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ORIGINAL PAPER

Fluorescence and Electrochemical Sensing of Pesticides Methomyl, Aldicarb and Prometryne by the Luminescent Europium-3-Carboxycoumarin Probe

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Abstract This work describes the application of time resolved fluorescence in microtiterplates and electrochemical methods on glassy carbon electrode for investigating the interactions of europium-3-carboxycoumarin with pesticides aldicarb, methomyl and prometryne. Stern-volmer studies at different temperatures indicate that static quenching dominates for methomyl, aldicarb and prometryne. By using Lineweaver-Burk equation binding constants were determined at 303 K, 308 K and 313 K. A thermodynamic analysis showed that the reaction is spontaneous with ΔG being negative. The enthalpy ΔH and the entropy ΔS of reactions were all determined. A time-resolved (gated) luminescence-based method for determination of pesticides in microtiterplate format using the long-lived europium-3-carboxycoumarin has been developed. The limit of detection is 4.80, 5.06 and 8.01 μmol L^{-1} for methomyl, prometryne and aldicarb, respectively. This is the lowest limit of detection achieved so far for luminescent lanthanide-based probes for pesticides. The interaction of the probe with the pesticides has been investigated using cyclic voltammetry (CV), differential pulse polarography (DPP), square wave voltammetry (SWV) and linear sweep voltammetry (LSV) on a glassy carbon electrode in I= 0.1 mol L^{-1} p-toluenesulfonate at 25 °C. The diffusion coefficients of the reduced species are calculated. The main properties of the electrode reaction occurring in a finite diffusion space are the quasireversible maximum and the splitting of the net SWV peak for Eu(III) ions in the ternary complex formed . It was observed that the increase of the cathodic peak currents using LSV is linear with the increase of pesticides concentration in the range 5×10^{-7} to 1×10^{-5} mol L^{-1}. The detection limit (DL) were about 1.01, 2.23 and 1.89 μmolL^{-1} for aldicarb, methomyl and prometryne, respectively. In order to assess the analytical applicability of the method, the influence of various potentially interfering species was examined. Influence of interfering species on the recovery of 10 μmol L^{-1} pesticides has been investigated.

Keywords Europium-3-carboxycoumarin · Time resolved fluorescence · Pesticides aldicarb · Methomyl · Prometryne · Cyclic voltammetry · Differential pulse voltammetry · Linear sweep voltammetry · Glassy carbon electrode

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Introduction

With the rapid growth of world population, there is an ever increasing demand for agricultural products, and a consequent need for pesticides. Because these substances are generally highly toxic, it is essential to have analytical methodology for monitoring their levels in the environment. The World Watch Institute estimates that over 700 different organic compounds, particularly pesticides and their breakdown products, surfactants, phenols and polycyclic aromatic hydrocarbons, may be found in environment, and that there are about 70,000 synthetic chemicals in everyday use, with between 500 and 1,000 new chemicals being added to the list each year [1]. Aldicarb belongs to the carbamate class of chemicals, is known as 2-methyl-2-(methylthio) propionaldehyde O-methylcarbamoyloxime. Aldicarb is a multi-use pesticide that controls populations of agriculturally-harmful insects and nematodes. Aldicarb inhibits the production of cholinesterase in these various organisms.

Methomyl is a highly toxic carbamate insecticide, is known as 1-(methylthio) ethylideneamino methylcarbamate. It is a cholinesterase inhibitor and is often most effective against pests that have developed a resistance to organophosphates.

Prometryne is a pre-emergency and post-emergency selective systemic herbicide. It is known as 2-methylthio-4,6-bis (isopropyl amino)-1,3,5-triazine. It is often used for the control of annual broadleaf and grass weeds in many cultivated plants. Its herbicide effects in target plants are based on the inhibition of photosynthetic transport electrons at the photosystem receptor site and inhibition of oxidative phosphorylation.

Recent estimates from the World Health Organization (WHO) indicated that one million serious accidental poisonings and two million suicide attempts involving pesticides occur worldwide each year. Their presence in water and food poses a potential hazard to human health [2], and fast, reliable and economically viable methods are required for their detection in the environment and in agro-food products. Many methods have been developed in the last few years for the determination of pesticides. Most of these are based on a separation by gas [3] or liquid chromatography [4]. The classical gas chromatographic methods have not been satisfactory in general, due to the thermal instability of the molecules of these compounds [5], and S- or P-detectors are required. In high performance liquid chromatography (HPLC), UV or electrochemical detectors are utilized in the analysis of water samples of different origin as well as pesticides molecular formula structure as of samples from soils and oils. However, the usual detectors cannot provide enough sensitivity to analyze the organophosphorus compounds in these samples; the detection limits were in the range of 40–600 gl^{-1} when a UV detector was used [6]. However they usually require a multi-step preparation procedure of sampling, extracting, clean-up and concentrating the pollutants before the quantitative evaluation. In some cases they also require chemical derivatization. Some of these methods are long and tedious, and they may also introduce significant errors caused by adsorption losses, contamination or even decomposition of the components of interest. Similar kinds of samples can also be analyzed with the use of the GC technique. However, in general, a pre-concentration step is needed to determine the analytes at ppb levels for both the instrumental techniques. Non-chromatographic methods have been described in the literature for the determination of several organophosphorus pesticides found in formulations, soils, crops or water. These are based on UV-visible [7] or infrared spectroscopy [8], solid phase extraction [9] or enzymatic techniques [10], sometimes in combination with mass spectrometry (MS) [11] or the flow injection technique [12].

Luminescence detection along with microtiter plate formats provides a high throughput technology for clinical assays with sensitive determination of low analyte concentrations [13–15]. The use of lanthanide chelates as luminescent indicators, rather than conventional fluorophores, can enable highly sensitive detection due to their specific properties. In particular, the large Stokes' shift of lanthanide chelates (mostly Eu(III) and Tb(III)) easily permits selection of the chelate-specific emission from scattered excitation light, even with filters. The narrow emission bands allow efficient separation of several luminescence signals in multicolor assays. Further, the very long luminescence life time permits gated detection on a micro- to millisecond timescale, to avoid typical short-lived non-specific background signals [16, 17]. In these systems, intense ion luminescence originates from the intramolecular energy transfer from the excited triplet-state of the ligand to the emitting level of the lanthanide (antenna effect) [18]. Voltammetric techniques are an important alternative to more complex procedures such as chromatography due to certain convenient features including sensitivity, selectivity, and simplicity in experimental procedure, low cost and short duration of analysis. Voltammetric pulse techniques were proposed by Barker and Jenkin [19] to improve the sensitivity of voltammetric measurements, allowing quantitative determinations at concentrations down to 10^{-7} to 10^{-8} mol L^{-1}. The improvement in sensitivity is achieved by a significant increase of the ratio between the faradic and capacitive currents [20].

The properties of the glassy carbon microelectrodes including their small size allowed their use in the study of the reactions and electrochemical process in pure water and lower conductivity solvents and in the absence of the support electrolyte; it minimizes the cost and the manipulation of the samples. The study of the quasireversible and reversible electronic processes and chemistry-coupled reactions is made easy by the use of the glassy carbon microelectrodes.

Recently, a number of innovative methods for the detection of pesticides based on optical chemosensors have been reported in the literature. The first fluorescent chemosensor for detection of OP compounds was reported by Van Houten et al. [21] where a series of non-emissive platinum 1,2-enedithiolate complexes with an appended primary alcohol were synthesized. Upon addition of electrophilic OP analyte to this compound and an activation agent (triazole) in dichloromethane, the alcohol was converted to a phosphate ester, which reacts intramolecularly to form a fluorescent cyclic product . The analysis was conducted with care of avoiding oxygen since the presence of oxygen quenched the fluorescence. Simonian's group [22] reported a fluorescence based sensor for OP pesticides based on coumarin derivative compound . This compound in the presence of p-nitrophenol-substituted OP compounds leads to fluorescence quenching due to fluorescence resonance energy transfer (FRET).The sensor is very effective in the detection of nitrophenyl substituted pesticides like methyl parathion and fenitrothion. Delattre and co-workers [23] reported a cyclodextrin (CD) based fluorescent sensor for the detection of pesticides in water. D-glucopyranose units in CDs form truncated cone-shaped molecules with a hydrophobic cavity, which can induce the inclusion phenomena of a guest. The dipole of the macromolecular system varies with the entry of a guest molecule. A modified β-cyclodextrin, pyridinoindolizin-β-cyclodextrin, was used to detect pesticides and herbicides, linadane, parathion, malathion, imidacloprid, atrazine, and simazine, through an inclusion complex between the pesticide or herbicide and the hydrophobic cavity of the macrocycle. This interaction leads to fluorescence quenching of the fluorophore. An advantage of this fluorescence sensor is the ability to quantify concentration data via fluorescence intensity concentration-dependence. The application of gold and carbon fiber microelectrodes allied to square-wave voltammetry for the study of the electrochemical behavior of the organophosphorous insecticides (methyl parathion and dichlorvos) and bipyridilium herbicides (paraquat and diquat), and the development of the sensitive methodology for their analytical determinations in natural water samples has been carried out [24]. The experimental and voltammetric conditions to obtain the best analytical signal, in terms of intensities and profile of the peak voltammetric, for four pesticides were optimized and the results were used to evaluate the type of the electrochemical redox process.

Molecules that provide optical and electrochemical signals are ideal for developing sensors that offer dual signal transductions [25].

Significant progress has been achieved toward the development of fluorescent chemosensors for toxic pesticides . These chemosensors have been demonstrated to be time-effective and more robust than biosensors. This work describes the application of time resolved fluorescence in microtiter plates and electrochemical methods on glassy carbon electrode for investigating the interactions of europium-3-carboxycoumarin with pesticides aldicarb, methomyl and prometryne. It also includes the development of methods for detection of the three investigated pesticides using time resolved fluorescence in microtiter plates and electrochemical methods. The work done in the present paper is a part of our ongoing research for the development of chemo sensors for determination of different types of pesticides based on moleculary imprinted polymers containing our new luminescent Eu(III) complexes.It is clear that future improvements in this area will require the design of new fluorescent chemosensors with additional modes for signal transduction as in our europium-3-carboxycoumarin complex where Eu(III) is an electroactive luminescent probe. Such sensors will play an important role in minimization or elimination of false-positives. Due to the structural similarity of pesticide compounds, it is also paramount that the designed sensors must be fabricated such that they are highly selective toward specific pesticide compounds.

