CATRINA (2013), 8 (1): © 11-19
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Spectroscopic Studies for the Interaction of Eu(III)- 3-oxo-3*H*-benzo-[f]chromene-2-Carboxylic Acid with Nucleosides, Nucleotides or DNA

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ABSTRACT

The absorption and fluorescence measurements are performed for the interaction of Eu(III) with synthesized coumarin derivative 3-oxo-3H-benzo[f]chromene-2-carboxylic acid (BCCA) in different solvents. The results showed that the most suitable solvents for tracing the emission bands for Eu(III) are methanol and DMSO. Confirmation of the formation of binary and ternary complexes Eu(III)-(BCCA), Eu(III)-(BCCA)-(NS), Eu(III)-(BCCA)-NU nucleosides (NS)= Adenosine, Guanosine, Inosine and Cytidine, nucleotides (NU)=5'-GMP, 5'-ATP and 5'-IMP in solution has been studied using UV-visible spectroscopic and luminescence measurements. Also the interaction of the binary complex Eu(III)-(BCCA) is performed with DNA using the same techniques. The binding parameters of the binary complex with DNA and its compartments are calculated and the mode of interaction with DNA is evaluated.

Keywords: Binding parameters, Fluorescence Measurements, Nucleosides, Nucleotides.

INTRODUCTION

The interaction of deoxyribonucleic acid (DNA) with small molecules is very important in the development of novel chemotherapeutics which are highly sensitive diagnostic agents, and in the design of new and more efficient drugs targeting DNA (Ambert *et al*., 1986; Barton *et al*., 1986; Hall *et al*., 1996; Jackson *et al*., 1999; Ji *et al*., 2001). The metal complexes can interact with nucleic acids in different binding modes which depend on the sizes and stereochemical properties of the metal complexes.

The binding modes are intercalation when the ligand contains planar ring systems, groove binding for large molecules or external electrostatic binding for cations. It's well known that the intercalation is the strongest binding modes. Eu(III) is one of the best studied lanthanide ions in fluorimetric measurements where it's excited states have long lifetimes and distinct narrow emission bands.

They are ideally suited for application as fluorescent probes (Robinson *et al*., 2000; Rutao *et al*., 2002) and as optical signal amplifiers (Steemers *et al*., 1995; Kim *et al*., 2003). Coumarin derivatives are of an interest due to their many biological activitys such as anti-tumor (Mohammad *et al*., 2011), anticoagulant, anti-inflammatory and antioxidant (Kokotos *et al*., 1997; Karaliota *et al*., 2001). It has been found that the binding of lanthanide metal ions to the coumarin moiety retains or even enhances its biological activity (Singh *et al*., 1980; Karaliota *et al*., 2001; Kostova *et al*., 2001).

The coordination compounds of rare earth ions have recently attracted much attention as a probe to study nucleic acids. 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) (Sato *et al*., 1970; Niyama *et al*., 2005; Azab *et al*., 2010). The antenna chromophore in our study is 3-oxo-3*H*-benzo[f]chromene-2-carboxylic acid (BCCA). In the present work, the interaction of Eu(III) metal ion with BCCA, nucleotides such as: guanosine 5′-monophosphate (5′-GMP), adenosine 5′-triphosphate (5′-ATP), Inosine 5'-triphosphate(5'-IMP), nucleosides as: Adenosine, Guanosine, Inosine and Cytidine.

DNA was studied by spectro-fluorimetric. The binary system Eu(III)-BCCA can be considered as a model for the development of fluorescent probes for DNA fragments. The structure of the synthesized ligand is shown in the figure (1).

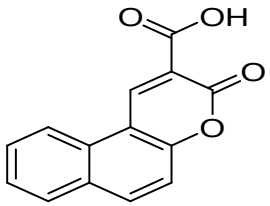

Figure (1): Structure of 3-oxo-3H-benzo[f]chromene-2-carboxylic acid (BCCA).

MATERIAL AND METHODS

Material and Solutions

All materials used in the present study were of A. R. Grade. They are as follows: $Eu(NO_3)_3 \cdot 6H_2O$, Calf thymus nucleic acid (CT-DNA), tris (hydroxyl-methyl) aminomethane (Tris), adenosine 5`-triphosphate (5`-ATP), guanosine5`-monophosphate (5`-GMP), inosin-5`monohosphate (5'- IMP), guanosine, inosine, adenosine and cytidine. They were used without purification.

