

Europium-(7-carboxymethoxy-4-methyl coumarin)₂ Complex based Electrochemical Probe for DNA based on the Interaction between Them

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Abstract: The interaction of Eu(III) ion with 7-carboxymethoxy-4-methylcoumarin (CMMC) has been investigated using the potentiometric method in the ethanol-water mixture solvent (0.15 volume fraction). The formation of the different binary, ternary complexes is confirmed by the corresponding pH-potentiometric curves. SUPERQUAD computer program has been used for the refinement of all the calculated constants in our present study. Electroanalytical techniques have been used to confirm the formation of different binary and ternary complexes under investigation. The binding constant of the ternary complex Eu(III)-CMMC-DNA calculated by cyclic voltammetry (CV) and differential pulse (DP) was found to be 1.8 and 2.5x 10⁵ M⁻¹ in Tris-HCl, respectively. The changes in the current intensity have been used for the quantitative determination of DNA over a linear concentration range with LOD of 1.0-1.3 µg/ml in 0.1 M Tris-HCl buffer.

Keywords: Potentiometry; Eu(III) -7- carboxymethoxy - 4- methyl coumarin (CMMC) ; voltammetry; DNA binding

1. INTRODUCTION

The interactions of DNA with different molecules can be used in the development of new drugs and diagnostic agents [1-5]. Several lanthanide complexes may bind to nucleic acids in different binding modes. The binding modes include intercalation or external electrostatic binding for cations. The intercalation is the most effective binding mode. Different Eu(III) fluorescent probes [6,7] and optical amplifiers [8,9] have been designed. Coumarin derivatives are interesting due to their many biological activities such as anti tumor [10], anticoagulant, anti-inflammatory, and antioxidant activity [11, 12]. The biological activity of the coumarin moiety has been enhanced or retained by binding of lanthanide metal ions [12-14].

The coordination complexes of rare earth ions have recently attracted much attention as probes for nucleic acids. The excited state of a luminescent Eu(III) Population of the excited state of a luminescent Eu(III) has been observed by energy transfer from the triplet state of the sensitizer [15-18]. Our current investigation

aims to study the interaction of Eu(III) ions with CMMC by pH-potentiometric and the ternary system of Eu(III)-CMMC-DNA using electroanalytical techniques. This work is a continuation of the author's work in the field of developing new lanthanide probes [19-24].

2. EXPERIMENTAL

2.1. Materials

Eu(NO₃)₃·6H₂O and nucleic acid (CT-DNA), were of the Sigma Chemical Co. products. CMMC was synthesized according to the literature [25, 26]. CMMC was recrystallized from ethanol (Mp 199-201) °C, lit.204-206 °C [25]. Confirmation of the structure of CMMC (C₁₂H₁₀O₅) has been carried out using NMR, elemental analysis, and IR spectra. ¹H-NMR (DMSO-d₆): δ (ppm) = 2.39 (3H, s, 4-CH₃), 4.66(2H, s, OCH₂), 6.22(1H, s, H3), 6.96-6.78 (2H, m, H6 and H8), 7.68 (1H, d, H5); ¹³C-NMR (DMSO-d₆): δ (ppm) = 169.65 (s, COOH), 160.97 (s, C=O, C2) 160.23 (s, C7) 154.51 (s, C9), 153.41 (s, C4), 126.50 (d, C5), 113.52 (d, C10), 112.33 (d, C6), 111.40(s, C3), 101.55 (d, C8), 64.83 (t, OCH₂), 18.16 (q, CH₃). CHN analysis; (calc.) C, 61.54; H, 4.3; found: C,

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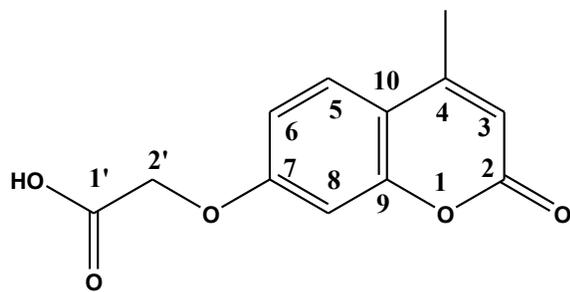


Figure 1. Structure of 7-carboxymethoxy-4-methylcoumarin (CMMC).

61.9; H, 4.9. IR (KBr, cm^{-1}) 3424, 3045, 2925, 1734, 1606. Structure of 7-carboxymethoxy-4-methylcoumarin (CMMC) is shown in Figure 1.

Complexometric titration with EDTA [27] has been used to check the concentration of the metal ions in stock solutions. $0.1 \text{ mol}\cdot\text{dm}^{-3} \text{ KNO}_3$ and the alcoholic solution of p-toluenesulfonate (Merck AG) were used to adjust the ionic strength of the solutions in potentiometric and electrochemical measurements, respectively. The purity of CT-DNA and the concentration of its solution were checked as reported in our previous work [28]. Aqueous 0.1 M Tris-HCl (pH 7.4) buffers were used as solvents for CT-DNA.