Our future generation of luminescent lanthanide sensors will seek to: (a) increase sensor multimodality,(b) enhance sensor selectivity between different types of pesticides, and (c) develop robust sensors with real world capability in complex matrices, including aqueous systems.

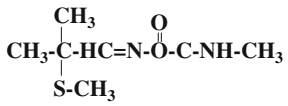
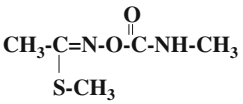
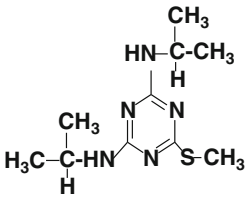
Experimental Section

Materials

Europium chloride hexahydrate ($EuCl_3.6H_2O$), gadalonium nitrate hexahydrate ($Gd(NO_3)_3.6H_2O$) and terbium chloride hexahydrate ($TbCl_3.6H_2O$) were purchased from Sigma-Aldrich and pesticides aldicarb, methomyl and prometryne were from Sigma Aldrich. Table 1 includes Chemical structures of the three pesticides under study.

3-carboxycoumarin was from Merck and all solvent used are of analytical grade quality from Sigma-Aldrich. Tris(2,2′-bipyridyl) dichlororuthenium(II) hexahydrate (Sigma) was dissolved in water to obtain a solution for quantum yield measurements. In electroanalytical technique, the ionic strength of the examined solutions was adjusted to 0.1 using solution of p-toluenesulfonate. This supporting electrolyte was purchased from Merck AG. The europium stock solution was prepared by dissolving 36.6 mg of $EuCl_3.6H_2O$ to give a final concentration 10^{-3} mol L^{-1}. For a stock solution of 10^{-3} mol L^{-1} of the 3-carboxycoumarin 19 mg of solid ligand were dissolved in 100 ml of DMF. The stock solutions of 10^{-3} mol L^{-1} of pesticides were obtained by dissolving of 19.03, 16.22 and 24.14 mg in ethanol for aldicarb, methomyl and prometryne, respectively.

Table 1 Chemical structure of the three pesticides under study

Pesticide	IUPAC chemical name	Class	Structure
Aldicarb	2-methyl-2-(methylthio) propanal O-(N-methylcarbamoyl) oxime	Carbamate	
Methomyl	1-(methylthio)ethylideneamino methylcarbamate	Carbamate	
Prometryne	2-Methylthio-4,6-bis(isopropyl amino)-1,3,5-triazine	Triazine	

Methods

Solutions of appropriate concentrations were added to a 10.0 ml volumetric flask then they were diluted with ethanol to the mark and kept at 25±1 °C for 10 min. Luminescence intensity was measured in a 1 cm quartz cell at an excitation wavelength of 320 nm and an emission wavelength of 614 nm then 150 μL of each solution were pipetted into a 96-well microtiterplate (each of 12 columns contained different concentrations of pesticides with eight replicates of the same concentration) and the time-resolved luminescence intensities were measured in a microtiterplate reader. Repeatability of the proposed method has been carefully investigated and the method proved to be reproducible.The same solutions were used for the electrochemical determination of pesticides by linear seep voltamettry(LSV) method on glassy carbon electrode using 0.1 mol L^{-1} p-toluensulfonate as a supporting electrolyte.

Instrumens

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 and emission filter of 614±10 nm, lag time 40 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 30 °C.

The studies of the interaction of the Eu(III)-3-carboxycoumarin with pesticides were obtained on A JASCO-FP6300 spectrofluorometer with 1 cm quartz cell used for the emission and spectral measurements.

UV-absorption spectra were recorded with A Shimadzu- UV Probe Version 2.33 UV-visible automatic recording spectrophotometer with 1 cm quartz cell.

Cyclic voltammetry (CV), square wave voltammetry (SWV), linear sweep voltammety and differential pulse voltammetry (DPP) are collected using EG and G Princeton applied research, potentiostat/galvanostat model 263 with a single compartment voltammetric cell equipped with a glassy carbon (GC) working electrode (area=0.1963 cm^2) embedded in a resin, a Pt-wire counter electrode, and Ag\AgCl electrode as reference electrode.

In cyclic voltammetry, the solution was purged with nitrogen for 120 s, and then the potential was scanned at scan rate 100 mV s^{-1} from −0.30 to −0.90 V. For square wave voltammetry,the samples were analyzed as in cyclic voltammetry, at pulse height 25 mV, SW frequency f=80 Hz and the scan increment 2.0 mV. In differential pulse voltammetry, the samples were analyzed also as in cyclic voltammetry, but at a scan rate=36.6 mV s^{-1},pulse height 25 mV, pulse width= 50 s, frequency 20 Hz and scan increment 2.0 mV. For linear sweep voltammetry the samples were analyzed also as in cyclic voltammetry, but at a scan rate=15 mV s^{-1} and scan increment 2.0 mV.

Determination of Quantum Yield

The quantum yield (QY) of Eu(III)-3-carboxycoumarin was determined in ethanol at concentration 5 μM. The QY was calculated with Tris (2,2′-bipyridyl) dichlororuthenium(II) hexahydrate (QY=0.13) as the reference. The different refractive indices of the ethanol solution and the solution of

the reference in water was considered using the following equation [26]:

$$Q_X = Q_R \frac{A_R.I_X.n^2_X}{A_X.I_R.n^2_R} \quad (1)$$

Where Q_R is the quantum yield of the reference, A_R, and A_X are absorbances of the reference (R) and Eu(III)-3-carboxycoumarin (X) at the excitation wavelength, I_R and I_X are the integrated areas under the corrected emission spectra of the reference and Eu(III)-3-carboxy coumarin, n_R and n_X are the refractive indices of the solutions of the reference and Eu(III)-3-carboxycoumarin, respectively.

Results and Discussion

Effect of Solvent on UV–vis Spectra and Fluorescence of Eu(III)- 3-Carboxycoumarin and its Interactions with Pesticides

Our research results concerning the effect of different solvents on UV–vis spectra for Eu(III)- 3-carboxycoumarin is shown in (Fig. 1). The л-л* band shifted to lower wavelength as the polarity of the solvent increases, following the order chloroform>acetonitrile>methanol~ethanol~isopropyl>water at 299 nm, 296 nm, 294.8 nm, 294.4 nm, 294.2 nm and 289.4 nm, respectively. The absorption spectrum of 3-carboxycoumarin in ethanol shows a maximum absorption band at 294 nm and another band around 320 nm due to the л-л* transition with high extinction coefficient 1.16×10^4 mol^{-1} cm^{-1} L. The addition of Eu (III) to the solution of 3-carboxycoumarin enhances the absorbance revealing the binding between 3-carboxycoumarin and Eu(III) ions. The molar absorbance for the Eu(III)—complex is 1.68×10^4 L. $mol^{-1}.cm^{-1}$ and the brightness (ε.Qy) of the probe is 840 L. $mol^{-1}.cm^{-1}$.

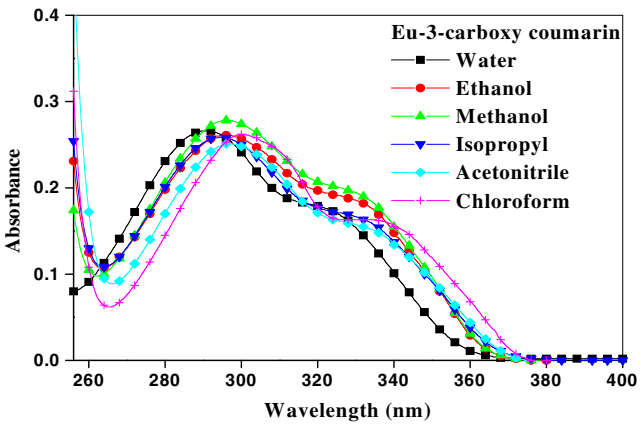


Fig. 1 UV–vis spectra for Eu(III)-3-carboxycoumarin in different solvents

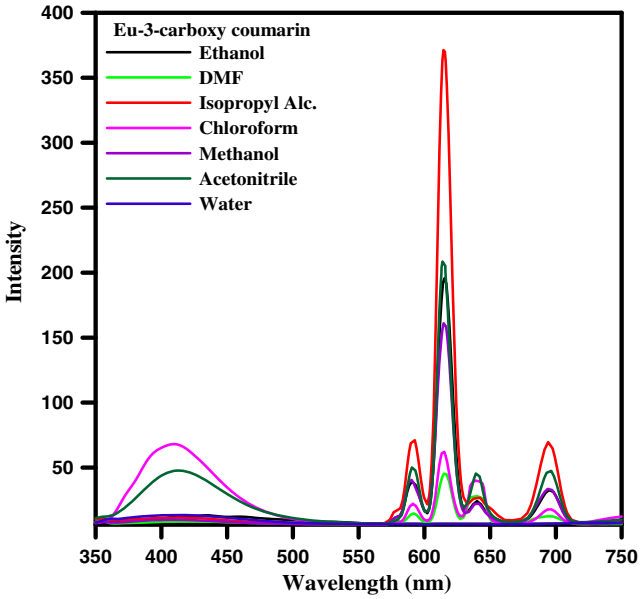


Fig. 2 Fluorescence spectra for Eu(III)-3-carboxycoumarin in different solvents

Solvents have remarkable effect on the fluorescence intensity of considered Eu(III)-complex as indicated in (Fig. 2). Upon using 5×10^{-6} mol L^{-1} of $EuCl_3.6H_2O$ and changing the concentration of 3-carboxycoumarin from 2.5×10^{-6} to 2×10^{-5} mol L^{-1} the stoichiometry for the interaction of 3-carboxycoumarin with Eu(III) ions was confirmed to be 1:2 as indicated in (Fig. 3).

