Eu(III)-Anthracene-9-carboxylic Acid as a Responsive Luminescent Bioprobe and Its Electroanalytical Interactions with *N*-Acetyl Amino Acids, Nucleotides, and DNA

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The interaction of Eu(9-ANCA)$_3$ (9-ANCA = anthracene-9-carboxylic acid) with DNA has been investigated by a fluorescence method. Potentiometric equilibrium measurements have been performed at (25.0 ± 0.1) °C and ionic strength $I = 0.1$ mol·dm^{-3} KNO$_3$ for the interaction of Eu(III) and 9-ANCA with adenosine 5′-diphosphate (ADP), adenosine 5′-triphosphate (ATP), *N*-acetyl glutamic acid (Nc-Glu), *N*-acetyl leucine (Nc-Leu), and *N*-acetyl lysine (Nc-Lys) in a 1:1:1 ratio. The formation of various mixed ligand complexes was inferred from the potentiometric titration curves. The formation constants of the binary and ternary complexes have been refined with the SUPERQUAD computer program. The interaction of Eu(III)-(9-ANCA) with Nc-Glu, Nc-Leu, and Nc-Lys has been investigated by electroanalytical methods including cyclic voltammetry (CV), differential pulse polarography (DPP), and square wave voltammetry (SWV) on a glassy carbon (GC) electrode.

1. Introduction

Nucleic acids and *N*-acetyl amino acids have an important functions in life processes, so their study has become an important research field of the life sciences. Since the fluorescence intensities of nucleic acid and *N*-acetyl amino acids are very weak, the direct use of their fluorescence emission properties has been limited.[1,2] Generally, fluorescent probes including organic dyes,[3–5] metal ions, and metal complexes[6,7] are employed to investigate nucleic acids.

Rare earth ions have luminescence characteristics such as narrow spectral width, long luminescence lifetime, large stocks shift, and strong binding with biological molecules, and they are used as fluorescent probes to study nucleic acids, which must be single-stranded and have low sensitivity.[7] However, the rare columinescence in nucleic acids has been found and used as a sensitive fluorescence probe to study nucleic acids including RNA and DNA.[7]

In recent years, the use of coordination complexes of rare earth ions as a probe to study nucleic acids has attracted much attention. One of the interesting organic ligands which has several spectral luminescence properties is anthracene-9-carboxylic acid (9-ANCA) in solution.[8] In the present work, the interaction of the synthesized complex Eu(III)-(9-ANCA)[9] with *N*-acetyl amino acids *N*-acetyl glutamic acid (Nc-Glu), *N*-acetyl leucine (Nc-Leu), and *N*-acetyl lysine (Nc-Lys), nucleotides adenosine 5′-diphosphate (5′-ADP) and adenosine 5′-triphosphate (5′-ADP), and DNA has been studied by fluorescence and electroanalytical methods including cyclic voltammetry (CV), differential pulse polarography (DPP), and square wave voltammetry (SWV) on a glassy carbon (GC) electrode.

2. Experimental Section

2.1. Materials and Apparatus. All materials used in the present investigation were of analytical reagent grade. The materials were purchased from the Sigma Chemical Co. They are as follows: 9-ANCA, EuCl$_3$·6H$_2$O, Nc-Glu, Nc-Leu, Nc-Lys, ADP, and ATP. To avoid hydrolysis prior to the potentiometric measurements, a known mass of the chromatographically pure sample of nucleotides as a solid was added to the reaction vessel just prior to performing the titration. Eu(NO$_3$)$_3$·5H$_2$O was from the Sigma Chemical Co. The concentrations of the metal ion stock solutions were determined complexometrically using ethylenediamine tetraacetic acid disodium salt (EDTA) and suitable indicators.[10]

A CO$_2$-free solution of potassium hydroxide (Merck AG) was prepared and standardized against multiple samples of primary standard potassium hydrogen phthalate (Merck AG) under CO$_2$ free conditions. HNO$_3$ solutions were prepared and standardized potentiometrically with tris(hydroxylmethyl)aminomethane. The ionic strength of the studied solutions was adjusted to 0.1 mol·dm^{-3} using a stock solution of KNO$_3$ in potentiometric and spectral measurements. KNO$_3$ was from Merck AG. In the electroanalytical measurements, the ionic strength of the examined solutions was adjusted to 0.1 mol·dm^{-3} using an alcoholic solution of *p*-toluene sulfonate. This supporting electrolyte was purchased from Merck AG.

CT-DNA was purchased from the Sigma Chemical Co. (USA) and used as received. The purity of CT-DNA was checked by monitoring the ratio of absorbance at (260 to 280) nm. The ratio was 1.89, indicating that the CT-DNA was free from protein.[11] CT-DNA was dissolved in aqueous tris-buffers (pH 7.2). These CT-DNA solutions were stored at 4 °C for more than 24 h with gentle shaking occasionally to get homogeneity and used within 5 days. The concentration of the CT-DNA stock solution was determined according to the absorbance at 260 nm by using an extinction coefficient of 6600 mol^{-1}·cm^{-1}·L.[12] Other regents used were purchased and used as analytical grade.

Melting points were determined on a MEL-TEMP II apparatus (thermometer uncorrected); elemental analysis was carried out by Elementar Vario EL. Thermogravimetric

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Table 1. Dissociation Constants of *N*-Acetyl Amino Acids, 9-ANCA, or Nucleotides at $I = 0.1\ \mathrm{mol^{-1}\cdot dm^{-3}}$ KNO_3 and (25.0 ± 0.1) °C[a]

pKa		
9-ANCA	5′-ATP	5′-ADP
6.33 ± 0.01	6.48 ± 0.01	6.38 ± 0.02

pKa					
Nc-Him	Nc-Glu	Nc-Asp	Nc-Leu	Nc-Lys	Nc-His
7.12 ± 0.01	4.60 ± 0.01	4.49 ± 0.02	3.94 ± 0.01	9.52 ± 0.01	7.02 ± 0.01

[a] Nc-Him, *N*-acetyl histamine; Nc-Asp, *N*-acetyl aspartic acid; Nc-His, *N*-acetyl histidine; Nc-Leu, *N*-acetyl leucine; Nc-Lys, *N*-acetyl lysine; Nc-Glu, *N*-acetyl glutamic acid.

analysis was carried out by (a Shimazdu TG-DTG), and fluorescence spectra were recorded on a Jasco FP-6300 spectrofluorometer with a 150W xenon lamp for excitation. Absorbance spectra were recorded on a Jasco FP-6300 attached UV unit using a 1.0 cm path length cell. The infrared spectra were obtained in the (4000 to 500) $\mathrm{cm^{-1}}$ region by using a Bruker α with KBr discs.

2.2. Synthesis of the Eu(9-ANCA)$_3$ Complex. The complex was synthesized and characterized by a method[9] similar to that reported by Hart and Laming.[12]

2.3. Procedure for the Potentiometric Measurements.

Apparatus and Procedure. The value of the electomotive force of the cell was taken with a commercial Fisher Accumet pH/ion meter model 825 MP. The potentiometric system was connected to a glass electrode (Metrohm 1028) connected against a double junction reference electrode (Orion 9020). The temperature was controlled by circulation of water through the jacket from a VEB model E3E ultrathermostat bath and maintained within (25.0 ± 0.1) °C. Purified nitrogen was bubbled through the solution to maintain an inert atmosphere. Efficient stirring of the solution was achieved with a magnetic stirrer. All solutions were prepared in a constant ionic medium, 0.1 $\mathrm{mol\cdot dm^{-3}}$ KNO_3, in a 0.10 volume fraction ethanol−water mixture. The concentration of hydrogen ion was decreased by the addition of potassium hydroxide, prepared in the ionic medium used for the solution. Gran's method[13] was used to determine $E^{\circ\prime}$ and E_j so that the hydrogen ion concentration, h, could be found from E, the measured potential by means of

$$E\,(\mathrm{mV}) = E^{o'} - 59.157 \log h + E_j \qquad (1)$$

Values of the ionic product of the different hydroorganic media were refined using the MAGEC program.[14] The protonation constants were then determined by use of the Bjerrum function.[15]

$$\bar{n} = (H_T - h + K_W/h)/A_T = (\beta_1 h + 2\beta_2 h^2)/(1 + \beta_1 h + \beta_2 h^2) \qquad (2)$$

which is calculated from the experimental quantities, h, the total concentration of titratable hydrogen ion, H_T, and the total reagent concentration, A_T. The pK_a values of the investigated ligands were determined in a 0.10 volume fraction ethanol−water mixture from the overall protonation constants β_1 and β_2 calculated by the linearization method of Irving and Rossotti.[16]

Initial estimates of the pK_a values were refined with the ESAB2M computer program[17] by minimizing the error square sum:

$$U_v = \Sigma_i W_i (V_i - V_{calcd,i})^2 \qquad (3)$$

where V_i and $V_{calcd,i}$ are the experimental and calculated volumes of the titrant for every point i of the titration curve.

The weight is calculated by

$$1/W_i = S_i^{\,2} = S_v^{\,2} + (\delta V_i/\delta E_i)^2 S_E^{\,2} \qquad (4)$$

where S_i, S_v, and S_E are estimated variances and estimates of standard deviations in titrant volume and potential, respectively. The titrant volume, V_{calcd}, can be calculated from an explicit equation.[18] The program ESAB2M minimizes eq 2 by using the Gauss−Newton nonlinear least-squares method with the very efficient Levenberg−Marquardt algorithm.[19,20] Our calculation has been performed with an assumption that $S_v = 0.005$.

For the stability constant determinations the solutions titrated can be presented according to the following scheme: (a) $4\cdot10^{-4}\ \mathrm{mol\cdot dm^{-3}}$ HNO_3 + $1\cdot10^{-4}\ \mathrm{mol\cdot dm^{-3}}$ 9-ANCA (as the first ligand); (b) $4\cdot10^{-4}\ \mathrm{mol\cdot dm^{-3}}$ HNO_3 + $1\cdot10^{-4}\ \mathrm{mol\cdot dm^{-3}}$ nucleotide or *N*-acetyl amino acids (5′-ADP, 5′-ATP, Nc-Lys, Nc-Leu, or Nc-Glu); (c) solution (a) + $1\cdot10^{-4}\ \mathrm{mol\cdot dm^{-3}}$ Eu(III); (d) solution (b) + $1\cdot10^{-4}\ \mathrm{mol\cdot dm^{-3}}$ Eu(III); and (e) $4\cdot10^{-4}\ \mathrm{mol\cdot dm^{-3}}$ HNO_3 + $1\cdot10^{-4}\ \mathrm{mol\cdot dm^{-3}}$ 9-ANCA + $1\cdot10^{-4}\ \mathrm{mol\cdot dm^{-3}}$ nucleotide or *N*-acetyl amino acids (5′-ADP, 5′-ATP, Nc-Lys, Nc-Leu, or Nc-Glu) + $1\cdot10^{-4}\ \mathrm{mol\cdot dm^{-3}}$ Eu(III).