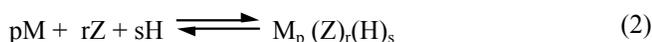
2.2. Apparatus and procedure

The procedures for potentiometric measurements, electrode calibrations, and determination of dissociation constants were used as reported in our previous paper [28, 29].

All the calculated formation constants of the systems under investigation have been refined using the SUPERQUAD [30] computer program. The stability constant can be represented by

$$K_{M(Z)} = \frac{[M_p(Z)_r]}{[M_p][Z]^r} \quad (1)$$

The overall complexation reaction involving protonation is



$$\beta_{pqrs} = \frac{M_p(Z)_r(H)_s}{[M]^p[Z]^r[H]^s} \quad (3)$$

in which Z = CMMC ligand, M = Eu(III) ions. Hydrolysis side reactions for Eu(III) ions have been considered during refinements.

Cyclic voltammetry, square wave voltammetry, and differential pulse voltammetry are collected using the methods and conditions described elsewhere [28].

3. RESULTS AND DISCUSSION

3.1. pH-potentiometric measurements

Potentiometric equilibrium measurements for the interaction between Eu(III) and CMMC have been carried out in 0.15 volume fraction ethanol-water mixture solvent, $I = 0.1 \text{ mol}\cdot\text{dm}^{-3} \text{ KNO}_3$. Representative titration curves are shown in Figure 2 for different

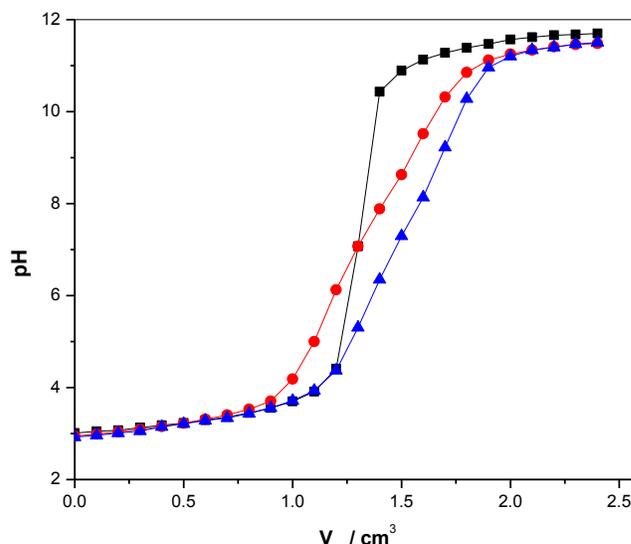


Figure 2. pH against volume of $0.022 \text{ mol}\cdot\text{dm}^{-3} \text{ KOH}$ for Eu(III) + CMMC system in 15%v/v ethanol-water mixture, $I=0.1 \text{ mol}\cdot\text{dm}^{-3} \text{ KNO}_3$ and at 25°C . (a) \blacksquare $2.86\cdot 10^{-3} \text{ mol}\cdot\text{dm}^{-3} \text{ HNO}_3 + 5\cdot 10^{-4} \text{ mol}\cdot\text{dm}^{-3} \text{ CMMC}$. (b) \bullet $2.86\cdot 10^{-3} \text{ mol}\cdot\text{dm}^{-3} \text{ HNO}_3 + 5\cdot 10^{-4} \text{ mol}\cdot\text{dm}^{-3} \text{ CMMC} + 5\cdot 10^{-4} \text{ mol}\cdot\text{dm}^{-3} \text{ Eu(III)}$. (c) \blacktriangle $2.86\cdot 10^{-3} \text{ mol}\cdot\text{dm}^{-3} \text{ HNO}_3 + 5\cdot 10^{-4} \text{ mol}\cdot\text{dm}^{-3} \text{ CMMC} + 1\cdot 10^{-3} \text{ mol}\cdot\text{dm}^{-3} \text{ Eu(III)}$.

complex systems under investigation. The acid dissociation constants for 7-carboxymethoxy-4-methylcoumarin (CMMC) are 3.90 ± 0.02 and 10.20 ± 0.02 referred to the deprotonation constant of the carboxylate group and oxygen of coumarin ring. The formation constants $\text{Log } K_1$ and $\text{Log } K_2$ of the Eu(III)-(CMMC) complex are 8.55 ± 0.02 and 7.84 ± 0.02 , respectively, indicating the binding between Eu(III) ions and the carboxylate group.

3.2. Electrochemical studies

In the present study electrochemical methods including differential pulse voltammetry (DP), cyclic voltammetry (CV), and square wave voltammetry (SWV) carried out on the glassy carbon electrode confirmed the formation of Eu(III) - CMMC and Eu(III)-CMMC-DNA in solution as shown in Figures 3 to 7. This observation agrees well with the potentiometric results. CV studies confirmed the reversibility of the electrochemical reactions for the systems under investigation. More than 80 mV as peak separation between the anodic and cathodic peaks indicates the quasi-reversible nature of the electrochemical reduction of the free Eu(III) ions as well as both the binary and ternary complexes formed in solution at the glassy carbon electrode.