The UV–vis, excitation and emission spectra for the interactions of Eu(III) -3-carboxycoumarin with pesticides in ethanol solvent are given in (Fig. 4). The shape and band maxima of absorption and fluorescence spectra remain unchanged, and no other emission band of the fluorophore towards longer wavelength is noticed . This observations suggest that the fluorophore-quencher interaction does not change the absorption and spectral properties. Also the

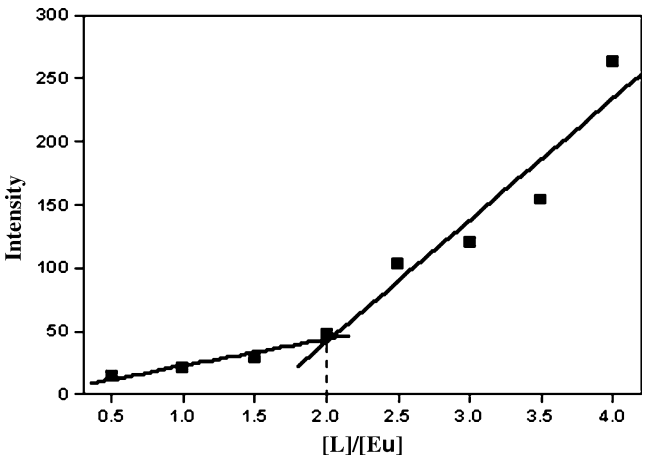


Fig. 3 [L]/[Eu] mole ratio plot at λ_{em}=616 nm, L/Eu=2/1

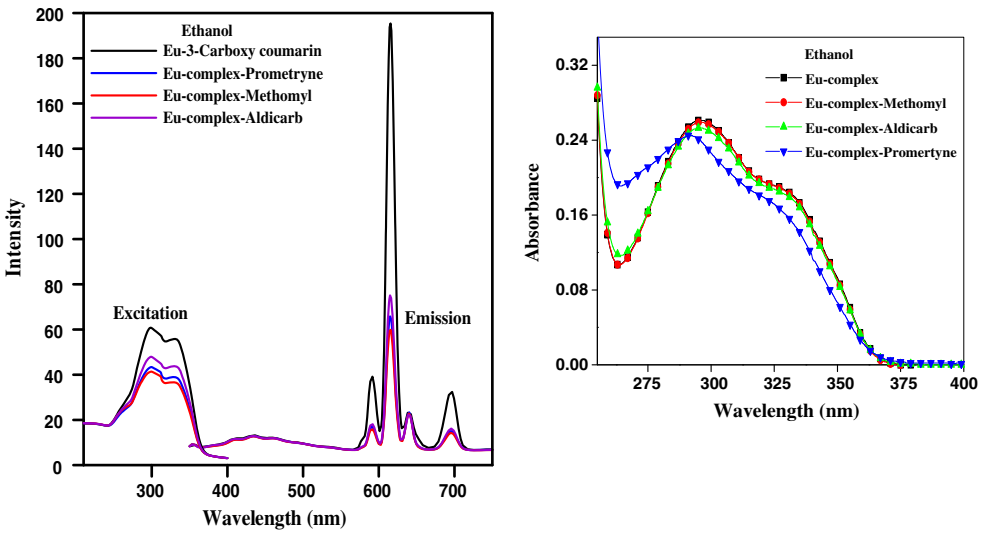


Fig. 4 UV–vis, excitation and emission spectra for the interactions of Eu(III) -3-carboxycoumarin with pesticides in ethanol solvent

formation of any emission exciplex may be discarded, since no new fluorescence peak appears at longer wavelength. No photochemical reaction between fluorophore and quencher have been observed.

Studies of the interaction of Eu(III)-3-carboxycoumarin with pesticides methomyl, aldicarb, and prometryne were conducted in different solvents. The solvents used were ethanol, DMF, isopropyl, acetonitrile, chloroform, methanol and water. Table 2 shows the I_o/I_F values determined from the ratio of fluorescence intensity I_o (Eu(III)-3-carboxycoumarin only) and fluorescence intensity I_F after addition of pesticides to the complex solution. This value is around 1.0 for all solvents but in ethanol has higher value (2.6, 2.97 and 3.25 for aldicarb, prometryne and methomyl, respectively). So the following studies were carried out in ethanol.

The excitation and emission maxima of the ligand were at 270 and 434 nm, respectively . The fluorescence of Eu (III) ion in solution is too weak to be observed, by introducing 3-carboxycoumarin, characteristic fluorescence of Eu (III) ion is increased by the intramolecular energy transfer process. The excitation maximum of Eu(III)-3-carboxycoumarin complex is at 320 nm and the emission maximum of the hypersensitive $^5D_4 \rightarrow {}^7F_2$ transition is recorded at 614 nm. As common for lanthanide complexes the Stokes' shift is large (about 294 nm). The quantum yield (QY) of the probe was determined to be 0.05 for Eu (III)-3-carboxycoumarin (10^{-5} mol L^{-1}).

In the present study only hypersensitive transitions were affected in the solution medium whereas other transitions did not show any effect in the solvent environments. In the spectra of Eu (III) -3-carboxycoumarin, the relative intensity of $^5D_0$-$^7F_2$ is stronger than that of $^5D_0$-$^7F_1$, showing that the Eu (III) ion is not in a centrosymmetric coordination site and no significant differences were observed in the $^5D_0$-$^7F_1$ emission transition in different solvents. The Laporte-forbidden transition of Eu(III) coordination compounds acquire a first-order electric dipole probability from transientdipoles induced in the ligand groups by an allowed even-multipole electric moment of the f-f excitation, and by the mixing of the f-f with f-d and f-g electron promotions under the electrostatic field of the pesticides aldicarb, methomyl and prometryne ligands. The electrostatic field and the ligand polarization mechanisms corresponding to different pesticides under investigation in the present study depending on the nature and polarity of the solvent make complementary intenisity contributions to the f-f transitions of Eu(III)-3-coumarin,dependent upon the nature of the leading electic-multipole moment. The polarization mechanism occurring during the interaction of the binary Eu(III) complex with the pesticides aldicarb, methomyl and prometryne may contribute principally to the intinsities of

Table 2 I_o/I_F ratio for the interaction of Eu(III)-3-carboxycoumarin with pesticides

Compound	I_o/I_F (ethanol)	I_o/I_F (acetonitrile)	I_o/I_F (isopropyl)	I_o/I_F (DMF)	I_o/I_F (chloroform)	I_o/I_F (methanol)	I_o/I_F (water)
Eu-complex-Prometryne	2.97	0.87	1.49	0.96	0.59	0.98	0.99
Eu-complex-Aldicarb	2.60	1.00	0.70	1.04	0.68	0.95	1.00
Eu-complex-Methomyl	3.25	0.98	0.94	1.04	0.80	0.91	1.00

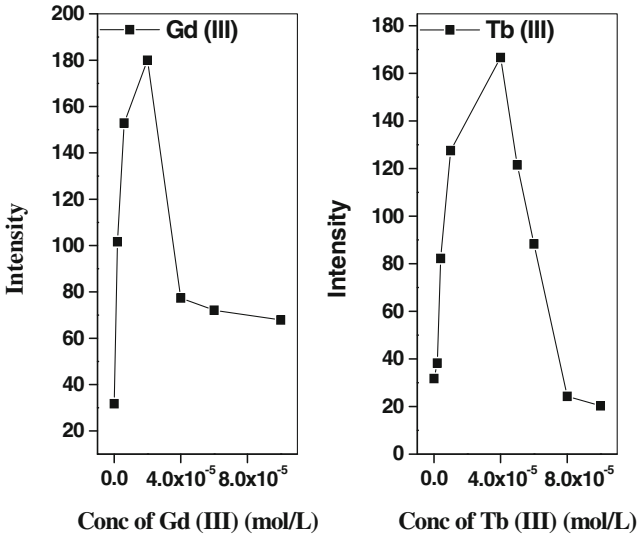


Fig. 5 Effect of different concentrations of Gd^{3+} and Tb^{3+} on the fluorescence intensity of Eu(III)-3-carboxycoumarin at λ_{em}= 616 nm

the ligand-hypersensitive 2-pole f-f transition, where as the electrostatic mechanism is predominant for the 2-pole transition intinsities, and makes the more important contribution 2-pole cases [27].

The intensity of hypersensitive band of Eu(III) ions in a complex is proportional to the ligand polarizability. Since for dissolved Eu(III)-3-coumarine the first coordination sphere of europium ion is formed by solvent ligands, the variation of oscillator strengths of the hypersensitive band in various solvents can be correlated vesus the polarizabilities of solvent molecules. Polarizabilities of the solvent molecules can be obtained from the mean molar refractivities of the solvents [28]. It is quite clear that in spite of the fact that the intensity of total fluorescence varies in a different manner throughout the series of solvents, the relative intensity of the hypersensitive fluorescence band generally increases with an increasing solvent molar refractivity in the same as in the the absorption spectrum.

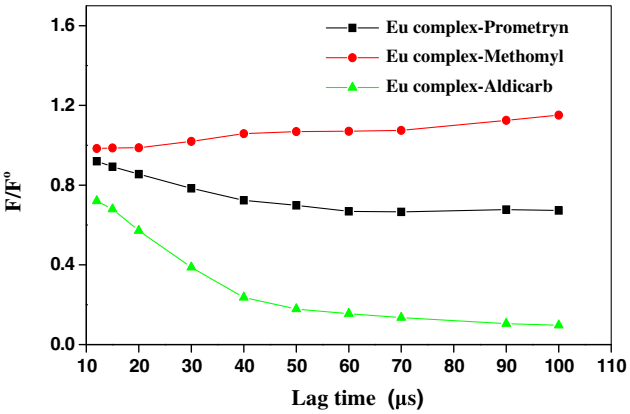


Fig. 7 Plot of F/F^{o} versus lag time for Eu(III)-3-carboxycoumarin – pesticide systems

Effect of Cofluorescence of Gd^{3+} and Tb^{3+} Ions on the Fluorescence of Eu(III)-3-Carboxycoumarin

As indicated in (Fig. 5) the fluorescence intensity of Eu (III)-3-carboxycoumarin increases with increasing Gd^{3+} and Tb^{3+} content. However, when Gd^{3+} and Tb^{3+} content is above 2×10^{-5} mol L^{-1} and 4×10^{-5} mol L^{-1}, respectively the fluorescence intensities of the complexes decrease with the increment of Gd^{3+} and Tb^{3+} content. Fluorescence enhancement of the Eu (III) -3-caboxycoumarin by Gd^{3+} and Tb^{3+},without other addetives and micelleformation, is quite clear in our present study which may be attributed to the binucleating complexeing agent ability of the ligand due to the presence of several oxygen atom centers in its chemical structure. In this solution, polymeric structure, where Eu(III) and Gd(III) or Tb(III) ions are linked together by ligands, has been generated. Luminescence enhancement