A constant ionic strength was obtained with 0.1 $\mathrm{mol\cdot dm^{-3}}$ KNO_3, and the total volume was kept at 25.0 $\mathrm{cm^3}$ in 0.10 volume fraction ethanol−water mixture solvent in all titrations.

2.4. Electrochemical Measurements. CV, SWV, and DPP were collected using EG and G Princeton applied research, potentiostat/galvanostat model 263 with a single compartment voltammetric cell equipped with a GC working electrode (area = 0.1963 $\mathrm{cm^2}$) embedded in a resin, a Pt-wire counter electrode, and an Ag/AgCl electrode as the reference electrode. In a typical experiment, a sample volume of 25 $\mathrm{cm^3}$ contains (a) $5\cdot10^{-4}\ \mathrm{mol\cdot dm^{-3}}$ Eu(III), (b) $5\cdot10^{-4}\ \mathrm{mol\cdot dm^{-3}}$ Eu(III) + $5\cdot10^{-4}\ \mathrm{mol\cdot dm^{-3}}$ 9-ANCA, (c) $5\cdot10^{-4}\ \mathrm{mol\cdot dm^{-3}}$ Eu(III) + $5\cdot10^{-4}\ \mathrm{mol\cdot dm^{-3}}$ Nc-Glu, Nc-Leu, or Nc-Lys, or (d) $5\cdot10^{-4}\ \mathrm{mol\cdot dm^{-3}}$ Eu(III) + $5\cdot10^{-4}\ \mathrm{mol\cdot dm^{-3}}$ 9-ANCA + $5\cdot10^{-4}\ \mathrm{mol\cdot dm^{-3}}$ Nc-Glu, Nc-Leu, or Nc-Lys.

The ionic strength of the studied solutions was adjusted to 0.1 $\mathrm{mol\cdot dm^{-3}}$ using *p*-toluene sulfonate solution.

Cyclic Voltammetry (CV). The solution was purged with nitrogen for 120 s, and then the potential was scanned at scan

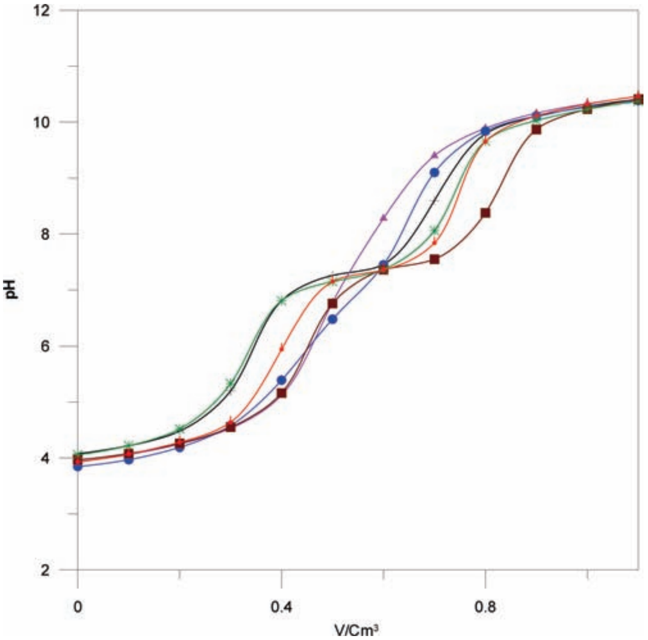

Figure 1. pH against a volume of 0.033 mol·dm^{-3} KOH for the Eu(III)-(9-ANCA) ternary complexes system at I = 0.1 mol·dm^{-3} KNO_3 and (25.0 ± 0.1) °C: +, Eu(III)-(9-ANCA); ▲, Eu(III)-(9-ANCA)-(5′-ATP); ●, Eu(III)-(9-ANCA)-(5′-ADP); ■, Eu(III)-(9-ANCA)-(Nc-Glu); ∗, Eu(III)-(9-ANCA)-(Nc-Leu); •, Eu(III)-(9-ANCA)-(Nc-Lys).

rates of (25, 50, 75, 100, 200, and 300) mV·s^{-1} from (− 0.30 to −0.90) V.

Square Wave Voltammetry (SWV). The samples were analyzed as in CV. The pulse height was 25 mV, and the SW frequency f = (20, 40, 60, 80, 100, 200, and 300) Hz, and the scan increment 2.0 mV.

Differential Pulse Voltammetry (DPP). The samples were analyzed also as in CV, but at a scan rate of (5, 15, 25, and 36) mV·s^{-1}. The pulse height was 25 mV, the pulse width 50 s, the frequency 20 Hz, and the scan increment 2.0 mV.

2.5. Spectrophotometric Measurements. UV−vis absorption spectra measurements of the interaction of the Eu-(9-ANCA) complex with DNA are as follows: (a) $2\cdot10^{-5}$ mol·dm^{-3} of the synthesized Eu-(9-ANCA) complex, dissolved in bidistilled water, and (b) solution (a) + ($2\cdot10^{-5}$, $4\cdot10^{-5}$, $6\cdot10^{-5}$, and 10^{-4}) mol·dm^{-3} DNA. The free Eu-(9-ANCA) complex solutions were scanned from (200 to 400) nm against a tris-buffer as a blank. For the binary Eu-(9-ANCA) complex with DNA the same procedure was operated.

2.6. Spectrofluorometric Measurements. Fluorescence spectroscopy measurements of interaction of Eu-(9-ANCA) complex with DNA are as follows: (a) $2\cdot10^{-5}$ mol·dm^{-3} of the synthesized Eu-(9-ANCA) complex, dissolved in bidistilled water, and (b) solution (a) + ($2\cdot10^{-5}$, $4\cdot10^{-5}$, $6\cdot10^{-5}$, and 10^{-4}) mol·dm^{-3} DNA. The excitation wavelength is 383 nm at room temperature.

3. Results and Discussion

3.1. Potentiometric Studies of the Interaction of Eu-Complexes with Some N-Acetyl Amino Acids and Nucleotides. The reaction of Eu(III) with *N*-acetyl amino acids (Nc-Lys, Nc-Leu, and Nc-Glu), 5′-ATP, 5′-ADP, and 9-ANCA has been carried out in a 0.10 volume fraction ethanol−water mixture, I = 0.1 mol·dm^{-3} KNO_3, and at (25.0 ± 0.1) °C. The calculated dissociation constants of the different ligands are collected in Table 1. The potentiometric titration curves are depicted in Figures 1 to 6. The dissociation constant of the −COOH group linked to the trinuclear aromatic ring has been determined (log K = 6.56 ± 0.02).

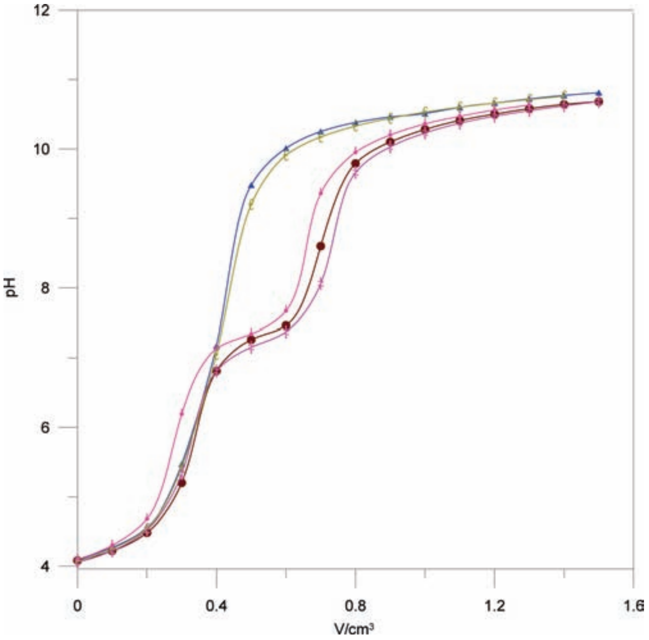

Figure 2. pH against a volume of 0.033 mol·dm^{-3} KOH for the Eu(III) + Nc-Lys + 9-ANCA system in a 0.10 volume fraction ethanol−water mixture at I = 0.1 mol·dm^{-3} KNO_3 and (25.0 ± 0.1) °C: (a) ▲, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} 9-ANCA; (b) ●, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} 9-ANCA + 0.0001 mol·dm^{-3} Eu(III); (c) €, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} Nc-Lys; (d) •, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} Nc-Lys + 0.0001 mol·dm^{-3} Eu(III); (e) ‡, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} Nc-Lys + 0.0001 mol·dm^{-3} 9-ANCA + 0.0001 mol·dm^{-3} Eu(III).

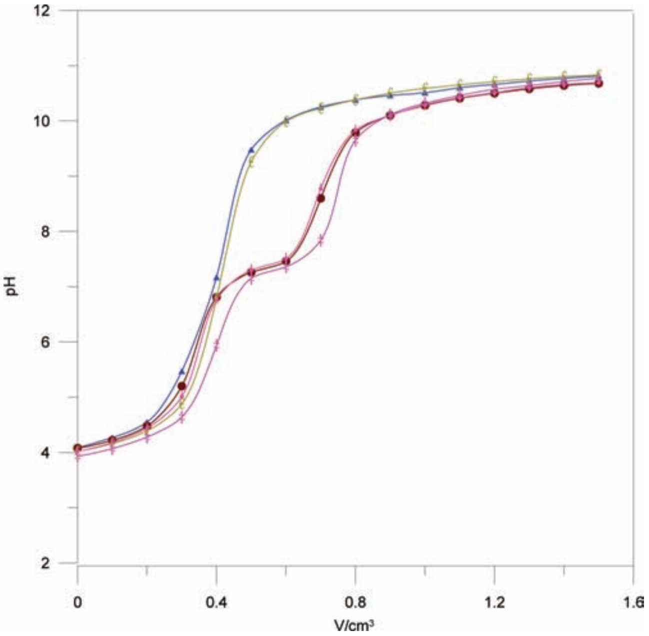

Figure 3. pH against a volume of 0.033 mol·dm^{-3} KOH for the Eu(III) + Nc-Leu + 9-ANCA system in a 0.10 volume fraction ethanol−water mixture at I = 0.1 mol·dm^{-3} KNO_3 and (25.0 ± 0.1) °C: (a) ▲, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} 9-ANCA; (b) ●, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} 9-ANCA + 0.0001 mol·dm^{-3} Eu(III); (c) €, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} Nc-Leu; (d) •, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} Nc-Leu + 0.0001 mol·dm^{-3} Eu(III); (e) ‡, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} Nc-Leu + 0.0001 mol·dm^{-3} 9-ANCA + 0.0001 mol·dm^{-3} Eu(III).

