Ternary Complexes Formed by the Fluorescent Probe Eu(III)−Anthracene-9-carboxylic Acid with Pyrimidine and Purine Nucleobases

Hassan A. Azab,*,† Ibrahim I. Abd El-Gawad,‡ and Rasha M. Kamel‡

Chemistry Department, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt, and Chemistry Department, Faculty of Science, Suez Canal University, Suez, Egypt

The formation of binary and ternary complexes of Eu(III) with nucleobases (NB), guanine (2-amino-6-oxypurine), thymine (2,4-dihydroxy 5-methyl pyrimidine), adenine (6-aminopurine), uracil (2,4-dioxypyrimidine), and anthracene-9-carboxylic acid (ANCA) has been studied potentiometrically at (25.0 ± 0.1) °C and ionic strength $I = 0.1$ mol·dm^{-3} (KNO_3) in 10 % ethanol−water mixture solvent. The acid−base properties of the ligands were investigated and discussed. The formation of the 1:1, 2:1 binary, and 1:1:1 and 2:1:1 ternary complexes is inferred from the corresponding titration curves. The ternary complexes formed are monoprotonated complexes. The stability constants of the binary and ternary systems were evaluated. Initial estimates of the formation constants of the resulting species and the protonation constants of the different ligands used have been refined with the SUPERQUAD computer program. The experimental conditions were selected such that self-association of the nucleobase and their complexes was negligibly small; that is, the monomeric normal and protonated complexes were studied. Confirmation of the formation of ternary complexes of the type Eu(III) (NB) (ANCA) in solution has been carried out using differential pulse polarography (DPP), square wave voltammetry (SWV), cyclic voltammetry (CV), UV−visible spectroscopic, and emission spectrofluorimetric measurements.

Introduction

Binding studies of small molecules to deoxyribonucleic acid (DNA) on a molecular level are very important in the development of novel chemotherapeutics and highly sensitive diagnostic agents.[1−4] The metal complexes can interact noncovalently with nucleic acids by intercalation when the ligand contains planar ring systems, groove binding for large molecules, or external electrostatic binding for cations. The binding modes are dependent on the sizes and stereochemical properties of the metal complexes.

Binding studies of small molecules with DNA are important in the design of new and more efficient drugs targeted to DNA.[5] Several aromatic hydrocarbons and their derivatives have been shown to be carcinogenic, and in several instances, the carcinogenicity was attributed to their activity at the DNA level.[6] Metal complexes, porphyrins, natural antibiotics, and a host of other planar heterocyclic cations have been investigated for their DNA binding affinity. Recently, the DNA sequence recognition by drugs as well as by small molecules that are conjugated to peptides or oligonucleotides has been of great interest. Binding studies with these various small molecules are valuable for the rational design of drugs as well as in understanding how proteins recognize and bind to specific DNA sequence.[7] With a view to explore the differences in the local environment of the four DNA bases within the double helix and to exploit these differences in the design of new and more efficient DNA binding agents, the DNA binding properties of a new hydrophobic probe, (9-anthrylmethyl)ammonium chloride (AMAC), have been tested.[8]

The lanthanide ions possess long-lived excited states, which can be populated by sensitizing antennae, and emit at long wavelengths in the visible and the near-infrared (NIR) regions. These are particularly desirable features for: (a) sensing as it overcomes drawbacks such as light scattering and auto fluorescence associated with short wavelength emitting sensors and (b) for probing metal directed synthesis of large supramolecular systems often formed between f−f or f−d metal ions.[9]

The metal complexes of purine and pyrimidine nucleobases play a dominant role in many biochemical systems. The participation of metal ions in biochemical reactions of nucleobases and nucleic acids has led to a great interest in determination of structures of metal nucleobase complexes. Heavy metal toxicity in our environment arises in part from the covalent interactions of heavy metal ions with nucleic acids. In addition, these heavy metals interfere with metalloregulatory proteins and in so doing disrupt gene expression.

Ternary complexes of some metal ions with purine and pyrimidine bases and secondary ligands have been investigated using several techniques.[10−22]

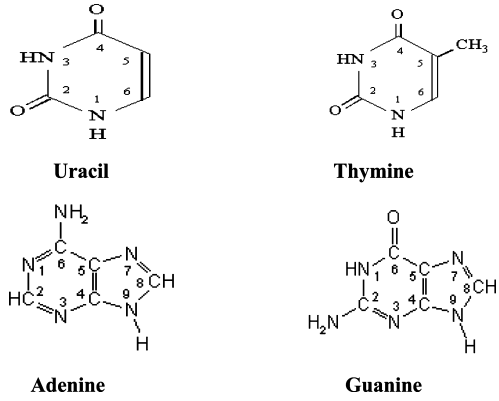
Table 1. Description of Solution Composition (a to h) Used in the Determination of the Stability Constants of Complex Species Formed in Solution

a	$4\cdot10^{-4}$ mol·dm^{-3} HNO_3 + $1\cdot10^{-4}$ mol·dm^{-3} 9-anthracene-carboxylic acid (as the first ligand)
b	$4\cdot10^{-4}$ mol·dm^{-3} HNO_3 + $1\cdot10^{-4}$ mol·dm^{-3} nucleobases (adenine, guanine, thymine, and uracil)
c	solution (a) + $1\cdot10^{-4}$ mol·dm^{-3} Eu(III)
d	solution (b) + $1\cdot10^{-4}$ mol·dm^{-3} Eu(III)
e	solution (a) + $2\cdot10^{-4}$ mol·dm^{-3} Eu(III)
f	solution (b) + $2\cdot10^{-4}$ mol·dm^{-3} Eu(III)
g	$4\cdot10^{-4}$ mol·dm^{-3} HNO_3 + $1\cdot10^{-4}$ mol·dm^{-3} anthracene-9-carboxylic acid + $1\cdot10^{-4}$ mol·dm^{-3} nucleobases + $1\cdot10^{-4}$ mol·dm^{-3} Eu(III)
h	$4\cdot10^{-4}$ mol·dm^{-3} HNO_3 + $1\cdot10^{-4}$ mol·dm^{-3} anthracene-9-carboxylic acid + $1\cdot10^{-4}$ mol·dm^{-3} nucleobases + $2\cdot10^{-4}$ mol·dm^{-3} Eu(III)

* Corresponding author. E-mail: azab2@yahoo.com.
† Suez Canal University, Ismailia 41522, Egypt.
‡ Suez Canal University, Suez, Egypt.

10.1021/je900149x CCC: $40.75
Published on Web 07/09/2009

Chart 1. Predominant Tautomeric Structures of the Purine and Pyrimidine Bases Used in This Study



Anthracene-9-carboxylic acid (ANCA) has been reported to show both potentiation and inhibitory effects on guinea-pig cardiac cAMP-activated chloride channels via two different binding sites, and inhibition of Mg^{2+}-sensitive proton phosphates