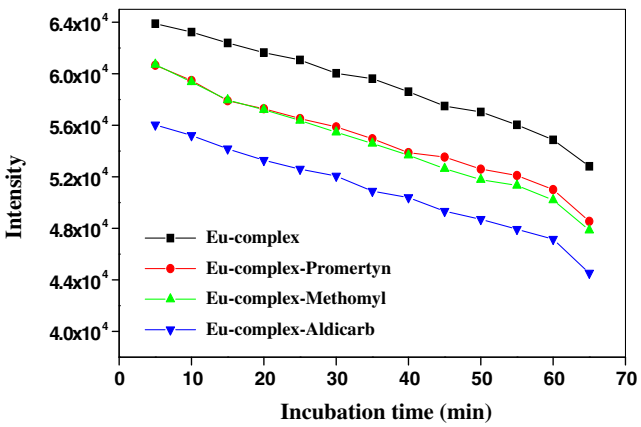


Fig. 6 Effect of incubation time on the fluorescence intensity of Eu (III)- 3-carboxycoumarin – pesticide systems at λ_{em}=614 nm

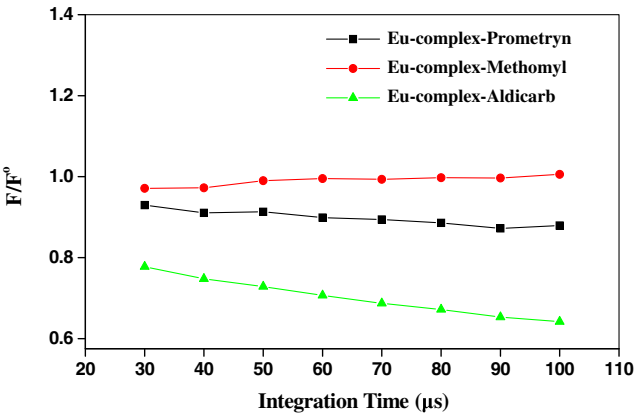


Fig. 8 Plot of F/F^{o} versus integration time for Eu(III)-3-carboxycoumarin—pesticide systems

of Eu(III) ions is a result of the transfer of absorbed energy along the polymer chain through several ligands [29]. The optimum content of Gd^{3+} and Tb^{3+} is 2×10^{-5} mol L^{-1} and 4×10^{-5} mol L^{-1}, respectively.

Effect of Incubation and Lag Times

Figure 6 indicates the effect of incubation time which has been tested in microtiter plates. First Eu(III)-3-carboxycoumarin luminescence intensity was recorded 8 replicates in the presence of pesticide. Eu(III)-3-carboxycoumarin was prepared by mixing the stoichimetric amounts of the ligand and $EuCl_3$ under stirring condition for 10 min. After addition of 5 μM of pesticide, the luminescence intensity of Eu(III)-3-carboxycoumarin was recorded every 5 min over 65 min period. Most of the luminescence signal (about 98%) decreases after the first 10 min, and after an additional 30 min period the decrease was 89%. Hence a 10 min incubation time was chosen for this assay to obtain the best sensitivity. As pointed out in the introduction, the triplet states of lanthanide complexes have a long lifetime. If the change of the luminescence lifetime depends on analyte concentration time-resolved (gated) measurements can help to significantly decrease the fast-decaying background fluorescence and to obtain better sensitivity [30]. The effect of different lag times on the values of F/F^o of the Eu(III)-3-carboxycoumarin solution in the absence and presence of 5 μM pesticide is shown in (Fig. 7). Here, F^o is the fluorescence of Eu(III)-3-carboxycoumarin in the absence of pesticide, and F is the intensity in the presence of pesticide. Data was acquired with a 40 μs integration time. The ratio of F/F^o strongly decreases until reached 40 μs, and then slightly decreases at longer lag times. Most of the interference from fluorescent substances is also eliminated by the use of this time-resolving method.

Effect of Integration Time

Integration time is the length of the time period the detector exposed to emission light. (Fig. 8) shows the effect of the integration time on F/F^o of Eu(III)-3-carboxycoumarin solutions in the presence of 5 μM of pesticide after a 40 μs lag time. If the integration time decreased from 30 μs to 100 μs, F/F^o decreases slowly. A 100 μs integration time was regarded to be appropriate .As indicated in (Fig. 8), it is obvious that the integration time is not really critical. After optemising the effect of incubation,lag and integeration times

Table 3 Parameters of calibrations curves obtained for the determination of aldicarb, methomyl and prometryne by time resolved fluorescence in microtiter plates, where r: correlation coefficients; S_b: standard deviations; s: slope of the working curves; *DL*: detection limits; and *QL*: quantification limits

Parameters	Aldicarb	Methomyl	Prometryne
r	0.9894	0.9724	0.9866
S_b	0.029	0.023	0.184
s $mol^{-1}.L$	1.08×10^4	2.28×10^4	1.09×10^5
DL (μmol L^{-1})	8.01	3.04	5.04
QL (μmol L^{-1})	26.68	10.14	16.81

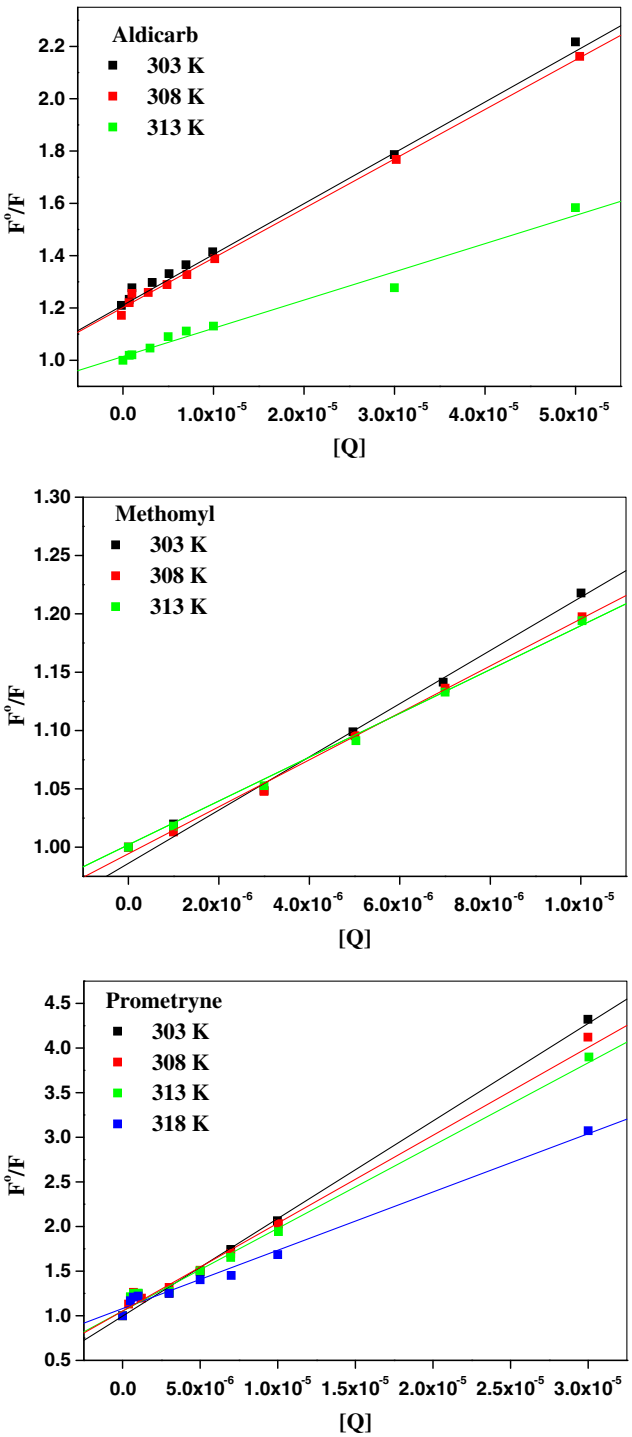


Fig. 9 Intensity—based (F^o/F) Stern-Volmer plots at different temperatures for pesticides under study

Table 4 Stern-Volmer constants (Ksv) of Eu(III)-3-carboxycoumarin with pesticides under study at different temperatures

Pesticide	Temperature	Ksv L.mol^{-1}
Prometryne	303 K	1.09×10^{5}
	308 K	1.02×10^{5}
	313 K	9.27×10^{4}
	318 K	6.44×10^{4}
Aldicarb	303 K	2.18×10^{4}
	308 K	2.17×10^{4}
	313 K	1.08×10^{4}
Methomyl	303 K	2.28×10^{4}
	308 K	2.01×10^{4}
	313 K	1.99×10^{4}

the fluorescence time resolved method in microtiter plates has been carried out for the determination of the pesticides under investigation. Parameters of calibrations curves obtained are given in Table 3.

Stern-Volmer Quenching Constant

Upon using a lag time of 40 μs and an integration time of 100 μs in concentration range of pesticide from 5×10^{-7} to 3×10^{-5} mol L^{-1},the fluorescence intensity of Eu(III)-3-carboxycoumarin decreased regularly with gradual increase in the concentration of pesticides under study. This indicates possible interaction between Eu(III)-3-carboxycoumarin and pesticides. The most likely reason for such Eu(III)-3-carboxycoumarin fluorescence quenching may be attributed to ground state Eu(III)-3-carboxycoumarin-pesticide complex formation or collisional quenching.

The relation between pesticide concentrations against the ratio F^o/F is plotted as shown in (Fig. 9), where F^o and F are the fluorescence intensities of Eu(III)-3-carboxycoumarin in the absence and presence of pesticide, respectively [26]. The ratio F^o/F increases linearly with the pesticide concentration and a linear regression equation following Stern-Volmer relation is obtained

$$F^o/F = 1 + Ksv[Q] = 1 + K_q\tau_o[Q] \quad (2)$$

One also observes that the Stern-Volmer plots do not show deviation towards the y-axis (under the studied experimental concentration range) which is an indication that either static or dynamic quenching is predominant.