BCCA was synthesized according to the following steps, Step1: Synthesis of ethyl 3-oxo-3H-benzo- [f]-chromene-2-carboxylate. A solution of (14.10 g, 0.082 mol of 2-hydroxy 1-naphthaldehyde, 14.4 g, 0.09 mol of diethyl malonate, 60 ml of absolute ethanol, 1 ml of piperidine and 10 drops of acetic acid), were stirred at 90 ^oC for 6hours. Then the mixture was poured into 100

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ml of ice-water mixture, and yellow precipitate was obtained. The precipitate was filtered and washed with water and 50% ethanol solution, respectively. The product was dried under reduced pressure and purified with recrystallization from 50% ethanol solution and 10g (50% yield) of brown crystals of the compound were obtained at m. p. 119 ^{o}C. Step2: Synthesis of 3-oxo-3H-benzo[f]chromene-2-carboxylic acid (BCCA). A solution of ethyl 3-oxo-3*H*-benzo[f]chromene-2-carboxylate (10.0 mmol) in ethanol (60 ml) and 5% KOH (25 ml) was stirred for 4 hours.

The solution was evaporated to dryness and the residue was dissolved in water and acidified with dil HCl. The white precipitate was filtered, dried and crystallized from ethanol to give 3-oxo-3*H*-benzo-[f]chromene-2-carboxylic acid in 85% yield at m.p. 235 ^{o}C. The concentrations of the lanthanide metal ions were determined complex metrically by titration against a standardized ethylene diamine tetra acetic acid disodium salt (EDTA). Purity of DNA was checked by monitoring the ratio of absorbance at 260 and 280 nm.

The ratio was 1.89, indicating that the DNA was free from protein. DNA was dissolved in bidistilled water with stirring for 24 hours and stored at 4^{o}C. Parallel solution of DNA was dissolved in aqueous Tris-HCl buffer (pH=7.4) with gentle shaking occasionally until complete homogeneity.

The solution was stored at 4 ^{o}C and used within 5 days. The exact molar concentration of DNA stock solution was determined according to the absorbance at 260 nm by using the molar extinction coefficient of 6600 mol^{-1} cm^{-1}. Other reagents used were purchased and used as analytical grade. High Spectroscopic grade of organic solvents (ethanol, methanol, acetone, acetonitrile, dimethyl sulf-oxide (DMSO), dimethyl formamide (DMF) and isopropyl alcohol) were used in fluorimetric measurements

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Fluorimetric measurements

- **Effect of solvents**

The fluorescence measurements were conducted for the free synthesized coumarin derivative (BCCA) and the lanthanide metal ion Eu(III) binary complex in different solvents (methanol, DMF, DMSO, acetone and acetonitrile). The concentration of the metal ion and the ligand are kept to be 2×10^{-5} mol dm^{-3}.

- **Stoichiometry of complexes**.

The best stoichiometry of the studied complex is evaluated using absorption and fluorescence measure-ments for Eu(III) with the synthesized ligand (BCCA) using various concentrations of ligands and finding the maximum fluorescence intensity corresponding to the characteristic emission bands which is 614 nm for Eu(III).

- **Interaction of the binary complex with nucleic acid compartments and DNA**

The best stoichiometry of the studied binary complex including Eu(III) and the coumarin derivative ligand (BCCA) is mixed with various concentrations of nucleosides (Adenosine, Guanosine, Inosine, Cytidine), or the nucleotides (5'-GMP, 5'-IMP, 5'-ATP).

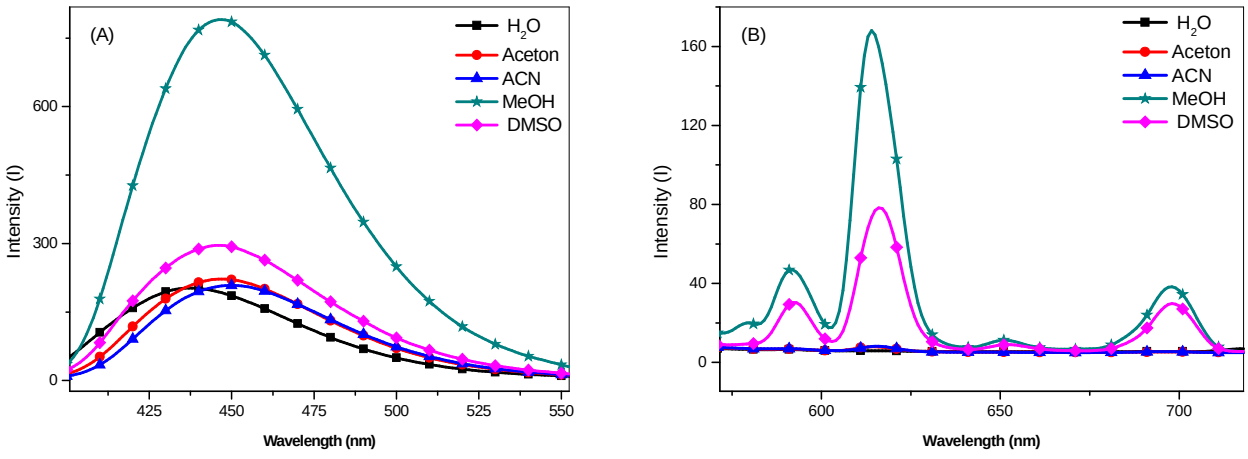


Figure (2): Effect of solvent on the excitation and emission spectra for Eu(III)-BCCA binary complex at λ_{ex}= 373 nm. $C_{Eu(III)}$ = 2×10^{-5} mol dm^{-3},C_{BCCA}=2×10^{-5} mol dm^{-3}. **(A)** Excitation spectra for Eu(III)-BCCA, **(B)** Emission spectra for Eu(III)-BCCA.