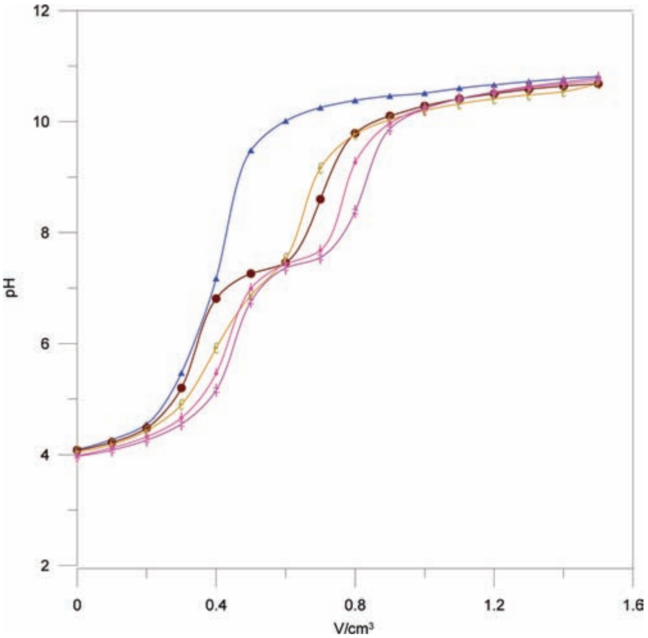

Figure 4. pH against a volume of 0.033 mol·dm^{-3} KOH for the Eu(III) + Nc-Glu + 9-ANCA system in a 0.10 volume fraction ethanol−water mixture at I = 0.1 mol·dm^{-3} KNO_3 and (25.0 ± 0.1) °C: (a) ▲, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} 9-ANCA; (b) ●, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} 9-ANCA + 0.0001 mol·dm^{-3} Eu(III); (c) €, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} Nc-Glu; (d) •, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} Nc-Glu + 0.0001 mol·dm^{-3} Eu(III); (e) ‡, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} Nc-Lys + 0.0001 mol·dm^{-3} 9-ANCA + 0.0001 mol·dm^{-3} Eu(III).

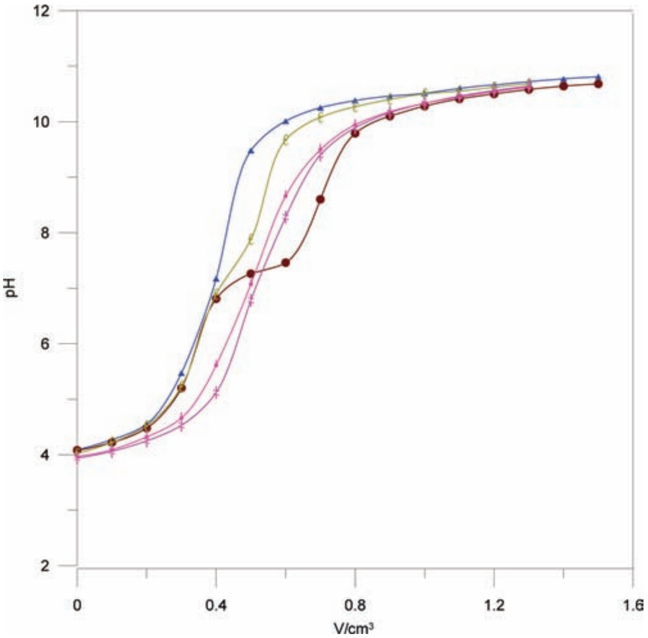

Figure 5. pH against a volume of 0.033 mol·dm^{-3} KOH for the Eu(III) + *N*-acetyl 5′-ATP + 9-ANCA system in a 0.10 volume fraction ethanol−water mixture at I = 0.1 mol·dm^{-3} KNO_3 and (25.0 ± 0.1) °C: (a) ▲, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} 9-ANCA; (b) ●, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 9-ANCA mol·dm^{-3} + 0.0001 mol·dm^{-3} Eu(III); (c) €, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} 5′-ATP; (d) •, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} 5′-ATP + 0.0001 mol·dm^{-3} Eu(III); (e) ‡, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} 5′-ATP + 0.0001 mol·dm^{-3} 9-ANCA + 0.0001 mol·dm^{-3} Eu(III).

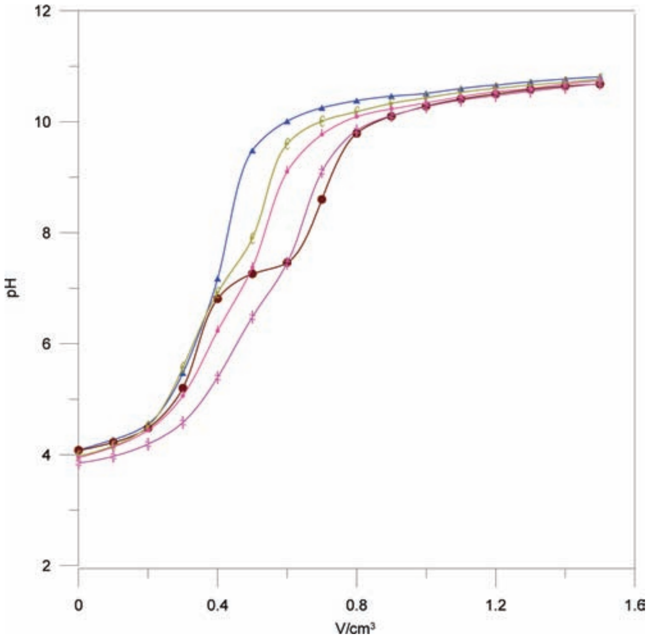

Figure 6. pH against a volume of 0.033 mol·dm^{-3} KOH for the Eu(III) + 5′-ADP + 9-ANCA system in a 0.10 volume fraction ethanol−water mixture at I = 0.1 mol·dm^{-3} KNO_3 and (25.0 ± 0.1)°C: (a) ▲, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} 9-ANCA; (b) ●, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 9-ANCA mol·dm^{-3} + 0.0001 mol·dm^{-3} Eu(III); (c) €, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} 5′-ADP; (d) •, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} 5′-ADP + 0.0001 mol·dm^{-3} Eu(III); (e) ‡, 0.0004 mol·dm^{-3} HNO_3 + 0.0001 mol·dm^{-3} 5′-ADP + 0.0001 mol·dm^{-3} 9-ANCA + 0.0001 mol·dm^{-3} Eu(III).

Table 2. Δ log $K_{M(Z)(9\text{-}ANCA)}$ for the 1:1:1 Ratios of Eu(III) + 9-ANCA + *N*-Acetyl Amino Acids, 5′-ATP, or 5′-ADP (Z) Ternary Complexes As Determined by Potentiometric pH-Titration in a 0.10 volume fraction Ethanol−Water Mixture at I = 0.1 mol·dm^{-3} (KNO_3)^a and (25.0 ± 0.1) °C

	Δ log $K_{M(Z)(9\text{-}ANCA)}$				
system	5′-ADP	5′-ATP	Nc-Glu	Nc-Leu	Nc-Lys
Eu(III) + (9-ANCA) + Z	+0.12	+0.03	+0.03	+0.02	+0.08

With respect to the titration curves of Eu(III) + 9-ANCA, Eu + Nc-Leu, Eu + Nc-Lys, Eu + Nc-Glu, Eu + 5′-ADP, and Eu + 5′-ATP binary complex solutions studied, one may deduce that these complexes begin to form at pH < 6.63 for Eu(III) + 9-ANCA, at pH < 6.89 for Eu + Nc-Leu, at pH < 7.2 for Eu + Nc-Lys, at pH < 4.515 for Eu + Nc-Glu, at pH < 3.94 for Eu + 5′-ADP, and at pH < 4.2 for Eu + 5′-ATP.

For the titration curves of the ternary systems studied [Eu(III) + 9-ANCA + *N*-acetyl amino acid or nucleotides] it was observed that curve (b) and curve (e) are well-separated at pH < 4.15 for Eu(III) + 9-ANCA + Nc-Leu, at pH < 4.17 for Eu(III) + 9-ANCA + Nc-Lys, at pH < 4.23 for Eu(III) + 9-ANCA + Nc-Glu, at pH < 3.79 for Eu(III) + 9-ANCA + 5′-ADP, and at pH < 4.04 for Eu(III) + 9-ANCA + 5′-ATP. The previous pH values indicate the points at which complexation starts.

To quantify the stability of the ternary complexes relative to the stability of the binary Eu(III) complexes, one may consider the following stepwise equilibria:

$$M_p(Z)_r + q(9\text{-ANCA}) \rightleftharpoons M_p(Z)_r(9\text{-ANCA})_q \qquad (1a)$$

$$M_p(9\text{-ANCA})_q + r(Z) \rightleftharpoons M_p(9\text{-ANCA})_q(Z)_r \qquad (2a)$$

in which M = Eu(III) ions and Z = Nc-Glu, Nc-Leu, Nc-Lys, ADP, and ATP. p, q, and r are the moles of M, 9-ANCA, and Z, respectively.

Equilibrium 1a holds for Z = Nc-Leu, N-Glu, 5′-ATP, and 5′-ADP, which refers to the addition of 9-ANCA to the binary

Table 3. Formation Constants for the Binary Complexes of Metal Ion Eu(III) + *N*-Acetyl Amino Acids, 9-ANCA, 5′-ATP, or 5′-ADP at $I = 0.1$ mol·dm^{-3} (KNO_3)[a] and (25.0 ± 0.1) °C

	log K_1(Z)					
metal ion	5′-ATP	5′-ADP	Nc-Glu	Nc-Leu	Nc-Lys	ANCA[b]
Eu(III)	6.33 ± 0.04	6.24 ± 0.04	4.77 ± 0.04	4.30 ± 0.02	4.29± 0.02	4.39 ± 0.02

[a] Uncertainties refer to three times the standard deviation (3δ). [b] The measurement was carried out in a 0.10 volume fraction ethanol−water mixture.

