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ARTICLE

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Fluorescence and Electrochemical Probing of *N*-Acetylamino Acids, Nucleotides, and DNA by the Eu(III)−Bathophenanthroline Complex

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ABSTRACT: The solid complex Eu(III)−bathophenanthroline was synthesized and characterized by elemental analysis, IR spectra, and thermal analysis. The interaction of the Eu(III)−bathophenanthroline solid complex with calf-thymus DNA has been investigated by fluorescence and electrochemical methods including cyclic voltammetery and differential pulse polarography on a glassy carbon electrode. The formation of binary and ternary complexes of Eu(III) with nucleotides guanosine 5′- monophosphate (5′-GMP), adenosine 5′-monophosphate (5′-AMP), inosine 5′-monophosphate (5′-IMP), cytidine 5′-monophosphate (5′-CMP), or *N*-acetylamino acids (*N*-acetylaspartic acid, *N*-acetylhistidine, and *N*-acetylhistamine), and bathophenanthroline (BPhen) has been studied potentiometrically at (25.0 ± 0.1) °C and an ionic strength of $I = 0.1$ mol·dm^{-3} (KNO_3) in 1.8 % v/v ethanol−water mixture solvent. The formation of the normal and protonated binary and ternary complexes is inferred from the corresponding titration curves. The experimental conditions were selected such that self-association of the nucleotides and their complexes was negligibly small, that is, the monomeric complexes were studied. 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. Confirmation of the formation of the ternary systems of the type Eu(III)−bathophenanthroline−*N*-acetylamino acids or nucleotides in solution has been carried out using UV−visible, cyclic voltammetry, square wave voltammetry, and emission spectrofluorometric measurements.

INTRODUCTION

Quantitative determination of nucleic acids is required in many fields, such as molecular biology, biotechnology, and medical diagnosis.

Bathophenanthroline (BPhen) is used for estimation of heavy metals which are considered as trace impurities that reduce the quality of production in pharmacy, food industry, etc.[1,2] Eu(III) complexes of bathophenanthroline are used in the fabrication of electroluminescent (EL) devices.[3,4] Iron, osmium, and samarium complexes of 4,7-diphenyl-1,10-phenanthroline are used in new sensitive, reliable, and reproducible fluorimetric methods for determining microgram amounts of nucleic acids.[5]

N-Acetylamino acids are important in neurochemistry, especially *N*-acetylaspartic acid (NAA) which is an unusual amino acid that is present in the vertebrate brain.[6] Its concentration is one of the highest of all free amino acids, and although NAA is synthesized and stored primarily in neurons, it cannot be hydrolyzed in these cells. Furthermore, neuronal NAA is dynamic and turns over more than once each day via extracellular fluids (ECF), between neurons and catabolic compartments in oligodendrocytes. While NAA has several functions in the central nervous system (CNS), an important role is the participation in the primary mechanism for the removal of metabolic water, against a water gradient, from myelinated neurons.[7] Also, *N*-acetylamino acids containing histidyl residue are considered as metal-binding sites in biological systems. Many such systems are enzymes and proteins that contain copper or iron as prosthetic groups and facilitate oxygen transport, the transport and storage of metal ions, electron transfer, and the dioxygen activation toward the oxidation and oxygenation of drugs and physiologically active substances.[8−11]

A relatively possible line of investigation for new anticancer agents includes studies on Eu(III) chemistry as an alternative metallopharmaceutical approach to platinum. The higher coordination number of Eu(III) compared with platinum provides additional coordination sites, which can potentially be used to fine-tune the properties of the complex, for example, by influencing the way the complex interacts with DNA. The different redox properties of Eu(III) can also play an important role in the transport mechanisms of the drug in the body, as well as in the interaction between the drug and several different biologically relevant proteins.[12] Eu(III) chemistry may also allow for photodynamic approaches to therapy.[13−15]

Hence, the aim of the present study is to study the interaction of the Eu(III)−bathophenanthroline complex with DNA, the nucleotides (5′-AMP, 5′-CMP, 5′-GMP, and 5′-IMP), and *N*-acetylamino acids (*N*-acetylaspartic acid, *N*-acetylhistidine, and *N*-acetylhistamine), to develop information regarding the possible electrochemical and fluorescence probing of the above-mentioned biologically important compounds by the Eu(III)−(BPhen) complex. Also, information about the structure of this interesting Eu(III) complex and the interaction with DNA may be gained.

EXPERIMENTAL SECTION

All materials employed in the present investigation were of A. R. grade. Guanosine 5′-monophosphate (5′-GMP), cytidine

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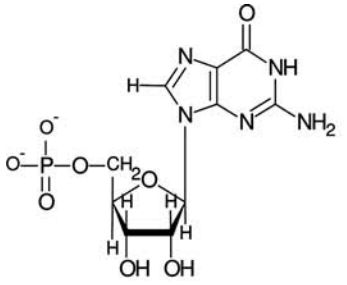
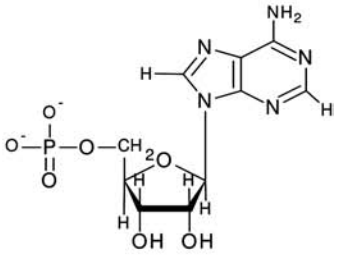
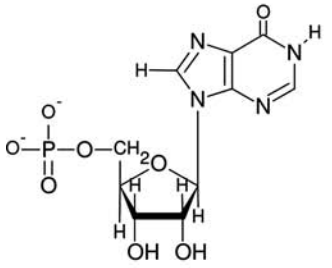
Scheme 1. Structures of the Studied Ligands



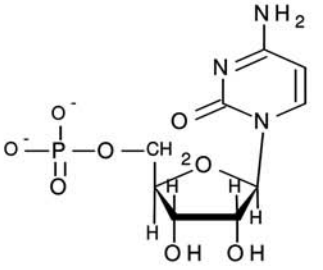
guanosine5`-monophosphate
(5`-GMP)



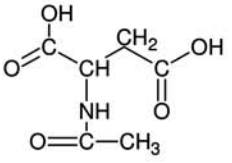
adenosine5`-monophosphate
(5`-AMP)



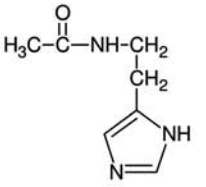
Inosine5`-monophosphate
(5`-IMP)



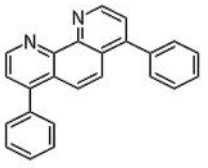
cytidine5`-monophosphate
(5`-CMP)



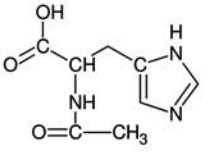
N-acetylaspartic acid



N-acetylhistamine



bathophenanthroline



N-acetylhistidine

5′-monophosphate (5′-CMP), adenosine 5′-monophosphate (5′-AMP), and inosine 5′-monophosphate (5′-IMP) were purchased from the Sigma Chemical Co. and were used without purification. Reagent grade *N*-acetylhistamine, *N*-acetylhistidine, and *N*-acetylaspartic acid were obtained from the Sigma Chemical Co., St. Louis, MO. The purity for these compounds averaged 99.5 %. Bathophenanthroline was obtained from Sigma. 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. KNO_3 was from Merck AG Darmstadt, Germany. KOH and nitric acid were of Pa grade. 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 measurements. Lanthanide metal salt $Eu(NO_3)_3 \cdot 6H_2O$ was from the Sigma Chemical Co. Stock solutions of lanthanide metal salt were prepared by dissolving precisely weighed amounts of the salts in bidistilled water and adjusting the pH values of the prepared solutions to be around pH = 4.5. The concentrations of the metal ion stock solutions were determined complexometrically by ethylenediamine tetraacetic acid (EDTA).[16] The structures of the studied ligands are depicted in Scheme 1.

Apparatus and Procedures. 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

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Table 1. Analytical Data for the Eu(III)−Bathophenanthroline (BPhen) Complex

complex	formula	mp/°C
$[Eu(BPhen)_2(NO_3)_3(H_2O)]$	$C_{48}H_{33}N_7O_{10}Eu$	220 °C

Elemental Analysis							
C %		H %		N %		Eu %	
calcd	found	calcd	found	calcd	found	calcd	found
56.42	*56.18*	3.33	*3.36*	9.60	*9.45*	14.89	14.80

Table 2. Dissociation Constants for the Studied Ligands Measured at I = 0.1 mol·dm^{-3} KNO_3 and at 25.0 °C in the Ethanol−Water Mixture (10 % v/v)

ligand	pK_a	pK_{a1}	pK_{a2}	pK_{a3}
bathophenanthroline (BPhen)	7.06 ± 0.03	−	−	−
N-acetylhistamine	−	−	7.12 ± 0.03	−
N-acetylhistidine	−	−	7.04 ± 0.03	9.92 ± 0.02
N-acetylaspartic acid	−	3.04 ± 0.02	4.49 ± 0.02	−
GMP			9.44 ± 0.03	
IMP			9.09 ± 0.03	
AMP		3.80 ± 0.02	6.15 ± 0.03	
CMP		4.33 ± 0.03	6.15 ± 0.02	

Table 3. Formation Constants of Eu^{3+} Binary Complexes with the Nucleotides (NU), *N*-Acetylamino Acids (NAA), and BPhen in a (10 % v/v) Ethanol−Water Mixture, at I = 0.1 mol·dm^{-3} KNO_3 and 25.0 °C

ligand	log K
bathophenanthroline (BPhen)	4.56 ± 0.02
N-acetylhistamine	6.19[a] ± 0.02
N-acetylhistidine	4.75 ± 0.02
N-acetylaspartic acid	4.45 ± 0.02
GMP	6.13 ± 0.03
IMP	6.94 ± 0.02
AMP	4.28 ± 0.02
CMP	4.29 ± 0.02

[a] log $K^{Eu}_{Eu(NAA)(H)}$ = log formation constant of the monoprotonated complex.

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 mol·dm^{-3} KNO_3, in a 10 % ethanol−water mixture because the Eu(III)−bathophenanthroline complex is not completely soluble in water. The concentration of 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.[17] Gran's method[18] was used to determine $E^{o\prime}$ and E_j so that the hydrogen ion

Table 4. Formation Constants of Eu(III) Ternary Complexes in a (10 % v/v) Ethanol−Water Mixture, at I = 0.1 mol·dm^{-3} KNO_3 and 25.0°C, with Bathophenanthroline

system	log K
Eu(III)−(*N*-acetylhistamine)−(BPhen)	4.63 ± 0.02
Eu(III)−(*N*-acetylaspartic acid)−(BPhen)	5.23 ± 0.03
Eu(III)−(GMP)−(BPhen)	4.51[a] ± 0.02
Eu(III)−(AMP)−(BPhen)	7.01 ± 0.03
Eu(III)−(CMP)−(BPhen)	4.69 ± 0.02
Eu(III)−(HIMP)−(BPhen)	5.08[b] ± 0.03

[a] log formation constant of monoprotonated complex. [b] log formation constant of diprotonated complex.