Table (1): Characteristic emission bands intensities for Eu(III)-3-oxo-3H benzo[f]chromene-2-carboxylic acid. (BCCA).

Solvent	λ1=590 nm $^5D_0 \rightarrow {}^7F_1$	λ2= 615 nm $^5D_0 \rightarrow {}^7F_2$
Methanol	45.2	168.11
DMSO	7.3	73.45

The florescence spectra (Fig. 2), Inspecting the results of the characteristic emission bands for Eu(III) are observed in only two solvents methanol and DMSO and the two bands appear at λ_1=590 nm ($^5D_0 \rightarrow {}^7F_1$) and λ_2= 615nm ($^5D_0 \rightarrow {}^7F_2$) as shown in Table (1). To study the interaction of Eu(III)-BCCA binary complex with nucleic acid compartments, the fluore-scence spectra of different metal ligand ratio is perfor-rmed in methanol as shown in figure 3.

The interaction of Eu(III)-BCCA binary complex in the ratio 1:2 in methanol was studied with the nucleosides (Adenosine, Inosine, Cytidine and Guan-osine), and nucleotides (5'-GMP, 5'-IMP and 5'-ATP) by the fluorescence measurements as shown in figures (4- 10).

The general feature for the interaction of such biomolecules with Eu(III)-BCCA binary complex is the quenching effect on the fluorescence intensity. The modified Stern-Volmer equation can be applied to calculate the binding constant values and the binding sites, as shown in figure 11, where the obtained data are collected (Table 2). The interaction of the binary Eu(III)-BCCA complex with DNA was studied by monitoring the change of the fluorescence intensity of Eu(III) at λ = 615 nm. The obtained spectra are shown in figure 12.

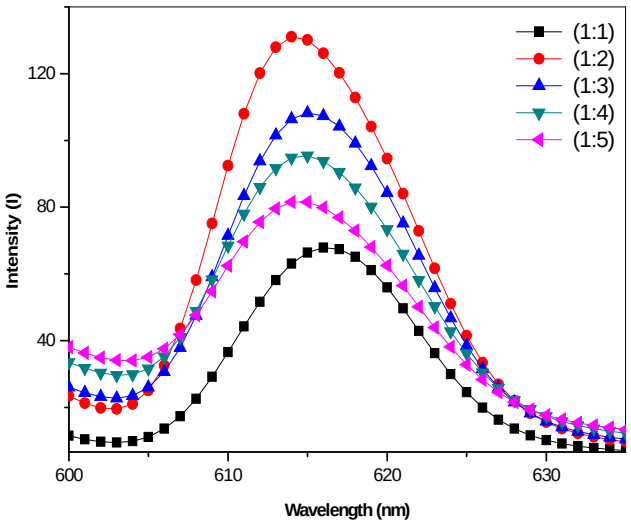


Figure (3): Effect of metal ligand ratio on emission spectra for Eu (III)-L_2 binary complex in methanol, at λex = 373 nm. $C_{Eu(III)}$ =2×10^{-5} mol dm^{-3}, C_{BCCA} =2×10^{-5} mol dm^{-3}.

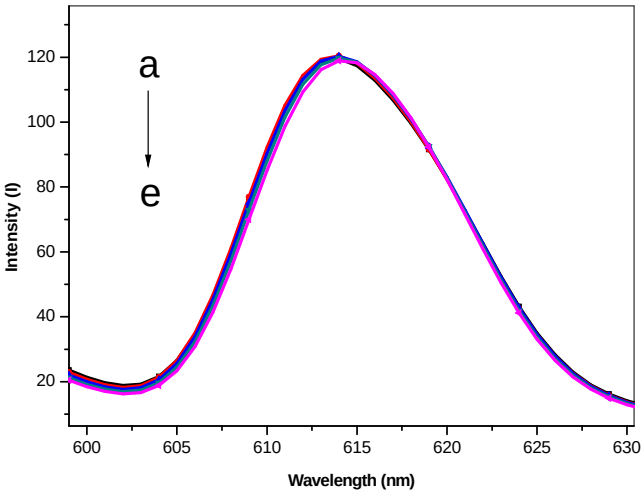


Figure (4): Effect of adenosine concentration on the emission spectra for Eu(III)-BCCA binary complex (1:2) in methanol at λ_{ex}=373 nm, λ_{em}= 614 nm. $C_{Eu(III)}$ = 2×10^{-5} mol dm^{-3}, of [Adenosine].