Table 4. Formation Constants for the Mixed Complexes of Metal Ions (M) + 9-ANCA + *N*-Acetyl Amino Acids, 5′-ATP, or 5′-ADP (Z) in a 0.10 volume fraction Ethanol−Water Mixture at $I = 0.1$ mol·dm^{-3} (KNO_3)[a] and (25.0 ± 0.1) °C

metal ion	log $K_{M(5'\text{-ATP})(N)}$	log $K_{M(5'\text{-ADP})(N)}$	log $K_{M(Nc\text{-Leu})(N)}$	log $K_{M(Nc\text{-Lys})(N)}$	log $K_{M(Nc\text{-Glu})(N)}$
Eu(III)	4.42 ± 0.04	4.52 ± 0.02	4.41 ± 0.02	4.37 ± 0.03	4.42± 0.04

[a] Uncertainties refer to three times the standard deviation (3δ).

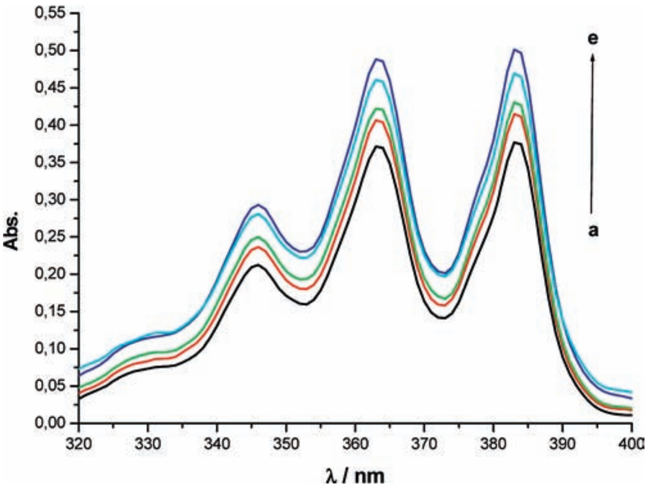

Figure 7. Absorption spectra of the Eu(III)-(9-ANCA) complex in different concentrations of DNA. [Eu(III)-(9-ANCA) complex] = 2.10^{-5} M; [DNA]/(10^{-5} M); (a−e) 0, 2, 4, 6, and 10.

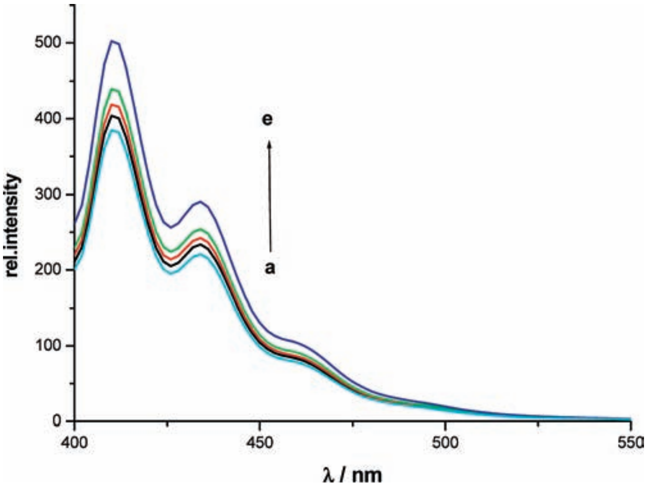

Figure 9. Effect of DNA concentration on the emission spectra of the Eu(III)-(9-ANCA) complex in aqueous solutions [Eu(III)-(9-ANCA) complex] = 2.10^{-5} M; [DNA]/(10^{-5} M); (a−e) 0, 2, 4, 6, and 10.

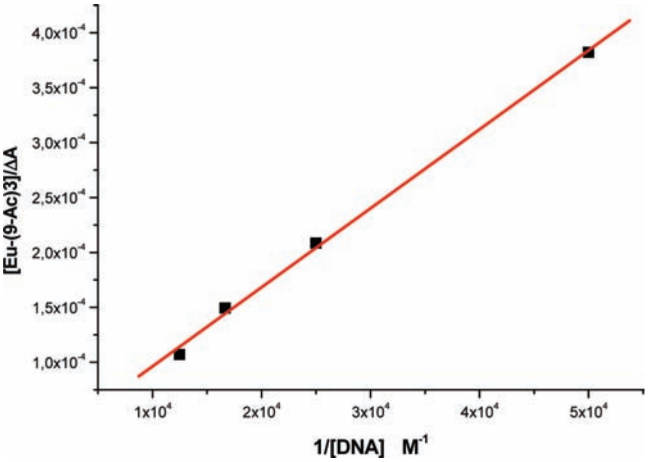

Figure 8. Effect of CT-DNA concentration on the absorbance of the Eu(III)-(9-ANCA) complex in aqueous solutions.

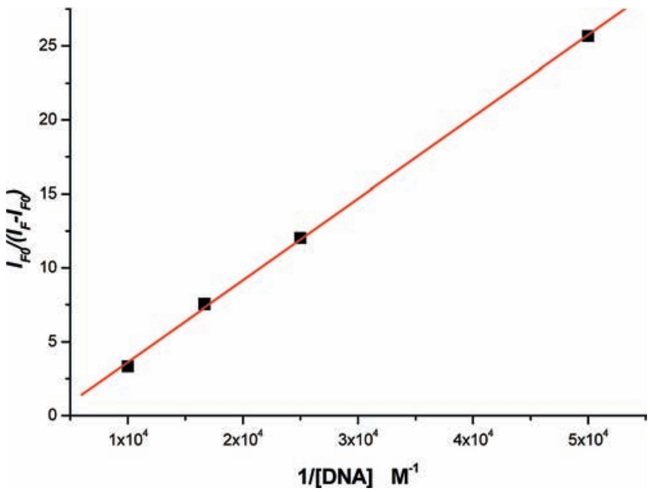

Figure 10. Effect of CT-DNA concentration on fluorescence intensity of the Eu(III)-(9-ANCA) complex in aqueous solution.

complex $M_p(Z)_r$, while equilibrium 2a holds for Z = Nc-Lys which refers to the addition of Z to the binary complex $M_p(9\text{-ANCA})_q$.

The corresponding equilibrium constant is defined by the equation:

$$10^{\Delta \log K_{M(Z)(9\text{-ANCA})}} = \frac{M_p(9\text{-ANCA})_q(Z)_r}{[M_p(Z)_r][9\text{-ANCA}]^q}$$

$$10^{\Delta \log K_{M(9\text{-ANCA})(Z)}} = \frac{M_p(9\text{-ANCA})_q(Z)_r}{[M_p(9\text{-ANCA})_q][Z]^r}$$

Values for $10^{\Delta K}{}_{M(Z)(9\text{-ANCA})}$ and $10^{\Delta K}{}_{M(9\text{-ANCA})(Z)}$ may be calculated according to:

$$\Delta \log K_{M(Z)(9\text{-ANCA})} = \log K_{M(Z)(9\text{-ANCA})} - \log K_{M(9\text{-ANCA})}$$
$$\Delta \log K_{M(9\text{-ANCA})(Z)} = \log K_{M(9\text{-ANCA})(Z)} - \log K_{M(Z)}$$

The results are given in Table 2 for all of the systems under investigation in this study.

Table 5. Thermodynamic Parameters of the Eu(III)-(9-ANCA)-DNA Complex

T K	K_A $L\cdot mol^{-1}$	ΔG^a $kJ\cdot mol^{-1}$	ΔH $kJ\cdot mol^{-1}$	ΔS $J\cdot mol^{-1}\cdot K^{-1}$	R^b	SD^c
298	$3.457\cdot 10^4$	− 6188				
318	$4.015\cdot 10^4$	− 6698	19874	52.3	0.999	0.27

[a] $\Delta G = -RT \ln K_c$. [b] Linear correlation coefficient. [c] Standard deviation.

Δ log K values are positive for all of the systems. The higher values for the formation constants of ternary complexes compared with those of binary systems may be attributed to the interligand interactions or some cooperativity between the coordinate ligands, possibly H-bond formation.

The formation constant of the binary complex Eu(III)-(9-ANCA) in a 1:1 molar ratio is 4.39 ± 0.02, where it indicates that there is a considerable binding between the lanthanide metal ion Eu(III) and the oxygen of the carboxylate group linked to the aromatic moiety. The three *N*-acetyl amino acids, Nc-Lys, Nc-Leu, and Nc-Glu, give considerably high stability complexes with Eu(III), where the latter forms the most stable complex species, which confirms the oxophilic nature of Eu(III) and the coordination of the dicarboxylic oxygen of Nc-Glu.

Initial estimates of the formation constants of the binary Eu(III) and ternary complexes have been refined using the SUPERQUAD computer program.[21] All side reactions due to metal hydrolysis have been included in the calculations.[22–24]

Inspecting the formation constant of the resulting binary and ternary species as collected in Tables 3 and 4 indicates that the ternary complex Eu(III)-(5′-ADP)-(9-ANCA) is slightly more stable than Eu(III)-(5′-ATP)-(9-ANCA). This