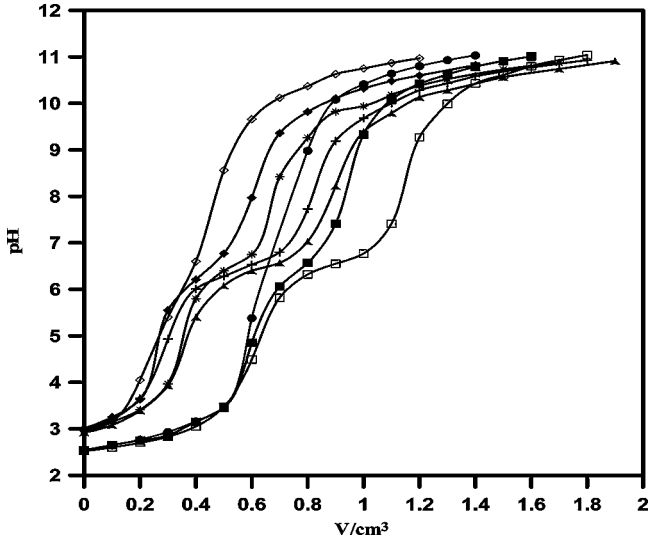


Figure 1. pH against volume of 0.033 mol·dm^{-3} KOH for the Eu(III)−ANCA−guanine system in 10 % (v/v) ethanol−water mixture at 0.1 mol·dm^{-3} KNO_3 and at 25 °C: ●, $4\cdot10^{-4}$ mol·dm^{-3} HNO_3 + $1\cdot10^{-4}$ mol·dm^{-3} ANCA; ◇, $4\cdot10^{-4}$ mol·dm^{-3} HNO_3 + $1\cdot10^{-4}$ mol·dm^{-3} guanine; ■, $4\cdot10^{-4}$ mol·dm^{-3} HNO_3 + $1\cdot10^{-4}$ mol·dm^{-3} ANCA + $1\cdot10^{-4}$ mol·dm^{-3} Eu(III); ◆, $4\cdot10^{-4}$ mol·dm^{-3} HNO_3 + $1\cdot10^{-4}$ mol·dm^{-3} guanine + $1\cdot10^{-4}$ mol·dm^{-3} Eu(III); □, $4\cdot10^{-4}$ mol·dm^{-3} HNO_3 + $1\cdot10^{-4}$ mol·dm^{-3} ANCA + $2\cdot10^{-4}$ mol·dm^{-3} Eu(III); +, $4\cdot10^{-4}$ mol·dm^{-3} HNO_3 + $1\cdot10^{-4}$ mol·dm^{-3} guanine + $2\cdot10^{-4}$ mol·dm^{-3} Eu(III); *, $4\cdot10^{-4}$ mol·dm^{-3} HNO_3 + $1\cdot10^{-4}$ mol·dm^{-3} ANCA + $1\cdot10^{-4}$ mol·dm^{-3} guanine + $1\cdot10^{-4}$ mol·dm^{-3} Eu(III); ▲, $4\cdot10^{-4}$ mol·dm^{-3} HNO_3 + $1\cdot10^{-4}$ mol·dm^{-3} ANCA + $1\cdot10^{-4}$ mol·dm^{-3} guanine + $2\cdot10^{-4}$ mol·dm^{-3} Eu(III).

Table 2. Formation Constants for the Binary Eu(III) + Nucleobase (NB) and Anthracene-9-carboxylic acid (ANCA) Complexes and Those for the Mixed Complexes of Eu(III) + NB + ANCA at (25.0 ± 0.1) °C and Ionic Strength I = 0.1 mol·dm^{-3} KNO_3 in 10 % (v/v) Ethanol−Water Mixture Solvent

ligand	log $K_{Eu(III)(NB)}$ and log $K_{Eu(III)(ANCA)}$	log $K_{Eu(III)(NB)(HANCA)}$
guanine	3.40 ± 0.02	14.32[a] ± 0.03
thymine	6.77 ± 0.02	15.72[a] ± 0.02
uracil	3.43 ± 0.02	14.20[a] ± 0.02
adenine	4.42 ± 0.01	14.29[a] ± 0.03
ANCA	4.71 ± 0.02	

[a] Log formation constants of the protonated ternary complex.

Table 3. Formation Constants for the Dimeric Binary Eu(III) + Nucleobase (NB) and Anthracene-9-carboxylic Acid (ANCA) Complexes and Those for the Dimeric Ternary Complexes of Eu(III) + NB + ANCA at (25.0 ± 0.1) °C and Ionic Strength I = 0.1 mol·dm^{-3} KNO_3 in 10 % (v/v) Ethanol-Water Mixture Solvent

ligand	log $K_{Eu(III)_2(NB)}$ and log $K_{Eu(III)_2(ANCA)}$	log $K_{Eu(III)_2(NB)(HANCA)}$
guanine	4.27 ± 0.02	4.26 ± 0.02
thymine	4.60 ± 0.02	4.31 ± 0.02
uracil	4.27 ± 0.01	4.26 ± 0.01
adenine	4.31 ± 0.01	4.44 ± 0.02
ANCA	4.32 ± 0.01	

has been proposed for the mechanism of the ANCA potentiation effect. The effect of ANCA on wild-type and mutant human cystic fibrosis transmembrane conductance regulator (CFTR) chloride channels expressed in NIH3T3 or CHO cells is examined. ANCA inhibits whole-cell CFTR current in a voltage-dependent manner, whereas the potentiation effects are not affected by membrane potentials. Anthracene 9-methanol, an electroneutral ANCA analogue, fails to block CFTR but shows a nearly identical potentiation effect, corroborating the idea that two chemically distinct sites are responsible, respectively, for potentiation and inhibitory actions of ANCA. In excised inside-out patches, ANCA increases channel open probability (P_o) by prolonging the mean burst durations and shortening the inter-burst durations.[23] This prompted us to investigate the ternary systems of the type Eu(III)−anthracene-9-carboxylic acid−nucleobases. These systems can be considered as models for the development of fluorescent probes for DNA fragments, containing the nucleobases under investigation.

Experimental Section

Material and Solutions. All materials employed in the present investigation were of A. R. grade. [6-Amino purine] $C_5H_5N_5$ (Adenine), [2-amino-6-oxypurine] $C_5H_5N_5O$ (Guanine), [5-methyl pyrimidine] $C_5H_6N_2O_2$ (Thymine), [2,4-dioxypyrimidine] $C_4H_4N_2O_2$ (Uracil), and anthracene-9-carboxylic acid were purchased from the Sigma Chemical Co. To avoid hydrolysis prior to the potentiometric measurements, a known mass of a chromatographically pure sample of nucleobase as a solid was added to the reaction vessel just prior to performing the titration. $Eu(NO_3)_3\cdot5H_2O$ was from the Sigma Chemical Co. The concentrations of the metal ion stock solutions were determined complexometrically by ethylenediamine tetracetic acid dissodium salt (EDTA) using suitable indicators.[17]

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(hydroxyl methyl) amino methane. 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 electroanalytical measurements, the ionic strength of the examined solutions was adjusted to 0.1 mol·dm^{-3} using an alcoholic solution of *p*-toluenesulfonate. This supporting electrolyte was purchased from Merck AG.

Apparatus and Procedure. The value of the EMF 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 with 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

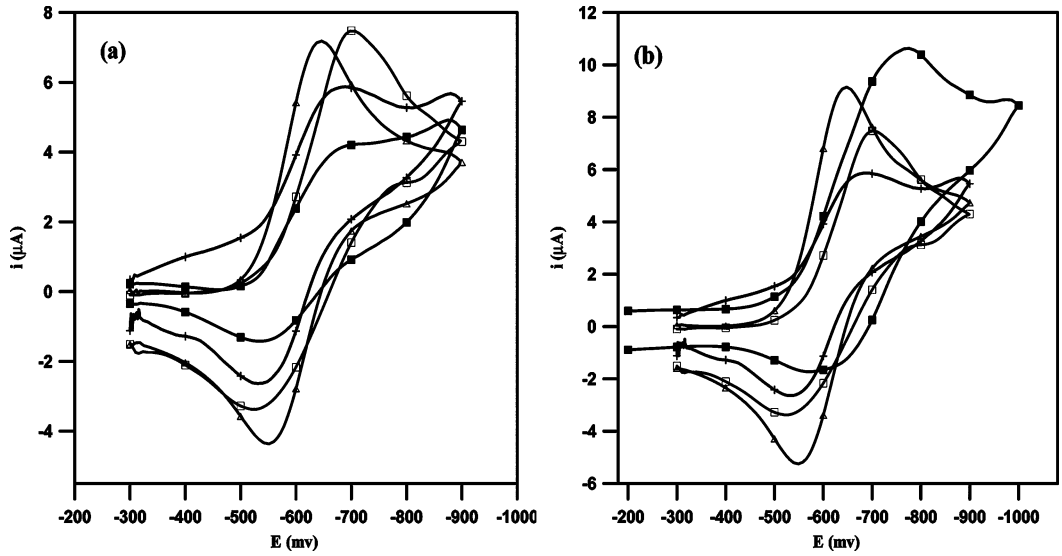


Figure 2. Cyclic voltammograms for the Eu(III) + ANCA + NB system in 10 % (v/v) ethanol−water mixture at 0.1 mol·dm^{-3} *p*-toluenesulfonate, scan rate = 100 mV·s^{-1} and at 25.0 °C: +, 1·10^{-3} mol·dm^{-3} Eu(III); □, 1·10^{-3} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} ANCA; ■, 1·10^{-3} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} NB; Δ, 1·10^{-3} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} ANCA + 5·10^{-4} mol·dm^{-3} NB. (a) NB = uracil and (b) NB = adenine.

± 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 mol·dm^{-3} KNO_3, in a 10 % ethanol−water mixture. The concentration of the hydrogen ion was decreased by the addition of potassium hydroxide, prepared in the ionic medium used for the solution.

The value for the K_w of water in the 10 % ethanol−water mixture has been taken from the literature.[12]

Gran's method[24] was used to determine $E^{o\prime}$ and E_j so that the hydrogen ion concentration, h, could be found from E, the measured potential, by means of

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

Values of the ionic product of the different hydroorganic media were refined using the MAGEC program.[25] The protonation constants were then determined by use of the Bjerrum function.[19]

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

which is calculated from the experimental quantities, h, the total concentration of titratable hydrogen ion H_T, and the total reagent concentration A_T. pK_a values of the investigated ligands were determined in the 10 % ethanol−water mixture from the overall protonation constants β_1 and β_2 calculated by the linearization method of Irving and Rossotti.[21]

Initial estimates of the pK_a values were refined with the ESAB2 M computer program.[26]

A detailed description of the solution composition used in the determination of the stability constants of complex species is shown in Table 1.