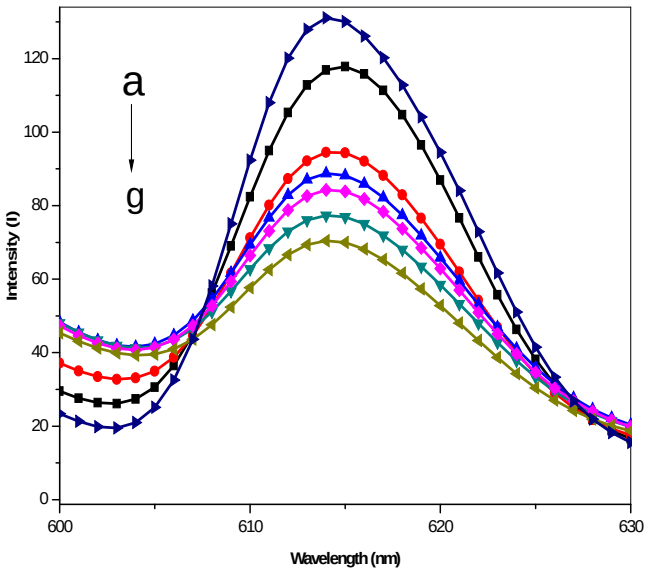


Figure (5): Effect of 5'-ATP concentration on the emission spectra for Eu(III)-BCCA binary complex (1:2) in methanol at λex= 373 nm, λem= 614 nm. CEu(III) = 2×10-5 mol dm-3, CBCCA= 4×10-5 mol dm-3 (a-g) = 0-5 × 10-5 mol dm-3 of [5'-ATP].

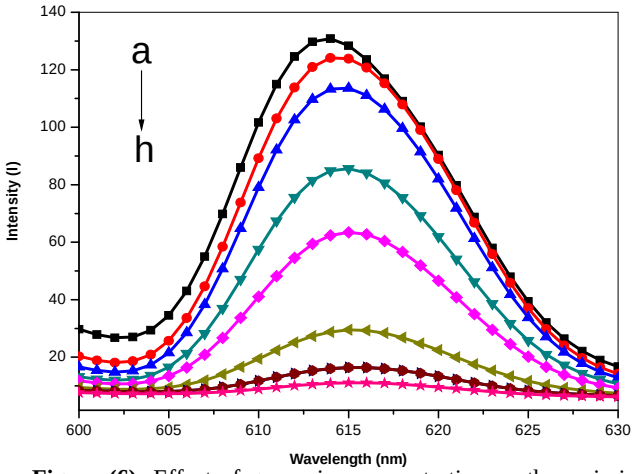


Figure (6): Effect of guanosine concentration on the emission spectra for Eu(III)-BCCA binary complex (1:2) in methanol at λ_{ex} = 373 nm, λ_{em} = 614 nm. $C_{Eu(III)}$ = 2×10^{-5} mol dm^{-3}, C_{BCCA}= 4×10^{-5} mol dm^{-3}. (a-h) = 0-5.14 × 10^{-4} mol dm^{-3} of [guanosine].

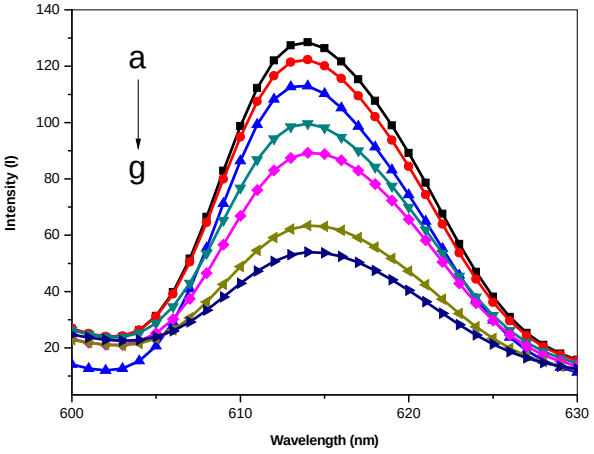


Figure (7): Effect of 5'-GMP concentration on the emission spectra for Eu(III)-BCCA binary complex (1:2) in methanol at λ_{ex}=373 nm, λ_{em}= 614nm. $C_{Eu(III)}$ =2×10^{-5} mol dm^{-3}, C_{BCCA}=4×10^{-5} mol dm^{-3}. (a-g) = 0-2.56 × 10^{-5} mol dm^{-3} of [5'-GMP].

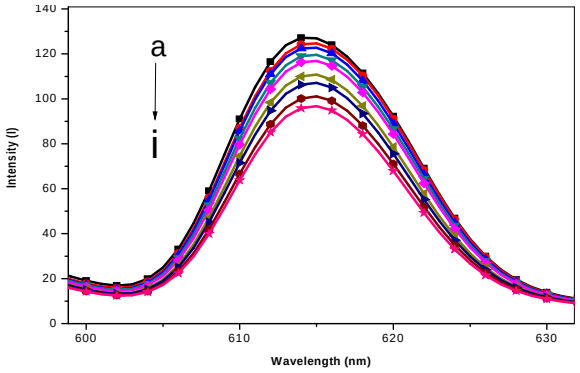


Figure (8): Effect of inosine concentration on the emission spectra for Eu(III)-BCCA binary complex (1:2) in methanol at λ_{ex}=373 nm, λ_{em}= 614 nm.$C_{Eu(III)}$ = 2 ×10^{-5} mol dm^{-3},C_{BCCA} = 4×10^{-5} mol dm^{-3} (a-i) = 0-5.71 × 10^{-4} mol dm^{-3} of [inosine].