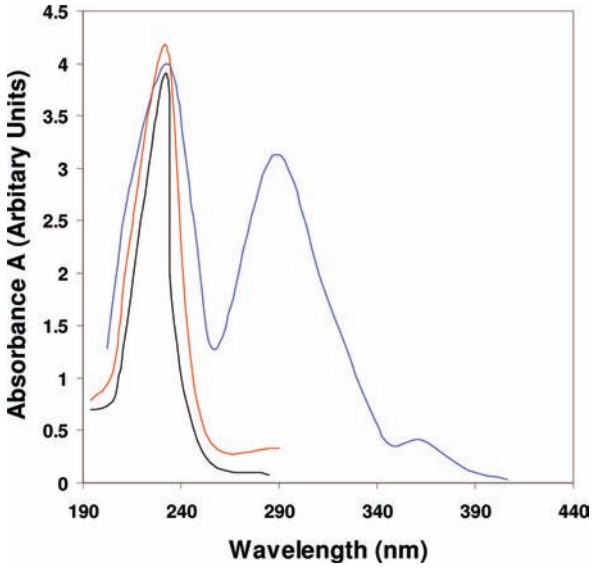

Figure 1. Absorption spectra for the Eu(III) + bathophenanthroline (BPhen) complex in 1:1 ratio in 1.8 % (v/v) ethanol−water mixture and at 25.0 °C. Black line, 1·10^{-4} mol·dm^{-3} Eu(III); red line, 1·10^{-4} mol·dm^{-3} bathophenanthroline;blue line, 1·10^{-4} mol·dm^{-3} bathophenanthroline + 1·10^{-4} mol·dm^{-3} Eu (III) (binary complex).

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.[19] The protonation constants were then determined by use of the Bjerrum function.[20]

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

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 1.8 % v/v 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 ESAB2M computer program.[22]

A detailed description of the solution composition used in the determination of the stability constants of the complex species is

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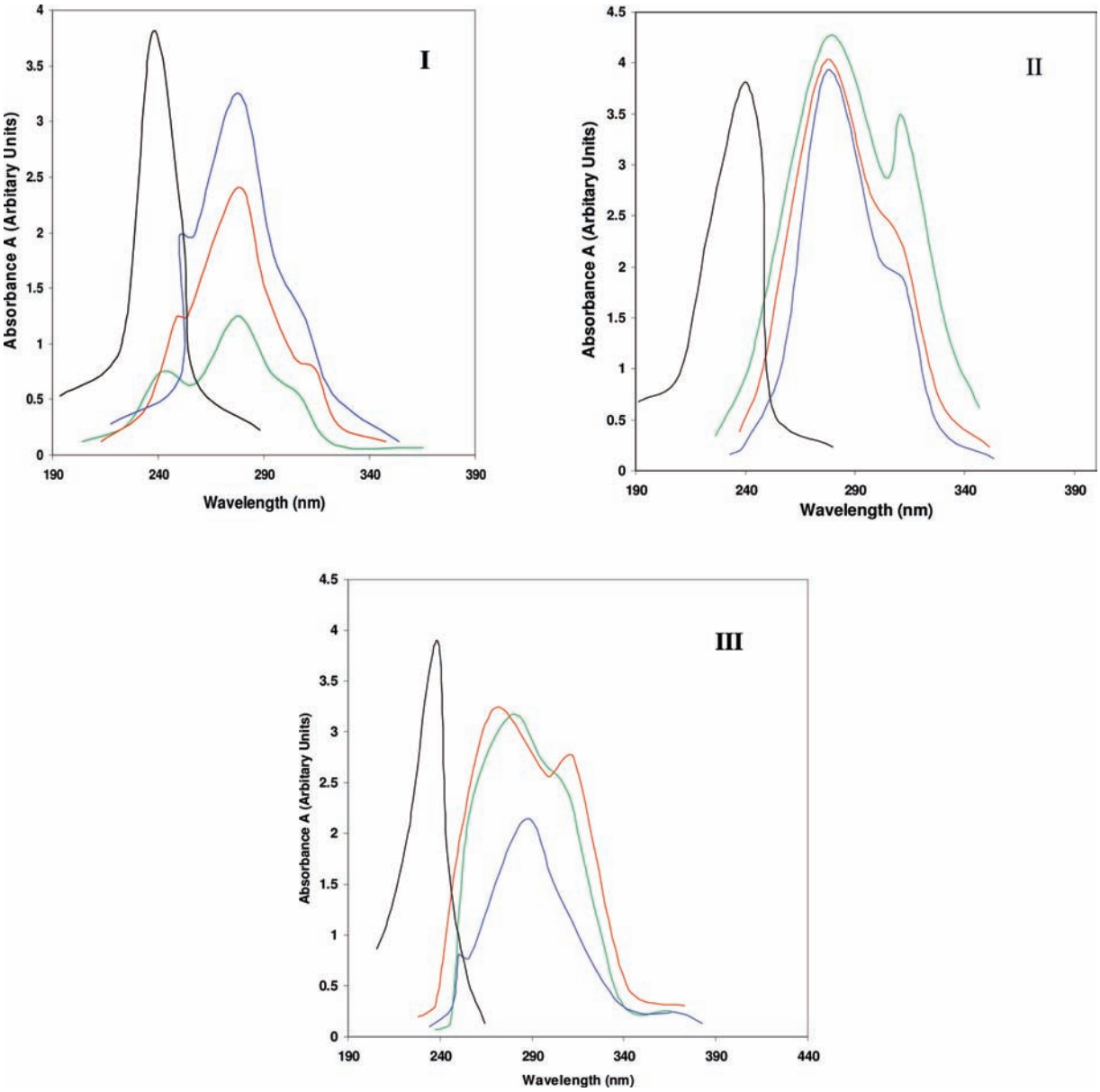

Figure 2. Absorption spectra for Eu(III) + *N*-acetylamino cids (ACA) + *n*-bathophenanthroline (BPhen) in 1.8 % (v/v) ethanol−water mixture and at 25.0 °C. Black line, $1 \cdot 10^{-4}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-4}$ mol·dm^{-3} (ACA); blue line, $1 \cdot 10^{-4}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-4}$ mol·dm^{-3} (ACA) + $1 \cdot 10^{-4}$ mol·dm^{-3} BPhen (1:1:1); red line, $1 \cdot 10^{-4}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-4}$ mol·dm^{-3} (ACA) + $1 \cdot 10^{-4}$ mol·dm^{-3} BPhen (1:1:2); green line, $1 \cdot 10^{-4}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-4}$ mol·dm^{-3} (ACA) + $1 \cdot 10^{-4}$ mol·dm^{-3} BPhen (1:1:3). (I) ACA = *N*-acetylhistamine, (II) ACA = *N*-acetylhistidine, (III) ACA = *N*-acetylaspartic acid.

shown as follows [nucleotides = (GMP, CMP, AMP, and IMP) and *N*-acetylamino acids = *N*-acetylhistamine, *N*-acetylhistidine, and *N*-acetylaspartic acid]:

(a) $2 \cdot 10^{-3}$ mol·dm^{-3} HNO_3 + $1 \cdot 10^{-4}$ mol·dm^{-3} *N*-acetylamino acids or nucleotides.

(b) $2 \cdot 10^{-3}$ mol·dm^{-3} HNO_3 + $1 \cdot 10^{-4}$ mol·dm^{-3} *N*-acetylamino acids or nucleotides + $5 \cdot 10^{-4}$ mol·dm^{-3} Eu(III) (binary complex).

(c) $2 \cdot 10^{-3}$ mol·dm^{-3} HNO_3 + $1 \cdot 10^{-4}$ mol·dm^{-3} bathophenanthroline.

(d) $2 \cdot 10^{-3}$ mol·dm^{-3} HNO_3 + $1 \cdot 10^{-4}$ mol·dm^{-3} bathophenanthroline + $5 \cdot 10^{-4}$ mol·dm^{-3} Eu(III) (binary complex).

(e) $2 \cdot 10^{-3}$ mol·dm^{-3} HNO_3 + $1 \cdot 10^{-4}$ mol·dm^{-3} bathophenanthroline + $1 \cdot 10^{-4}$ mol·dm^{-3} *N*-acetylamino acids or nucleotides + $5 \cdot 10^{-4}$ mol·dm^{-3} Eu(III).

The total volume was kept constant at 10 cm^3 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 significant. Initial estimates of the formation constants of ternary complexes in a 1:1:1 ratio and the stability constants of the binary 1:1 and 1:2 complexes have been refined using the SUPERQUAD computer program.[23]

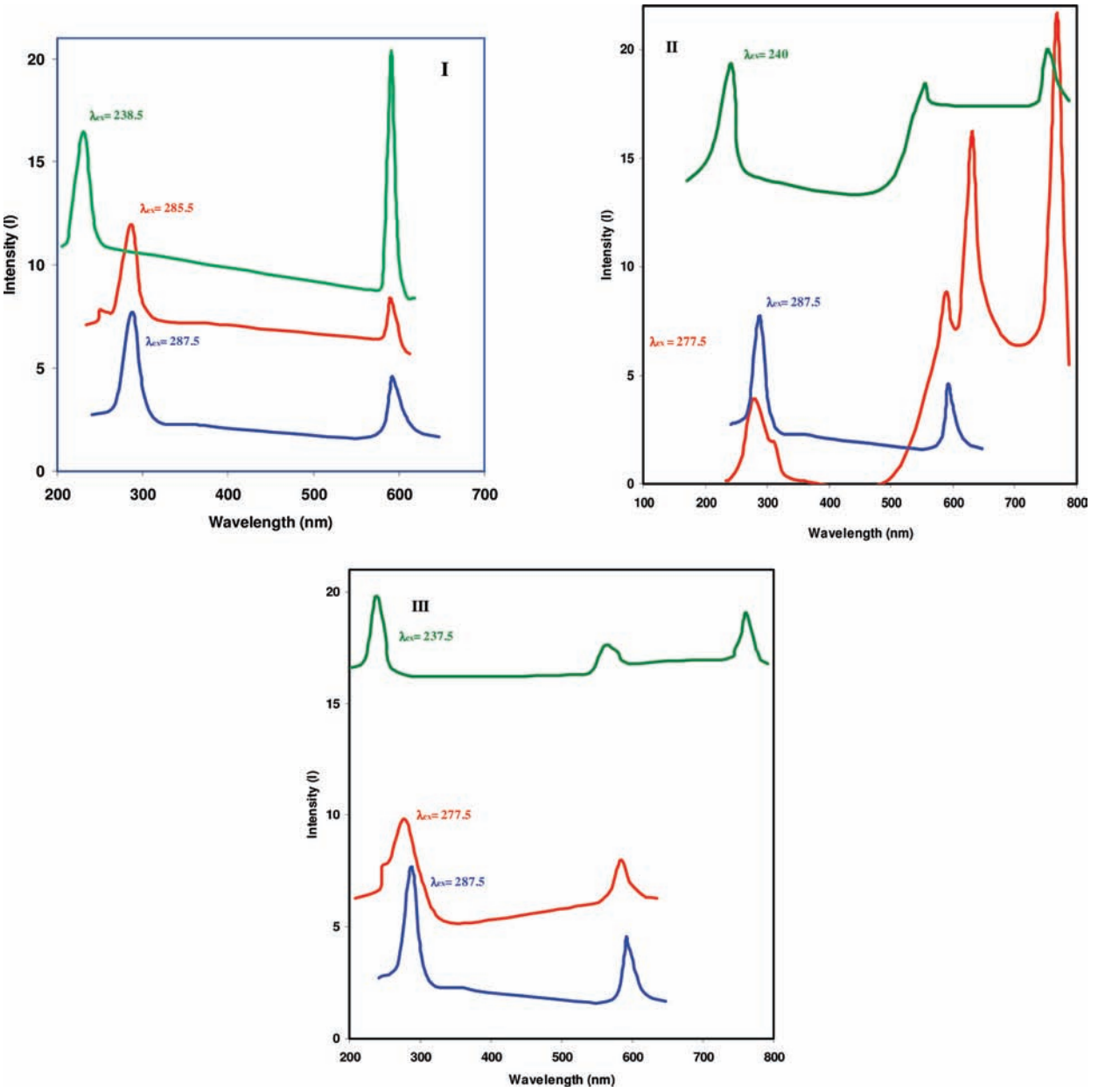

Figure 3. Excitation and emission spectra for Eu(III) + bathophenanthroline (BPhen) + *N*-acetylamino acid (NAC) in 1.8 % v/v ethanol−water mixture and at 25.0 °C [Eu(III)] = [BPhen] = [NAC] = $1 \cdot 10^{-5}$ mol·dm^{-3}. Black line, $1 \cdot 10^{-5}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-5}$ mol·dm^{-3} BPhen; blue line, $1 \cdot 10^{-5}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-5}$ mol·dm^{-3} (NAC); red line, $1 \cdot 10^{-5}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-5}$ mol·dm^{-3} (NAC) + $1 \cdot 10^{-5}$ mol·dm^{-3} BPhen. (I) NAC = *N*-acetylaspartic acid, (II) NAC = *N*-acetylhistidine, (III) NAC = *N*-acetylhistamine.

