the interaction of S_1 with Tb(III) ions was confirmed using the molar ratio method and was found to be 1:2 as indicated in Figure 4. The QY of the probe were determined to be 0.015 in ethanol for Tb(III)-S_1 (1 μM).

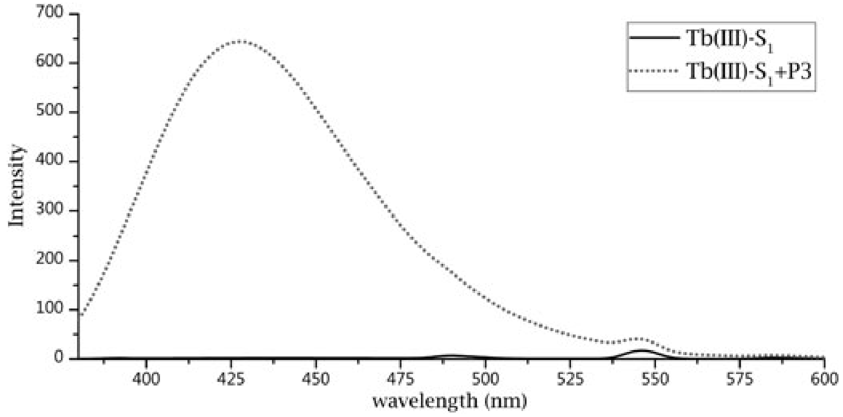

FIGURE 6 Fluorescence spectra for the interaction of [10 μM] Tb(III)-S_1 (1:2) complex with [10 μM] of Crotoxyphos (P3) in ethanol, excitation at 350 nm with medium sensitivity

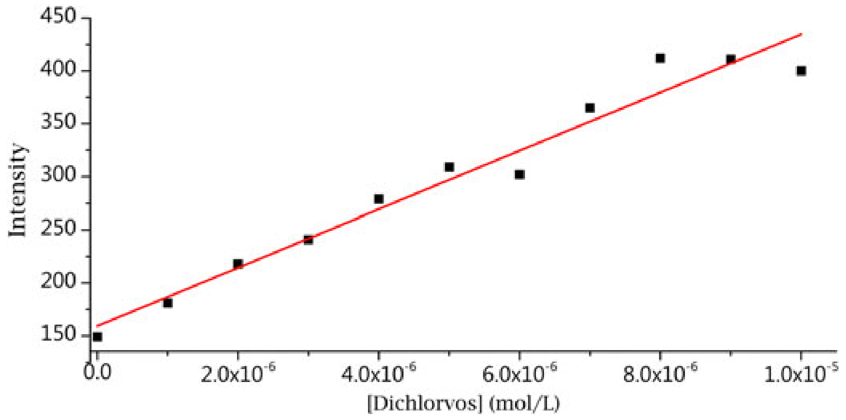

FIGURE 7 Calibration plot for the interaction of P1 with [1:2] Tb(III)-S_1 at room temperature

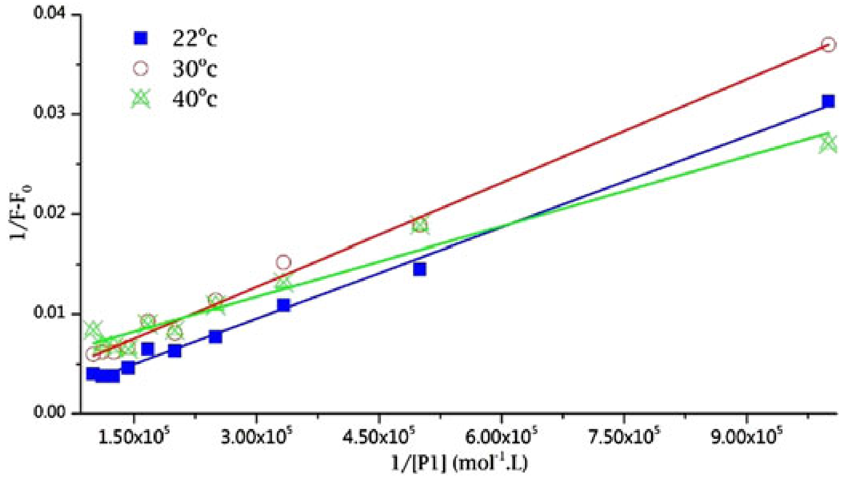

FIGURE 8 Benesi – Hildebrand and relation plot 1/F – F_0 versus 1/[P1] in ethanol