Temperature Dependence of Ksv

The effect of temperature has been investigated to distinguish between both static and dynamic mechanisms for quenching process. One would expect an increase of F^o/F of Eu(III) -3-carboxycoumarin fluorescence with quencher concentration at high temperatures if collisional quenching predominates. This is because higher temperatures result in faster diffusion and hence larger amounts of collisional quenching as given in (Fig. 9) which shows Stern-Volmer plots for quenching of Eu(III)-3-carboxycoumarin fluorescence by the pesticides at different temperatures. Table 4 shows that the Stern Volmer quenching constant for pesticides is inversely proportional to temperature. This behavior can be considered as an evidence for probable quenching of pesticide-Eu(III)-3-carboxycoumarin through binding reaction which is initiated by ground state compound formation rather than by dynamic collision

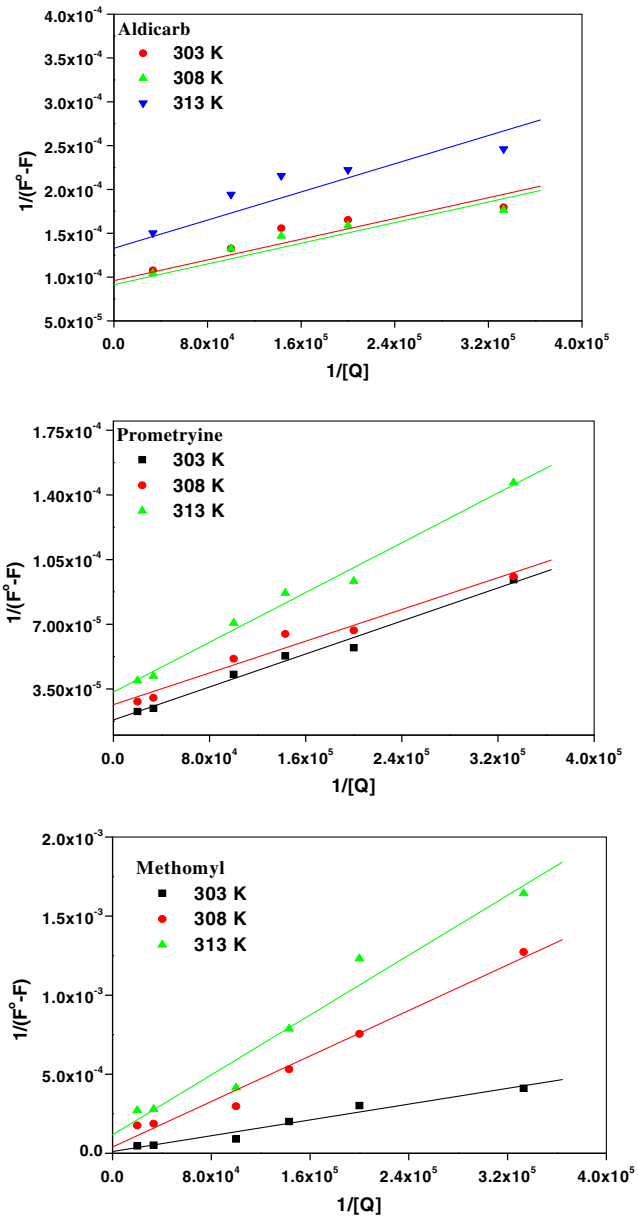


Fig. 10 Lineweaver-Burk curves for quenching of Eu(III)-3-carboxycoumarin with pesticides

Table 5 Binding constants and thermodynamic parameters for the interaction of Eu(III)-3-carboxycoumarin with the pesticides under study

	Temp	Binding constant K($Lmol^{-1}$)	R	ΔH (kJ/mol)	ΔS (J/mol.k)	ΔG^o (kJ/mol)
Methomyl	303 K	8.70×10^3	0.9806	81.8	344.4	−22.85
	308 K	1.10×10^4	0.9905			−23.83
	313 K	2.47×10^4	0.9794			−26.32
Prometryne	303 K	8.15×10^4	0.9931	20.8	162.6	−28.49
	308 K	9.89×10^4	0.9845			−29.45
	313 K	1.23×10^5	0.9933			−30.50
Aldicarb	303 K	3.10×10^5	0.9806	4.90	121.1	−31.85
	308 K	3.24×10^5	0.9905			−32.49
	313 K	3.27×10^5	0.9794			−33.04

[26]. The quenching plots illustrate that the quenching of the emission of Eu(III) complex is in good agreement with the linear Stern-Volmer equation.

Binding Constant at Different Temperatures

The change in the fluorescence intensity of Eu(III)-3-carboxycoumarin in ethanol in the presence of different concentrations of pesticides was used to calculate the binding constant of Eu(III)-3-carboxycoumarin-pesticide systems as given in (Fig. 10). The values of the binding constants determined at different temperature using Lineweaver-Burk (Eq. 3) together with the thermodynamic parameters for the interaction between Eu(III)-carboxycoumarin with the pesticides under investigation are given in Table 5 where K_D is the binding constant as given by the ratio of the intercept to the slope [31].

$$\frac{1}{F^o - F} = \frac{1}{F^o K_D [Q]} + \frac{1}{F^o} \tag{3}$$

From the thermodynamic standpoint ΔH>0 and ΔS>0 implies a hydrophobic interaction; ΔH<0 and ΔS<0 reflects the Van der Waals force or hydrogen bond formation; and ΔH≈0 and ΔS>0 suggesting an electrostatic force [32].

The thermodynamic parameters for the dependence on temperatures were analyzed in order to further characterize the acting forces between the Eu(III)-3-carboxycoumarin and pesticides by applying the Van't Hoff equation at three different temperatures (303, 308, and 313 K)

$$\ln K = -\Delta H^o/RT + \Delta S^o/R \tag{4}$$

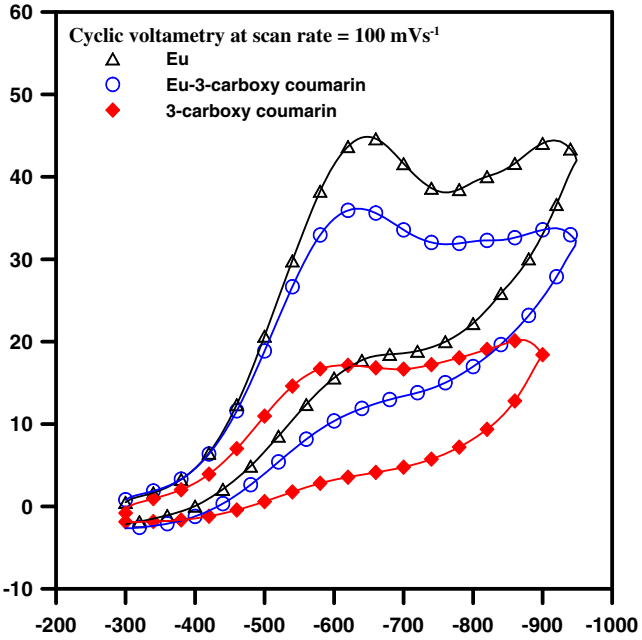


Fig. 11 Cyclic voltammograms for Eu(III)-3-carboxycoumarin in I=0.1 mol/L p-toluenesulfonate, scan rate=100 mV/s and at 25.0 °C

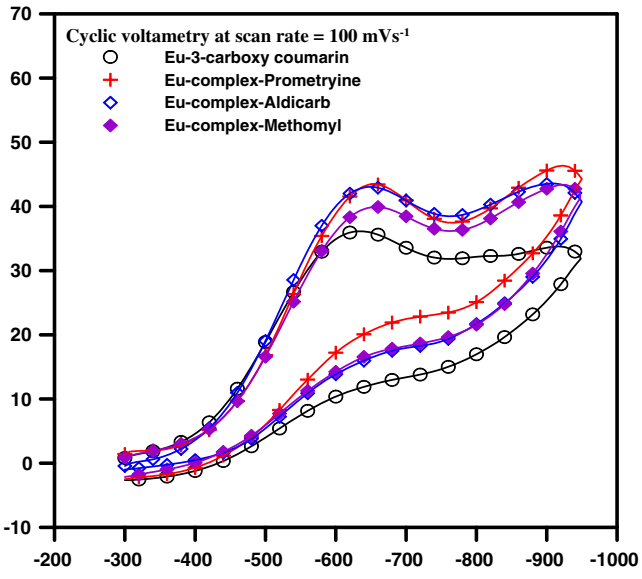


Fig. 12 Cyclic voltammograms for the interactions of Eu(III)-3-carboxycoumarin with pesticides in I=0.1 mol/L p-toluenesulfonate, scan rate=100 mV/s and at 25.0 °C

$$\Delta G^o = \Delta H^o - T\Delta S^o = -RT\ \ln K \quad (5)$$

where K is the binding constant at corresponding temperature and R is the gas constant, ΔH and ΔS of reaction could be determined from the linear relationship between lnK and the reciprocal absolute temperature. The free energy (ΔG^o) could be calculated by Eq. 5.

The reaction of Eu(III)-3-carboxycoumarin with pesticide is spontaneous as indicated from the negative value for ΔG^o, both ΔH^o and ΔS^o are positive. This attributed to