The formation of the ternary complex species occurs via two different mechanisms, the first one being the stepwise mechanism, where it follows two steps

$$pM + q(NU) \rightleftharpoons (M)_p(NU)_q$$

$$(M)_p(NU)_q + r(AAC) \rightleftharpoons (M)_p(NU)_q(AAC)_r$$

During this refinement, the stepwise stability constants is

$$K_{M(NU)(AAC)} = \frac{M_p(NU)_q(AAC)_r}{[M]_p(NU)_q[AAC]^r} \quad (3)$$

which refers to the addition of AAC to the binary complex $M_p(NU)_q$; i.e., the lanthanide metal ion reacts with the primary ligand (nucleotide). Hence, the second step is the reaction of the formed binary complex with the *N*-acetylamino acid as the secondary ligand.

The overall complexation reaction involving protonation is

$$pM + q(NU) + r(AAC) + sH \rightleftharpoons (M)_p(NU)_q(AAC)_r(H)_s \quad (4)$$

$$\beta_{pqrs} = \frac{M_p(NU)_q(AAC)_r(H)_s}{[M]^p[NU]^q[AAC]^r[H]^s} \quad (5)$$

in which AAC = *N*-acetylamino acids, NU = nucleotide ligands (GMP, AMP, CMP, and IMP), and M = Eu(III).

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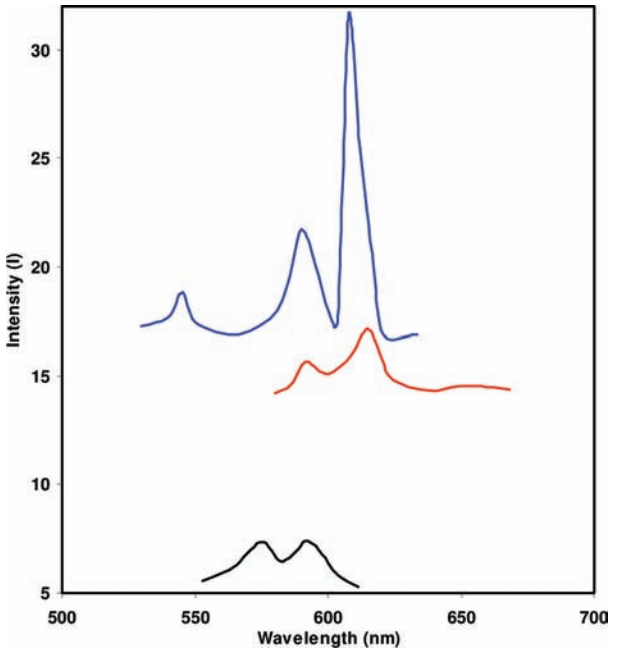

Figure 4. Comparative fluorescence spectra for Eu(III)−bathophenanthroline (BPhen)−nucleotides (NU) [Eu(III)] = [BPhen] = [NU] = $1\cdot10^{-5}$ mol·dm^{-3}. (a) Black line, $1\cdot10^{-5}$ mol·dm^{-3} Eu(III) + $1\cdot10^{-5}$ mol·dm^{-3} BPhen + $1\cdot10^{-5}$ mol·dm^{-3} 5′-GMP;(b) red line, $1\cdot10^{-5}$ mol·dm^{-3} Eu(III) + $1\cdot10^{-5}$ mol·dm^{-3} BPhen + $1\cdot10^{-5}$ mol·dm^{-3} 5′-CMP;(c) blue line, $1\cdot10^{-5}$ mol·dm^{-3} Eu(III) + $1\cdot10^{-5}$ mol·dm^{-3} BPhen + $1\cdot10^{-5}$ mol·dm^{-3} 5′-AMP.

The second mechanism is the simultaneous type where both ligand molecules react at the same time with the lanthanide metal ion as depicted in the following equilibrium

$$pM + q(NU) + sH + r(AAC) \rightleftharpoons (M)_p(NU)_q(H)_s(AAC)_r \quad (6)$$

where p, q, r, and s are the number of moles of M, NU or AAC, and H in the formed ternary complex.

All side reactions due to metal ion hydrolysis have been included in the calculations.[24−26]

Electrochemical Measurements. Cyclic voltammetry (CV), square wave voltammetry (SWV), and differential pulse voltammetry (DPP) were collected using EG and G Princeton applied research, potentiostat/galvanostat model 263 with a single compartment voltammetric cell equipped with a glassy carbon (GC) working electrode (area = 0.1963 cm^2) embedded in a resin, a Pt-wire counter electrode, and Ag\AgCl electrode as reference electrode. A typical experiment had a sample volume of 25 cm^3 with 1.8 % v/v ethanol−water mixture solvent containing:

(a) $5\cdot10^{-4}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} bathophenanthroline (1:1).
(b) $5\cdot10^{-4}$ mol·dm^{-3} Eu(III) + $1\cdot10^{-3}$ mol·dm^{-3} bathophenanthroline (1:2).
(c) $5\cdot10^{-4}$ mol·dm^{-3} Eu(III) + $1.5\cdot10^{-3}$ mol·dm^{-3} bathophenanthroline (1:3).
(d) $5\cdot10^{-4}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} nucleotide or *N*-acetylamino acids + $5\cdot10^{-4}$ mol·dm^{-3} bathophenanthroline (1:1:1).
(e) $5\cdot10^{-4}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} nucleotide or *N*-acetylamino acids + $1\cdot10^{-3}$ mol·dm^{-3} bathophenanthroline (1:1:2).
(f) $5\cdot10^{-4}$ mol·dm^{-3} Eu(III) + $5\cdot10^{-4}$ mol·dm^{-3} nucleotide or *N*-acetylamino acids + $1.5\cdot10^{-3}$ mol·dm^{-3} bathophenanthroline (1:1:3).

The ionic strength of the studied solutions was adjusted at 0.1 mol·dm^{-3} using an alcoholic solution of *p*-toluenesulfonate.

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 −(0.40 to 0.90) V, which is the suitable potential window for Eu(III).

Spectrophotometric Measurements. The ultraviolet (UV) spectra of the solutions in a 1.8 % v/v ethanol−water mixture of the binary and ternary complexes were scanned on a Shimadzu-1601PC UV−visible automatic recording spectrophotometer with 1 cm quartz cells for the 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 $1\cdot10^{-4}$ mol·dm^{-3} in the 1.8 % v/v 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 in a 1:1 ratio were scanned against a 1.8 % v/v 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 to be $1\cdot10^{-4}$ mol·dm^{-3} and at 0.1 mol·dm^{-3} KNO_3 in a 1.8 % v/v ethanol−water mixture solvent. 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 water, of the binary and ternary complexes were scanned on a JASCO-FP6300 spectrofluorometer with 1 cm quartz cells. 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 $1\cdot10^{-5}$ mol·dm^{-3}. All the studied solutions were diluted with bidistilled water, after pH adjustment to pH 6.5 to 7 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 to be $1\cdot10^{-5}$ mol·dm^{-3} in water.

Preparation of Solid Complex. The Eu(III)−bathophenanthroline binary complex was prepared according to a method[27] priviously described. In this method, 2.00 mmol (0.6648 g) of bathophenanthroline was dissolved in 20.00 mL of absolute ethanol with vigorous stirring and 1.00 mmol of Eu(III) nitrate hexahydrate [$(Eu(NO_3)_3\cdot6H_2O)$ (0.36641 g)] dissolved in 5 mL of absolute ethanol which was added to the first solution. After stirring for about 30 min, the pH value was adjusted to 5.5 by adding an aqueous sodium hydroxide solution slowly under stirring with heating until the precipitate appeared and the reaction system was then cooled to ambient temperature. The resulting product was precipitated from the system, the precipitate washed with ethanol, and the product collected and identified by elemental analysis, thermal analysis, and IR spectra.

RESULTS AND DISCUSSION

The deprotonation constants for the studied ligands were calculated from the potentiometric titration curves of each ligand

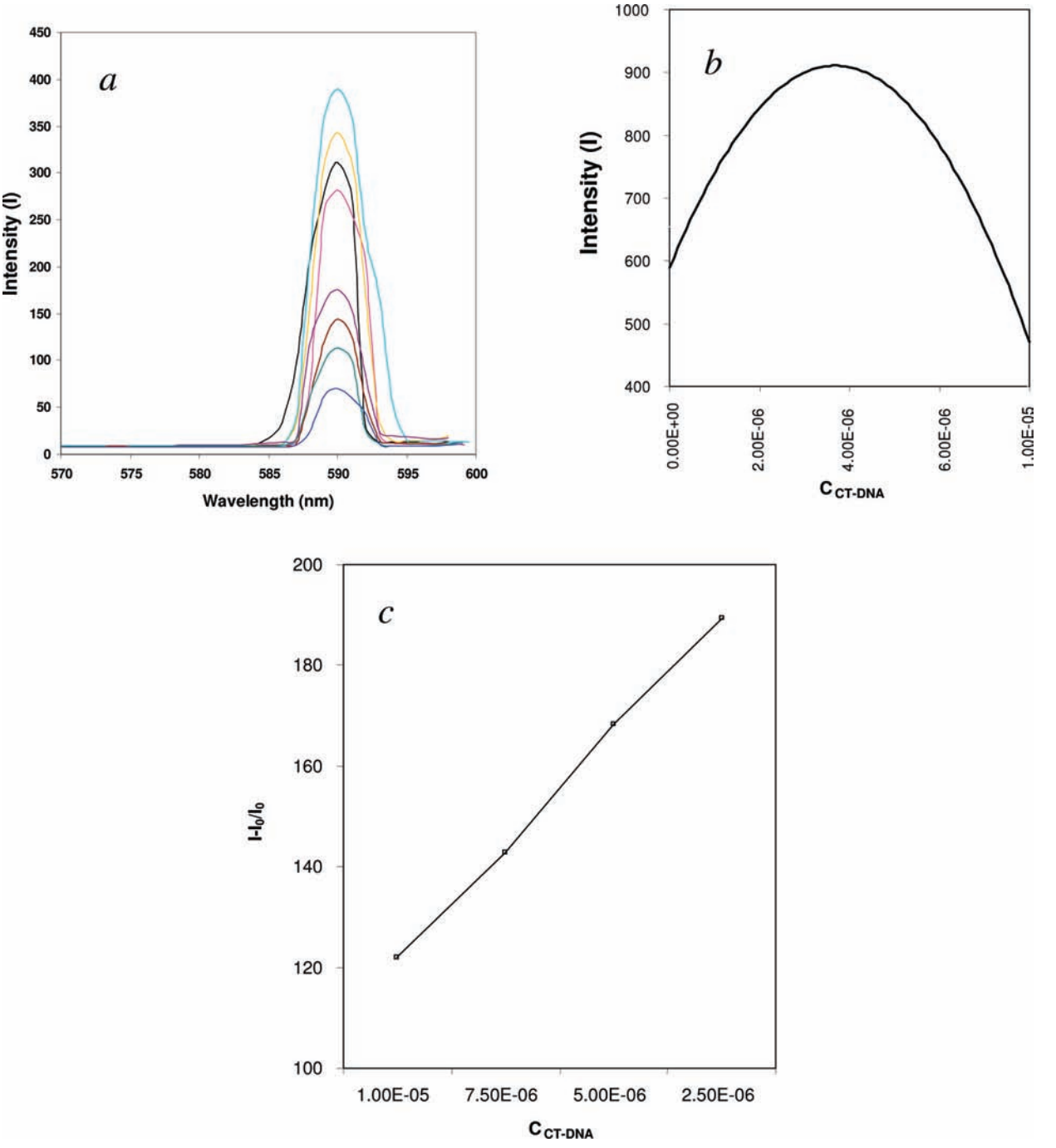

Figure 5. Fluorescence spectra for Eu(III)−bathophenanthroline (BPhen)−CT-DNA in various concentrations, at 25.0 °C. (a) Fluorescence spectra fo Eu(III)−bathophenanthroline (BPhen)−(CT-DNA) in various concentrations in the range $1 \cdot 10^{-5}$ mol·dm^{-3} to $0.5 \cdot 10^{-8}$ mol·dm^{-3}, at 25.0 °C. (b) Fluorescence intensity correlation with CT-DNA concentration. (c) Relative fluorescence intensity correlation with CT-DNA concentration.