TABLE 1 Thermodynamic parameters involve the interaction of Tb(III)-S_1 with dichlorvos

Thermodynamic parameter	Temperature		
	295 K	303 K	313 K
K_b (L·mol^{-1})	1.4×10^4	6.8×10^4	20.3×10^4
ΔH (KJ.mol^{-1})	103.3		
ΔS (J·mol^{-1} K^{-1})	430		
ΔG (KJ.mol^{-1})	−23.53	−26.97	−31.27

Studies of the interaction of pesticides included in this work with the1:2 Tb(III)-S_1 probe in ethanol are illustrated in Figures 5 and 6. All tested pesticides exhibited enhancement effects to Tb^{3+} bands with very close variation, except for a noticeable enhancement brought by dichlorvos (P1) pesticide. Crotoxyphos (P3) showed a strong emission band at 482 nm with the disappearance of Tb^{3+} bands. Conversely, a small quenching was shown by chlorpyrifos (P4).

The fluorescence of Tb(III)-S_1 probe showed strong sensitivity towards the presence of dichlorvos (P1) pesticide in solution, when the hypersensitive transition of Tb(III) at 546 nm is enhanced by tangible amounts via the gradual addition of P1. The calibration curve was developed and is depicted in Figure 7, in which the correlation coefficient (R^2) is 0.962, which indicated that the regression line perfectly fits the data. The limit of detection (LOD) is 1.18 μM, while the limit of quantitation (LOQ) is 3.94 μM. All these data were extracted from the calibration plot.[11]

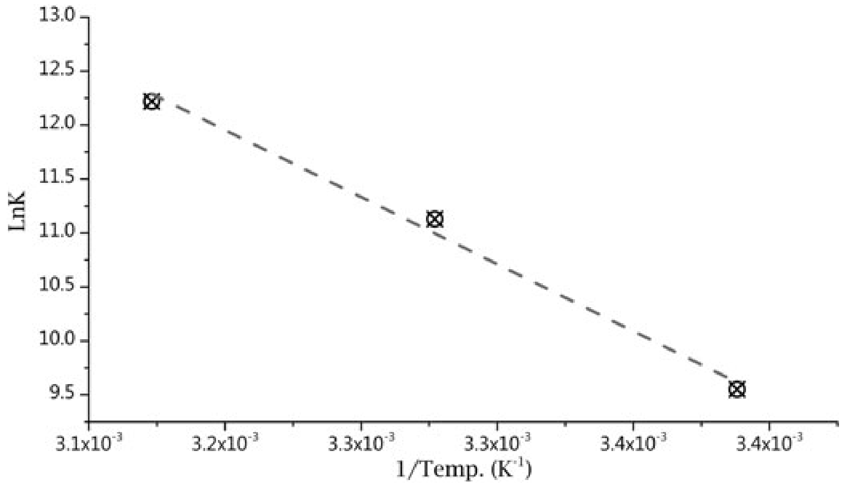

FIGURE 9 Van 't Hoff plot for the interaction of Tb(III)-L_1 with dichlorvos (P1) in ethanol

TABLE 2 Effect of interfering anions, cations, and pesticides on fluorescence spectra of Tb(III)-S_1 complex with dichlorvos

Interfering anion tolerance 5% (μg/L)		Interfering cation tolerance 5% (μg/L)		Interfering pesticides tolerance 5% (μM)	
HPO_4^{2-}	30	Ni^{2+}	26	Malathion	0.5
NO_3^-	10	NH_4^+	27		
CO_3^{2-}	40	Na^+	38	Chlorpyrifos	1
$H_2PO_4^-$	70	K^+	112		
Br^-	100	Pb^{2+}	83	Paraoxon	3.5
I^-	170	Cd^{2+}	59		
		Ca^{2+}	333	Profenofos	0.5