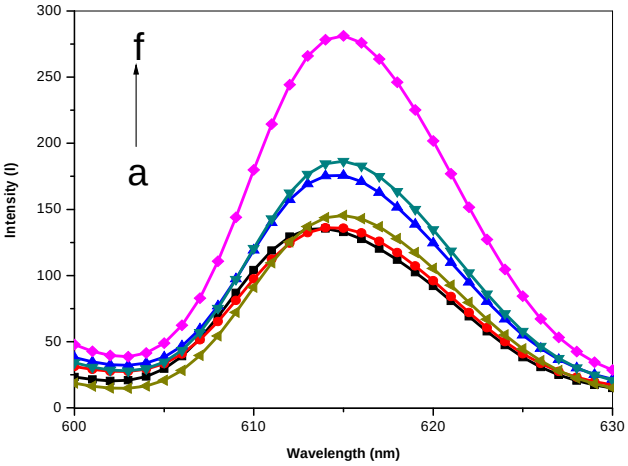


Figure (9): Effect of 5'-IMP concentration on the emission spectra for Eu(III)-BCCA binary complex (1:2) in methanol at λ_{ex}=373 nm, λ_{em}= 614nm. $C_{Eu(III)}$ = 2 ×10^{-5} mol dm^{-3}, C_{BCCA}= 4×10^{-5} mol dm^{-3}(a-f) = 0-8 × 10^{-5} mol dm^{-3} of [5'-IMP].

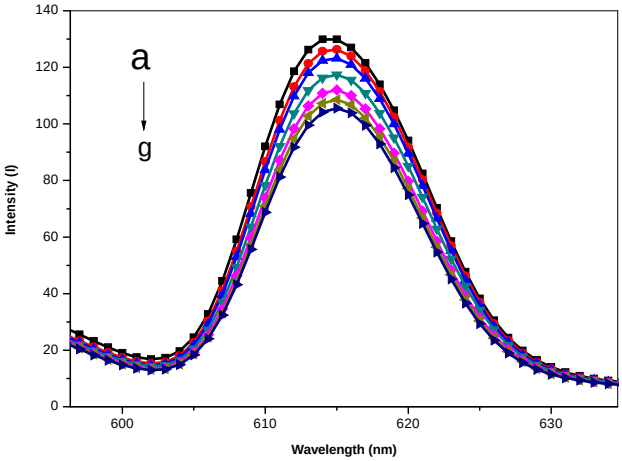


Figure (10): Effect of cytidine concentration on the emission spectra for Eu(III)-BCCA binary complex (1:2) in methanol at λ_{ex}=373 nm, λ_{em}= 614 nm. CEu(III) = 2×10^{-5} mol dm^{-3},C_{BCCA}=4×10^{-5} mol dm^{-3} , (a-g) = 0-4.5 × 10-4 mol dm^{-3} of [cytidine].

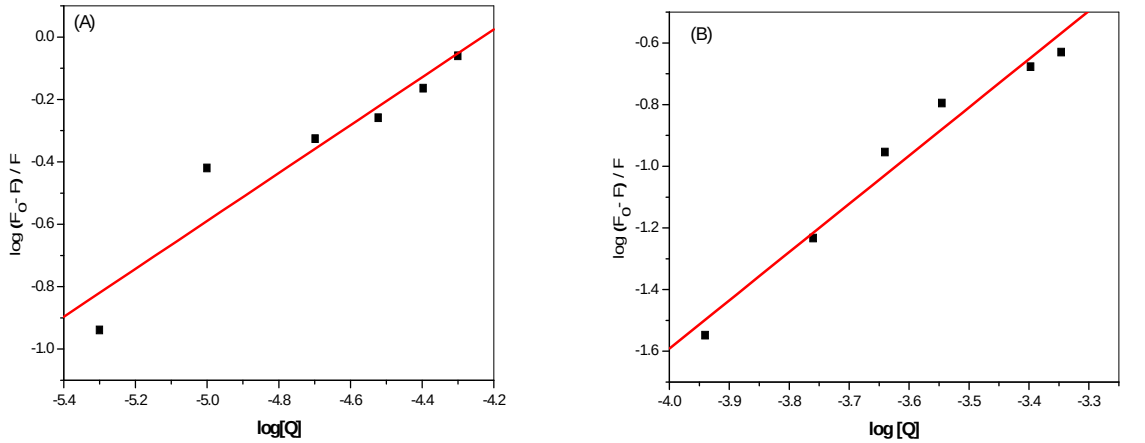
RESULTS

Spectroscopic studies of the binary and ternary systems:

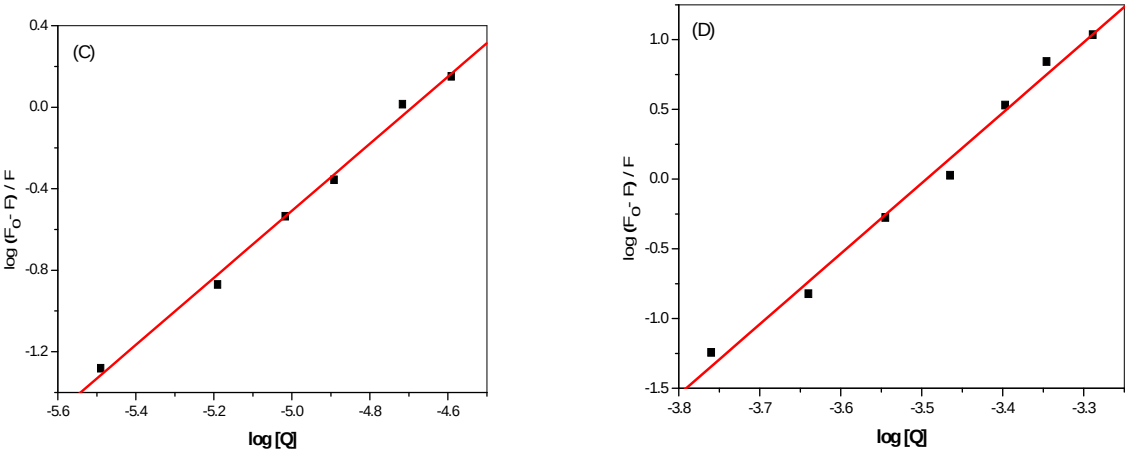
The interactions of Eu(III) with the concerned ligand are examined using florescence measurements in different solvents (Methanol, Acetone, Acetonitrile, DMSO and water) at the ratio of 1:1 in metal legand.

The successive addition of DNA to the binary complex causes quenching of the characteristic emission bands for Eu (III) in the complex at λ =615 nm. The binding constant and binding sites are calculated using the application of modified SternVolmer equation (Fig. 13). The calculated values are collected in table 3.

(A)



(B)



(C)

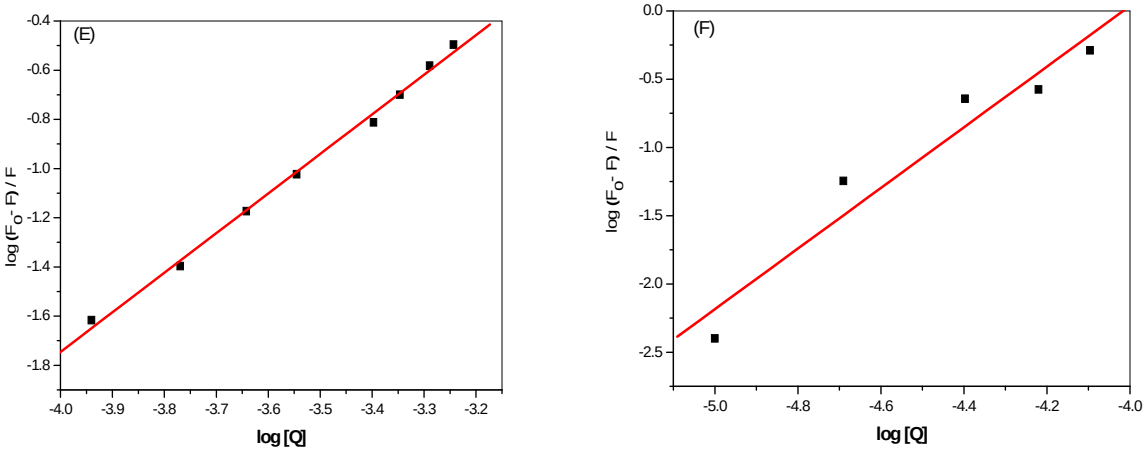


Figure (11) (A): The plot of log (Fo-F)/F versus log[Q] for quenching of Eu(III)-BCCA (1:2) with (A) 5'-ATP , (B) cytidine, $C_{Eu(III)}$ =2×10^{-5} mol dm^{-3},C_{BCCA}=4×10^{-5} mol dm^{-3}.

(B): The plot of log (Fo-F)/F versus log[Q] for quenching of Eu(III)-BCCA (1:2) with (C)5'- GMP , (D)Guanosine $C_{Eu(III)}$ =2×10^{-5} mol dm^{-3}, C_{BCCA}=4×10^{-5} mol dm^{-3}.

(C): The plot of log (Fo-F)/F versus log[Q] for quenching of Eu(III)-BCCA (1:2) with (E) Inosine , (F) 5'-IMP, $C_{Eu(III)}$ = 2×10^{-5} mol dm^{-3},C_{BCCA}= 4×10^{-5} mol dm^{-3}.