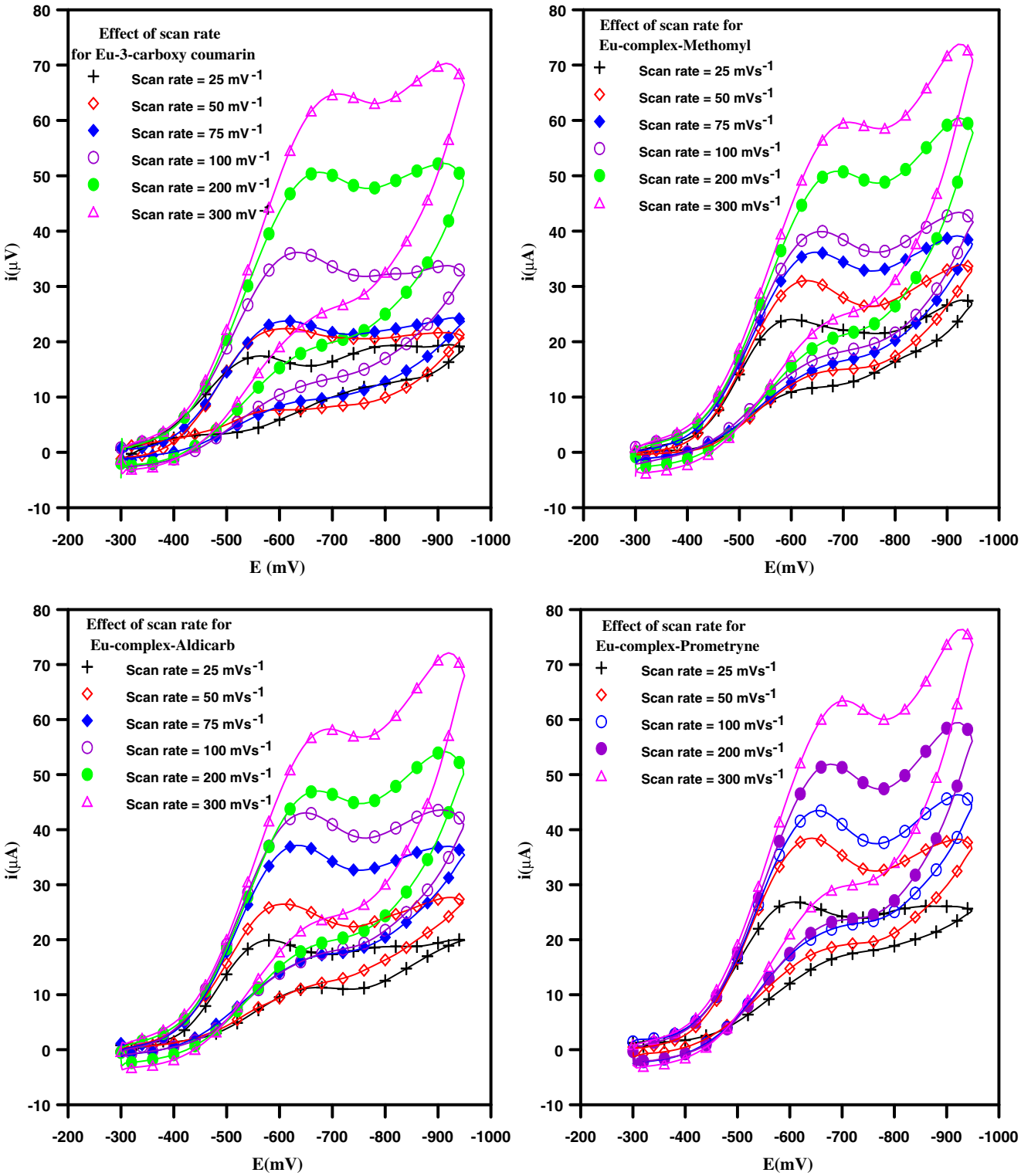


Fig. 13 Effect of scan rate on the cyclic voltammograms for systems under study

hydrophobic interactions being the leading contributor to the binding. A positive value for ΔS^o is also associated with electrostatic interactions occurring during the complex formation. The large value for the entropy change also suggests that the binding process is mostly entropy driven.

The binding constant increase with temperature suggesting that some covalent type interactions may contribute in the binding process.

Electrochemical Investigation for the Interaction Between Eu(III)-3-Carboxycoumarin and Pesticides

Our complete analysis for the electrochemical reduction of Eu (III) ions confirmed that the reduction process for Eu (III) on the glassy electrode surface occurs via one electron step to produce Eu (II). The complexation of Eu (III) with 3-carboxycoumarin is accompanied by a shift in the reduction potential to more positive value, and decrease in i_p which mean the decrease in the concentration of free metal ion due to complexation with 3-carboxycoumarin as indicated in the cyclic voltammograms shown in (Fig. 11).

The interaction of Eu (III)-3-carboxycoumarin binary complex with pesticides under study are shown in (Fig. 12), where for all pesticides the reduction potential are shifted to more negative value with respect to Eu(III)-3-carboxycoumarin curve by 35, 18.7 and 33.7 mV for methomyl, aldicarb and prometryne, respectively. The cyclic voltamettry response for the binary and ternary complexes containing Eu(III) on the GC electrode reveals a one-electron reduction process with the following electrochemical features in the scan rate υ range of (25 to 300) mVs^{-1}: the ip,a/ip,c ratio decreases by increasing υ; (ΔE_p) ($E_{p,c}$ - $E_{p,a}$) increases by increasing υ with slopes in the range (0.459 mV) for $E_{p,c}$ (ip versus $\upsilon^{1/2}$ plots), which agrees very well with the theory for a typical quasi-reversible process. We have carried out an exhaustive determination of ΔE_p values at different scan rates finding a linear behavior between ΔE_p and the square root of the scan rate for Eu(III) binary and ternary systems,which agrees very well with the theory for a typical quasireversible process.

The reduction peak potential shifted from −0.55 V to −0.68 V with increase in the scan rates from 25 to 300 mVs^{-1} for Eu(III)-3-carboxycoumarin binary complex, which indicates the quasireversible nature of the electrochemical process as confirmed by complete analysis for the obtaind cyclic voltammograms under our experimental conditions. For the reactions that are 'slow' (so called quasi-reversible or irreversible electron transfer reactions) the voltage applied does result in the generation of the concentrations at the electrode surface predicted by the Nernst equation as shown in (Fig. 13). This takes place because the kinetics of the reaction is 'slow' and thus the equilibrium is not established rapidly as comparison to the voltage scan rate. In this kind of

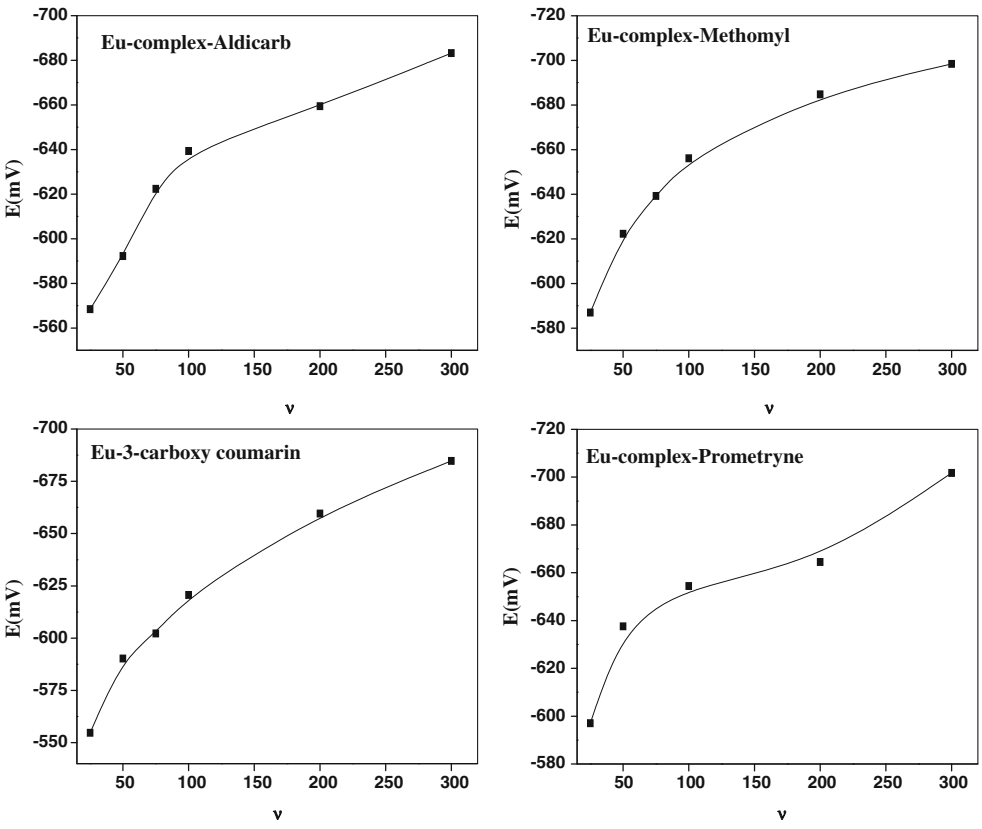


Fig. 14 Dependence of E_p on ν for Eu(III)-3-carboxycoumarin in I=0.1 $mol.L^{-1}$ p-toluensulfonate and at 25 °C. Graphical calculation of the critical potential scan rate ν_o

Table 6 Values of electrochemical parameters for studied systems via cyclic voltammetry technique

System	α	D (cm^2/s)	K^o (s^{-1})
Eu(III)-3-carboxycoumarin	0.49	9.71×10^{-5}	4.40
Eu(III)-3-carboxycoumarin-Aldicarb	0.54	7.02×10^{-5}	4.84
Eu(III)-3-carboxcoumarin-Methomyl	0.56	6.70×10^{-5}	5.02
Eu(III)-3-carboxycoumarin-Prometryne	0.69	5.74×10^{-5}	6.19

situation the overall form of the voltammogram remains similar to the reversible system but position of the current maximum, peak potential shifts depending upon the reduction rate constant and also the applied voltage scan rates. This occurs because the current takes more time to respond to the applied voltage than the reversible case [33].

The correlation between ip and $\upsilon^{1/2}$ were performed for all the systems using Randles-Sevick equation [34].

$$i_p = 2.99 \times 10^5 F(\alpha\, n)^{1/2} C D^{1/2} A \nu^{1/2} \quad (6)$$

where, i_{pc} is the peak cathodic current in ampere (μA), C the bulk concentration of the active species (mol L^{-1}), A is the area of the electrode (cm^2), F is Faradays per mole of the substrate electrolyzed, D is the diffusion coefficient (cm^2 s^{-1}), υ the potential scan rate (mV s^{-1}) and n is the number of electrons involved in the rate determining step.

Plots of the peak current are linearly increased with the square root of the scan rate (Fig not shown) suggesting that at sufficient overpotential the reaction is diffusion controlled. The deviation from origin of i_p vs. $\nu^{1/2}$ dependence shows that the electrode reaction is not under pure diffusion control and adsorption part may take place during electrochemical reaction occurring at the surface of glassy carbon electrode [33].

The relationship between the peak potential and scan rate for Eu(III)-3-carboxycoumarin-pesticide systems has been analyzed using Eq. 7 [35]

$$E_p = \text{const} + (2.303RT/\alpha\, n\, F)\, \log \nu \quad (7)$$

where E_p is the peak potential, α is the transfer coefficient, n is the number of electrons involved in the rate determining step, ν (mV/s) is the potential scan rate, ip (μA) is the peak current, F is the Faradays per mole of the substrate electrolyzed. From the slope of the linear relationship observed between E_p and log ν, the value of α (the cathodic transfer coefficient) at glassy carbon electrode was calculated according to Eq. 8, where T=298 K and n=1.

$$E_p - E_{p/2} = 1.857(RT/\alpha\, n\, F) \quad (8)$$

Table 5 shows values of electrochemical parameters for studied systems via cyclic voltammetry technique .Values of the symmetry coefficient (α) were determined from the difference of peak and half-peak cathodic potentials [35], and were found to be more than 0.5 when n=1 confirming the quasireversible nature of the reduction process.