A constant ionic strength was obtained with 0.1 mol·dm^{-3} KNO_3, and the total volume was kept at 25.0 cm^3 in the 10 % ethanol−water mixture solvent in all titrations.

For both ligand protonation and metal complex formation equilibria, data were collected over the largest possible pH interval, although a number of experimental points were frequently discarded for the final stability constant calculations, especially within the range where the complexation observed was insignificant.

Initial estimates of the formation constants of the ternary complexes and the stability constants of binary 1:1 complexes have been refined using the SUPERQUAD computer program.[10] During this refinement, the stepwise stability constant

$$K_{M(ANCA)(NB)} = \frac{[M_p(ANCA)_q(NB)_r]}{[M_p(ANCA)_q][NB]^r} \qquad (3)$$

which refers to the addition of NB to the binary complex $M_p(ANCA)_q$ is determined.

The overall complexation reaction involving protonation is

$$pM + qANCA + rNB + sH \leftrightarrow M_p(ANCA)_q(NB)_r(H)_s \qquad (4)$$

$$\beta_{pqrs} \frac{M_p(ANCA)_q(NB)_r(H)_s}{[M]^p[ANCA]^q[NB]^r[H]^s} \qquad (5)$$

in which ANCA = anthracene-9-carboxylic acid, NB = nucleobase ligand (guanine, thymine, adenine, and uracil), and M = Eu(III). All side reactions due to metal ion hydrolysis have been included in the calculations.[18,27,28]

Electrochemical Measurements. Cyclic voltammetry (CV), square wave voltammetry (SWV), 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 a Ag\AgCl electrode as the reference electrode. In a typical experiment, a sample volume of 25 cm^3 containing

(a) 5·10^{-4} mol·dm^{-3} Eu(III);

(b) 1·10^{-3} mol·dm^{-3} Eu(III);

(c) 5·10^{-4} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} 9-anthracene-carboxylic acid;

(d) 5·10^{-4} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} nucleobase (adenine, guanine, thymine, and uracil);

(e) 1·10^{-3} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} 9-anthracene-carboxylic acid;

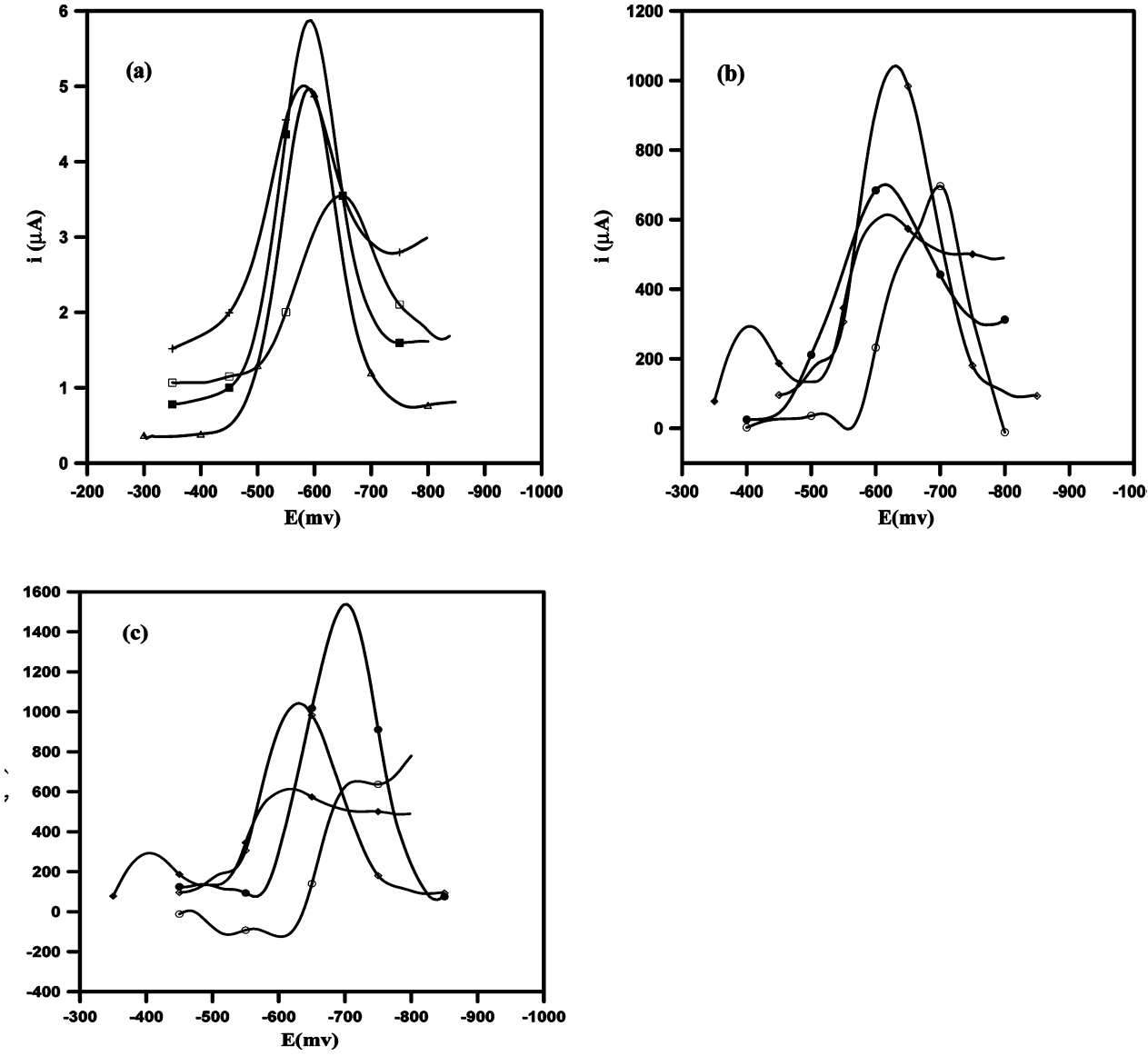


Figure 3. Differential pulse polarograms for the Eu(III) + ANCA + NB system in 10 % (v/v) ethanol−water mixture at 0.1 mol·dm^{-3} *p*-toluenesulfonate, scan rate = 25 mV·s^{-1} and at 25.0 °C: ◇, 5·10^{-4} mol·dm^{-3} Eu(III); ◆, 5·10^{-4} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} ANCA; ○, 5·10^{-4} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} NB; ●, 5·10^{-4} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} ANCA + 5·10^{-4} mol·dm^{-3} NB; +, 1·10^{-3} mol·dm^{-3} Eu(III); □, 1·10^{-3} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} ANCA; ■, 1·10^{-3} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} NB; Δ, 1·10^{-3} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} ANCA + 5·10^{-4} mol·dm^{-3} NB. (a) NB = uracil, (b) NB = adenine, and (c) NB = guanine.

(f) 1·10^{-3} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} nucleobase (adenine, guanine, thymine, and uracil);

(g) 5·10^{-4} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} 9-anthracene-carboxylic acid + 5·10^{-4} mol·dm^{-3} nucleobase (adenine, guanine, thymine, and uracil); or

(h) 1·10^{-3} mol·dm^{-3} Eu(III) + 5·10^{-4} mol·dm^{-3} 9-anthracene-carboxylic acid + 5·10^{-4} mol·dm^{-3} nucleobase (adenine, guanine, thymine, and uracil)

were used.

The ionic strength of the studied solutions was adjusted to 0.1 mol·dm^{-3} using *para*-toluenesulfonate solution.

Cyclic Voltammetry. The solution was purged with nitrogen for 120 s, and then the potential was scanned at a scan rate of 100 mV·s^{-1} from −(0.30 to 0.90) V.

Square Wave Voltammetry. The samples were analyzed as in cyclic voltammetry; the pulse height was 25 mV; the SW frequency was f = 20 Hz; and the scan increment was 2.0 mV.

Differential Pulse Voltammetry. The samples were analyzed also as in cyclic voltammetry, but at a scan rate = 36.6 mV·s^{-1}. The pulse height was 25 mV; the pulse width = 50 s; frequency was 20 Hz; and the scan increment was 2.0 mV.