Table (2): Binding parameters of Eu(III)-BCCA binary complex with nucleosides and nucleotides

Compound	Log K	K(M^{-1})	n	r	SD
Adenosine	-	-	-	-	-
5'-ATP	3.24	$1.73x10^{3}$	3.24	0.949	0.108
Guanosine	17.67	$4.67x10^{17}$	5.05	0.995	0.092
5'-GMP	7.72	$5.24x10^{7}$	1.64	0.997	0.043
Inosine	4.69	$4.89x10^{4}$	4.69	0.998	0.027
5'-IMP	9.03	$1.07x10^{9}$	2.24	0.968	0.243
Cytidine	4.66	$4.57x10^{4}$	1.56	0.984	0.07

Table (3): Binding parameters for Eu(III)-BCCA binary complex with DNA.

Compound	Log K	K(M^{-1})	n	r	SD
DNA (dissoluted in H_2O)	5.96	$8.70x10^{5}$	1.28	0.967	0.122
DNA (dissoluted in Tris buffer pH=7.4)	7.53	$3.38x10^{7}$	1.54	0.965	0.1414

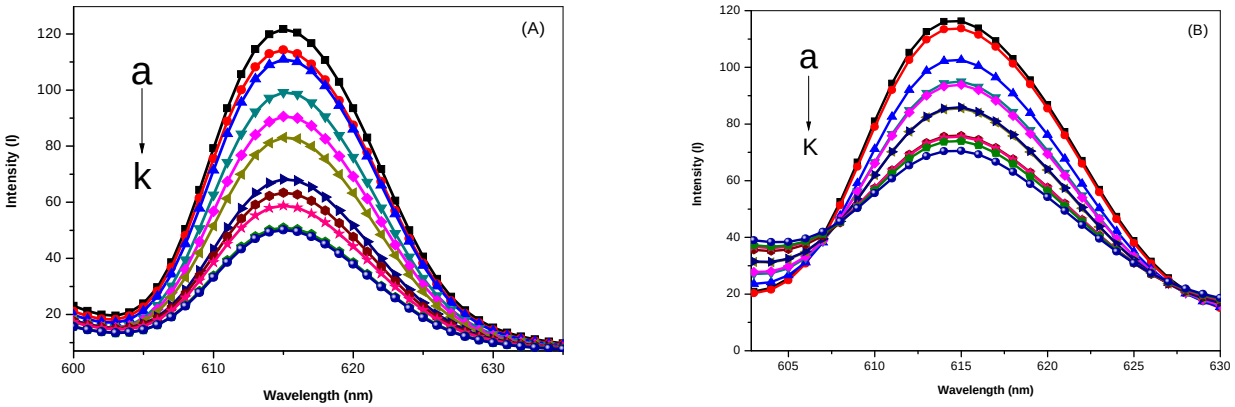


Figure (12) A: Effect of DNA concentration on the emission spectra for Eu(III)-BCCA binary complex (1:2) in methanol at λ_{ex}= 373 nm, λ_{em}= 614 nm $C_{Eu(III)}$ = 2×10^{-5} mol dm^{-3},C_{BCCA}= 4×10^{-5} mol dm^{-3}.DNA is dissolute in Tris-buffer.(a-k) = 0-2 $\times$ 10^{-5} mol dm^{-3} of [DNA].

B: Effect of DNA concentration on the emission spectra for Eu(III)-BCCA binary complex (1:2) in methanol at λ_{ex}=373 nm, λ_{em}= 614 nm. CEu(III) =2×10^{-5} mol dm^{-3},C_{BCCA}=4×10^{-5} mol dm^{-3}. DNA is dissoluted in H_2O. (a-k) = 0-2 $\times$ 10^{-5} mol dm^{-3} of [DNA].

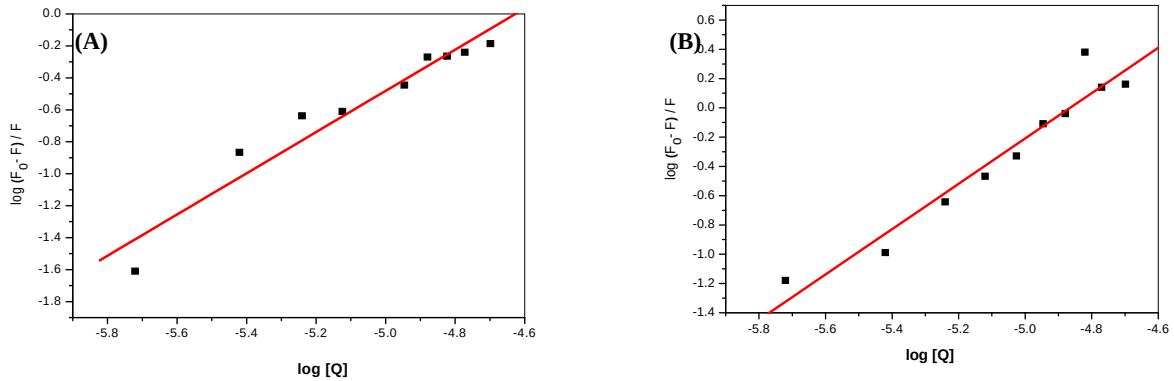


Figure (13) A: Modified Stern-Volmer equation for the interaction of Eu(III)- BCCA (1:2) with DNA is dissoluted in H_2O. $C_{Eu(III)}$ = 2×10^{-5} mol dm^{-3}, CBCCA= 4×10^{-5} mol dm^{-3} .