3.2.1. Electrochemical studies on the interaction of Eu(III) with CMMC

The cyclic voltammograms for the free ligand (7-carboxymethoxy-4-methylcoumarin) as shown in Figure 3 using glassy carbon working electrode at $I = 0.1 \text{ mol}\cdot\text{dm}^{-3}$ p-toluenesulfonate and at 25°C . The free ligand doesn't exhibit reduction or oxidation peak within the studied potential range -0.30 to -0.90 V .

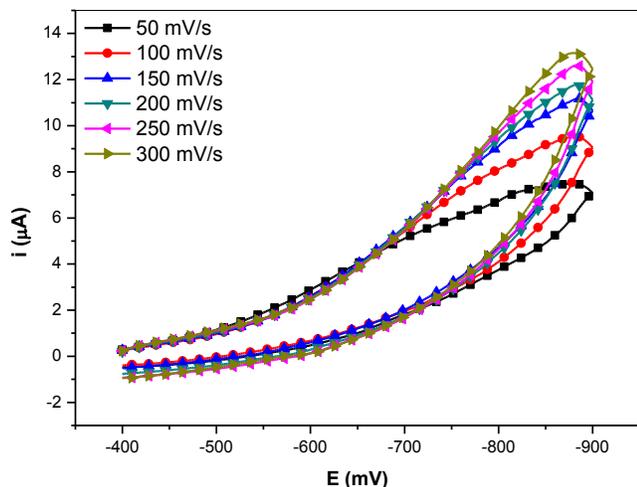


Figure 3. Effect of scan rate on the cyclic voltammograms for the free ligand (2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetic acid) CMMC in 0.15 volume fraction ethanol-water mixture solvent, $I=0.1\text{ mol}\cdot\text{dm}^{-3}$ p-toluenesulfonate and at $25.0\text{ }^\circ\text{C}$

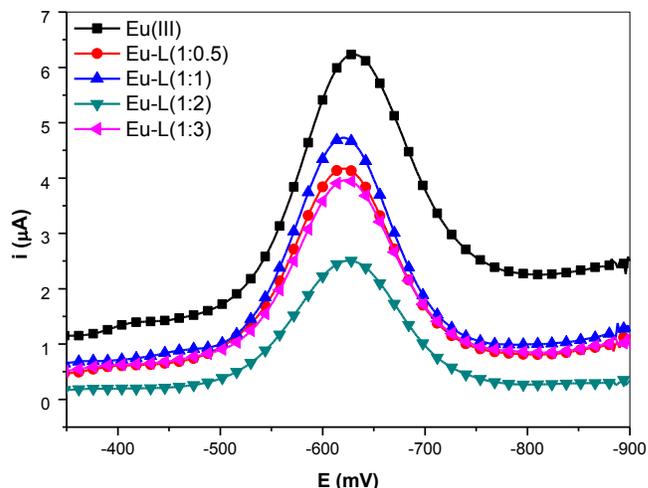


Figure 5. Differential pulse voltammograms for the Eu(III)–CMMC binary complexes in different metal: ligand ratio in 0.15 volume fraction ethanol-water mixture solvent, $I=0.1\text{ mol}\cdot\text{dm}^{-3}$ p-toluenesulfonate, $n = 15\text{ mVs}^{-1}$ and at $25.0\text{ }^\circ\text{C}$

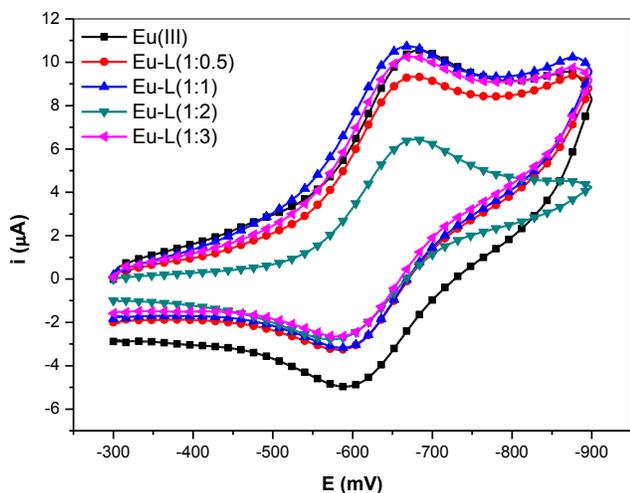


Figure 4. Cyclic voltammograms for the Eu(III)–CMMC binary complex in different metal: ligand ratio, in 0.15 volume fraction ethanol-water mixture solvent, $I=0.1\text{ mol}\cdot\text{dm}^{-3}$ p-toluenesulfonate, $n = 100\text{ mVs}^{-1}$, and at $25.0\text{ }^\circ\text{C}$.