($5 \cdot 10^{-4}$ mol·dm^{-3}) in 1.8 % v/v ethanol−water mixture, I = 0.1 mol·dm^{-3} KNO_3, and at 25 °C. The deprotonation constants for *N*-acetylhistidine and *N*-acetylhistamine are 7.04 and 7.12, respectively, due to the deprotonation of N_3H.[24]

The two calculated dissociation constants of *N*-acetylaspartic acid ($pK_1 = 3.04 \pm 0.02$, $pK_2 = 4.49 \pm 0.02$, Table 2) correspond to the α,β-carboxylate group ionizations and are in good agreement with those found in the literature.[28]

The deprotonation constants of the nucleotide molecules were found to be ($pK_{a3} = 9.44 \pm 0.02$, $pK_{a2} = 6.38 \pm 0.04$, $pK_{a1} = 2.45 \pm 0.05$) for 5′-GMP and (9.09 ± 0.03) for N_1H, N7, and O(PO) group in 5′-IMP. The adenosine nucleotide 5′-AMP has ($pK_1 = 6.15 \pm 0.03$, $pK_2 = 3.80 \pm 0.03$), while for 5′-CMP the deprotonation constants are ($pK_1 = 4.33 \pm 0.02$, $pK_2 = 6.19 \pm 0.02$).

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 ligands combine with the binary 1:1 Eu(III)−nucleotide or *N*-acetylamino acid complexes. Thus, the initial estimation of the stability constants of the normal and protonated ternary complexes formed

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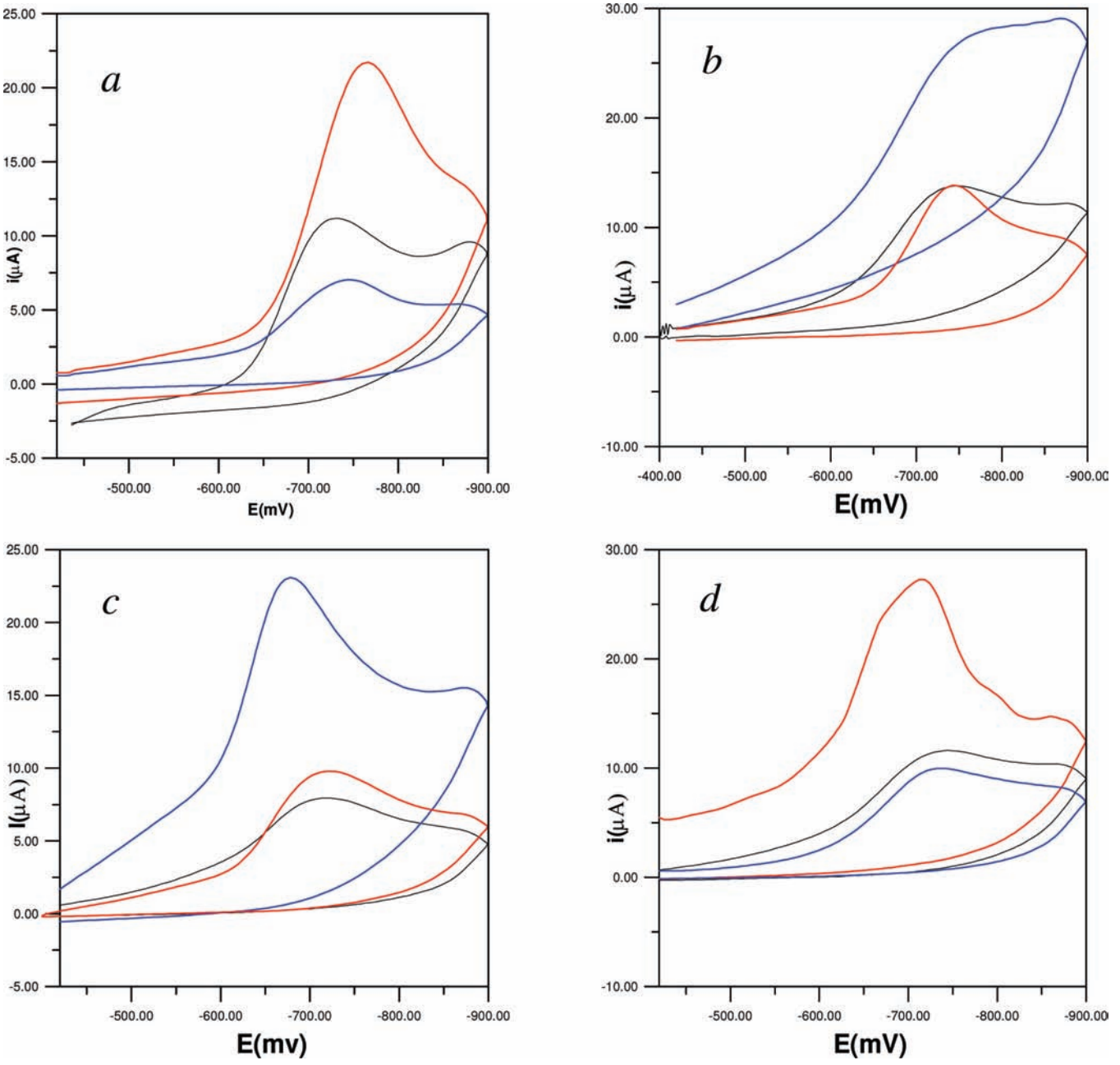

Figure 6. Cyclic voltammograms for the Eu(III) + bathophenanthroline (BPhen) + nucleotides (NU) system, in 1.8 % v/v ethanol−water mixture, at I = 0.1 mol·dm^{-3} *p*-toluenesulfonate, ν = 100 mV·s^{-1}, and 25.0 °C. Black line, 5·10^{-4} mol·dm^{-4} Eu(III) + 5·10^{-4} mol·dm^{-4} (NU) + 5·10^{-4} mol·dm^{-4} BPhen (1:1:1); blue line, 5·10^{-4} mol·dm^{-4} Eu(III) + 5·10^{-4} mol·dm^{-4} (NU) + 1·10^{-3} mol·dm^{-4} BPhen (1:1:2); red line, 5·10^{-4} mol·dm^{-4} Eu(III) + 5·10^{-4} mol·dm^{-4} (NU) + 1.5·10^{-3} mol·dm^{-4} BPhen (1:1:3). (a) NU = 5′-GMP; (b) NU = 5′- IMP; (c) NU = 5′-AMP; (d) NU = 5′CMP.

in solution has been determined using half point neutralization. Initial estimates of the stability constants of different normal and protonated binary (Table 3) and ternary complexes formed in solution have been refined with the SUPERQUAD computer program.[23] The quality of the fit during this refinement was judged by the values of the sample standard deviations and the goodness of fit X^2 (Pearson's test). At σ_E = 0.1 mV (0.001 pH error) and σ_E = 0.005 mL, the values of S in different sets of titrations were between 1.0 and 1.7, and X^2 was between 12.0 and 13.0. Our calculations fit the model in which nucleotides or *N*-acetylamino acids displace one bathophenanthroline molecule during the formation of ternary complexes. 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 suggested for the actual species formed under our experimental conditions. Examination of the different formation constant values listed in Table 4 reveals that the order of the stability constants of different ternary complexes in the systems Eu(III)−NU−bathophenanthroline in terms of nucleotides follows generally the trend AMP > IMP > CMP > GMP. In the systems Eu(III)−*N*-acetylamino acid−bathophenanthroline, the trend

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Table 5. Voltammetric Data and Transfer Coefficient (α) from the Cyclic Voltammograms of the Eu(III) + Bathophenanthroline (BPhen) + *N*-Acetylhistamine System, in the 1.8 % v/v Ethanol + Water Mixture, at I = 0.1 mol·dm^{-3} *p*-Toluenesulfonate and at 25.0 °C ± 0.1 °C

system	E_p^c (mV)	$-E_{p/2}^c$ (mV)	$-(E_p^c - E_{p/2}^c)$	i_p^c (μA)	α	D_{red} (cm^2·s^{-1})
$5\cdot10^{-4}$ mol·dm^{-3} Eu(III)	704.00	604.00	100.00	2.25	0.49	$2.2\cdot10^{-5}$
Eu(III) + BPhen (1:1)	733.3	567.2	166.1	6.26	0.2865	$2.5\cdot10^{-6}$
Eu(III) + BPhen (1:2)	696.00	528.00	168	0.746	0.258	$3.02\cdot10^{-11}$
Eu(III) + BPhen (1:3)	760.00	576.6	183.4	9.8	0.259	$1.08\cdot10^{-9}$
Eu(III) + *N*-acetylhistamine (1:1)	681.9	490.9	191	4.86	0.249	$3.12\cdot10^{-4}$
Eu(III) + BPhen + *N*-acetylhistamine (1:1:1)	706.6	577.37	129.23	9.47	0.368	$2.84\cdot10^{-10}$
Eu(III) + BPhen + *N*-acetylhistamine (1:2:1)	702.7	550.00	152.7	32.00	0.312	$3.83\cdot10^{-9}$
Eu(III) + BPhen + *N*-acetylhistamine (1:3:1)	760.4	576.6	183.8	9.8	0.259	$4.3\cdot10^{-10}$

Table 6. Voltammetric Data and Transfer Coefficient (α) from the Cyclic Voltammograms of the Eu(III) + Bathophenanthroline (BPhen) + *N*-Acetylhistidine System, in the 1.8 % v/v Ethanol + Water Mixture, at *I* = 0.1 mol·dm^{-3} *p*-Toluenesulfonate and at 25.0 °C ± 0.1 °C

system	E_p^c (mV)	$-E_{p/2}^c$ (mV)	$-(E_p^c - E_{p/2}^c)$	i_p^c (μA)	α	D_{red} (cm^2·s^{-1})
$5\cdot10^{-4}$ mol·dm^{-3} Eu(III)	704.00	604.00	100.00	2.25	0.49	$2.2\cdot10^{-5}$
Eu(III) + BPhen (1:1)	733.3	567.2	166.1	6.26	0.2865	$2.5\cdot10^{-6}$
Eu(III) + BPhen (1:2)	696.00	528.00	168	0.746	0.258	$3.02\cdot10^{-11}$
Eu(III) + BPhen (1:3)	760.00	576.6	183.4	9.8	0.259	$1.08\cdot10^{-9}$
Eu(III) + *N*-acetylhistidine (1:1)	681.8	486.36	195.44	6.31	0.243	$2.864\cdot10^{-11}$
Eu(III) + BPhen + *N*-acetylhistidine (1:1:1)	746.00	577.00	169.00	6.42	0.2719	$8.83\cdot10^{-11}$
Eu(III) + BPhen + *N*-acetylhistidine (1:2:1)	722.00	557.37	164.63	7.68	0.289	$1.19\cdot10^{-10}$
Eu(III) + BPhen + *N*-acetylhistidine (1:3:1)	740	563.93	176.06	3.66	0.2733	$2.89\cdot10^{-11}$