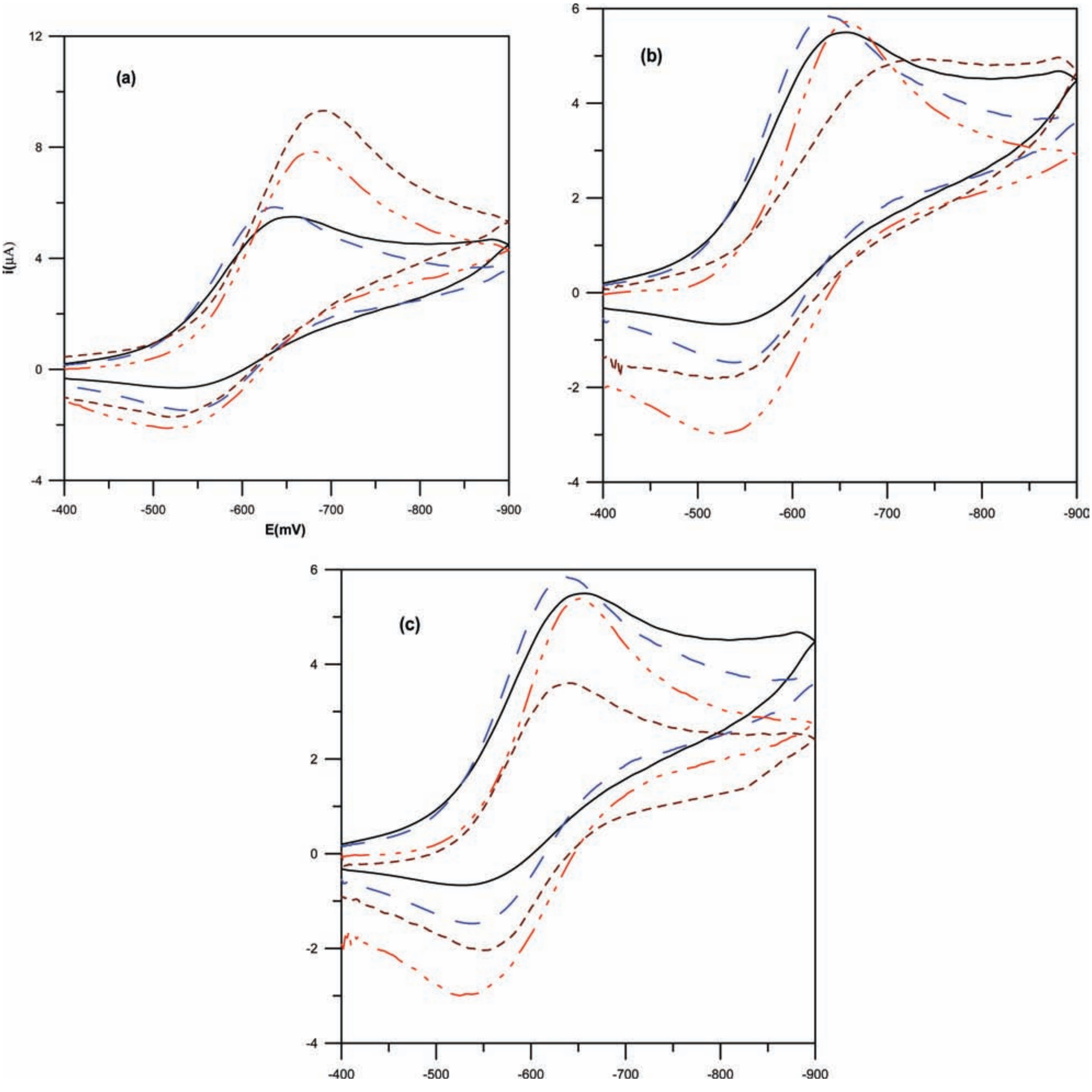

Figure 11. Cyclic voltammograms for the Eu(III) + ANCA + *N*-acetyl amino acids (NAA) system in a 0.10 volume fraction ethanol−water mixture at 0.1 $mol\cdot dm^{-3}$ *p*-toluene sulfonate, pH 6.0, scan rate = 200 $m\cdot (V\cdot s)^{-1}$ and at t = (25.0 + 0.1) °C: black line, $5\cdot 10^{-4}$ $mol\cdot dm^{-3}$ Eu(III); blue line, $5\cdot 10^{-4}$ $mol\cdot dm^{-3}$ Eu(III) + $5\cdot 10^{-4}$ $mol\cdot dm^{-3}$ ANCA; purple line, $5\cdot 10^{-4}$ $mol\cdot dm^{-3}$ Eu(III) + $5\cdot 10^{-4}$ $mol\cdot dm^{-3}$ NAA; red line, $5\cdot 10^{-4}$ $mol\cdot dm^{-3}$ Eu(III) + $5\cdot 10^{-4}$ $mol\cdot dm^{-3}$ ANCA + $5\cdot 10^{-4}$ $mol\cdot dm^{-3}$ NAA. (a) NAA = Nc-Glu, (b) NAA = Nc-Leu, (c) NAA = Nc-Lys.

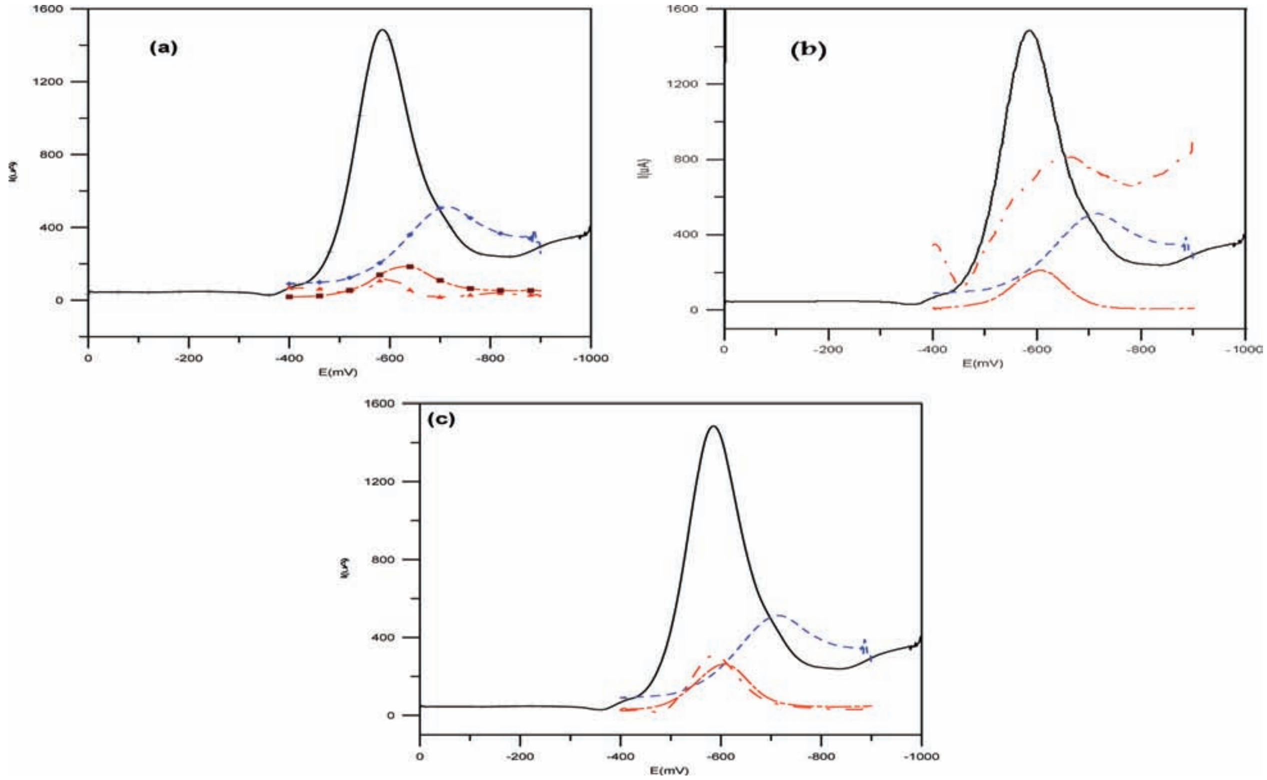

Figure 12. Differential pulse polarograms for the Eu(III) + 9-ANCA + *N*-acetyl amino acids (NAA) system in a 0.10 volume fraction ethanol−water mixture at 0.1 mol·dm^{-3} *p*-toluene sulfonate, pH 6.0, scan rate = 25 m·(V·s)$^{-1}$ and at t = (25.0 + 0.1) °C: black solid line, 5·10^{-4} mol·dm^{-3} Eu(III); blue dashed line, 5·10^{-4} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} 9-ANCA; red dashed line, 5·10^{-4} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} NAA; purple dashed line, 5·10^{-4} mol·dm^{-3} Eu (III) + 5·10^{-4} mol·dm^{-3} ANCA + 5·10^{-4} mol·dm^{-3} NAA. (a) NAA = Nc-Glu, (b) NAA = Nc-Leu, (c) NAA = Nc-Lys.

observation could be attributed to the steric hindrance effect of 5′-ATP with respect to 5′-ADP. Irrespective to the type of *N*-acetyl amino acid used, the formation constants of the ternary complexes Eu(III)-(*N*-acetyl amino acid)-(9-ANCA) acquire nearly the same value, which indicates that the complex species are isostructural, or in other words the same mode of coordination takes place for the three amino acids under investigation.

3.2. Fluorescence Studies for the Interaction of Eu(III)-(9-ANCA) Complex with DNA. The interactions of metal complexes with DNA have been the subject of interest for the development of effective chemotherapeutic agents. Eu(III) centers are particularly attractive moieties for such research since they exhibit well-defined coordination geometries and also often possess distinctive electrochemical and photophysical properties, thus enhancing the functionality of the binding agent. Electronic absorption spectroscopy was an effective method in examining the binding mode of DNA with the metal complex.[25] If the binding mode was intercalation, the π^* orbital of the intercalation ligand can couple with the π orbital of the base pairs, thus decreasing the $\pi \rightarrow \pi^*$ transition energy and resulting in bathochromism. If the coupling π orbital is partially filled by electrons, it results in decreasing the transition probabilities and concomitantly results in hypochromism.[26]

The spectroscopic variations are strongly indicative that the Eu(III)-(9-ANCA) can insert into DNA base pairs depending on its selectivity toward the ADP and ATP nucleotides as indicated by potentiometric equilibrium measurements in our present study. The absorption spectra of the Eu(III)-(9-ANCA) complex in aqueous solutions containing varying concentrations of CT-DNA are shown in Figure 7. It is clear that the absorption peak gradually increases without a wavelength shift, indicating the formation of some sort of binding most probably simple groove binding between the complex including Eu(III)-(9-ANCA) and CT-DNA.

3.2.1. Binding Constant K and the Stoichiometry of the Eu(III)-(9-ANCA)-DNA System. Assuming that DNA forms a 1:1 complex with the Eu-complex, the following expression can be written:

$$\text{Eu-complex} + \text{DNA} \overset{K}{\leftrightarrow} \text{Eu-complex} - \text{DNA}$$

For this equilibrium, the association constant of the formed complex (K) is given by a Benesi−Hildebrand plot:[27]

$$\frac{[\text{Eu-complex}]_0}{\Delta A} = \frac{1}{\Delta\varepsilon} + \frac{1}{K[\text{DNA}]_0\Delta\varepsilon}$$

where ΔA is the difference between the absorbance of Eu(III)-(9-ANCA) complex in the presence and absence of CT-DNA and $\Delta\varepsilon$ is the difference between the molar absorption coefficients of Eu(III)-(9-ANCA) complex and Eu(III)-(9-ANCA)-(CT-DNA). [Eu-complex]$_0$ and [DNA]$_0$ are the initial concentrations of the Eu-complex and CT-DNA, respectively. Figure 8 depicts a plot of 1/ΔA as a function of 1/[CT-DNA] for the (Eu-complex)-(CT-DNA) system. Good linear correlations were obtained, confirming the formation of a 1:1 Eu(III)-(9-ANCA)-DNA complex. From the intercept and slope value of this plot, K is evaluated at room temperature (25 °C). The association constant at room temperature was determined to be (3.39·10^4 ± 100) M^{-1} through the regression fit.