Figure 4 shows the interaction of Eu(III) with different concentrations of the ligand in different molar ratios of Eu(III): CMMC (1:0.5, 1:1, 1:2 and 1:3). The Eu(III) - CMMC in 1:2 ratio has a considerable decrease in the cathodic current which could be attributed to the decrease in the concentration of Eu(III) ions in the bulk solution due to the higher affinity of complexation with two molecules of the ligand. The cyclic voltammogram for the 1:3 molar ratio metal complex has a slight change in cathodic current and reduction potential due to the instability of this complex and the steric effect renders itself through the observed behavior. This behavior is also confirmed through the data obtained from the differential pulse

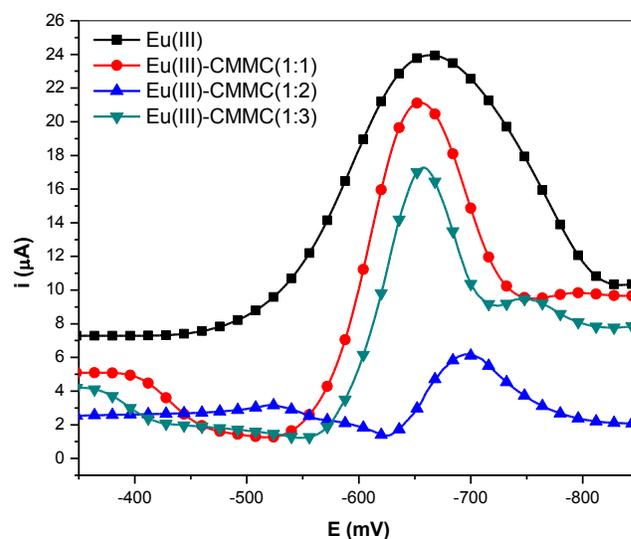


Figure 6. Square wave voltammograms for the Eu(III)–CMMC binary complexes in different metal: ligand ratios in 0.15 volume fraction ethanol-water mixture solvent, $I=0.1\text{ mol}\cdot\text{dm}^{-3}$ p-toluenesulfonate, $f=40\text{ Hz}$, and at $25.0\text{ }^\circ\text{C}$

voltammograms as shown Figure 5. The reduction of Eu(III) ions in solution shows a cathodic peak (E_p) at 0.630 V using scan rate $100\text{ mV}\cdot\text{s}^{-1}$ which is corresponding to Eu(III)/Eu(II) redox system. This peak can be assigned to the reduction of Eu(III) to Eu(II) via a one electron transfer process. Generally, the addition of CMMC to Eu(III) solution results in a shift of the cathodic peak to a more negative potential indicating the formation of the binary complexes in solution.

The square wave voltammetry for the formation of Eu(III) - CMMC has been investigated at different metal/ ligand ratio and

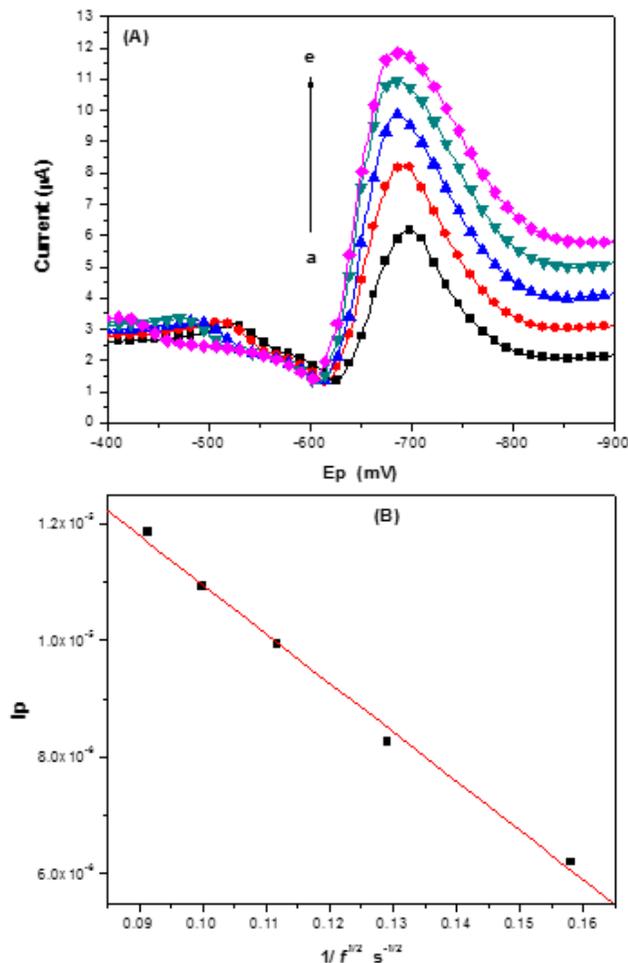


Figure 7. (A) SWV of Eu(III)-CMMC (1:2) in 0.15 volume fraction ethanol-water mixture solvent, $I=0.1 \text{ mol}\cdot\text{dm}^{-3}$ p-toluene sulfonate and at 25 °C at (a) 40, (b) 60, (c) 80, (d) 100, and (e) 120 Hz; (B) Cathodic peak current (i_{pc}) as a function of the reciprocal of square root of frequency ($f^{1/2}$)

frequencies as displayed in Figures 6 and 7. The Eu(III) - CMMC complex shows the maximum wave at the potential of -0.696 mV which is shifted to more negative potential with increasing CMMC concentration, indicating a considerable interaction between the Eu(III) metal ion and CMMC under experimental conditions. The straight line correlation between (i_{pc}) on the square root of frequency ($f^{1/2}$) is shown in Figure 7 B.