Diffusion coefficient (D) of the investigated systems were determined from the slope of the ip vs $\sqrt{\upsilon}$ plot. The values obtained for the diffusion coefficients of Eu(III)-3-carboxycoumarin after addition of pesticides follow the order aldicarb>methomyl>prometryne.

A mean value of k^o for the cathodic reaction was evaluated from the experimental data as shown in (Fig. 14) applying the equation

$$k^o = 2.303\, \alpha\, n\, F\, \nu_o/RT \quad (9)$$

where k^o is electrochemical rate constant, ν_o is the critical potential scan rate, α is the transfer coefficient, n is the number of electrons involved in the rate determining step, T is temperature in Kelvin and F is the Faradays per mole of the substrate electrolyzed. Kinetic parameters,as indicated in Table 6, have been calculated for the binary and ternary Eu(III) complexes with the aim to probe their electron transfer ability when used as a basis for biosensors for the electrochemical detection of the pesticides aldicarb, methomyl and prometryne. So the results obtained in the present work concerning the electrochemical reduction and kinetic parameters for the Eu(III)-3-carboxycoumarin ternary

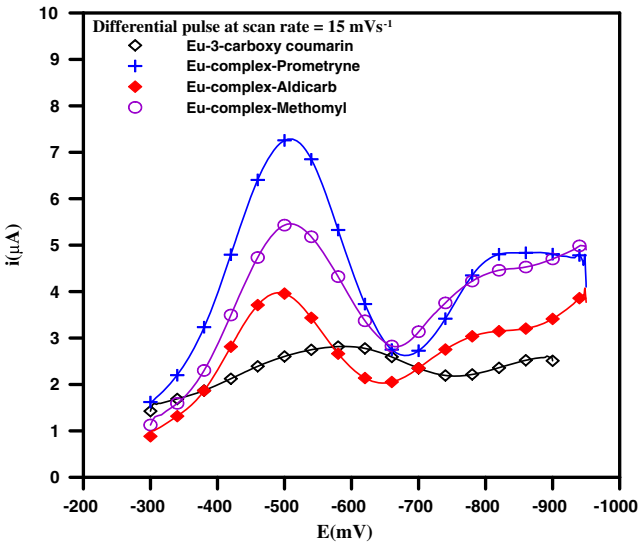


Fig. 15 Differential pulse polarograms for Eu(III)-3-carboxycoumarin-pesticide systems in I=0.1 mol/L p-toluenesulfonate, scan rate=15 mV/s and at 25.0 °C

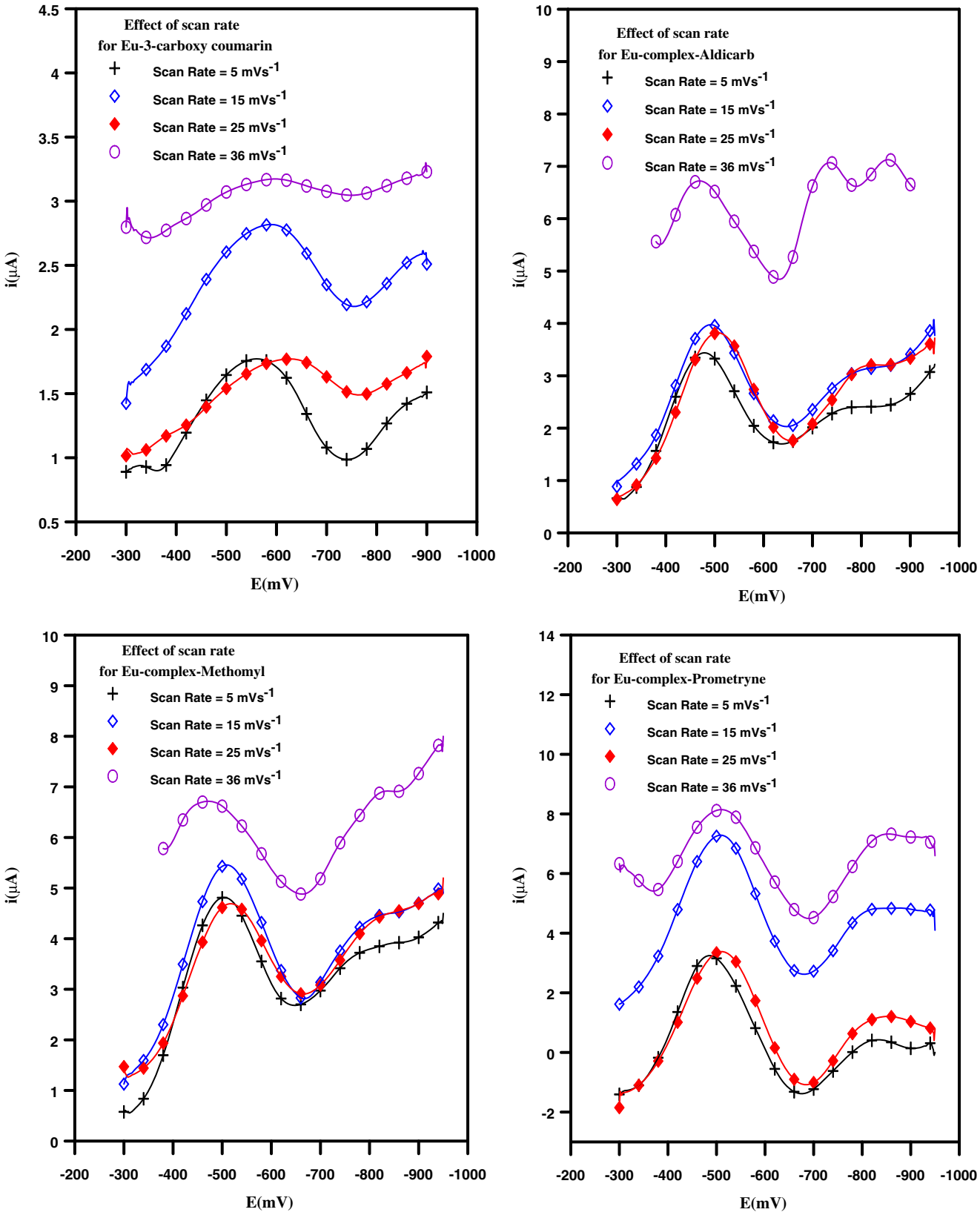


Fig. 16 Effect of scan rate on differential pulse polarograms for Eu(III)-3-carboxycoumarin-pesticide systems under study

systems can be considered as a basis for the future development of novel chemo sensors for the trace determination of the pesticides. Even an electrochemluminescence method can be developed on the basis of the interesting luminescent properties of Eu(III) ions.

The complexation of Eu (III) with 3-carboxycoumarin is confirmed using differential pulse technique. The reduction peak of Eu (III) was shifted to more negative value by −73 mV with reduction in cathodic current by 1.67 μA which means strong interaction of Eu (III) with 3-carboxycoumarin.

The interaction of Eu (III)-3-carboxycoumarin with pesticides is shown in Fig. 15. Considerable shifts to the more positive value with respect to Eu (III)-3-carboxycoumarin curve by +84.8, + 65 and +63 mV have been observed for pesticides aldicarb, methomyl and prometryne, respectively. This behavior which may be attributed to the high degree of binding with aldicarb pesticide agrees with the high value of diffusion coefficient.

Effect of scan rate on differential pulse polarograms for Eu (III)-3-carboxycoumarin-pesticide systems shown in (Fig. 16) has been carried out in order to optimize the condition for differential pulse polarographic measurements.

Besides cyclic voltammetry, square-wave voltammetry (SWV) is the second powerful voltammetric tool for studying both the mechanism and kinetics of electrode reactions in a thin film. Because it is one of the most advanced voltammetric techniques, the usefulness of SWV for studying the electrode reactions occurring in restricted diffusion conditions has been proven in particular with three-phase electrodes.

As shown in (Fig. 17) the binary complex Eu (III)-3-carboxycoumarin exhibits two reduction peaks, the first one shifted to more positive values by 100, 30 and 26.7 mV after addition of methomyl, prometryne and aldicarb, respectively.

The effect of frequency for Eu (III)-3-carboxycoumarin-pesticide systems has been studied in the range 20–100 Hz as shown in (Fig. 18). Linearity between the peak current and the square root of the frequency has been found for the redox process controlled by diffusion.

The peak potential is almost unaffected by the SW frequency. For instance it shifts for only 30 mV in positive direction with changing frequency from 25 to 100 Hz. However the SW peak current is severely sensitive to the SW frequency. The separation between the split peaks is sensitive to both the SW amplitude and the redox kinetic parameter, whereas the relative heights of the split peaks are solely determined by the electron transfer coefficient. Our findings are in agreement with literature regarding both properties, the quasireversible maximum and the splitting of the net SW peak, which can be exploited for complete kinetic characterization of the systems under investigation [36].

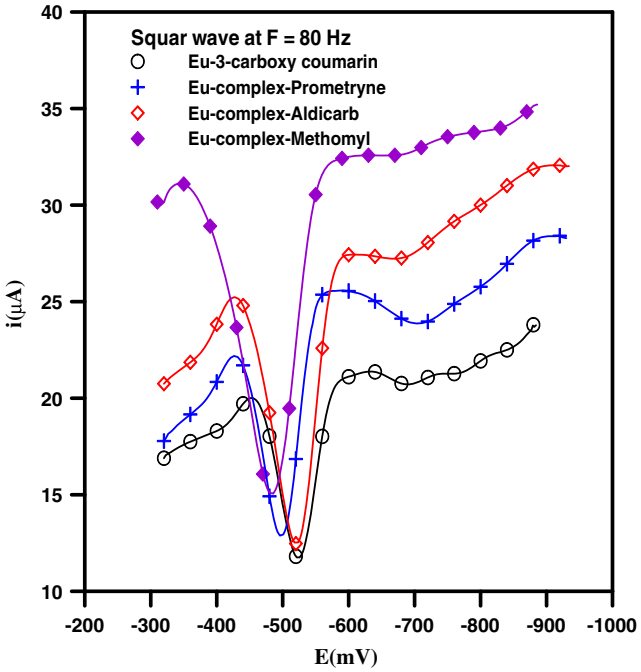


Fig. 17 Square wave voltammograms for the interactions of Eu(III)-3-carboxycoumarin with pesticides in I=0.1 mol/L p-toluenesulfonate, frequency=80 Hz, and at 25.0 °C

Electrochemical Determination of Pesticides Using LSV

It was observed that the increase of the cathodic peak currents using LSV, is linear with the increase of pesticides concentration in the range 5×10^{-7} mol L^{-1} to 1×10^{-5} mol L^{-1}. Calibration curves for the determination of the different pesticides under investigation were constructed based on the following regression equations: $y = 1.099 + 9.22.10^{4}x, y = 1.354 + 1.12.10^{5}x, y = 1.22 + 6.15.10^{4}x$ for pesticides aldicarb,methomyl and prometryne, respectively. Parameters of calibration curves obtained for the determination of pesticides aldicarb, methomyl and prometryne by LSV on GC are shown in Table 7. In the intervals studied a good linearity between peak current and concentration was obtained for the pesticides aldicarb, methomyl and prometryne with the detection limits 1.01,2.23 and 1.89 μmol L^{-1}, respectively. This method can be used for the analytical determination of the trace amounts of these pesticides in environmental (water and soil) and food samples.