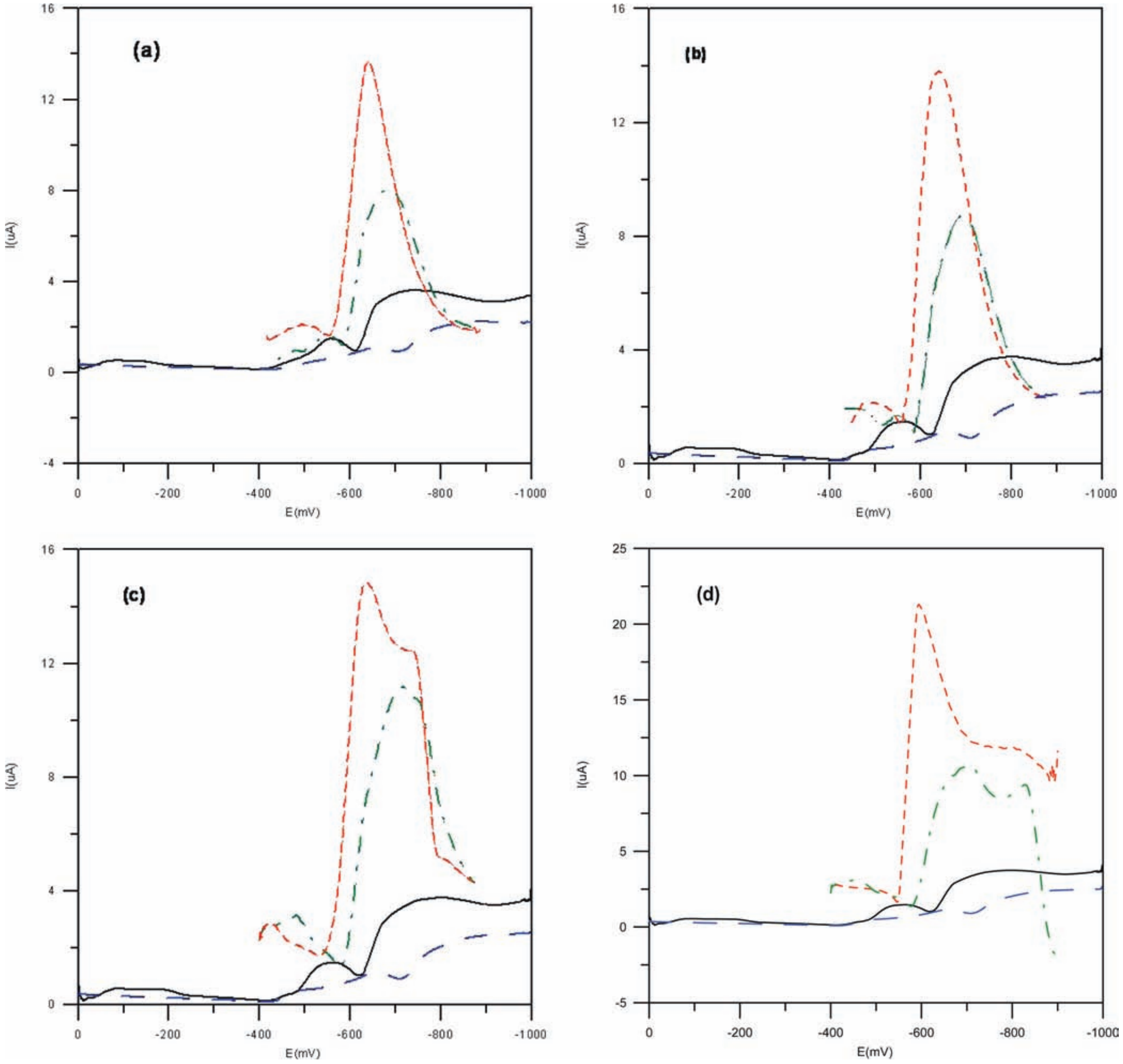

Figure 13. Square wave voltammograms for the Eu(III) + 9-ANCA + Nc-Glu system in a 0.10 volume fraction ethanol−water mixture at 0.1 mol·dm^{-3} *p*-toluene sulfonate, pH 6.0, scan rate = 100 m·(V·s)$^{-1}$, at (a) frequency = 80 Hz, (b) frequency = 100 Hz, (c) frequency = 200 Hz, (d) frequency = 300 Hz and at t = (25.0 + 0.1) °C: black solid line, 5·10^{-4} mol·dm^{-3} Eu(III); blue dashed line, 5·10^{-4} mol·dm^{-3} Eu (III) + 5·10^{-4} mol·dm^{-3} ANCA; red dashed line, 5·10^{-4} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} Nc-Glu; green dashed line, 5·10^{-4} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} ANCA + 5·10^{-4} mol·dm^{-3} Nc-Glu.

The results of the fluorescence emission titrations as indicated in Figure 9 suggest that the Eu(III)-(9-ANCA) complex molecules are protected from solvent water molecules by the hydrophobic environment inside the CT-DNA helix and that the complex can be protected more efficiently than the free ligand. This implies that both the free ligand and the complex can insert between CT-DNA base pairs deeply and that the complex can bind to CT-DNA more strongly than just the free ligand which interacts only through stacking interactions with the aromatic rings of the nucleobases of CT-DNA. Since the hydrophobic environment inside the CT-DNA helix reduces the accessibility of solvent water molecules to the compound and the compound mobility is restricted at the binding site, a decrease of the vibrational modes of relaxation results. The binding of Eu(III)-(9-ANCA) to CT-DNA leads to a marked increase in emission intensity which also agrees with those observed for other intercalations (Figure 10).

Fluorescence experiments were carried out to determine the binding constants of the Eu(III)-(9-ANCA)-(CT-DNA) complex. The wavelength of the excitation light was selected as 383 nm, which is equal to its corresponding absorption maximum. It is observed that the shape and positions of the fluorescence emission spectra in aqueous solutions did not change at various concentrations of CT-DNA. The fluorescence intensity was determined by the measurement of the relative intensity at the fluorescence maxima, which were found at 410 nm.

Thermodynamic parameters for the Eu(III)-(9-ANCA)-DNA complex have been investigated to further characterize the acting

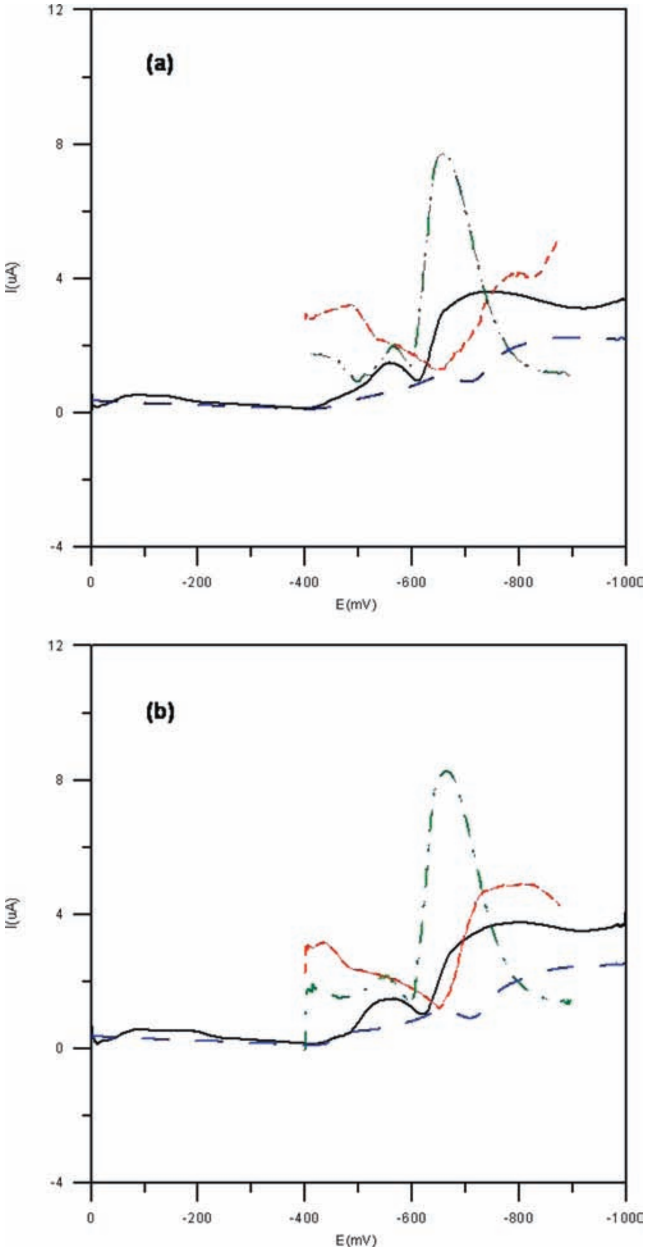

Figure 14. Square wave voltammograms for the Eu(III) + ANCA + Nc-Leu system in a 0.10 volume fraction ethanol−water mixture at 0.1 $\text{mol}\cdot\text{dm}^{-3}$ *p*-toluene sulfonate, pH 6.0, scan rate = 100 $\text{m}\cdot(\text{V}\cdot\text{s})^{-1}$, at (a) frequency = 80 Hz, (b) frequency = 100 Hz, at $t = (25.0 + 0.1)$°C: black solid line, $5\cdot10^{-4}$ $\text{mol}\cdot\text{dm}^{-3}$ Eu(III); blue dashed line, $5\cdot10^{-4}$ $\text{mol}\cdot\text{dm}^{-3}$ Eu(III) + $5\cdot10^{-4}$ $\text{mol}\cdot\text{dm}^{-3}$ ANCA; red dashed line, $5\cdot10^{-4}$ $\text{mol}\cdot\text{dm}^{-3}$ Eu(III) + $5\cdot10^{-4}$ $\text{mol}\cdot\text{dm}^{-3}$ Nc-Leu; green dashed line, $5\cdot10^{-4}$ $\text{mol}\cdot\text{dm}^{-3}$ Eu(III) + $5\cdot10^{-4}$ $\text{mol}\cdot\text{dm}^{-3}$ ANCA + $5\cdot10^{-4}$ $\text{mol}\cdot\text{dm}^{-3}$ Nc-Leu.

forces between Eu(9-ANCA)$_3$ and CT-DNA. The acting forces between a small molecule substance and macromolecule mainly include hydrogen bonding, van der Waals forces, electrostatic forces, and hydrophobic interaction forces. From Table 5, it was observed that the negative sign for free energy ΔG means that the interaction process of Eu(9-ANCA)$_3$ and DNA is spontaneous. The thermodynamic parameters were calculated according to the following thermodynamic equations.

$$\ln\left(\frac{K_{A2}}{K_{A1}}\right) = \left(\frac{1}{T_1} - \frac{1}{T_2}\right)\frac{\Delta H}{R} \qquad (5)$$

and

$$\Delta G = -RT \ln K_A = \Delta H - T\Delta S \qquad (6)$$

The values of enthalpy change ΔH, free energy change ΔG, and entropy change ΔS can be obtained, where K_{A1} and K_{A2} are the binding constants at temperature T_1 and T_2, respectively, and can be calculated by linear fitting plots of eq 5. The enthalpy change ΔH could be a constant and be obtained using eq 5. The thermodynamic parameters (ΔH, ΔG, and ΔS) obtained are listed in Table 5.