The binding constants (K_b) at different temperatures were calculated based on the Benesi – Hildebrand equation (equation 1):[12,13]

$$\frac{1}{F-F_0} = \alpha + \frac{\alpha}{K_b[Q]}, \text{where } \alpha = \frac{1}{F_L-F_0} \quad (2)$$

where [Q] represents the analytical concentration of P1, F_0 and F are the fluorescence intensities in the absence and presence of dichlorvos pesticide and

$$\alpha = \frac{1}{F_L-F_0}.$$

Figure 8 depicts the relationship between 1/[P1] versus 1/F − F_0 at three temperatures; 22, 30 and 40°C, in which the correlation coefficients were 0.998, 0.993 and 0.963 respectively. From slope and intercept values, the binding constant was calculated at each temperature. K_b are 1.4×10^4, 6.8×10^4 and 20.3×10^4 $L.mol^{-1}$ at 22, 30 and 40°C respectively (Table 1).

The binding constant values dependence on temperature was calculated in order to get more information about the forces acting between P1 and Tb(III)-S_1. The thermodynamic parameters, enthalpy change (ΔH), entropy change (ΔS) and Gibbs energy change (ΔG) are the main quantities utilized to determine the binding mode. The thermodynamic parameters were deduced using equations 3 and 4. The ΔH and ΔS of the binding reaction were the main thermodynamic parameter to determine binding modes. From the thermodynamic standpoint, where $\Delta H > 0$ and $\Delta S > 0$ reflects a hydrophobic interaction; $\Delta H < 0$ and $\Delta S < 0$ reflects the van der Waals force or hydrogen bond formation and $\Delta H < 0$ and $\Delta S > 0$ suggests an electrostatic force.[14] The binding constant K_b was deduced utilizing the Benesi – Hildebrand equation, then using the van 't Hoff equation (equation 3):[15]

$$\mathrm{LnK_b} = -\left[{}^{\Delta H}/_{RT}\right] + \left[{}^{\Delta S}/_{R}\right] \quad (3)$$

The van 't Hoff plot in our study is a linear relationship with a negative slope (Figure 9). and implies that the reaction is of an endothermic nature between the probe and P1. Thermodynamic parameters values were ΔH = 103.3 $KJ.mol^{-1}$ and ΔS = 430 $J.mol^{-1}$ K^{-1}. In this case $\Delta H^o > 0$ and $\Delta S^o > 0$, concluding that the interaction is to of a hydrophobic type. Gibbs free energy was calculated at different temperatures using standard Gibbs free energy equation (equation 4):[10]

$$\Delta G = \Delta H - T\Delta S \quad (4)$$

The reaction between Tb(III)-S_1 and P1 is a spontaneous reaction and became more favoured at higher temperatures (Table 1).

The Tb(III)-3-ally-salicylohydrazide of fluorescence assay was tested with several metals and anions as found mostly in the environment e.g. soil and water[16], in the presence of 5 μM of dichlorvos pesticide, the interfering species were tested by increasing its concentration, and recording the concentration at which an error of <5.0% on the luminescence determination of the P1 occurred, and this concentration was taken as the tolerance level. Table 2 summarize the data obtained from studying the most important anion, cation and also the interference of other pesticides. Repeatability of the proposed method was investigated and the method proved to be reproducible. The probe used can distinguish dichlorvos in presence of other pesticides with high sensitivity towards low concentration other pesticides, as 5% error occurs at low concentrations of other pesticides.

4 | CONCLUSION

In this work we have prepared 3-Ally-salicylohydrazide (S_1) and proven its structure using CHN, IR, 1H NMR and LC–MS, Tb(III)-S_1 exhibits a very stable and good luminescence in ethanol. Also, the complex has shown quick interaction with pesticides. Tb(III)-3-ally-salicylohydrazide is a promising luminescent probe, especially the high sensitivity towards dichlorvos in the micromolar range, thermodynamic studies showed that the interaction between probe and P1 was endothermic and the reaction was more favoured in high temperatures. Finally, the probe response to P1 is sensitive to the presence of other pesticides.

For future studies, this study revealed that the probe in its current state cannot detect dichlorvos in an environmental sample, this flaw can be overcome in future studies by developing a molecularly imprinted polymer (MIP), taking advantage of the allyl moiety on the ligand, which may further be integrated into a microarray for pesticide instant detection in the environment.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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