Spectrophotometric Measurements. The ultraviolet (UV) spectra of the solutions in the 10 % ethanol−water mixture of the binary and ternary complexes were scanned on a Shimadzu-1601PC UV−visible automatic recording spectrophotometer with a 1 cm quartz cell for absorbance and spectral measurements. The required volume of the stock metal ion salt is mixed with that of the ligand solution, keeping the total concentration of each to be 1·10^{-4} mol·dm^{-3}. The ionic strength of each solution was adjusted to 0.1 mol·dm^{-3} KNO_3 in the 10 % ethanol−water mixture. All the studied solutions were diluted with bidistilled water, after pH adjustment to the required value using diluted solutions of either HNO_3 or KOH. The binary complex solutions at a 1:1 ratio were scanned against the 10 % ethanol−water mixture as a blank in a 1 cm quartz cell.

The ternary complex solutions were prepared in a 1:1:1 ratio keeping the concentration of each species at 1·10^{-4} mol·dm^{-3} and at 0.1 mol·dm^{-3} KNO_3 in the 10 % ethanol−water mixture

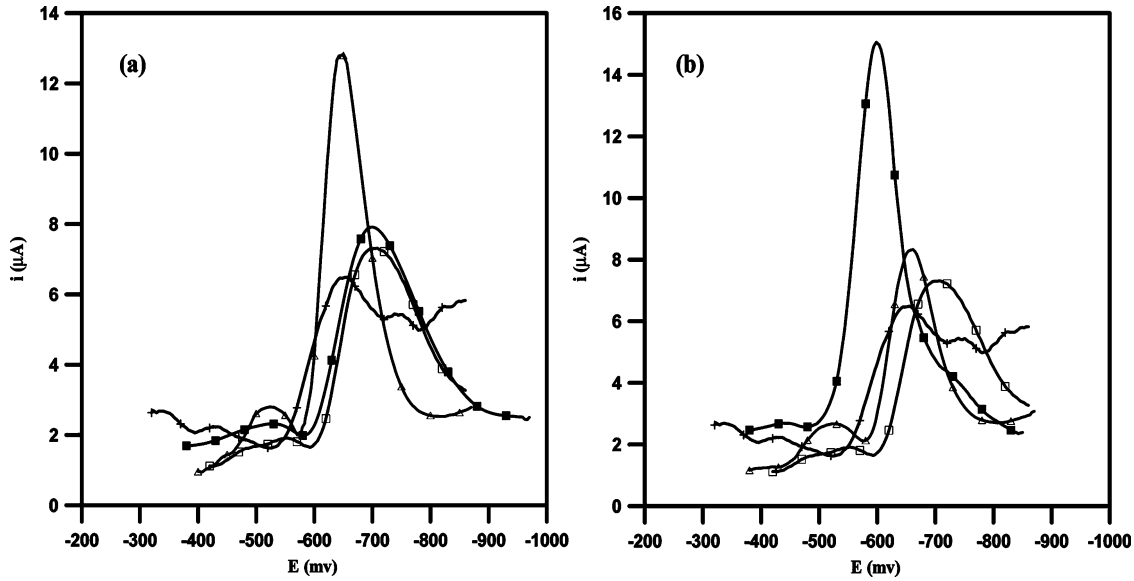


Figure 4. Square wave voltammograms for the Eu(III) + ANCA + NB system in 10 % (v/v) ethanol−water mixture at 0.1 mol·dm^{-3} *p*-toluenesulfonate, frequency = 60 Hz and at 25.0 °C: +, $1\cdot10^{-3}$ mol·dm^{-3} Eu(III); □, $1\cdot10^{-3}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} ANCA; ■, $1\cdot10^{-3}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} NB; Δ, $1\cdot10^{-3}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} ANCA + $5\cdot10^{-4}$ mol·dm^{-3} NB. (a) NB = thymine, (b) NB = uracil.

Table 4. Voltammetric Data, Transfer Coefficient (α), and Heterogeneous Rate Constant (k_o) from Cyclic Voltammetry for Eu(III) and Its Binary and Ternary Complexes with Nucleobases and Anthracene-9-carboxylic Acid in 1:1:1 and 2:1:1 Ratio, I = 0.1 mol·dm^{-3} *p*-Toluenesulfonate in 10 % (v/v) Ethanol−Water Mixture at 25 °C

system	$-E_{p,c}$ (mV)	$-E_{p,a}$ (mV)	$-(E_{p,c} - E_{p,a})$ (mV)	$i_{p,c}$ (μA)	$-i_{p,c}$ (μA)	$-E_p/2$ (mV)	$-E^o$ (mV)	α	K_o (s^{-1})
$1\cdot10^{-3}$ mol·dm^{-3} Eu(III)	679	533	146	5.85	2.64	598	61	0.54	1.17
$5\cdot10^{-4}$ mol·dm^{-3} Eu(III)	658	521	164	2.54	0.72	604	60	0.28	0.074
$1\cdot10^{-3}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} ANCA	697	524	173	7.50	3.32	626	610	0.67	1.19
$5\cdot10^{-4}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} ANCA	700	588	112	3.70	2.18	614	640	0.55	0.51
$1\cdot10^{-3}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} adenine	773	591	182	10.60	1.70	632	680	0.34	0.24
$1\cdot10^{-3}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} uracil	676	543	154	4.18	1.43	594	610	0.56	0.36
$5\cdot10^{-4}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} uracil	679	570	109	2.60	0.62	592	620	0.55	0.109
$1\cdot10^{-3}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} thymine	785	597	188	10.18	0.73	651	700	0.36	0.618
$5\cdot10^{-4}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} thymine	743	557	186	3.36	2.21	645	650	0.49	0.57
$1\cdot10^{-3}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} ANCA + $5\cdot10^{-4}$ mol·dm^{-3} adenine	642	552	90	9.20	5.27	578	600	0.75	1.06
$5\cdot10^{-4}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3}ANCA + $5\cdot10^{-4}$ mol·dm^{-3} adenine	652	546	106	4.33	2.18	584	600	0.70	0.693
$1\cdot10^{-3}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3}ANCA + $5\cdot10^{-4}$ mol·dm^{-3} uracil	639	548	91	7.15	4.42	578	590	0.78	0.649
$5\cdot10^{-4}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} ANCA + $5\cdot10^{-4}$ mol·dm^{-3} uracil	646	552	94	4.21	1.55	571	600	0.65	0.076
$1\cdot10^{-3}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} ANCA + $5\cdot10^{-4}$ mol·dm^{-3} thymine	652	548	104	8.21	5.73	584	600	0.70	3.88
$5\cdot10^{-4}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} ANCA + $5\cdot10^{-4}$ mol·dm^{-3} thymine	643	561	82	3.64	1.52	576	600	0.71	0.157
$1\cdot10^{-3}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3}ANCA + $5\cdot10^{-4}$ mol·dm^{-3}guanine	649	558	91	4.56	2.56	578	600	0.67	0.214
$5\cdot10^{-4}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} ANCA + $5\cdot10^{-4}$ mol·dm^{-3} + $5\cdot10^{-4}$ mol·dm^{-3} guanine	758	655	103	4.14	2.22	677	710	0.59	0.671

solvent. The desired pH value is maintained using HNO_3 or KOH diluted solutions. Each ternary complex solution was scanned against the binary complex containing the metal ion and the primary ligand corresponding to each system.

Spectrofluorometric Measurements. The emission spectra of the solutions in the 10 % ethanol−water mixture of the binary and ternary complexes were scanned on a JASCO-FP6300 spectrofluorometer with a 1 cm quartz cell. The required volume of the stock metal ion salt is mixed with that of the ligand solution, keeping the total concentration of each at $1\cdot10^{-5}$ mol·dm^{-3}. All the studied solutions were diluted with bidistilled water, after pH adjustment to pH 7 to 8 using diluted solutions of either HNO_3 or KOH. The ternary complex solutions were prepared in a 1:1:1 ratio keeping the concentration of each species at $1\cdot10^{-5}$ mol·dm^{-3} in the 10 % ethanol−water mixture solvent. The desired pH value is maintained using HNO_3 or KOH diluted solutions.

Results and Discussion

The acid formation constant values for guanine, thymine, uracil, adenine, anthracene-9-carboxylic acid, and the stability constants of their Eu(III) complexes were determined from the titration curves and were in good agreement with those found in the literature.[29]

Chart 1 shows the predominant tautomeric structures of the purine and pyrimidine bases used in this study.