3.3. Electrochemical Investigation of the Interaction of Eu(III)-(9-ANCA) with N-Acetyl Amino Acids at the GC Electrode. Confirmation of the formation of binary and ternary complexes of the type Eu(III)-(9-ANCA), Eu(III)-(Nc-Lys), Eu(III)-(Nc-Leu), Eu(III)-(Nc-Glu), Eu(III)-(9-ANCA)-(Nc-Lys), Eu(III)-(9-ANCA)-(Nc-Leu), or Eu(III)-(9-ANCA)-(Nc-Glu) in solution has been carried out using CV, DPP, and SWV on a GC electrode. Figures 11 to 15 show the electrochemical behavior (CV, SWV, and DPP) of some of the ternary complexes formed in solution during this study. All of the voltammetric diagrams confirm the formation of different binary and ternary complexes that have been found using potentiometric titrations. It is quite interesting to observe that changing the frequency resulted in quite clear changes in the shapes of the square wave voltammograms of the ternary complexes formed in solution, which may be attributed to changing the mechanistic behavior of the electrochemical reduction of the resulting ternary complex on the GC electrode. The reversibility of the electrochemical reaction of binary or ternary complexes in the systems Eu(III)-(9-ANCA)-(Nc-Lys), Eu(III)-(9-ANCA)-(Nc-Leu), and Eu(III)-(9-ANCA)-(Nc-Glu) has been investigated by CV. The peak separation between the anodic and the cathodic peaks is more than 30 mV. These values indicate that the electrochemical reduction in the case of free Eu(III) ions and Eu(III) binary and ternary complexes under investigation is quasi-reversible on the GC electrode. The electrochemical behaviors of the complexes containing the electroactive metal ion Eu(III) in all of its species are reduced through Eu(III)/Eu(II), that is, a one-electron transfer process. It is clearly observed through the reoxidation of such species that the stability of the ternary complexes including *N*-acetyl amino acids follows the order: Nc-Glu > Nc-Leu > Nc-Lys. The different electrochemical characteristics and some kinetic parameters of the systems under investigation are given in Table 6. The CV 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 (v) range of (25 to 300) mVs^{-1}: the $i_{p,a}/i_{p,c}$ ratio decreases by increasing v; ($\Delta E_p = E_{p,c} - E_{p,a}$) increases by increasing v with slopes in the range (0.459 mV) for $E_{p,c}$ (i_p versus $v^{1/2}$ plots), which agrees very well with the theory for a typical quasi-reversible process. Kinetic parameters for the above-mentioned binary and ternary Eu(III) complexes have been calculated with the aim to probe their electron transfer ability when used as a basis for biosensors for the electrochemical detection of the biologically important Nc-Glu, Nc-Lys, and Nc-Leu investigated in this study. So the results obtained in the present work concerning the electrochemical reduction and kinetic parameters calculation for the Eu(III) ternary systems can be considered as a basis for the future development of novel biosensors for the trace determination of these biologically important compounds. Even an electrochemluminescence method can be developed on the basis of the interesting luminescent properties of Eu(III) ions. We have carried out

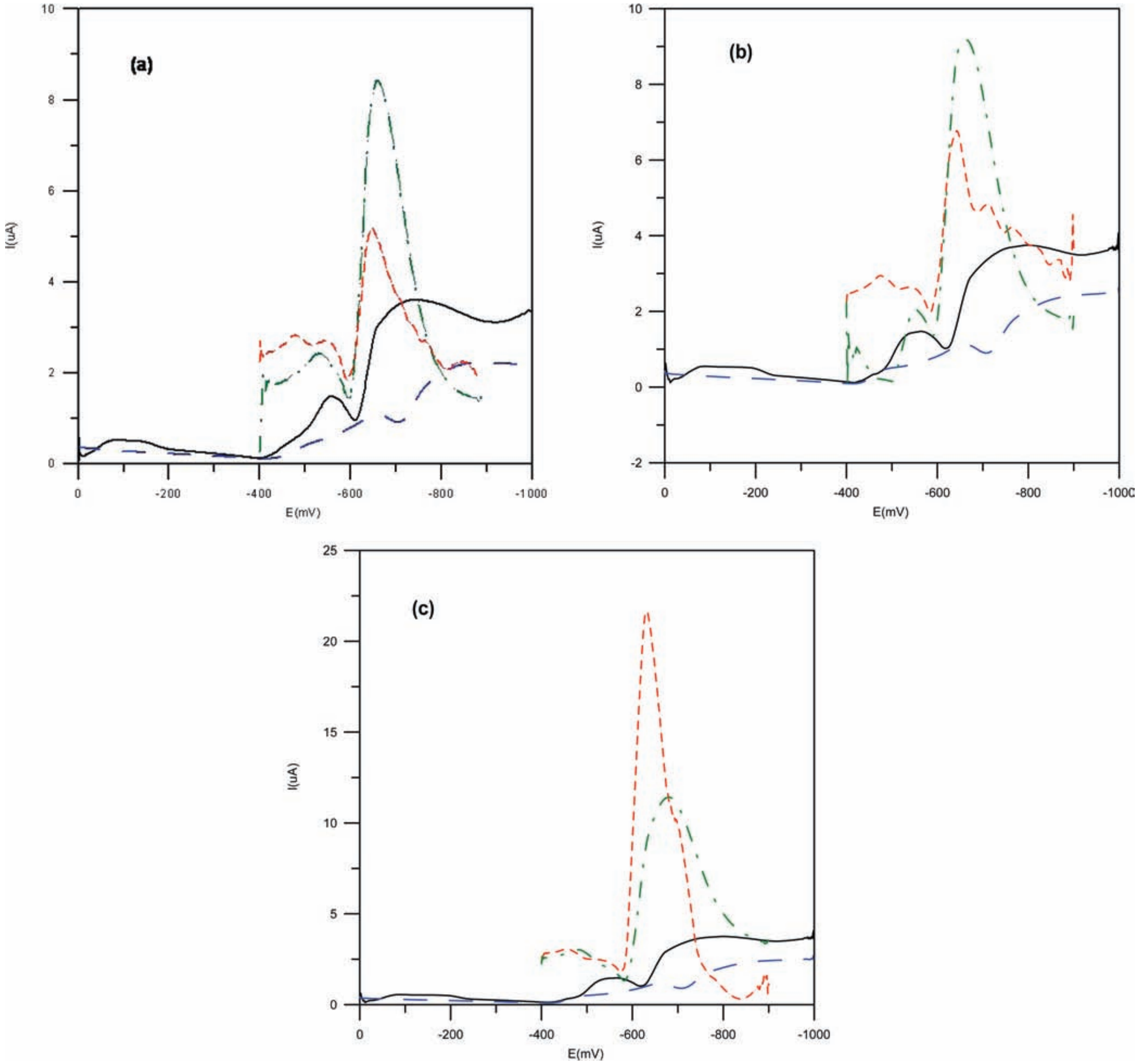

Figure 15. Square wave voltammograms for the Eu(III) + ANCA + Nc-Lys system in a 0.10 volume fraction ethanol−water mixture at 0.1 mol·dm^{-3} p-toluene sulfonate, pH 6.0, scan rate = 100 m·(V·s)$^{-1}$, at (a) frequency = 80 Hz, (b) frequency = 100 Hz, (c) frequency = 200 Hz, at t = (25.0 + 0.1) °C: black solid line, 5·10^{-4} mol·dm^{-3} Eu(III); blue dashed line, 5·10^{-4} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} ANCA; red dashed line, 5·10^{-4} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} Nc-Lys; green dashed line, 5·10^{-4} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} ANCA + 5·10^{-4} mol·dm^{-3} Nc-Lys.

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 quasi-reversible process. In fact, according to this theory, the kinetic parameter Ψ varies lineary with $\upsilon^{1/2}$, and ΔE_p approaches linearity for small Ψ. Consequently, in this zone ΔE_p should vary linearly with $\upsilon^{1/2}$. Because of the quasi-reversibility for the electrochemical reduction of the binary and ternary Eu(III) systems under investigation, it may be possible to study the kinetics of the electrode reaction. The separation of the peak potentials, ΔE_p, should be a measure of the standard rate constant for the electron transfer. These ΔE_p values were introduced in the working curve described by Nicholson[28] for obtaining the transfer parameter, Ψ, and then the standard heterogeneous charge-transfer rate constant (K^0) for the electron-transfer process at the GC electrode according to the following equation:

$$\Psi = \frac{(D_O/D_R)^{(\frac{\alpha}{2})}K^0}{D_O\pi\nu\left(\frac{nF}{RT}\right)^{1/2}} \tag{7}$$

where D_O and D_R are the diffusion coefficients for oxidized and reduced species, respectively, and can be calculated from the equation

$$i_p = (2.69{\cdot}10^5)n^{3/2}AD^{1/2}\nu^{1/2}C \tag{8}$$

where (i_p) is the peak current (A), n is the number of the electron transfer, A is the electrode area (cm^2), D is the diffusion coefficient (cm^2·s^{-1}), υ is the scan rate (V·s^{-1}), and C is the