Interference Study

The Eu(III)-3-carboxycoumarin time resolved fluorescence assay was tested with several metals and anions as found mostly in soil and irrigation water in presence of 10 μmol L^{-1} of pesticide under study. Results are summarized in Table 8. The interference of carbonate and phosphate were tested, since these anions are known to have a strong tendency to

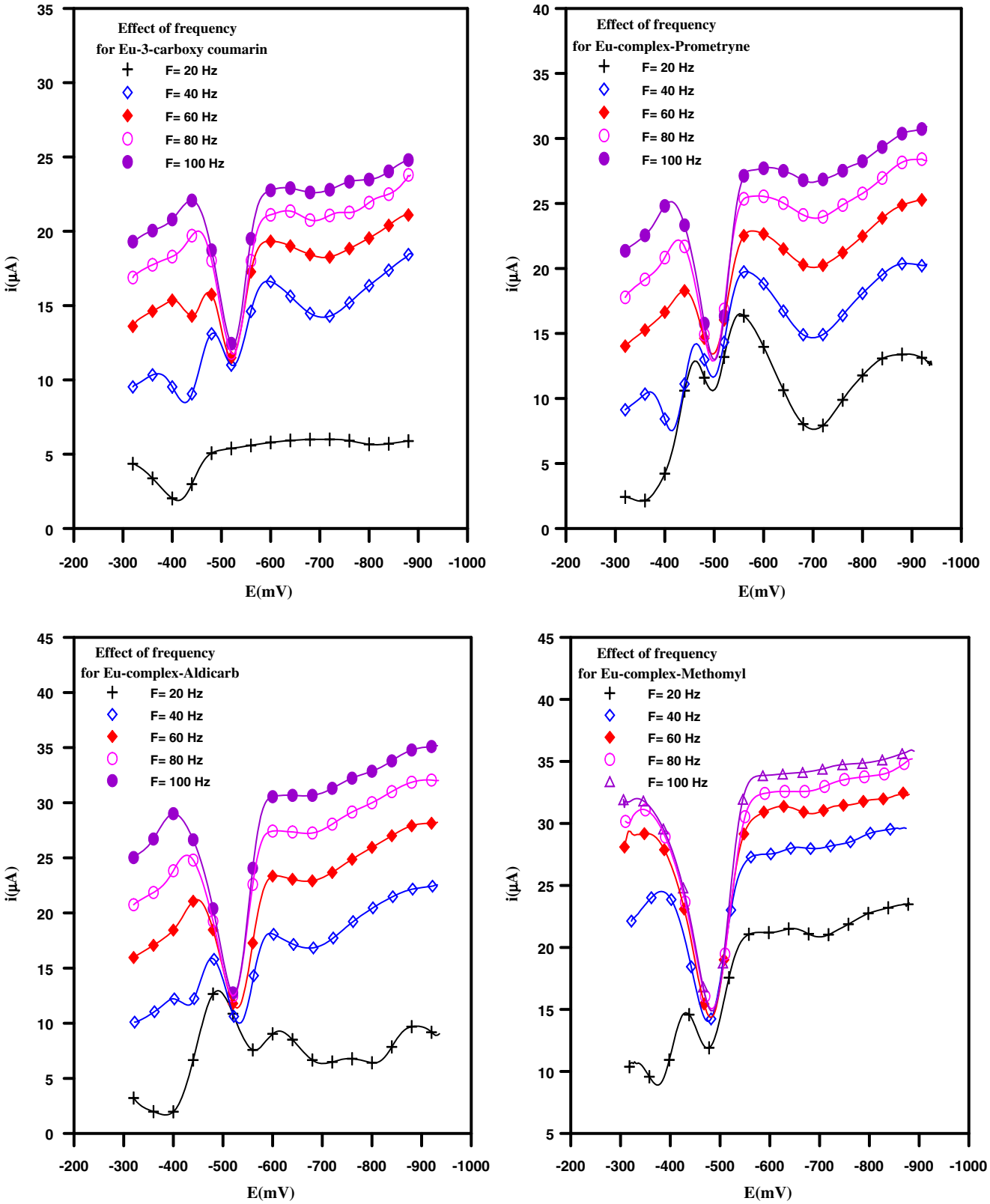


Fig. 18 Effect of frequency on the square wave voltammograms for Eu(III)-3-carboxycoumarin systems under study

form insoluble compounds with Eu(III) ions [37, 38]. The influence of some other commonly used pesticides e.g., azinphos-ethyl, chlorofeniviphos, diazinon and isofenphos on the determination of pesticide under study was also

Table 7 Parameters of calibration curves obtained for the determination of pesticides aldicarb, methomyl and prometryne by LSV on GC electrode, where r: correlation coefficients; S_b: standard deviations; *s*: slope of the working curves; *DL* detection limits and *QL* quantification limits

Parameters	Aldicarb	Methomyl	Prometryne
r	0.9767	0.9706	0.9786
S_b (A)	3.11×10^{-2}	8.31×10^{-2}	3.88×10^{-2}
s mol^{-1}.L	9.22×10^{4}	1.12×10^{5}	6.15×10^{4}
DL (μmol L^{-1})	1.01	2.23	1.89
QL (μmol L^{-1})	3.37	7.42	6.31

examined. The selectivity of the proposed electrochemical method using LSV for aldicarb, methomyl, and prometryne was also investigated in the presence of some inorganic ions mostly in soil and irrigation water. Some of these co-existing ions are electro active (produce a peak current), e.g., Ni^{2+}, Cu^{2+}, Pb^{2+}, Co^{2+}, Cd^{2+} and some electro-inactive, e.g., Na^{+} and K^{+}. Nitrate salts were used to obtain all the remaining interfering ions. The degree of interference effects were treated as the recoveries (by percentage) of 10 μmol L^{-1} of pesticide under study in the presence of the interfering ions. The results are summarized in Table 9.All these results indicate the possible use of the proposed methods for environmental applications.

Conclusion

A new method for simple and rapid determination of pesticides in microplate format has been developed employing the Eu(III)-3-carboxycoumarin complex, with limit of detection in the μM-range. In this work, the nature of the interaction between the Eu(III)-complex and methomyl, prometryne and aldicarb pesticides was studied. The fluorescence of Eu(III)-complex is strongly quenched in ethanol solvent. The experimental results indicate that the probable quenching mechanism of Eu(III)-complex fluorescence is mainly static quenching. The corresponding binding constant is increased as temperature increase; the thermodynamic parameters demonstrated that the binding was a spontaneous process and hydrophobic force played an important role.

Linear sweep voltammetry with glassy carbon electrode was suitable for determination of aldicarb, methomyl and prometryne by employing the Eu(III)-3-carboxycoumarin complex, with limit of detection in the μM-range. In this work, the reduction potential is shifted for all pesticides to the more negative value with respect to Eu(III)-3-carboxycoumarin curve by 35, 18.7 and 33.7 mV for methomyl, aldicarb and prometryne, respectively. Values of the symmetry coefficient (α) were found to be more than 0.5 when $n=1$, confirming the quasireversible nature of the reduction process. Based on the diffusion coefficient of Eu(III)-3-carboxycoumarin, the values obtained after addition of pesticides under study follow the order aldicarb>methomyl>prometryne. The selec-

Table 8 Concentrations of interferences tolerated in the presence of pesticides (10 μmol L^{-1})

Interferent	Aldicarb (μmol L^{-1})	Methomyl (μmol L^{-1})	Prometryne (μmol L^{-1})
NH_4^+	100	100	100
CO_3^{2-}	10	10	10
NO_3^-	1,000	1,000	1,000
PO_4^{3-}	100	100	100
K^+	100	100	10
Ca^{2+}	5	5	5
Na^+	1,000	100	100
Cd^{2+}	10	10	10
Pb^{2+}	100	50	100
Cu^{2+}	5	10	10
Ni^{2+}	5	5	5
Co^{2+}	5	5	5
Azinphose-ethyl	30	30	30
chlorofenivphos	30	30	30
Diazinon	5	5	5
Isofenphos	30	30	30

Table 9 Influence of interfering species on the recovery of 10 μmol L^{-1} pesticides

Interferent	Concentration (μmol L^{-1})	Recovery (%) of Aldicarb	Recovery (%) of Methomyl	Recovery (%) of Prometryne
Ni^{2+}	10	92.9%	94.5%	99.9%
	100	84.4%	89.2%	84.7%
Cd^{2+}	10	100.0%	107.6%	99.9%
	100	93.2%	109.2%	83.9%
Pb^{2+}	10	103.7%	92.0%	97.5%
	100	94.8%	82.9%	89.1%
Cu^{2+}	10	106.1%	102.2%	98.5%
	100	112.1%	110.6%	93.5%
Ca^{2+}	10	95.4%	101.4%	99.9%
	100	88.7%	95.2%	100.8%
	500	86.7%	–	90.9%
K^+	10	101.6%	98.9%	97.4%
	100	94.7%	94.1%	95.3%
	500	94.7%	–	94.9%
Azinphose-ethyl	10	87.9%	92.0%	96.3%
	100	83.9%	85.7%	78.2%
chlorpyrifos	10	96.8%	91.3%	87.9%
	100	89.4%	91.3%	84.7%
Diazinon	10	94.0%	96.6%	97.8%
	100	92.2%	93.0%	96.2%
Isofenphos	10	90.1%	88.0%	83.9%
	100	84.8%	86.0%	77.0%

tivity of the proposed methods for pesticides under study was investigated in the presence of some inorganic ions and some other commonly used pesticides found mostly in soil and irrigation water.

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