3.2.2. Electrochemical parameters

The ratio of the anodic and cathodic peak currents shows that the kinetics of the oxidation and reduction of the Eu (III) binary and the ternary system is not reversible but nevertheless quasi-reversible. The correlation between the cathodic and anodic peak currents (i_{pc} and i_{pa}) and the square root of scan rate $n^{1/2}$ are depicted in Figure 8 which reveals that the electrochemical processes are controlled by a diffusion mechanism.

Also, the correlation between the (E_{pc}) and log scan rate ($\log n$) are plotted as shown in Figure 9. The obtained straight lines are of

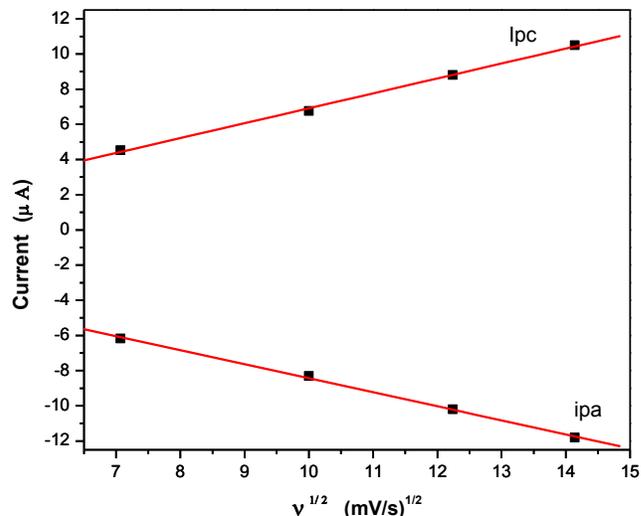


Figure 8. Correlation between cathodic peak current (i_{pc}), anodic peak current (i_{pa}) with square root of scan rate ($n^{1/2}$) for Eu(III)-CMMC(1:2) binary complex in 0.15 volume fraction ethanol-water mixture solvent, $I= 0.1 \text{ mol}\cdot\text{dm}^{-3}$ p- toluenesulfonate, $n =100 \text{ mVs}^{-1}$, and at 25.0 °C, $C_{\text{Eu(III)}} = 5\cdot 10^{-4} \text{ mol}\cdot \text{dm}^{-3}$

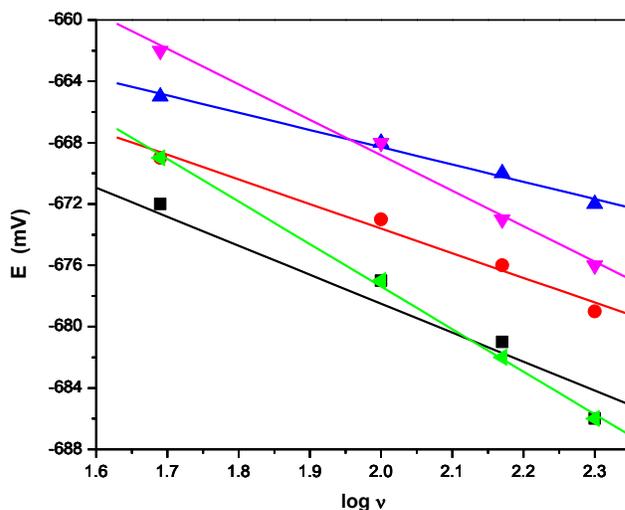


Figure 9. Correlation between cathodic peak potential (E_{pc}) and $\log n$ for ■ Eu (III) free metal ion, 1:0.5, ▲ 1:1, ▼ 1:2, and ◀ 1:3 Eu(III)-CMMC in 0.15 volume fraction ethanol-water mixture solvent, $I= 0.1 \text{ mol}\cdot\text{dm}^{-3}$ p- toluenesulfonate, $n = 100 \text{ mVs}^{-1}$, and at 25.0 °C.

slopes ranging from 0.011 to 0.027 which confirm the quasi-reversible behavior for the studied systems.

Randles Sivick equation [29] can be used for calculation of diffusion coefficients for different species during the oxidation reduction reaction

$$i_p = (2.69 \cdot 10^5) n^{3/2} A D^{1/2} n^{1/2} C \quad (4)$$

where $2.69 \cdot 10^{-5}$ is a collection of constants at 25 °C, (i_p), n , A , D , n , and C are the peak current, the number of electrons transferred, the electrode area (cm^2), the diffusion coefficient ($\text{cm}^2 \cdot \text{s}^{-1}$), the scan rate ($\text{V} \cdot \text{s}^{-1}$), and the concentration of species, respectively. The value of D_{red} of Eu(III) - CMMC (1:1) is higher than that for the free metal ion Eu(III).