Table 6. Voltammetric Characteristics of $5\cdot10^{-4}$ mol·dm^{-3} Eu(III) and Their Binary and Ternary Complexes with 9-ANCA and *N*-Acetyl Amino Acids in a 0.10 volume fraction Ethanol−Water Mixture at $I = 0.1$ mol·dm^{-3} *p*-Toluene Sulfonate and (25 ± 0.1) °C

	scan rate	$E_{pc/2}$	$-E_{pc}$	$E_{pc} - E_{pc/2}$	E_{pa}	$E_{pc} - E_{pa}$		i_{pc}	i_{pa}		D_{ox}	D_{red}		K^0	$-\Delta G^0_{25}$
system	$mV\cdot s^{-1}$	mV	mV	mV	mV	mV	E^0	μA	μA	i_{pa}/i_{pc}	$cm^2\cdot s^{-1}$	$cm^2\cdot s^{-1}$	α	$cm\cdot s^{-1}$	$kJ\cdot mol^{-1}$
Eu(III)	25	516.3	630	113.77	530	100	580	1.69	1.93	1.14	$2.60\cdot10^{-25}$	$1.98\cdot10^{-25}$	0.42	$1.45\cdot10^{-15}$	3.05
	50	549.3	642	92.75	558	84	600	2.84	3.39	1.19	$3.26\cdot10^{-25}$	$2.29\cdot10^{-25}$	0.52	$1.62\cdot10^{-15}$	3.04
	75	558	644	86	550	94	597	3.66	3.80	1.03	$2.53\cdot10^{-25}$	$2.35\cdot10^{-25}$	0.56	$9.26\cdot10^{-16}$	3.09
	100	566.7	656	89.34	528	128	592	5.26	5.26	1.00	$3.78\cdot10^{-25}$	$3.78\cdot10^{-25}$	0.54	$2.72\cdot10^{-15}$	2.99
	200	567.5	652	84.5	550	102	601	5.98	6.48	1.08	$2.71\cdot10^{-25}$	$2.31\cdot10^{-25}$	0.57	$4.13\cdot10^{-15}$	2.96
	300	572.7	660	87.25	542	118	601	7.39	8.10	1.09	$2.92\cdot10^{-25}$	$2.43\cdot10^{-25}$	0.55	$4.41\cdot10^{-15}$	2.95
Eu(III) + 9-ANCA	25	564.9	648	83.08	534	114	591	2.89	2.86	0.98	$4.16\cdot10^{-25}$	$4.27\cdot10^{-25}$	0.58	$1.69\cdot10^{-15}$	3.04
	50	564.1	642	77.91	536	106	589	4.31	4.00	0.92	$3.81\cdot10^{-25}$	$4.43\cdot10^{-25}$	0.62	$2.57\cdot10^{-15}$	3.00
	75	560.6	636	75.41	538	98	587	5.84	5.08	0.87	$3.97\cdot10^{-25}$	$5.24\cdot10^{-25}$	0.64	$3.63\cdot10^{-15}$	2.97
	100	557.1	632	74.91	540	92	586	6.95	6.31	0.90	$4.56\cdot10^{-25}$	$5.54\cdot10^{-25}$	0.64	$1.86\cdot10^{-15}$	3.03
	200	586.6	670	83.41	522	148	596	7.83	7.25	0.93	$3.35\cdot10^{-25}$	$3.91\cdot10^{-25}$	0.58	$2.84\cdot10^{-15}$	2.99
	300	603.1	694	90.91	512	182	603	9.06	8.64	0.95	$3.46\cdot10^{-25}$	$3.80\cdot10^{-25}$	0.53	$1.46\cdot10^{-15}$	3.05
Eu(III) + Nc-Lys	25	548.4	608	59.58	474	134	541	2.20	3.26	1.48	$3.87\cdot10^{-25}$	$1.76\cdot10^{-25}$	0.81	$9.21\cdot10^{-16}$	3.09
	50	574.5	642	67.50	562	80	602	2.25	2.88	1.28	$1.71\cdot10^{-25}$	$1.04\cdot10^{-25}$	0.71	$1.29\cdot10^{-15}$	3.06
	75	568.4	634	65.58	556	78	595	3.10	3.71	1.19	$1.84\cdot10^{-25}$	$1.28\cdot10^{-25}$	0.73	$1.84\cdot10^{-15}$	3.03
	100	571.8	642	70.16	554	88	598	3.60	4.47	1.24	$2.14\cdot10^{-25}$	$1.39\cdot10^{-25}$	0.68	$1.39\cdot10^{-15}$	3.05
	200	573.6	642	68.41	552	90	597	5.09	6.23	1.22	$2.03\cdot10^{-25}$	$1.36\cdot10^{-25}$	0.7	$1.67\cdot10^{-15}$	3.04
	300	576.2	646	69.83	554	92	600	6.34	7.72	1.21	$2.12\cdot10^{-25}$	$1.43\cdot10^{-25}$	0.69	$1.81\cdot10^{-15}$	3.03
Eu(III) + Nc-Leu	25	533.7	656	122.33	536	120	596	3.02	3.73	1.23	$1.04\cdot10^{-24}$	$6.83\cdot10^{-25}$	0.39	$2.29\cdot10^{-15}$	3.01
	50	576.2	678	101.83	534	144	606	3.80	4.25	1.12	$5.64\cdot10^{-25}$	$4.51\cdot10^{-25}$	0.47	$1.77\cdot10^{-15}$	3.03
	75	585.8	694	108.25	528	166	611	4.20	5.29	1.26	$6.19\cdot10^{-25}$	$3.90\cdot10^{-25}$	0.44	$1.40\cdot10^{-15}$	3.05
	100	599.7	716	116.33	530	186	623	4.90	6.73	1.37	$8.07\cdot10^{-25}$	$4.27\cdot10^{-25}$	0.41	$9.05\cdot10^{-16}$	3.09
	200	625.7	766	140.33				6.60				$4.68\cdot10^{-25}$	0.34		
	300														
Eu(III) + Nc-Glu	25	571	646	75	546	100	596	5.01	3.98	0.79	$7.29\cdot10^{-25}$	$1.15\cdot10^{-24}$	0.64	$2.96\cdot10^{-15}$	2.99
	50	583.1	672	88.83	564	108	618	6.67	4.59	0.69	$5.73\cdot10^{-25}$	$1.21\cdot10^{-24}$	0.54	$3.62\cdot10^{-15}$	2.97
	75	597.9	674	76.08	548	126	611	7.83	5.71	0.73	$5.06\cdot10^{-25}$	$9.52\cdot10^{-25}$	0.63	$3.40\cdot10^{-15}$	2.97
	100	602.2	692	89.75	516	176	604	9.31	7.01	0.75	$6.75\cdot10^{-25}$	$1.19\cdot10^{-24}$	0.53	$1.66\cdot10^{-15}$	3.04
	200	622.1	716	93.83	520	196	618	12.47	9.62	0.77	$6.64\cdot10^{-25}$	$1.11\cdot10^{-24}$	0.51	$3.99\cdot10^{-15}$	2.96
	300	630.9	730	99.08				14.9				$1.12\cdot10^{-24}$	0.48		
Eu(III) + 9-ANCA + Nc-Lys	25	556.5	636	79.75	530	106	583	3.31	3.75	1.13	$6.86\cdot10^{-25}$	$5.37\cdot10^{-25}$	0.6	$2.17\cdot10^{-15}$	3.01
	50	563.2	636	72.83	538	98	587	4.53	4.42	0.97	$4.35\cdot10^{-25}$	$4.58\cdot10^{-25}$	0.66	$2.87\cdot10^{-15}$	2.99
	75	579.7	644	64.33	540	104	592	4.93	5.00	1. 01	$3.28\cdot10^{-25}$	$3.19\cdot10^{-25}$	0.75	$2.82\cdot10^{-15}$	2.99
	100	586.6	652	65.41	538	114	595	5.38	5.73	1.06	$3.28\cdot10^{-25}$	$2.90\cdot10^{-25}$	0.73	$2.84\cdot10^{-15}$	2.99
	200	594.4	662	67.58	524	138	593	7.92	7.64	0.96	$3.02\cdot10^{-25}$	$3.24\cdot10^{-25}$	0.71	$3.07\cdot10^{-15}$	2.98
	300	598.7	670	71.25	514	156	592	9.80	9.24	0.94	$3.10\cdot10^{-25}$	$3.49\cdot10^{-25}$	0.67	$2.86\cdot10^{-15}$	2.99
Eu(III) + 9-ANCA + Nc-Leu	25	555.3	630	74.66	546	84	588	3.86	3.36	0.87	$5.18\cdot10^{-25}$	$6.82\cdot10^{-25}$	0.64	$1.72\cdot10^{-15}$	3.03
	50	576.2	644	67.83	536	108	590	4.43	4.44	1.00	$4.09\cdot10^{-25}$	$4.07\cdot10^{-25}$	0.71	$2.49\cdot10^{-15}$	3.00
	75	584.9	650	65.08	528	122	589	4.92	5.00	1.01	$3.32\cdot10^{-25}$	$3.21\cdot10^{-25}$	0.74	$2.34\cdot10^{-15}$	3.01
	100	590.9	656	65.08	524	132	590	5.72	5.90	1.03	$3.47\cdot10^{-25}$	$3.25\cdot10^{-25}$	0.74	$2.42\cdot10^{-15}$	3.00
	200	598.7	668	69.25	512	156	590	7.90	7.72	0.97	$3.15\cdot10^{-25}$	$3.30\cdot10^{-25}$	0.69	$2.30\cdot10^{-15}$	3.01
	300	604	678	74	494	184	586	9.83	9.34	0.95	$3.29\cdot10^{-25}$	$3.64\cdot10^{-25}$	0.65	$1.33\cdot10^{-15}$	3.06
Eu(III) + 9-ANCA + Nc-Glu	25	544.9	626	81.08	546	80	586	5.23	4.09	0.78	$8.31\cdot10^{-25}$	$1.35\cdot10^{-24}$	0.59	$2.75\cdot10^{-15}$	2.99
	50	576.2	650	73.83	528	122	589	6.34	5.01	0.78	$5.66\cdot10^{-25}$	$9.10\cdot10^{-25}$	0.65	$2.95\cdot10^{-15}$	2.99
	75	593.6	668	74.41	536	132	602	7.05	5.58	0.79	$4.72\cdot10^{-25}$	$7.55\cdot10^{-25}$	0.65	$2.92\cdot10^{-15}$	2.99
	100	600.5	678	77.50	514	164	596	7.84	6.43	0.82	$4.90\cdot10^{-25}$	$7.28\cdot10^{-25}$	0.62	$1.90\cdot10^{-15}$	3.03
	200	614.4	696	81.58	502	194	599	10.89	9.01	0.82	$5.06\cdot10^{-25}$	$7.39\cdot10^{-25}$	0.59	$3.45\cdot10^{-15}$	2.97
	300	623.1	708	84.91	498	210	603	13.17	11.09	0.84	$5.32\cdot10^{-25}$	$7.50\cdot10^{-25}$	0.57	$3.98\cdot10^{-15}$	2.96

concentration of species ($\text{mol}\cdot\text{cm}^{-3}$). The calculated values are collected in Table 6, where the data reveals that the diffusion coefficient values and the standard rate constants (K_s) follow the order: Nc-Glu > Nc-Lys > Nc-Leu.

To convert experimentally determined values of Ψ to K^0, good agreement between the values of the kinetic parameters calculated by the Butler equation is obtained.[29] This work can be considered as a continuation of the author's work in the field of bioinorganic chemistry.[30–34]

The Eu(III) ternary systems studied in the present work can be considered as a basis for the future development of novel biosensors for the trace determination of biologically important compounds as stated by the authors in the papers.[35,36]

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