The proton binding sites of the purine nucleobases have been established (N3 for cytosine and uracil; protonation of N between the keto groups in thymine and between N1 and N7

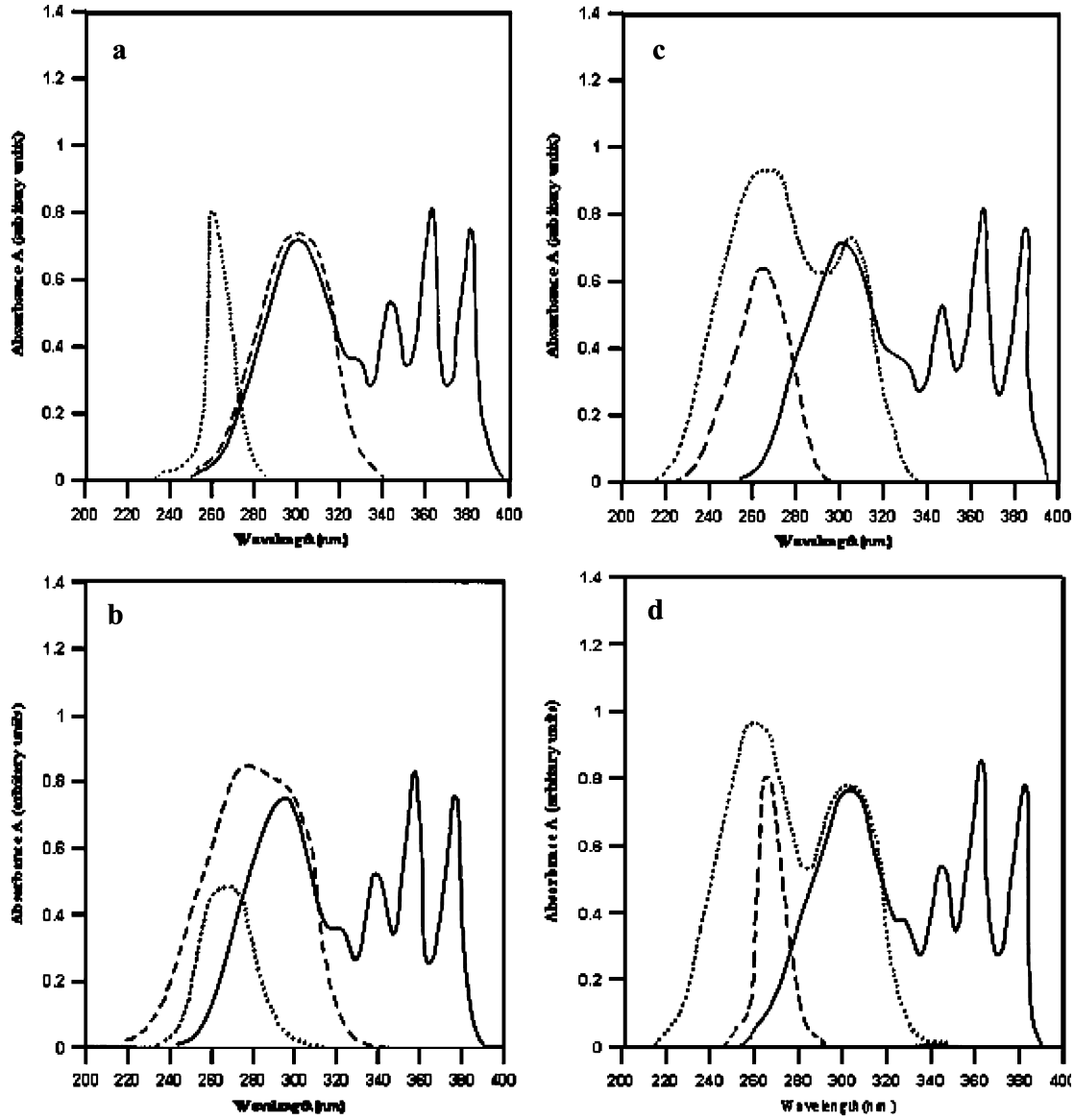


Figure 5. Absorption spectra for the Eu(III) + anthracene-9-carboxylic acid + nucleobase system in 10 % (v/v) ethanol−water mixture at 25 °C and at 0.1 mol·dm^{-3} KNO_3: —, 1·10^{-4} mol·dm^{-3} Eu(III) + 1·10^{-4} mol·dm^{-3} ANCA; - - - -, 1·10^{-4} mol·dm^{-3} Eu(III) + 1·10^{-4} mol·dm^{-3} NB; ····, 1·10^{-4} mol·dm^{-3} Eu(III) + 1·10^{-4} mol·dm^{-3} ANCA + 1·10^{-4} mol·dm^{-3} NB. (a) NB = adenine, (b) NB = guanine, (c) NB = thymine, and (d) NB = uracil.

Table 5. Absorption Spectra Characteristics for Eu(III) + Anthracene-9-carboxylic Acid + Uracil Systems at (25.0 ± 0.1) °C in 10 % (v/v) Ethanol−Water Mixture Solvent and I = 0.1 mol·dm^{-3} KNO_3

	1		2		3		4		5	
system	λ (nm)	$\varepsilon\cdot10^2$	λ (nm)	$\varepsilon\cdot10^2$	λ (nm)	$\varepsilon\cdot10^2$	λ (nm)	$\varepsilon\cdot10^2$	λ (nm)	$\varepsilon\cdot10^2$
1·10^{-4} mol·dm^{-3} Eu(III)	301.8	7.78	–	–	–	–	–	–	–	–
2·10^{-4} mol·dm^{-3} Eu(III)	301.6	3.96	–	–	–	–	–	–	–	–
1·10^{-4} mol·dm^{-3} Eu(III) + 1·10^{-4} mol·dm^{-3} ANCA	384.2	7.85	364.2	8.49	346.6	5.58	302.2	7.58	–	–
2·10^{-4} mol·dm^{-3} Eu(III) + 1·10^{-4} mol·dm^{-3} ANCA	384.2	4.75	364.2	5.14	346.4	3.37	328.4	2.17	301.8	4.03
1·10^{-4} mol·dm^{-3} Eu(III) + 1·10^{-4} mol·dm^{-3} uracil	301.6	7.53	–	–	–	–	–	–	–	–
2·10^{-4} mol·dm^{-3} Eu(III) + 1·10^{-4} mol·dm^{-3} uracil	301.6	3.79	–	–	–	–	–	–	–	–
1·10^{-4} mol·dm^{-3} Eu(III) + 1·10^{-4} mol·dm^{-3} ANCA + 1·10^{-4} mol·dm^{-3} uracil	260.6	7.58	–	–	–	–	–	–	–	–
2·10^{-4} mol·dm^{-3} Eu(III) + 1·10^{-4} mol·dm^{-3} ANCA + 1·10^{-4} mol·dm^{-3} uracil	265.2	2.70	–	–	–	–	–	–	–	–

for guanine and adenine). The N1 site in the six-membered ring is at least 100 times more basic than the N7 site in the five-membered ring. While both N1 and N7 in purine bases bind metal ions, they exhibit different features.

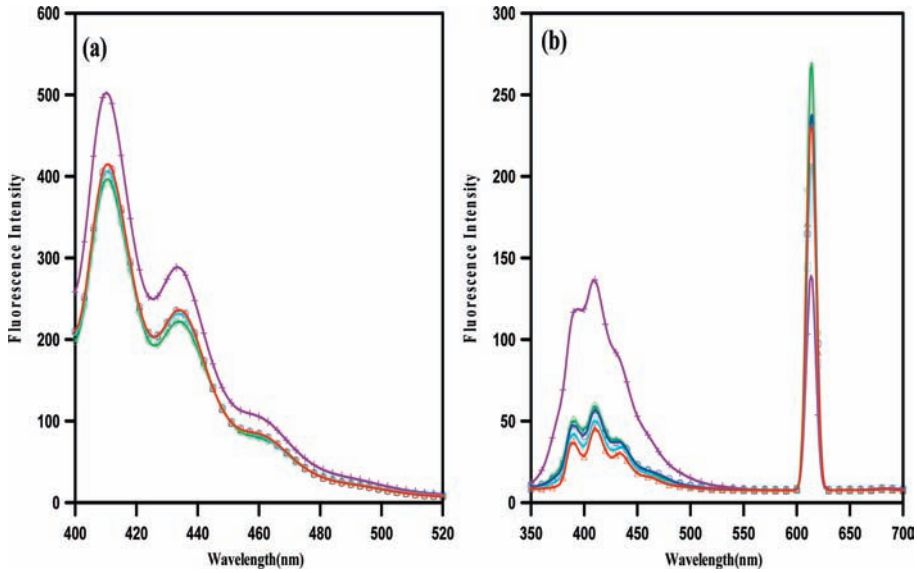


Figure 6. Fluorescence spectra for the Eu(III) + anthracene-9-carboxylic acid + nucleobase system in 10 % (v/v) ethanol−water mixture at 25 °C: +, $1 \cdot 10^{-5}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-5}$ mol·dm^{-3} ANCA; ◇, $1 \cdot 10^{-5}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-5}$ mol·dm^{-3} ANCA + $1 \cdot 10^{-5}$ mol·dm^{-3} uracil; □, $1 \cdot 10^{-5}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-5}$ mol·dm^{-3} ANCA + $1 \cdot 10^{-5}$ mol·dm^{-3} adenine; ○, $1 \cdot 10^{-5}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-5}$ mol·dm^{-3} ANCA + $1 \cdot 10^{-5}$ mol. dm^{-3} guanine; Δ, $1 \cdot 10^{-5}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-5}$ mol·dm^{-3} ANCA + $1 \cdot 10^{-5}$ mol·dm^{-3} thymine. (a) $\lambda_{ex} = 385$ nm, (b) $\lambda_{ex} = 308$ nm.