Table 7. Voltammetric Data and Transfer Coefficient (α) from the Cyclic Voltammograms of the Eu(III) + Bathophenanthroline (BPhen) + *N*-Acetylaspartic Acid System, in the 1.8 % v/v Ethanol + Water Mixture, at *I* = 0.1 mol·dm^{-3} *p*-Toluenesulfonate and at 25.0 °C ± 0.1 °C

system	E_p^c (mV)	$-E_{p/2}^c$ (mV)	$-(E_p^c - E_{p/2}^c)$	i_p^c (μA)	α	D_{red} (cm^2·s^{-1})
$5\cdot10^{-4}$ mol·dm^{-3} Eu(III)	704.00	604.00	100.00	2.25	0.49	$2.2\cdot10^{-5}$
Eu(III) + BPhen (1:1)	733.3	567.2	166.1	6.26	0.2865	$2.5\cdot10^{-6}$
Eu(III) + BPhen (1:2)	696.00	528.00	168	0.746	0.258	$3.02\cdot10^{-11}$
Eu(III) + BPhen (1:3)	760.00	576.6	183.4	9.8	0.259	$1.08\cdot10^{-9}$
Eu(III) + *N*-acetylaspartic acid (1:1)	672.72	536.36	136.36	3.63	0.349	$2.527\cdot10^{-11}$
Eu(III) + BPhen + *N*-acetylaspartic acid (1:1:1)	738.00	567.21	170.79	11.2	0.278	$2.62\cdot10^{-10}$
Eu(III) + BPhen + *N*-acetylaspartic acid (1:2:1)	746.00	573.77	172.23	6.46	0.276	$8.8\cdot10^{-11}$
Eu(III) + BPhen + *N*-acetylaspartic acid (1:3:1)	738.00	570.49	167.51	4.06	0.284	$3.38\cdot10^{-11}$

N-acetylaspartic acid > *N*-acetylhistamine was found. The observed trend of overall formation constants of the different ternary complexes formed in this study in terms of nucleotide or *N*-acetylamino acid may be attributed to the nature of the interaction of the Eu(III)− bathophenanthroline complex with the different nucleotides or *N*-acetylamino acids. To the auther's knowledge, no data for the ternary complex of the secondry ligand bathophenanthroline with nucleotides guanosine 5′-monophosphste, adenosine 5′-monophosphste, inosine 5′-monophosphste, cytidine 5′-monophosphste, or *N*-acetylaspartic acid or *N*-acetylhistamine are available in the literature for comparison. The higher values of the stability constants of the ternary complexes of the type Eu(III)−AMP−bathophenanthroline and Eu(III)−CMP−bathophenanthroline compared with those of the binary systems may be attributed to the interligand interactions or some coordinated ligands and possibly H-bond formation. It may also 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 of AMP, causing strengthening of the Eu(III)−N bond. Due to the back-donation from metal to the adenine base of AMP, the f electron content on the metal decreases, which renders the metal

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Table 8. Voltammetric Data and Transfer Coefficient (α) from the Cyclic Voltammograms of the Eu(III) + Bathophenanthroline (BPhen) + AMP System, in the 1.8 % v/v Ethanol + Water Mixture, at I = 0.1 mol·dm^{-3} p-Toluenesulfonate and at 25.0 °C ± 0.1 °C

system	E_p^c (mV)	$-E_{p/2}^c$ (mV)	$-(E_p^c - E_{p/2}^c)$	i_p^c (μA)	α	D_{red} (cm^2·s^{-1})
$5\cdot10^{-4}$ mol·dm^{-3} Eu(III)	704.00	604.00	100.00	2.25	0.49	$2.2\cdot10^{-5}$
Eu(III) + BPhen (1:1)	733.3	567.2	166.1	6.26	0.2865	$2.5\cdot10^{-6}$
Eu(III) + BPhen (1:2)	696.00	528.00	168	0.746	0.258	$3.02\cdot10^{-11}$
Eu(III) + BPhen (1:3)	760.00	576.6	183.4	9.8	0.259	$1.08\cdot10^{-9}$
Eu(III) + AMP (1:1)	737.00	554.00	183.00	6.00	0.26	$5.179\cdot10^{-11}$
Eu(III) + BPhen + AMP (1:1:1)	718.00	550.81	167.19	3.93	0.2846	$6.32\cdot10^{-11}$
Eu(III) + BPhen + AMP (1:2:1)	678.00	537.7	140.3	9.8	0.339	$3.3\cdot10^{-10}$
Eu(III) + BPhen + AMP (1:3:1)	720.00	554.9	165.1	5.76	0.288	$1.34\cdot10^{-10}$

Table 9. Voltammetric Data and Transfer Coefficient (α) from the Cyclic Voltammograms of the Eu(III) + Bathophenanthroline (BPhen) + IMP System, in the 1.8 % v/v Ethanol + Water Mixture, at I = 0.1 mol·dm^{-3} p-Toluenesulfonate and at 25.0 °C ± 0.1 °C

system	E_p^c (mV)	$-E_{p/2}^c$ (mV)	$-(E_p^c - E_{p/2}^c)$	i_p^c (μA)	α	D_{red} (cm^2·s^{-1})
$5\cdot10^{-4}$ mol·dm^{-3} Eu(III)	704.00	604.00	100.00	2.25	0.49	$2.2\cdot10^{-5}$
Eu(III) + BPhen (1:1)	733.3	567.2	166.1	6.26	0.2865	$2.5\cdot10^{-6}$
Eu(III) + BPhen (1:2)	696.00	528.00	168	0.746	0.258	$3.02\cdot10^{-11}$
Eu(III) + BPhen (1:3)	760.00	576.6	183.4	9.8	0.259	$1.08\cdot10^{-9}$
Eu(III) + IMP (1:1)	700.00	549.00	151.00	3.58	0.3152	$1.844\cdot10^{-11}$
Eu(III) + BPhen + IMP (1:1:1)	726.00	557.37	168.63	4.88	0.282	$9.83\cdot10^{-11}$
Eu(III) + BPhen + IMP (1:2:1)	756.00	565.57	190.43	8.94	0.2499	$1.9\cdot10^{-10}$
Eu(III) + BPhen + IMP (1:3:1)	744.00	563.93	180.07	9.4	0.264	$1.95\cdot10^{-10}$

Table 10. Voltammetric Data and Transfer Coefficient (α) from the Cyclic Voltammograms of the Eu(III) + Bathophenanthroline (BPhen) + CMP System, in the 1.8 % v/v Ethanol + Water Mixture, at I = 0.1 mol·dm^{-3} p-Toluenesulfonate and at 25.0 °C ± 0.1°C

system	E_p^c (mV)	$-E_{p/2}^c$ (mV)	$-(E_p^c - E_{p/2}^c)$	i_p^c (μA)	α	D_{red} (cm^2·s^{-1})
$5\cdot10^{-4}$ mol·dm^{-3} Eu(III)	704.00	604.00	100.00	2.25	0.49	$2.2\cdot10^{-5}$
Eu(III) + BPhen (1:1)	733.3	567.2	166.1	6.26	0.2865	$2.5\cdot10^{-6}$
Eu(III) + BPhen (1:2)	696.00	528.00	168	0.746	0.258	$3.02\cdot10^{-11}$
Eu(III) + BPhen (1:3)	760.00	576.6	183.4	9.8	0.259	$1.08\cdot10^{-9}$
Eu(III) + CMP (1:1)	757.37	574.19	186.18	8.2	0.2598	$1.934\cdot10^{-10}$
Eu(III) + BPhen + CMP (1:1:1)	740.00	572.13	167.87	6.315	0.2835	$8.19\cdot10^{-11}$
Eu(III) + BPhen + CMP (1:2:1)	734.00	563.93	170.07	9.368	0.2798	$1.83\cdot10^{-10}$
Eu(III) + BPhen + CMP (1:3:1)	730.00	567.21	162.27	17.36	0.2924	$6.00\cdot10^{-10}$

more electrophilic. The interaction of the π electrons of the secondary ligands with the metal will increase to a greater extent, and that sequentially enhances the formation of the mixed ligand complex.

The titration curve (figure not shown) for Eu(III)−5′-GMP−bathophenanthroline (BPhen) lies between Eu(III)−5′-GMP and Eu(III)−BPhen, which idicates that the mixed ligand complex occurs via the coordination of the protonated form of BPhen with the binary Eu(III)−5′-GMP, the formation constant of which is relatively high. On the other hand, the titration curve (figure not shown) for Eu(III)−5′-IMP−BPhen lies beyond the corresponding binary complex titration curves Eu(III)−5′-IMP and Eu(III)−BPhen, where the two ligands coordinate to the central metal ion in their unionized form, where the formed complexes are in the diprotonated form Eu(III)(H-5′-IMP)(HBPhen).

Spectrophotometric Measurement. As shown in Figure 1, the aqua $Eu(H_2O)_9^{3+}$ complex acquires an intense absorption peak (λ = 225 nm), and the free bathophenanthroline ligand exhibits a high absorption peak with a slight shift to blue due to the complexation between bathophenanthroline and Eu(III). A new band appears at λ = 290 nm due to the perturbation of the

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Table 11. Voltammetric Data and Transfer Coefficient (α) from the Cyclic Voltammograms of the Eu(III) + Bathophenanthroline (BPhen) + GMP System, in the 1.8 % v/v Ethanol + Water Mixture, at I = 0.1 mol·dm^{-3} *p*-Toluenesulfonate and at 25.0 °C ± 0.1°C

system	E_p^c (mV)	$-E_{p/2}^c$ (mV)	$-(E_p^c - E_{p/2}^c)$	i_p^c (μA)	α	D_{red} (cm^2·s^{-1})
$5\cdot10^{-4}$ mol·dm^{-3} Eu(III)	704.00	604.00	100.00	2.25	0.49	$2.2\cdot10^{-5}$
Eu(III) + BPhen (1:1)	733.3	567.2	166.1	6.26	0.2865	$2.5\cdot10^{-6}$
Eu(III) + BPhen (1:2)	696.00	528.00	168	0.746	0.258	$3.02\cdot10^{-11}$
Eu(III) + BPhen (1:3)	760.00	576.6	183.4	9.8	0.259	$1.08\cdot10^{-9}$
Eu(III) + GMP (1:1)	664.5	529.00	135.5	10.19	0.3512	$7.47\cdot10^{-11}$
Eu(III) + BPhen + GMP (1:1:1)	756.66	576.6	180.06	12.33	0.264	$3.35\cdot10^{-10}$
Eu(III) + BPhen + GMP (1:2:1)	824.00	600.00	224.00	3.8	0.2124	$3.96\cdot10^{-11}$
Eu(III) + BPhen + GMP (1:3:1)	760.00	557.04	202.96	17.8	0.2344	$7.87\cdot10^{-10}$

peak which confirms the formation of a stable complex. The complexation of bathophenanthroline to the Eu(III)−*N*-acetylhistamine binary complex will shift the absorption peak into a small shoulder and a well-resoluted peak at a longer wavelength. A decrease in the absorptivity occurs upon reacting with a second molecule of bathophenanthroline with a slight shift to a longer wavelength. A simultaneous decrease in the absorbance value is observed upon addition of the third molecule of bathophenanthroline to form a ternary complex of a 1:1:3 ratio for the Eu(III)−*N*-acetylhistamine−bathophenanthroline mixed ligand complex as shown in Figure 2(I).