The standard rate constant for the electron transfer can be represented by the separation of the peak potentials, ΔE_p . Values of ΔE_p calculated during our extensive analysis for the different voltammograms were used for the calculation of transfer parameter, Ψ by the aid of the working curve described by Nicholson [30]. Then standard heterogeneous charge-transfer rate constant (K_s) for the electron-transfer process has been calculated by the following equation:

$$\Psi = \frac{(D_o/D_r)^{\frac{\alpha}{2}} K_s}{D_o \pi \nu \left(\frac{nF}{RT}\right)^{1/2}} \quad (5)$$

In which the diffusion coefficients for oxidized and reduced species are represented by D_o and D_r , respectively. Equation (19) was used to calculate the transfer coefficient (α) [30]

$$\alpha = \frac{1.857 RT}{nF [E_p - E_{p/2}]} \quad (6)$$

where E_p is the peak potential (V) and $E_{p/2}$ is the half-peak potential, (F) is Faraday's constant, (R) is the gas constant ($\text{J mol}^{-1} \text{K}^{-1}$), and (T) is the temperature (K). Electrochemical characteristics and kinetic parameters of the systems studied are listed in Table 1.

3.2.3. Electrochemical investigation of the interaction of Eu(III) –CMMC with DNA

The interaction of different concentrations of DNA with the binary Eu(III)- CMMC complex in 0.15 volume fraction ethanol-water mixture solvent, $I = 0.1 \text{ mol} \cdot \text{dm}^{-3}$ p-toluene sulfonate and at 25 °C is depicted in Figure 10. The cathodic current is decreased by the gradual addition of DNA which confirm the binding of the complex to DNA with a dramatic decrease in the values of the diffusion coefficients of the reduced species and the standard heterogeneous rate constant (K_s) as depicted in Table 2. Increasing the concentration of DNA shifts the reduction potential to the more positive value which indicates the increase of binding probability of DNA to Eu (III) - CMMC complex, while the oxidation potential is shifted to less positive values. Thus, the binding of the Eu(III) - CMMC to the nucleic acid may occur via an intercalation interaction with base pairs of the DNA [31].

The binding constant and sites of the ternary complex of the type Eu(III)-CMMC- (DNA)_n can be calculated using the following

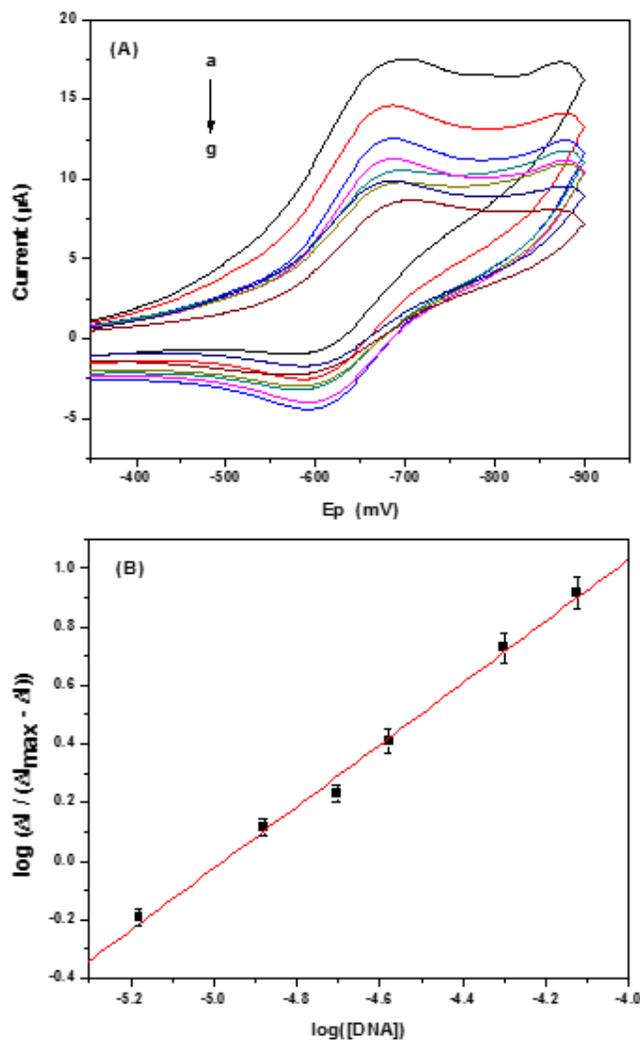


Figure 10. (A) Cyclic voltammograms of $2.0 \cdot 10^{-5} \text{ mol} \cdot \text{dm}^{-3}$ Eu(III) - CMMC + DNA in $0.1 \text{ mol} \cdot \text{dm}^{-3}$ acetate buffer solution (pH 5.0) in the presence of (a) 0, (b) 0.698, (c) 1.3, (d) 2.64, (e) 5.0, 7.5 and (g) $10 \cdot 10^{-5} \text{ M}$ DNA. Scan rate: 80 mVs^{-1} . (B) Double logarithms of $\log(\Delta i_p / (\Delta i_{p,\text{max}} - \Delta i_p))$ vs $\log[\text{DNA}]$.

equation [32-34]

$$\log \frac{\Delta i_p}{\Delta i_{p,\text{max}} - \Delta i_p} = \log K + n \log [\text{DNA}] \quad (7)$$

where Δi_p is the peak current difference in the absence and pres-

Table 1. Voltammetric data for Eu(III) and Eu(III)-CMMC complexes in different metal: ligand ratios in 0.15 Volume fraction ethanol-water mixture solvent, $I=0.1 \text{ mol} \cdot \text{dm}^{-3}$ p-toluenesulfonate, and at 25 °C.