Table 6. Fluorescence Spectral Features of Eu(III) + Anthracene-9-carboxylic Acid + Nucleobase Systems in 10 % (v/v) Ethanol−Water Mixture Solvent and at 25 °C

system	intensity at λ_{ex} (385)	intensity at λ_{ex} (308)
$1 \cdot 10^{-5}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-5}$ mol·dm^{-3} ANCA	502.71	139.30
$1 \cdot 10^{-5}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-5}$ mol·dm^{-3} ANCA + $1 \cdot 10^{-5}$ mol·dm^{-3} uracil	396.16	269.48
$1 \cdot 10^{-5}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-5}$ mol·dm^{-3} ANCA + $1 \cdot 10^{-5}$ mol·dm^{-3} adenine	397.29	208.65
$1 \cdot 10^{-5}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-5}$ mol·dm^{-3} ANCA + $1 \cdot 10^{-5}$ mol·dm^{-3} guanine	406.08	238.47
$1 \cdot 10^{-5}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-5}$ mol·dm^{-3} ANCA + $1 \cdot 10^{-5}$ mol·dm^{-3} thymine	414.74	230.56

The amino ($-NH_2$) groups are located at C(6) in adenine and C(2) in guanine. These primary amino groups have been proposed many times as metal ion binding sites. However, these amino groups are not basic. The flat $-NH_2$ group is nearly coplanar with the ring, and the amino nitrogen to carbon bond length is about 0.06 Å shorter in the nucleobases than in aniline. This combination of properties indicates appreciable double bond and extensive delocalization of electron density into the ring. Consistent with their appreciable positive charge density, the primary amino group serves as hydrogen bond donors in specific base pairing. Moreover, in strongly acidifying solvents, even the divalent cations of cytosine and the trivalent cations of adenine and guanine still have not undergone amino group protonation as the other nitrogens and O(2) in cytosine and O(6) in guanine protonate before the amino group.

In Figure 1, a representative set of the experimental titration curves obtained according to the sequence described in the Experimental Section are displayed for the system Eu(III)−guanine−ANCA. Generally, the complex titration curves show an inflection after addition of 2 mol of base per mole of guanine nucleobase. This indicates the stoichiometry of the final product formed in this buffer region.

At the experimental pH values used in the calculations in this work, the interfering effects of hydroxyl complexes are negligible for Eu(III). Thus, the protonated secondary ligand combines with the binary 1:1 Eu(III)−nucleobase complexes [Eu(III)−guanine, Eu(III)−thymine, Eu(III)−uracil, Eu(III)−adenine]. Thus, the initial estimation of the stability constants of the protonated ternary complexes formed in solution has been determined using the half point neutralization. Initial estimates of the stability constants of different normal and protonated binary and ternary complexes formed in solution have been refined with the SUPERQUAD computer program.[10] The quality of the fit during this refinement was judged by the values of the sample standard deviations and the goodness of fit χ^2 (Pearson's Test). At $\sigma_E = 0.1$ mV (0.001 pH error) and $\sigma_V = 0.005$ mL, the values of S in different sets of titrations were between 1.0 and 1.7 and χ^2 was between 12.0 and 13.0. The scatter of residuals ($E_{obs} - E_{calc}$) versus pH is reasonably random, without any significant systematic trends, thus indicating a good fit of the experimental data of the expected model systems under our experimental conditions.

Examination of the different formation constant values listed in Tables 2 and 3 reveals that the order of stability constants of different ternary complexes in the systems Eu(III)−NB + ANCA in terms of NB follows generally the trend thymine > guanine > adenine > uracil.

The observed trend for the overall formation constant of the different ternary complexes formed in this study in terms of nucleobases may be attributed to the nature of the interaction of the ANCA−Eu(III) complex with the different nucleobases. At neutral pH, the affinity of the metal ion Eu(III) binding of the sites available in the nucleobases in single-stranded nucleic acids decreases along the series[30] N7G > N3C > N7A > N1A > N3A > N3G.

As stated previously,[31] most ions favor mixed oxygen and nitrogen doners. Although N1 is more basic than N7, it was confirmed that macrochelate formation with N1 of the purine moiety is not possible for steric reasons. This behavior may account for the nature of interaction during the formation of Eu(III)−ANCA−thymine, Eu(III)−ANCA−guanine, or Eu(III)−ANCA−adenine, which may include dimeric Eu(III) complexes, while those including uracil may contain macrochelate formation with a lower stability constant.

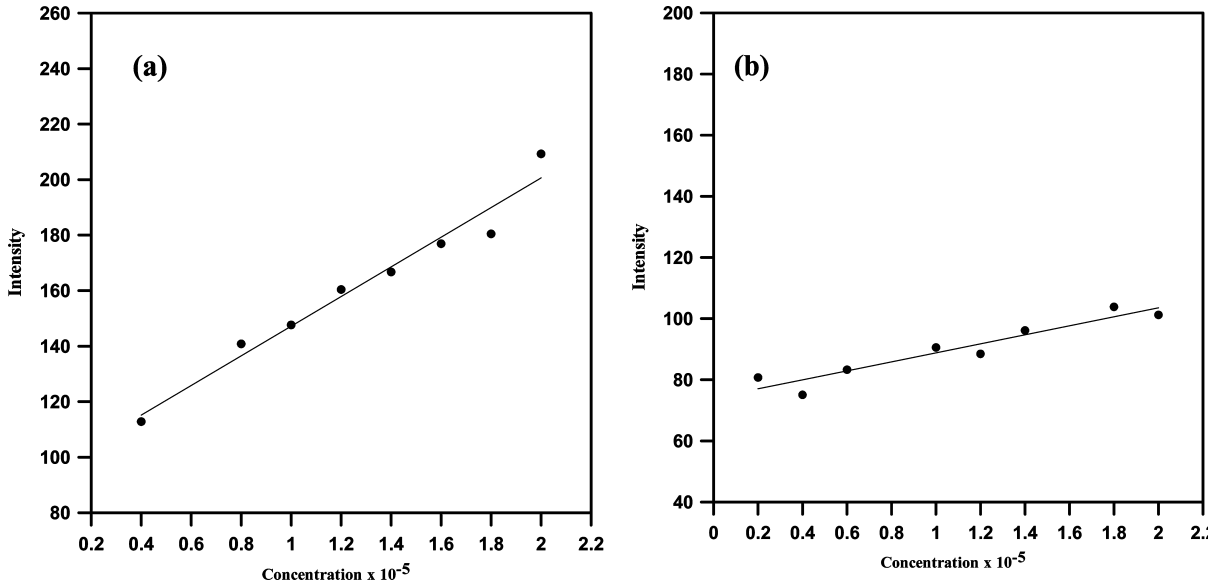


Figure 7. Effect of concentration of nucleobases on the fluorescence intensity of the $1 \cdot 10^{-5}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-5}$ mol·dm^{-3} anthracene-9-carboxylic acid system in 10 % (v/v) ethanol−water mixture at 25 °C. (a) = guanine, (b) = thymine.

To the author's knowledge, no data for the ternary complex of the secondary ligand anthracene-9-carboxylic acid with the nucleobases guanine, uracil, thymine, and adenine are available in the literature for comparison.

The higher values for the stability constants of ternary complexes compared with those of the binary systems may be attributed to the interligand interactions or some cooperativity between the coordinated ligands, possibly H-bond formation. This also may be explained on the basis of the π-electron-donating tendency of the Eu(III) ion to the antibonding π^* orbital of the heteroaromatic N base, such as the adenine base, causing strengthening of the Eu(III)−N bond. Due to the back-donation from metal to adenine base the f electron content on the metal decreases, which renders the metal more electrophilic. The interaction of the π electrons of the secondary ligands with the metal will increase to a greater extent and consequently enhance the formation of the mixed ligand complexes.