The coordination of bathophenanthroline with the Eu(III)−*N*-acetylhistidine binary complex shifts the absorption peak slightly to a longer wavelength with increasing absorptivity. The absorption peak does not change greatly with variation of the number of moles added of BPhen; i.e., there is hindering of attack of this ligand to Eu(III)−*N*-acetylhistidine. This observation is in a good agreement with the potentiometric results as in Figure 2(II).

The interaction of one mole of BPhen with Eu(III)−*N*-acetylaspartic acid shifts the absorption peak of the latter to red with a subsequent decrease in absorption intensity. Increasing the number added of moles of BPhen to two or three results in an increase of the absorbance value, but a steric hindrance factor between the third molecule of BPhen and Eu(III)−*N*-acetylaspartic acid−$(BPhen)_2$ has been indicated as shown in Figure 2(III).

Fluorometric Measurement. The bathophenanthroline (BPhen) ligand in the mixed ethanol−water mixture exhibits one sharp emission band which is located at λ = 647 nm (high intensity, 531.44 au) and one broad band at λ = 758 nm of intensity 33.71 au.

The interaction of the BPhen free ligand with Eu(III) results in the formation of the corresponding binary complex, which acquires an emission peak at λ = 595 nm, which is attributed to a $^5D_0 \rightarrow {}^7F_1$ transition type but of relatively low intensity (4.6 au); i.e., the Eu(III) metal ion acts as a quencher for the ligand of high aromaticity. Excitation wavelengths are given in the figures showing emission spectra of all complexes. Generally, all the Eu(III) complexes should show four emission peaks over the range examined in this study for emission to the 7F1, 7F2, 7F3, and 7F4 levels. This is not true in our case. Some of these emission peaks already exist as shown in the figures given for different binary and ternary Eu(III) complexes. In some cases, ligand-based excitations cause structural emission of Eu(III) complexes, and the ligand fluorescence is quenched showing that ligand-to-metal energy transfer occurs. This behavior is clearly the case in the emission spectra for Eu(III)−*N*-acetylamino acid binary complexes. In some Eu(III)−*N*-acetylamino acid binary complexes, there are no luminescence spectra that show Eu(III) ion based luminescence in the present work. This behavior may be attributed to a nonradiative quenching process through energy transfer to vibrational modes that match the energy of the excited state of the Eu(III) ion.

A comparative study is performed among three types of *N*-acetylamino acids (*N*-acetylhistidine, *N*-acetylhistamine, *N*-acetylaspartic acid) as binary complexes in addition to the corresponding ternary ones with BPhen as shown in Figure 3.

Considering that *N*-acetylamino acid is the primary ligand and BPhen is the secondary ligand according to the following equilibria

$$Eu^{3+} + NAC \rightarrow [Eu(NAC)]^{n+} \quad (7)$$

$$[Eu(NAC)]^{n+} + BPhen \rightarrow [Eu(NAC)(BPhen)]^{n+}$$

The ternary complex of the type Eu(III)−*N*-acetylaspartic acid−BPhen has an emission peak at λ = 585 nm of intensity 8.38 au. This transition could be assigned to $^5D_0 \rightarrow {}^7F_1$ type here again, and it is clearly observed that there is a decrease in the emission peak at λ = 592 nm ($^5D_0 \rightarrow {}^7F_1$ transition).

The mixed ligand complex containing Eu(III)−*N*-acetylhistidine−BPhen has three emission bands at λ_1 = 592 nm (8.32 au), λ_2 = 615 nm (15.82 au), and λ_3 = 759 nm (23.42 au), respectively. The *N*-acetylhistidine binary complex with Eu(III) exhibits two bands located at λ = 595 nm of intensity 18.00 au and at λ = 752 nm of 20.20 au.

The band observed at λ = 615 nm for the ternary complex is not located in the binary complexes, and this band could be assigned to a $^5D_0 \rightarrow {}^7F_2$ transition type. The band at 595 nm for the binary Eu(III)−*N*-acetylhistidine is lowered upon reacting with the BPhen ligand molecule, where the peak located at λ = 752 nm increases in its intensity in addition to the formation of the new band at λ = 615 nm.

The Eu(III)−*N*-acetylhistamine binary complex exhibits two emission bands. The first is located at λ_1 = 559 nm which could be attributed to a $^5D_0 \rightarrow {}^7F_0$ transition of intensity 17.50 au, while the second is observed at λ_2 = 762 nm which could be attributed to a $^5D_0 \rightarrow {}^7F_5$ transition of intensity 19.00 au.

The ternary complex containing BPhen has only one emission band at λ = 584 nm which could be assigned to a $^5D_0 \rightarrow {}^7F_1$ transition of intensity equal to 7.75 au.

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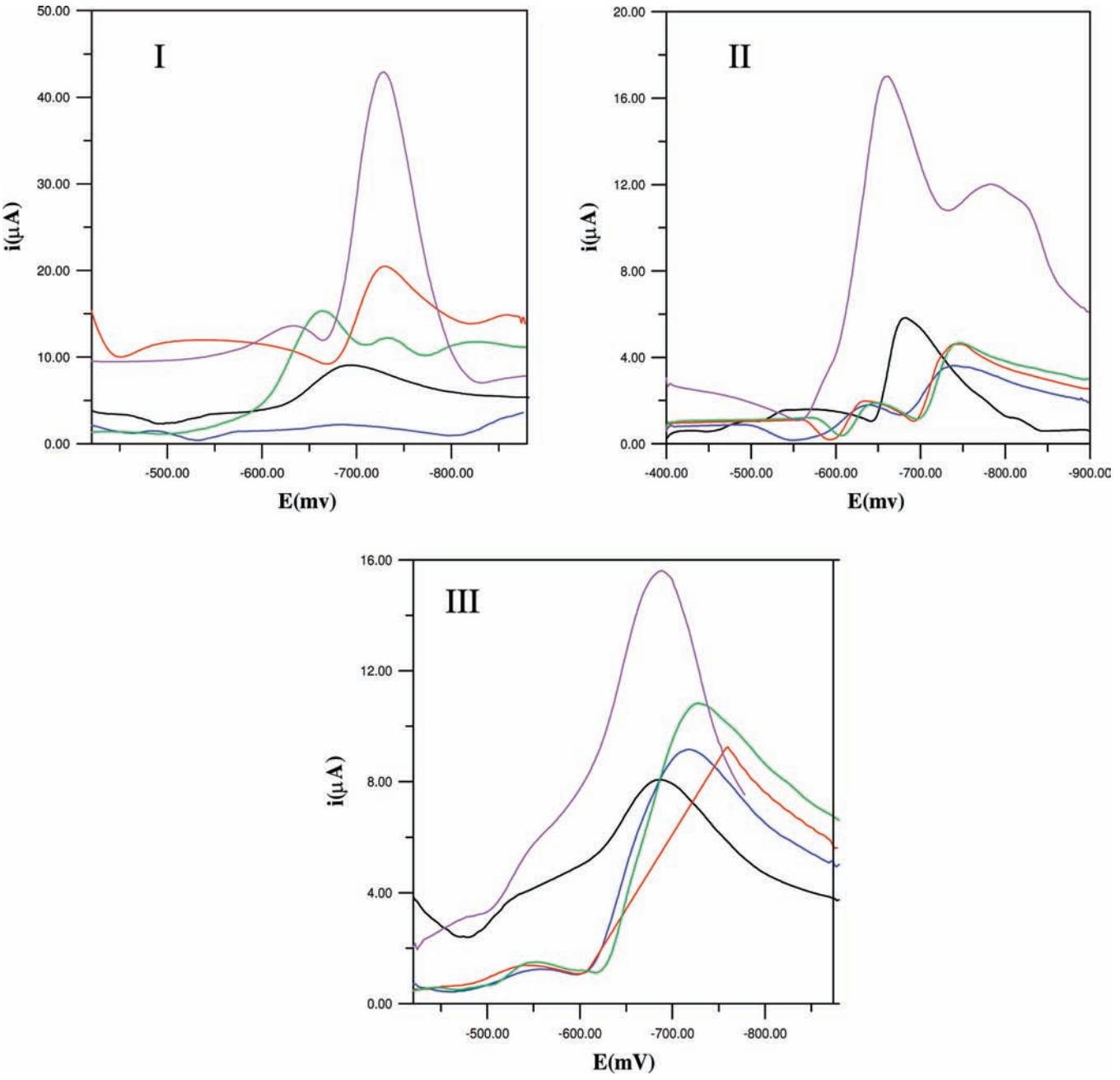

Figure 7. Effect of frequency on the square wave voltammograms of Eu(III) + *N*-acetylamino acid (NAC) + bathophenanthroline (BPhen) ternary systems at I = 0.1 mol·dm^{-3} *p*-toluenesulfonate and at 25.0 °C. $C_{Eu(III)} = 5 \cdot 10^{-4}$ mol·dm^{-3}, $C_{NAC} = 5 \cdot 10^{-4}$ mol·dm^{-3}, and $C_{Batho} = 5 \cdot 10^{-4}$ mol·dm^{-3}. (a) Black line, f = 20 (Hz); (b) blue line, f = 40 (Hz); (c) red line, f = 60 (Hz); (d) green line, f = 80 (Hz); (e) purple line, f = 100 (Hz). (I) NAC = *N*-acetylaspartic acid; (II) NAC = *N*-acetylhistamine, (III) NAC = *N*-acetylhistidine.

The comparative study is extended to the ternary complexes containing the nucleotides (5′-GMP, 5′-AMP, and 5′-CMP), where the first two represent purine type nucleotides while the third is of a pyrimidine type as shown in Figure 4.

The mixed ligand complexes including Eu(III)−5′-GMP−BPhen have a completely different behavior than the two binary complexes, where the Eu^{3+}−5′-GMP complex has one characteristic emission band at λ = 692 nm which could be assigned to a $^5D_0 \rightarrow {}^7F_4$ transition, with an intensity of 19.26 au. The ternary complex shows different behavior where two emitted bands are observed at λ_1 = 576 nm (7.36 au) and λ_2 = 590 nm (7.38 au), which could be assigned to a $^5D_0 \rightarrow {}^7F_1$ transition. The ternary complex containing Eu(III)−5′-CMP−BPhen exhibits four emission bands at (559, 614, 655, and 672) nm of intensities (15.62, 17.25, 14.45, and 14.39) au, respectively. This emission spectrum is completely different from that of Eu(III)−5′-CMP and Eu(III)−BPhen which confirm the formation of the ternary complex, and the nature of bonding is of a completely different nature, since Eu(III)−5′-CMP exhibits only one emission peak at λ = 610 nm which may be attributed to a $^5D_0 \rightarrow {}^7F_2$ transition of relatively high intensity (95 au).