System	E_{pc} mv	E_{pa} mv	$E_{p/2}$ mv	E^0 mV	i_{pa}/i_{pc}	ΔE mV	α	$D_{ox} \cdot 10^{-7}$ $\text{cm}^2 \cdot \text{s}^{-1}$	$D_{red} \cdot 10^{-7}$ $\text{cm}^2 \cdot \text{s}^{-1}$	$K_s \cdot 10^{-6}$ $\text{cm} \cdot \text{s}^{-1}$
Eu(III)	-684	-590	-619	637	1.53	94	0.73	12.95	5.47	1.62
Eu-CMMC (1:0.5)	-678	-582	-602	630	1.20	96	0.62	9.48	6.55	0.604
Eu-CMMC (1:1)	-668	-586	-596	627	1.19	82	0.66	10.19	7.09	0.6
Eu-CMMC (1:2)	-678	-574	-607	626	0.94	104	0.67	4.1	4.61	0.279
Eu-CMMC (1:3)	-674	-576	-599	625	1.105	98	0.63	8.46	6.93	0.56

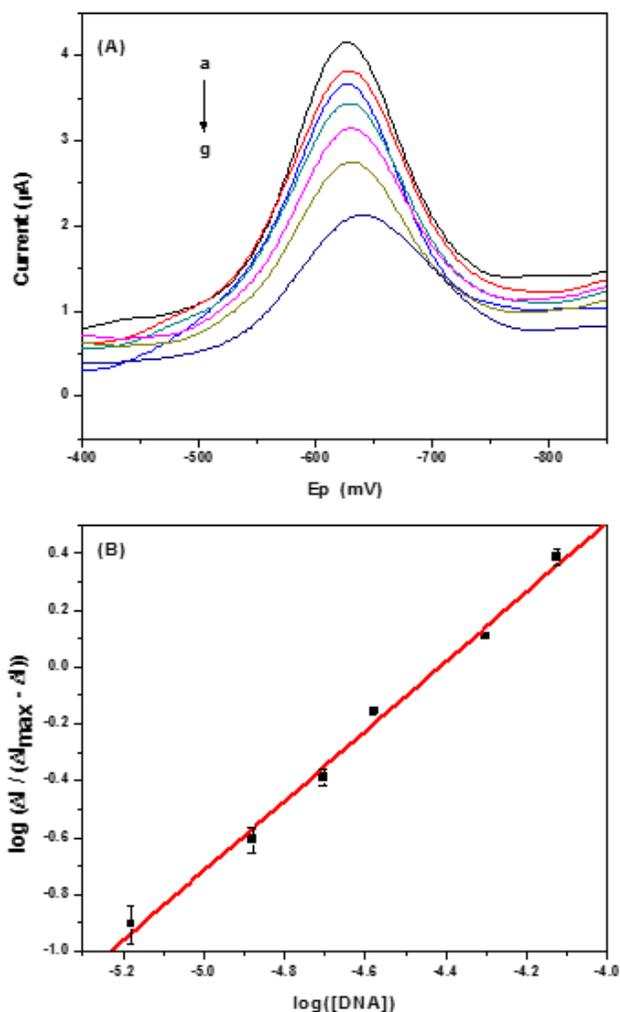


Figure 11. (A) DPV of Eu(III)-CMMC in 0.15 volume fraction ethanol-water mixture solvent, $I=0.1 \text{ mol}\cdot\text{dm}^{-3}$ p-toluene sulfonate and at 25°C in the presence of (a) 0, (b) 0.698, (c) 1.3, (d) 2.64, (e) 5.0, 7.5 and (g) 10. $\cdot(10^{-5} \text{ M})$ DNA; (B) Double logarithms of $\log(\Delta I_p/(\Delta I_{p,\max} - \Delta I_p))$ vs $\log[\text{DNA}]$.

ence of CT-DNA, $\Delta i_{p,\max}$ corresponds to the maximum difference of the peak currents. According to equation (7), the relationship curve of $\log(\Delta I_p/(\Delta I_{p,\max} - \Delta I_p))$ and $\log[\text{DNA}]$ should be a straight line with the suitable n , if there is only one complex formed. Figure 10B depicts a plot of $\log(\Delta I_p/(\Delta I_{p,\max} - \Delta I_p))$ as a function of $\log[\text{DNA}]$ for Eu(III)-CMMC-DNA ternary system. Values of K and n are evaluated at room temperature (25°C) using this plot. The calculated association constant is $1.8 \cdot 10^5 \pm 500 \text{ M}^{-1}$ with 1:1 stoi-