The design of molecular systems that combine binding abilities and useful photophysical properties for the construction of efficient photoluminescent Eu(III) complexes continues to be an active area of research. The long lifetimes of the exited states of Eu(III) ions and their distinct narrow emission bands ranging from UV−visible to the near-infrared region are ideally suited for application as fluorescent probes[32,33] and as optical signal amplifiers.[34] The long luminescent lifetimes of Eu(III) ions are due to the forbidden character of their intra 4f transitions, which unfortunately also result in low absorption coefficients (typically 1 to 10 $M^{-1} \cdot cm^{-1}$).[35] For this reason, the excited state of a luminescent Eu(III) ion is generally populated by energy transfer from the triplet state of an organic antenna chromophore (the sensitizer).[36] The antenna chromophore in our study is anthracene-9-carboxylic acid. The quantum yield of the overall process, which involves the excitation of the antenna chromophore and intersystem crossing to the triplet state, energy transfer to the lanthanide ion, and subsequent lanthanide emission, depends on the efficiency of these individual steps: the intersystem crossing efficiency, the energy transfer efficiency, and the lanthanide(III) luminescence quantum yield. Eu(III) is one of the best studied lanthanide ions, and research of the sensitization process of this ion has resulted in a better understanding of the sensitization process of other lanthanide ions, in particular, the near-infrared emitting lanthanide ions.[37,38]

Confirmation of the formation of ternary complexes of the type Eu(III)−nucleobase−anthracene-9-carboxylic acid in solution has been carried out using differential pulse polarography (DPP), cyclic voltammetry (CV), and square wave voltammetry (SWV). Figures 2 to 4 show the electrochemical behavior of some representative systems.

All the voltammetric diagrams confirm the formation of different binary and ternary complexes that have been found using potentiometric titrations.

The differential pulse polarography of Eu(III) solution shows a cathodic peak $E_p = 0.406$ V·s^{-1} at scan rate = 5 mV·s^{-1}. This peak may be described as a result of the reduction of Eu(III) to Eu (II) in a one electron transfer process at the glassy carbon electrode. The addition of primary ligand (nucleobases) caused a shift of the cathodic peak to a more negative potential indicating the formation of the binary complexes in solution.

SWV is an effective and rapid electroanalytical technique with well-established advantages, including good discrimination against double-layer charging current and low concentration detection limits. To eliminate the possibility of the electrochemical interference from the electroreduction of the primary ligand nucleobases during the investigation of the voltammetric behavior of the ternary systems in solutions, the cyclic voltammetry of both ligands has been carried out in the same potential windows.

The reversibility of the electrochemical reaction for the ternary systems under investigation has been confirmed by CV studies. The peak separation between the anodic and cathodic peaks is more than 30 mV. These values indicate that the electrochemical reduction in the case of free Eu(III) ions, Eu(III) binary, or Eu(III) ternary complexes under investigation is quasi-reversible at the glassy carbon electrode.

It is quite interesting to observe that changing the frequency from (30 to 60) Hz resulted in a quite clear change in the shape of the SWV of the ternary complex formed in solution, which may be attributed to changing the mechanistic behavior of the electrochemical reduction of the resulting ternary complex at the glassy carbon electrode. The different electrochemical

characteristics and some kinetic parameters of the systems under investigation are given in Table 4.

The CV response for the binary and ternary complexes of the type Eu(III)−NB−ANCA on the glassy carbon electrode reveal a one electron reduction process with the following electrochemical features in the scan rate (υ) range of (150 to 300) $mV \cdot s^{-1}$: $i_{p,a}/i_{p,c}$ ratio decreases by increasing scan rate υ; ($\Delta E_p = E_{p,a} - E_{p,c}$) increases by increasing υ with slopes in the range (0.459) mV for $E_{p,c}$ (i_p vs $\upsilon^{1/2}$ plots), which agree very well with the theory for a typical quasi-reversible process. According to this theory, the kinetic parameter Ψ varies linearly with $\upsilon^{1/2}$, and ΔE_p approaches linearity for small Ψ. Consequently, in this zone ΔE_p should vary linearly with $\upsilon^{1/2}$.

The kinetics of the electrode reaction at the glassy carbon electrode can be studied under our condition of quasi-reversibility for the electrochemical reduction of the binary and ternary systems under investigation.

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[39] for obtaining the transfer parameter, Ψ, and then the standard heterogeneous charge-transfer rate constant (K^o) for the electron-transfer process at the glassy carbon electrode according to the following equation

$$\Psi = \frac{(D_o/D_R)^{a/2} K^o}{D_o \pi \nu (nF/RT)^{1/2}} \quad (6)$$

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

$$i_p = (2.69 \cdot 10^5) n^{3/2} A D_o^{1/2} \upsilon^{1/2} C_o \quad (7)$$

where ν is the scan rate and A is the surface area of the electrode. To convert experimentally determined values of Ψ to K^o, good agreement between the values of the kinetic parameters calculated using this method and those calculated by the Butler equation is obtained.[40]

Absorption spectra of some investigated complexes of Eu(III) binary and ternary complexes were measured in the range (200 to 400) nm (see Figure 5). Deprotonation and coordination of the carboxylic acid group of ANCA resulted in the complex formation reactions with investigated Eu(III) ions. This binary and ternary complex formation has been confirmed by a small shift of the band due to the free metal ions in solution. Absorption spectral characteristics for the Eu(III)−uracil−ANCA ternary system are given in Table 5.

Fluorescence Properties of the Complexes. The fluorescence spectra of the Eu(III) complexes were recorded at room temperature. The emission spectra of the complexes are shown in Figure 6. Ligand-based excitations cause structural emission of rare earth complexes, and the ligand fluorescence is quenched showing that ligand-to-metal energy transfer occurs. Meanwhile, when excited at 308 nm, there is a characteristic emission peak of Eu(III) at 617 nm corresponding to the $^5D_0 \rightarrow {}^7F_2$ electronic transition (see Table 6). The emission spectrum of the Eu(III) complex recorded from (400 to 700) nm, by monitoring the excitation at 385 nm, shows a strong band peak at 410 nm indicating that the bands in higher energies can be attributed to the fluorescence due to the $S_1(\pi, \pi^*$ and $n, \pi^*) \rightarrow S_0$ transitions centered in the anthracene-9-carboxylic acid. These complexes could be anticipated as potential fluorescent materials which can be used for probing different types of DNA fragments containing the nucleobases under investigation.

The effect of concentration of the nucleobases was studied with the Eu−ANCA binary complex as shown in Figure 7. The results indicated the possibility of analytical determination of guanine and thymine in the concentration ranges $0.4 \cdot 10^{-5}$ $mol \cdot dm^{-3}$ to $2 \cdot 10^{-5}$ $mol \cdot dm^{-3}$ and $0.2 \cdot 10^{-5}$ $mol \cdot dm^{-3}$ to $1.8 \cdot 10^{-5}$ $mol \cdot dm^{-3}$, respectively.

This work is a continuation of the author's work in the field of bioinorganic chemistry.[41−45]

Literature Cited

(1) Jackson, B. A.; Alekseyev, V. Y.; Barton, J. K. A versatile mismatch recognition agent: specific cleavage of a plasmid DNA at a single base mispair. *Biochemistry* **1999**, *38*, 4655–4662.

(2) Hall, D. B.; Holmlin, R. E.; Barton, J. K. Oxidative DNA damage through long range electron transfer. *Nature* **1996**, *382*, 731.

(3) Ji, L. N.; Zou, X. H.; Liu, J. G. Shape and enantioselective interaction of Ru(II)/Co(III) polypyridyl complexes with DNA. *Coord. Chem. Rev.* **2001**, *513*, 216–217.

(4) Barton, J. K.; Goldberg, J. M.; Kumar, C. V.; Turro, N. J. Binding modes and base specificity of tris(phenanthroline) ruthenium(II) enantiomers with nucleic acids tuning the stereoselectivity. *J. Am. Chem. Soc.* **1986**, *108*, 2081.

(5) Lambert, B.; LePecq, J. B. *DNA-Ligand Interactions, from Drugs to Proteins*; Guschibaver, W., Saenger, W., Eds.; Plenum: New York, 1986; p 141.

(6) Heidelberger, C. Polycyclic Hydrocarbons and Carcinogenesis. *Annu. Rev. Biochem.* **1977**, *44*, 79.

(7) Porschke, D. *DNA-Ligand Interactions, Specificity and Dynamics of protein-Nucleic Acid Interactions*; Guschlbaver, W., Saenger, W. Eds.; Plenum: New York, 1986; p 85.

(8) Kumar, C. V.; Asuncion, E. H. DNA Binding Studies and Site Selective Fluorescence Sensitization of an Anthryl Probe. *J. Am. Chem. Soc.* **1993**, *115*, 8547–8553.

(9) Cidália, M. G.; Andrew, J. H.; Susan, J. Q. Recent developments in the field of supramolecular lanthanide luminescent sensors and self-assemblies. *Coord. Chem. Rev.* **2008**, *252*, 2512–2527.