The formation of the ternary complex of the type Eu(III)−BPhen−5′-AMP is accompanied by a slight increase in the emission band at λ = 544 nm of intensity 18.66 au. The emission band at λ = 575 nm which is a $^5D_0 \rightarrow {}^7F_1$ transition is shifted

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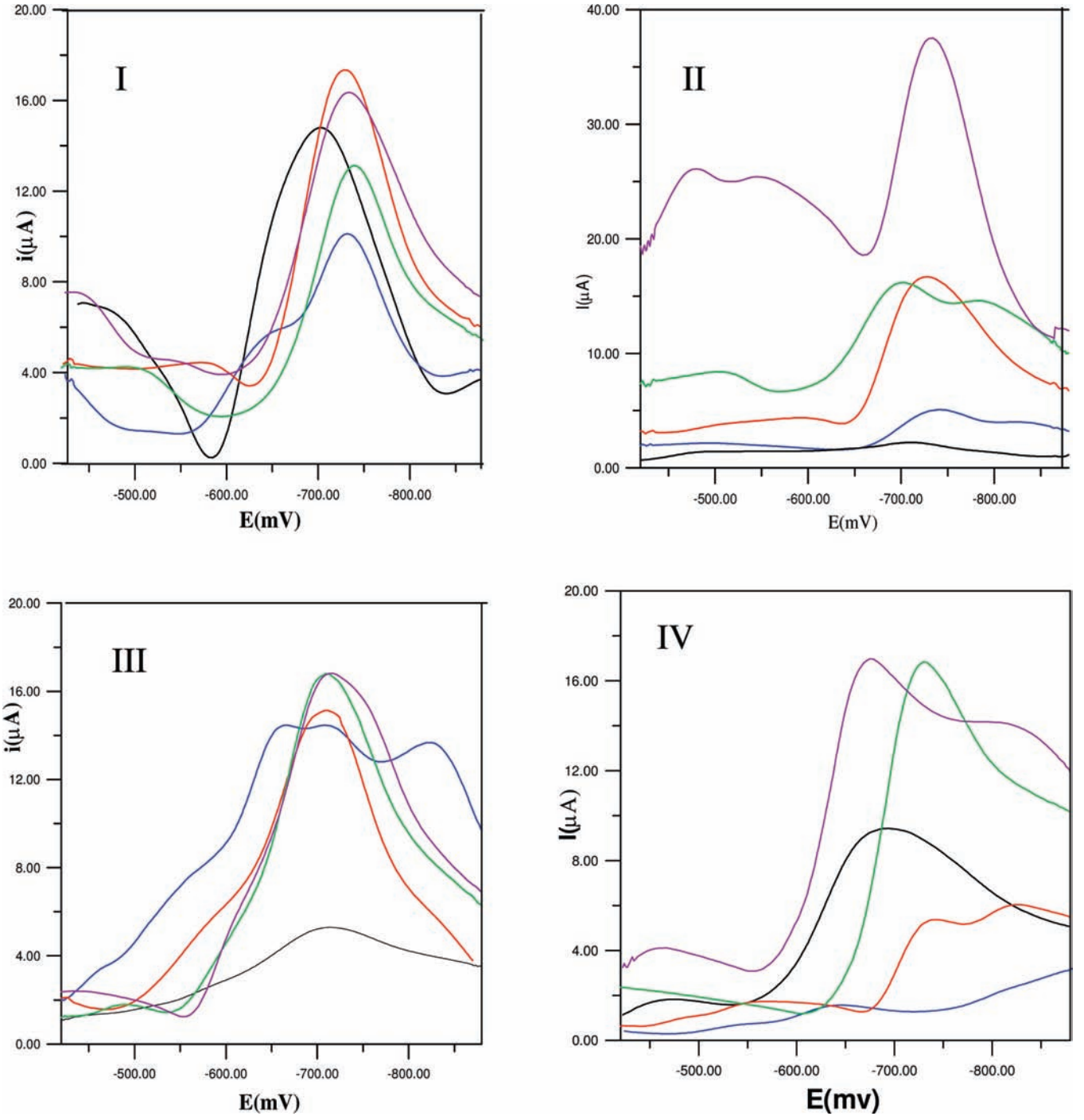

Figure 8. Effect of frequency f on the square wave voltammograms of Eu(III) + nucleotide (Nu) + bathophenanthroline (BPhen) ternary systems at I = 0.1 mol·dm^{-3} p-toluenesulfonate and at 25.0 °C. $C_{Eu(III)} = 5 \cdot 10^{-4}$ mol·dm^{-3}, $C_{NAC} = 5 \cdot 10^{-4}$ mol·dm^{-3}, and $C_{Batho} = 1.5 \cdot 10^{-3}$ mol·dm^{-3}. (a) Black line, f = 20 (Hz); (b) blue line, f = 40 (Hz); (c) red line, f = 60 (Hz); (d) green line, f = 80 (Hz); (e) purple line, f = 100 (Hz). (I) Nu = GMP, (II) Nu = IMP, (III) Nu = CMP, (IV) Nu = AMP.

to a new band at λ = 591 nm of intensity 21.58 au. Two new bands are observed at λ = 614 nm ($^5D_0 \rightarrow {}^7F_2$ transition) of intensity 31.51 au and at λ = 694 nm ($^5D_0 \rightarrow {}^7F_4$ transition) of intensity 19.24 au.

Figure 5a,b illustrates the fluorescence spectra for the Eu(III)−bathophenanthroline complex with Calf Thymus DNA (CT-DNA) in a concentration range of $1 \cdot 10^{-5}$ mol·dm^{-3} to $0.5 \cdot 10^{-8}$ mol·dm^{-3}. Where the characteristic emission peak of Eu(III)−BPhen is located at λ = 590 nm, this transition $^5D_0 \rightarrow {}^7F_1$ is increased in intensity to a limit value of $0.25 \cdot 10^{-5}$ mol·dm^{-3}. The effect of CT-DNA concentration on the intensity of the Eu(III)−BPhen complex emission is performed in Figure 5c which explains the linear behavior between $I - I_0/I_0$ and the molar concentration of the CT-DNA, where I is the intensity of the emitted peak with DNA and I_0 is the intensity of the Eu(III)−BPhen binary complex.

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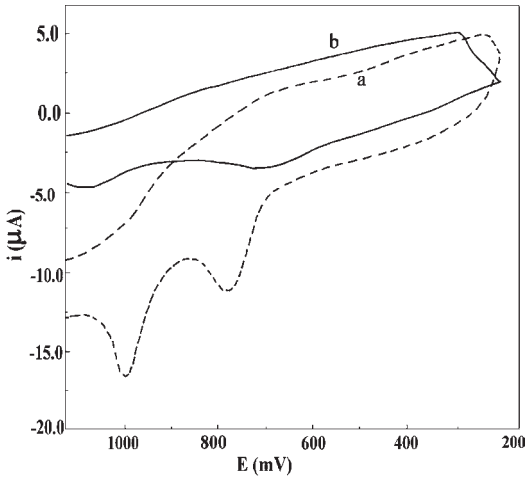

Figure 9. Cyclic voltammogram for CT-DNA interaction with the Eu(III)−bathophenanthroline (BPhen) complex, in 1.8 % v/v ethanol−water mixture, $\nu = 100\ mV \cdot s^{-1}$ and at 25.0 °C. (a) - - -, $2.58 \cdot 10^{-4}$ mol·dm^{-3} CT-DNA. (b) —, $2.58 \cdot 10^{-4}$ mol·dm^{-3} CT-DNA + $1 \cdot 10^{-4}$ mol·dm^{-3} Eu(III)− BPhen complex.

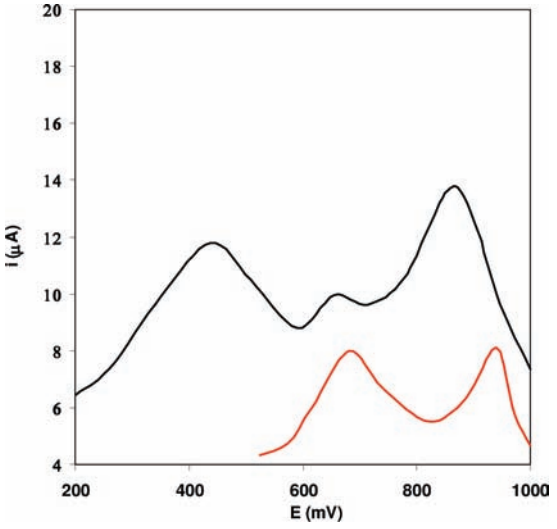

Figure 10. Differential pulse voltammetry for the CT-DNA interaction with the Eu(III)−bathophenanthroline (BPhen) complex, in the 1.8 % v/v ethanol−water mixture, $\nu = 36.6\ mV \cdot s^{-1}$, and at 25.0 °C. (a) Red line, $2.58 \cdot 10^{-4}$ mol·dm^{-3} CT-DNA; (b) black line, $2.58 \cdot 10^{-4}$ mol·dm^{-3} CT-DNA + $1 \cdot 10^{-4}$ mol·dm^{-3} Eu(III)−BPhen complex.

Electrochemical Behavior of the Eu(III)−Bathophenanthroline Complex. Confirmation of the formation of the ternary complexes of the type Eu(III)−bathophenanthroline−nucleotide and Eu(III)−bathophenanthroline−*N*-acetylamino acids in solution has been carried out using cyclic voltammetry and square wave voltammetry on a glassy carbon electrode. Figure 6 shows the electrochemical behavior of some representative systems. All the cyclic voltammetric diagrams confirm the formation of the different binary and ternary complexes that have been found using potentiometric titrations. The irreversible nature of the electrochemical reaction for the binary and ternary systems under investigation is quite clear from the shape of their cyclic voltammograms obtained at the glassy carbon electrode. The CV response for the binary and ternary complexes under investigation on the glassy carbon electrode reveals a one-electron reduction process.

The diffusion coefficients of the binary complexes Eu(III)−nucleotide and Eu(III)−*N*-acetylamino acid have been calculated using the equations[29]

$$i_p^c = 0.496nFCAD^{1/2}(\alpha n_\alpha F\nu/RT)^{1/2} \quad (8)$$

$$E_p^c - E_{p/2}^c = -1.857RT/\alpha n_\alpha F \quad (9)$$

where i_p^c is the peak cathodic current; A is the area of the electrode (cm^2); C is the bulk concentration of the active species (mol·dm^{-3}); D is the diffusion coefficient (cm^2·s^{-1}); ν is the potential sweep rate (V·s^{-1}); n is the number of electrons involved in the reaction; α is the transfer coefficient; E_p^c is the peak cathodic potential; $E_{p/2}{}^c$ is the half peak potential of the cathodic wave; n_α is the number of electrons at the rate-determining step; R is the gas constant; F is the faraday; and T is the absolute temperature. Voltammetric data, transfer coefficients (α), and diffusion coefficients from the cyclic voltammograms of different ternary systems investigated are given in Tables 5 to 11.

As indicated in the above-mentioned tables, for the ternary complexes Eu(III)−nucleotides−bathophenanthroline in a 1:1:1 ratio, the diffusion coefficient values follow the order 5′-GMP > 5′-IMP > 5′-CMP > 5′-AMP; i.e., the complex species containing the purine G form acquires the higher rate of diffusion to the electrode surface, while the species including the purine A form has the lowest value.

The reverse trend is observed for the mixed ligand complexes of a 1:1:2 ratio (upon increasing the concentration of bathophenanthroline from one to two). Again for ternary complexes containing 1:1:3 where a third molecule of bathophenanthroline is added, the complexes containing the 5′-GMP nucleotide molecule have the highest diffusion coefficient value.

The square wave voltammograms shown in Figures 7 and 8 confirm the formation of different ternary systems. It is quite interesting to observe that changing the frequency from (20 to 100) 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 oxidation of the CT-DNA on the surface of the glassy carbon electrode was studied in 0.1 M phosphate buffer at 25.0 °C, where two oxidation peaks are observed at (0.78 and 1.10) V with respect to SCE in the cyclic voltammogram. The two anodic peaks are attributed to the oxidation of guanine and adenine bases. The obtained results are in good agreement with the values found in the literature.[30] As depicted in Figure 9, the two oxidation peaks disappear due to the binding of the complex to DNA.

This behavior may be attributed to the possible formation of ternary complexes of the type Eu(III)−bathophenanthroline−guanine or Eu(III)−bathophenanthroline−adenine on the glassy carbon electrode with different redox properties. The differential pulse polarogram (DPP) for the DNA oxidation on the glassy carbon electrode surface also displays two well-resoluted oxidation peaks at (0.70 and 0.95) V which may be attributed to the characteristic anodic oxidation of guanine and adenine nucleobases in the DNA molecule.