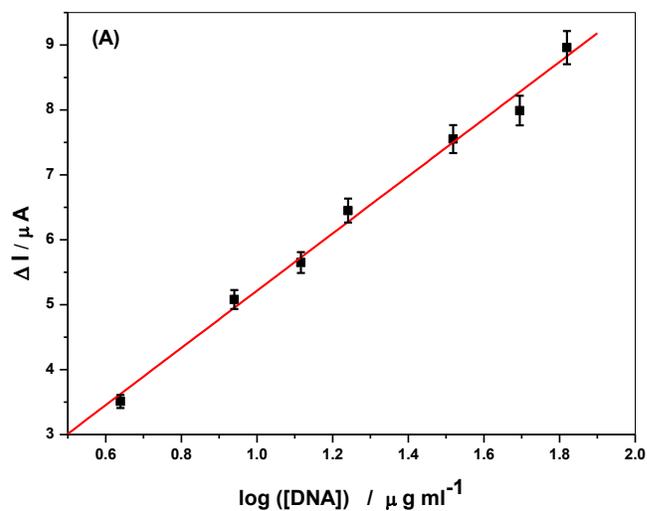


Figure 12. Calibration curve of CT-DNA interaction with Eu(III)-CMMC complex (a) CV and (b) DPV experiments.

chiometric molar ratio.

Differential pulse voltammograms for the interaction of Eu(III)-CMMC complex with different concentrations of DNA are shown in Figure 11. The cathodic peak potential for Eu(III)-CMMC appeared at -0.626 V . By the addition of DNA a decrease of cathodic peak current has been observed. According to equation (7), the association constant of Eu(III)-CMMC with DNA was determined from differential pulse voltammetry curves to be $2.5 \cdot 10^5 \pm 500 \text{ M}^{-1}$ with 1:1.12 stoichiometric molar ratio. The previously results agree well with that obtained from cyclic voltammograms.

3.3. Calibration curve and limits of detection

In this paper, the cyclic voltammetry (CV) and differential pulse voltammetry (DP) techniques have been employed to determine the concentration of CT-DNA. A linearity regression equations of $DI = a + m \log [\text{DNA}]$ have been obtained. Addition CT-DNA to Eu(III)-CMMC complex results in a decrease of the current of the Eu(III)-complex. The decrease in the current of Eu(III)-CMMC complex was proportional to the DNA concentrations as shown in Fig. 12. The detection limits obtained from CV and DPV experiments were found to be 1.3 and 1.04 $\mu\text{g}/\text{ml}$, respectively. The influences of the foreign substances such as protein, glycine, leucine, glucose, and metal ions have been tested. Small amounts of the most foreign substances used cannot alter the determination of DNA while the small amount of bovine serum albumin alters the measurements of DNA.

Table 2. Voltammetric data for the Interaction of different concentrations of DNA with Eu(III) + CMMC binary complex in 0.15 volume fraction ethanol-water mixture solvent, $I = 0.1 \text{ mol dm}^{-3}$ p-toluenesulfonate, $n=100 \text{ mVs}^{-1}$ and at 25°C .

System	E_{p_c} (mV)	E_{p_a} (mV)	i_{pa}/i_{pc}	$E_{p/2}$ (mV)	E^0 (mV)	ΔE_p (mV)	α	$D_{ox} \cdot 10^{-7}$ $\text{cm}^2 \text{s}^{-1}$	$D_{red} \cdot 10^{-7}$ $\text{cm}^2 \text{s}^{-1}$	$K_s \cdot 10^{-6}$ $\text{cm}\cdot\text{s}^{-1}$
Eu-CMMC	-687	-595	0.82	-602	641	92	0.56	1.94	2.84	0.296
Eu-CMMC-DNA($6.6 \cdot 10^{-6}$)	-682	-591	1.02	610-	636.5	91	0.66	12.79	12.19	1.84
Eu-CMMC-DNA($1.32 \cdot 10^{-5}$)	-684.5	-597	1.26	-619	640.8	87.6	0.72	16.47	10.32	2.45
Eu-CMMC-DNA($1.98 \cdot 10^{-5}$)	-686.3	-593	1.59	-613	639.6	93.3	0.65	17.82	7.03	1.856
Eu-CMMC-DNA($2.64 \cdot 10^{-5}$)	-702.13	-572	0.99	-627	637	130	0.63	5.97	6.08	0.34

4. CONCLUSION

In this work, the electrochemical properties of the Eu(III)-(CMMC) complex with DNA have been studied by cyclic voltammetry (CV) and differential pulse (DP) on glassy carbon electrode. Also, the formation constants of Eu(III)-(CMMC) complex have been evaluated potentiometrically with the SUPERQUAD computer program. The binding constant and stoichiometric molar ratio of Eu(III)-CMMC with DNA was found to be $1.8 \times 10^5 \pm 500 \text{ M}^{-1}$ and 1:1.1, respectively.

5. ACKNOWLEDGMENTS

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