(10) Gans, P.; Sabatini, A.; Vacca, A. SUPERQUAD: An improved general program for computation of formation constants from potentiometric data. *J. Chem. Soc., Dalton Trans.* **1985**, 1195–1200.

(11) Good, N. E.; Winget, G. D.; Winter, W.; Connoly, T. N.; Izwa, S.; Singh, R. M. M. Hydrogen ion Buffers for Biological Research. *Biochemistry* **1966**, *5*, 467–477.

(12) Bates, G. R.; Roy, N. R.; Robinson, A. R. *Determination of pH: Theory and practice*; John Wiley and Sons: New York, 1964.

(13) Ghose, R. Ternary transition metal chelates of 1,10-phenanthroline and a biological buffer (bicine). *Indian J. Chem.* **1984**, *23A*, 493–495.

(14) Corfù, A. N.; Song, B.; Ji, L. N. Metal ion/buffer interactions. Stability of binary and ternary metal ion complexes containing the anion of N,N-bis(2-hydroxyethyl)glycine (bicine) and adenine 5′-triphosphates (ATP). *Inorg. Chim. Acta* **1992**, *192*, 243–251.

(15) Azab, H. A.; El-Nady, A. M.; El-Korashy, S. H.; Hamed, M. M. A. Ternary complexes of Co(II) with adenosine 5′-mono, 5′-di, and 5′-triphosphates as primary ligands and some biologically important Zwitterionic buffers as secondary ligands. *J. Chem. Eng. Data* **1995**, *40*, 83–87.

(16) Azab, H. A.; Anwar, Z. M.; Sokar, M. Metal Ion Complexes Containing Nucleobases and Some Zwitterionic Buffers. *J. Chem. Eng. Data* **2004**, *49*, 62–72.

(17) Welcher, F. J. *The Analytical Uses of Ethylene diaminetetraacetic acid*; D.Von. Nostrand Co., Inc.: Princeton, 1965.

(18) Ringbom, A. *Complexation in Analytical Chemistry*; Wiley-Interscience: New York, 1963.

(19) Bjerrum, J. *Metal Amine Complex Formation in Aqueous Solution*; Haase: Copenhagen, 1941.

(20) Calvine, M.; Wilson, K. W. Stability of Chelate Compounds. *J. Am. Chem. Soc.* **1945**, *67*, 2003–2007.

(21) Irving, H.; Rossotti, H. S. Methods for computing successive stability constants from experimental formation curves. *J. Chem. Soc.* **1953**, 3397–3405.

(22) Irving, H.; Rossotti, H. S. The calculation of formation curves of metal complexes from pH-titration curves in mixed solvents. *J. Chem. Soc.* **1954**, 2904–2910.

(23) Ai, T.; Bompadre, S.; Yoshiro, S.; Xiaohui, W.; Li, M.; Tzyh-Chang, H. Direct Effects of 9-Anthracene Compounds on Cystic Fibrosis Transmembrane Conductance Regulator Gating. *Pflugers Arch. Eur. J. Physiol.* **2004**, *449*, 88–95.

(24) Gran, G. Determination of the Equivalence Point in Potentiometric Titration Part II. *Analyst* **1952**, *77*, 661.

(25) May, P. M.; Williams, D. R. *Computational Methods for the determination of Formation Constants*; Leggett, D. J., Ed.; Plenum Press: New York, 1985; pp 37−70.
(26) Smith, R. M.; Martell, A. E.; Motekaitis, R. J. Critically Selected Stability Constants of Metal Complexes. *NIST Data Base 46*, version 8.0, NIST: Gaithersubrg, MD, 2004.
(27) De Stefano, C.; Princi, P.; Rigano, C.; Sammartano, S. Computer analysis of equilibrium data in solution. ESAB2M: An improved version of the ESAB program. *Ann. Chim.(Rome)* **1987**, *77*, 643–675.
(28) Perrin, D. D.; Dempscy, B. *Buffer for pH and Metal Ion Control*; Chapman and Hall: London, 1979.
(29) Martell, A. E.; Sillen, L. G. *Stability Constants of Metal Ion Complexes*; The Chemical Society, London, 1971.
(30) Sigel, H. Interaction of Metal Ions with Nucleotides and Nucleic Acids and their Constituents. *Chem. Soc. Rev.* **1993**, *22*, 255.
(31) Martin, R. B.; Mariam, Y. H. Nucleotides and derivatives; their ambivalency. In *Metal Ions in Biological Systems*; Sigel, H., Ed.; Marcel Dekker: New York, 1979; Vol. 8, p 57ff.
(32) Mukkala, V.-M.; Helenius, M.; Hemmilä, I.; Kankare, J.; Takalo, H. Development of Luminescent Europium(III) and Terbium(III) Chelates of 2, 2′:6′, 2″-Terpyridine Derivatives for Protein Labelling. *Helv. Chim. Acta* **1993**, *76*, 1361.
(33) Steemers, F. J.; Verboom, W.; Reinhoudt, D. N.; Van der Tol, E. B.; Verhoeven, J. W. New Sensitizer Modified Calix[4]arenas Enabling Near UV Excitation of Complexed Luminescent Lanthanide Ions. *J. Am. Chem. Soc.* **1995**, *117*, 9408.
(34) Slooff, L. H.; Polman, A.; Klink, S. I.; Hebbink, G. A.; Grave, L.; van Veggel, F. C. J. M.; Reinhoudt, D. N.; Hofstraat, J. W. Optical Properties of Lissamine Functionalized Nd^{+3} Complexes in Polymer Waveguides and Solution. *Opt. Mater.* **2000**, *14*, 101.
(35) Gschneidner, K. A.; Eyring, L. R. *Handbook on the Physics and Chemistry of Rare Earths*; North-Holland Publishing Company: Amsterdam, 1979.
(36) Sato, S.; Wada, M. Relation between Interamolecular Energy Transfer Efficiencies and Triplet State Energies in Rare Earth β-Diketone Chelates. *Bull. Chem. Soc. Jpn.* **1970**, *43*, 1955.
(37) Klink, S. I.; Keizer, H.; Van Veggel, F. C. J. M. Transition Metal Complexes as Photosensitizers for Near Infrared Lanthanide Luminescence. *Angew. Chem., Int. Ed.* **2000**, *39*, 4319.
(38) Werts, M. H. V.; Woudenberg, R. H.; Emmerink, P. G.; Van Gassel, R.; Hofstraat, J. W.; Verhoeven, J. W. A Near Infrared Luminescent Label Based on Yb (III) Ions and its Application in a Fluoroimmunoassay. *Angew. Chem., Int. Ed.* **2000**, *39*, 4542.
(39) Nicholson, R. S. Theory and Application of Cyclic Voltammetry for Measurement of Electrode Reaction Kinetics. *Anal. Chem.* **1965**, *37*, 1351.
(40) Gosser, D. K., Jr. *Cyclic Voltammetry: Simulation and Analysis of Reaction Mechanisms*; VCH Publishers: New York, 1993.
(41) Azab, H. A.; Deghaidy, F. S.; Orabi, A. S.; Farid, N. Y. Comparison of the Effectiveness of Various Metal ions on the Formation of the Ternary Complexes Containing adenosine 5′-mono, 5′-di-, and 5′-Triphosphate and Some Zwitterionic Buffers for Biochemical and Physiological Research. *J. Chem. Eng. Data* **2000**, *45*, 709–715.
(42) Azab, H. A.; Orabi, A. S.; Abdel-Salam, E. T Role of Biologically Important Zwitterionic Buffer Secondary Ligands on the Stability of the Mixed Ligand Complexes of Divalent Metal Ions and Adenosine5′-mono-, 5′-di-, and 5′-Triphosphate. *J. Chem. Eng. Data* **2001**, *46*, 346–352.
(43) Anwar, Z. M.; Azab, H. A. Ternary Complexes in Solution. Comparison of the Coordination Tendency of Some Biologically Important Zwitterionic Buffers toward the Binary Complexes of Some Transition Metal Ions and Some Amino Acids. *J. Chem. Eng. Data* **1999**, *44*, 1151–1157.
(44) Anwar, Z. M.; Azab, H. A. Role of Biologically Important Zwitterionic Buffer Secondary Ligands in the Stability of the Ternary Complexes Containing Some Metal Ions and Guanosine5′-Monophosphate, Inosine 5′-Monophosphate and Cytidine 5′-Monophosphate. *J. Chem. Eng. Data* **2001**, *46*, 34–40.
(45) Anwar, Z. M.; Azab, H. A. Ternary Complexes Formed by Trivalent Lanthanide Ions, Nucleotides, and Biological Buffers. *J. Chem. Eng. Data* **2001**, *46*, 613–618.

Received for review February 3, 2009. Accepted June 20, 2009.

JE900149X