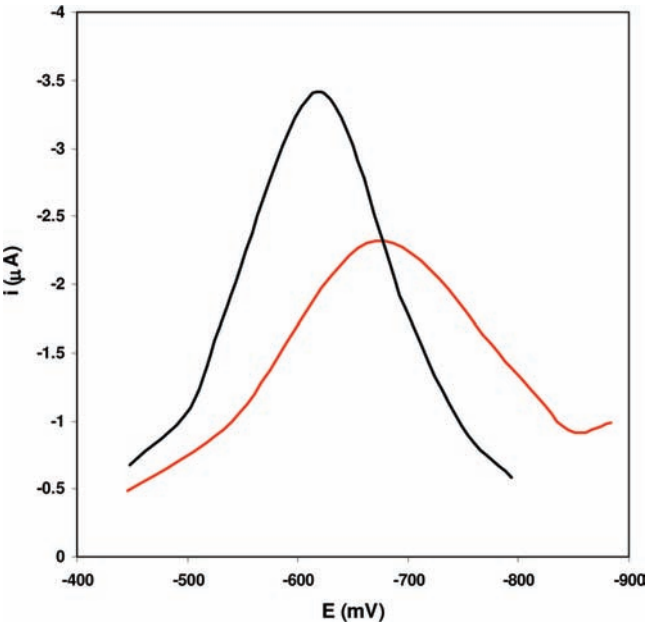

Figure 11. Differential pulse voltammetry for the Eu(III)−bathophenanthroline (BPhen) complex solution interaction with CT-DNA, in the 1.8% v/v ethanol−water mixture, $\nu = 5\ mV \cdot s^{-1}$, and at 25.0 °C. (a) Red line, $1 \cdot 10^{-4}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-4}$ mol·dm^{-3} BPhen complex solution; (b) black line, $2.58 \cdot 10^{-4}$ mol·dm^{-3} CT-DNA + $1 \cdot 10^{-4}$ mol·dm^{-3} Eu(III) + $1 \cdot 10^{-4}$ mol·dm^{-3} BPhen.

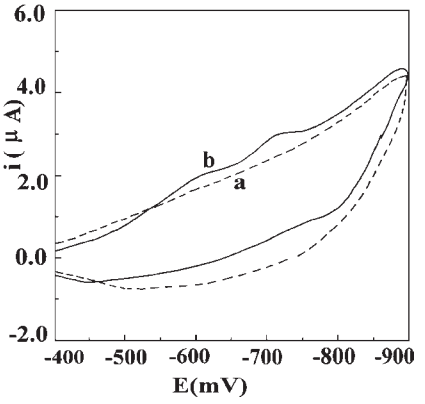

Figure 12. Cyclic voltammogram for the Eu(III)−bathophenanthroline (BPhen) complex solution interaction with CT-DNA, in the 1.8 % v/v ethanol + water mixture, $\nu = 100\ mV \cdot s^{-1}$, and at 25.0 °C. (a) Red line, $1 \cdot 10^{-4}$ mol·dm^{-3} Eu(III)−BPhen complex solution. (b) Black line, $1 \cdot 10^{-4}$ mol·dm^{-3} Eu(III) + BPhen + $2.58 \cdot 10^{-4}$ mol·dm^{-3} CT-DNA.

The interaction of the solid complex dissolved in ethanol with the CT-DNA molecule will perturb the two peaks, where the oxidation peak of guanine is split into two oxidation peaks at (663.12 and 463.12) mV. A considerable shift in the anodic oxidation peak of adenine from (942.08 to 863.12) mV is observed. The interaction of the complex with CT-DNA is accompanied by an increase of the oxidation current; i.e., the combination of the complex with the DNA gives a product of high adsorption capability on the electrode surface as shown in Figure 10.

The electrochemical behavior of the Eu(III)−bathophenanthroline complex in ethanol indicates that a reduction peak at 650 mV occurs in the interaction of the CT-DNA with the complex.

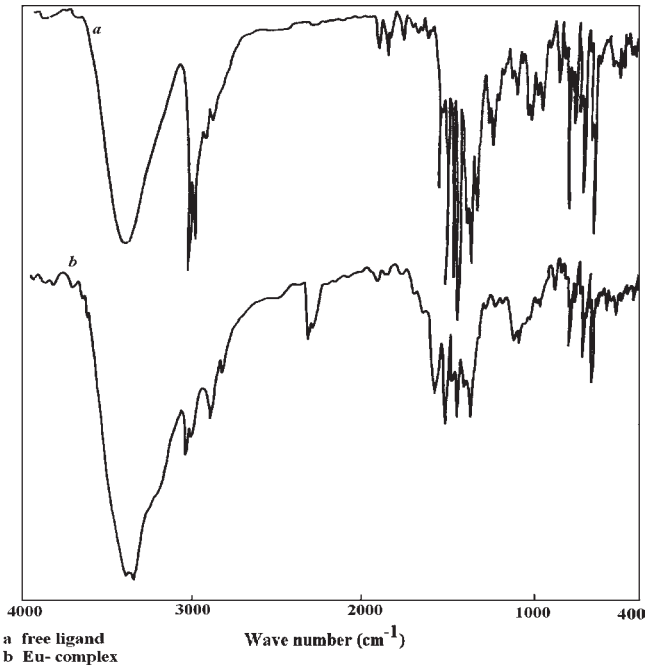

Figure 13. IR spectra for the synthesized Eu(III)−bathophenanthroline complex: (a) free ligand, (b) Eu(III)−Bphen complex.

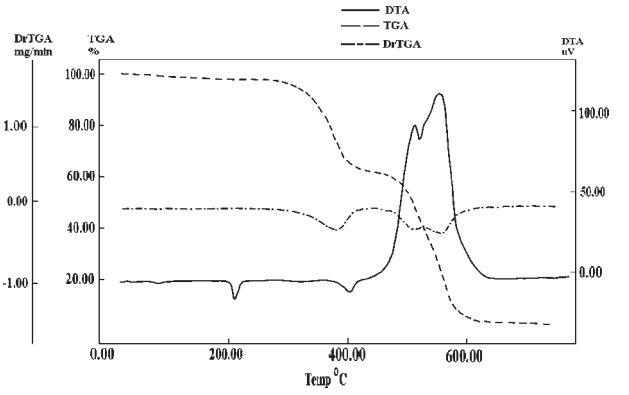

Figure 14. Thermal analysis of the synthesized Eu(III)−bathophenanthroline complex. —, DTA; - - -, TGA; -·-·-, DRTGA.

This will shift the cathodic peak to a less negative value indicating a considerable interaction between the complex with CT-DNA, as depicted in the differential pulse polarogram shown in Figure 11.

As shown in Figure 12, the cyclic voltammogram for the complex dissolved in the mixed ethanol−water solvent shows two small reduction peaks at −(650 and 725) mV. The reaction of the complex with CT-DNA is accompanied by a diminishing of the cathodic peaks characterizing the Eu(III)−bathophenanthroline complex. This result confirms the binding of the complex to the CT-DNA molecule. There is enhancement of the fluorescence after addition of CT-DNA. On the basis of our comparative fluorescence spectra for Eu(III)−bathophenanthroline−nucleotides, there is a great selectivity for complexation toward the 5′-AMP nucleotide. This result coupled with the data obtained by differential pulse voltammmetry and cyclic voltammetry may indicate the coordination mode of interaction of our Eu(III)−bathophenanthroline complex with CT-DNA. The in vitro cytotoxicity assays of six new complexes including Eu(III)−bathophenanthroline against cell lines A2780

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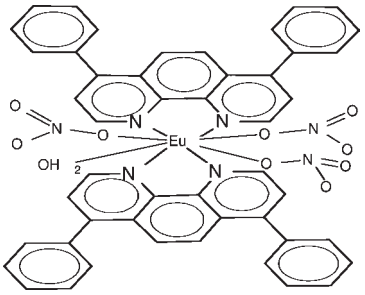

Figure 15. Proposed structure of Eu(III)−BPhen.

(human ovarian carcinoma), A2780R cisplatin-resistant, cisplatin-sensitive L1210/0, and cisplatin-resistant mouse leukemia L1210/2 are now under investigation in one of our collaborations and will be the subject of a further publication. Experimental results are summarized to show that the appropriate use of the regulatory effects of Ln would be useful in therapeutical application. The perforation of cell membranes and apoptosis induced by Ln as well as their influence on ROS-mediated oxidative damages and on the assembly and stability of the cytoskeleton may be considered as the potential pharmacological action.[31] The carcogenic cells that respond to the attacking Eu(III)−bathophenanthroline complex can be considered as a multiple-target system, in which various reactions with various targets are organized in a sequence of events. The ultimate biological effect is actually the integrated effects of these different events including possible high stacking interactions with the three bathophenanroline moieties of the complex under investigation. The mode of interactions when the Eu(III)−bathophenanthroline complex attacks a cancer cell and finally induces apoptosis might be considered as the core of its expected anticancer activity as our preliminary results indicate.

Synthesis and Characterization of the Eu(III)−bathophenanthroline Complex. The solid complex of Eu(III)−bathophenanthroline synthesized according to the experimental procedure is well characterized and acquires the formula $C_{48}H_{33}N_7O_{10}Eu$ according to elemental analysis, molar conductivity and thermal analysis. The analytical data for this complex are collected in Table 1.

Inspecting the IR spectra shown in Figure 13 for the synthesized complex, it is clearly observed that the free ligand acquires a strong absorption band at υ = 1605 cm^{-1} which is attributed to the stretching frequency of υC = N. This band is shifted to a longer wavenumber for the complex which indicates the binding of the heteroatom (nitrogen) of the aromatic moiety to the Eu metal ion. The band observed at 1480 cm^{-1} could be assigned to the monodentate coordinated nature of the nitrate anion where the characteristic band of the bidentate behavior of the nitrate anion is absent[32] (in the range of 1700−1800 cm^{-1}). The band observed at 3369.03 cm^{-1} could be attributed to the presence of a coordinated water molecule.

Thermal Analysis. The thermal behavior of the Eu(III)−BPhen complex is shown in Figure 14. As depicted in the differential thermal analysis curve, two endothermic peaks are observed: the first takes place at 215.98 °C which is accompanied by a weight loss of 1.56 %. This weight loss could be assigned to the loss of one coordinated water molecule (the theoretical value is 1.76 %). A second endothermic change occurs at 410.62 °C with a weight loss of 3.46 % due to loss of a NO_2 molecule arising from decomposition of the nitrate anion (theoretical value = 4.58 %). Two simultaneous exothermic peaks are observed at (519.68 and 559.72) °C which may be attributed to the decomposition of the complex into the metal oxide Eu_2O_3 and a carbon residue.

The structure of the synthesized complex can be speculated in Figure 15, where it acquires a nonelectrolytic nature.

CONCLUSION

The solid complex of Eu(III)−bathophenanthroline has been synthesized and characterized. The interaction of the complex with CT-DNA has been investigated by fluorescence and electrochemical methods including cyclic voltammetry and differential pulse polarography on a glassy carbon electrode. The formation of binary and ternary complexes of Eu(III)−nucleotides guanosine 5′-monophosphate (5′-GMP), adenosine 5′-monophosphate (5′-AMP), inosine 5′-monophosphate (5′-IMP), cytidine 5′-monophosphate (5′-CMP), or *N*-acetylamino acids (*N*- acetylaspartic acid, *N*-acetylhistidine, *N*-acetylhistamine), and bathophenanthroline (BPhen) has been confirmed using UV−visible spectrophotometry, cyclic voltammetry (CV), square wave voltammetry (SWV), and emission spectrofluorimetric measurements.

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