B: Modified Stern-Volmer equation for the interaction of Eu (III)- BCCA (1:2) with DNA is disoluted in Tris-buffer. $C_{Eu(III)}$ = 2×10^{-5} mol dm^{-3},C_{BCCA}= 4×10^{-5} mol dm^{-3}.

DISCUSSION

The fluorescence spectrum of the binary system Eu(III) ion with ligand in different solvent is shown in figure 2. The high intensity of the Eu(III) emission band in methanol and DMSO confirms the strong interaction in such media. The data reveal that the most suitable stoichiometry for this complex is 1:2 through the maximum emission intensity obtained at the characteristic band for Eu(III)in the complex at λ =614 nm in figure 3.

The interaction of Eu(III)-BCCA complex with the nucleosides (Adenosine, Inosine, Cytidine, Guanosine), and nucleotides (5'-GMP, 5'-IMP and 5'-ATP) was studied by the fluorescence measurements. Inspecting the obtained data, it is clearly observed that there is no significant binding between the binary complex and adenosine nucleoside, while there is a somewhat considerable binding between Eu(III)-BCCA binary complex and 5'-ATP molecule with three binding sites most probably with the nucleobase and phosphate group. The interaction of the binary complex with guanosine is very promising due to the high binding constant and binding sites. This behavior could be explained to the preferential attack of the complex to the guanosine molecule through the *N7* and *6C=O* of the nucleobase and the OH of the sugar moiety. The high values of the binding sites can be attributed to that the complex molecule acts as a bridging center to two molecules of guanosine i.e. one molecule of the complex binds to two molecules of guanosine. For the interaction of 5'-GMP with the binary complex the binding constant is also high but less than guanosine with binding site nearly equals 2, where the binding of Eu(III)-BCCA may be attributed to be with the nucleobase and phosphate group. The very strong binding is not observed in inosine nucleoside which confirm the selective inter-action of Eu(III)-BCCA complex with guanosine rather than inosine. On the other hand, for the corresponding nucleotide molecule, the binding strength of the complex with 5'-IMP is about one hundred times greater than 5'-GMP keep in the same binding sites to be also two.

The extra value of binding for the complex with the 5'-IMP may be attributed to interligand and interactions between BCCA and 5'-IMP molecule through NH_2 in C6 position rather than C=O in 5'-GMP. The cytidine nucleoside has about nearly the same binding constant as inosine with two sites of binding most probably C=O of the nucleobase and OH of sugar moiety.

The obtained data indicate that the binding of Eu(III)-BCCA binary complex with DNA which is dissoluted in Tris buffer, is higher than that with DNA which dissoluted in water. The presence of Tris molecule will enhance the binding of the binary complex with the double helix DNA. Also the calculated binding site for the complex with DNA in water (n = 1.28) is less than in the case of DNA in tris buffer (n = 1.54), so this can be attributed to the presence of tris molecule which facilitated the binding of the complex towards DNA and also strengthened the bonding between them.

This work can be considered as a continuation of the author's work in the field of bioinorganic chemistry (Anwar and Azab, 1999; Anwar and Azab, 2001a; Anwar and Azab, 2001b; Azab *et al*., 2001; Azab *et al*., 2009). The Eu(III) ternary systems studied in the present work can be considered as a basis for the future development of novel biosensors for the trace determination of biologically important compounds as stated by the authors in the papers (Azab *et al*., 2010).

CONCLUSION

The new fluorescent probe containing Eu(III) with BCCA can be used to determine nucleosides (Adenosine, Guanosine, Inosine and Cytidine), nucleotides (5'-GMP, 5'-ATP and 5'-IMP) and DNA. The effect of biomolecules on the fluorescence intensity was used to evaluate the binding constant and sites

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Received July 16, 2012
Accepted March 16, 2013

دراسات طيفية لمتراكب الأيروبيوم بنزوكومارين Eu(III)- 3-oxo- 3*H*-benzo-[f]chromene-2-carboxylic acid مع النيوكلوزيدات و النيوكلوتيدات والحمض النووي

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دراسة طيف إمتصاص الأشعة فوق البنفسجية والإنبعاث الفلورسينى لليجاندات مشتقات، مختلفة (الكحول الميثيلى، الكحول الايثيلى، الكحول الايزوبروبيلى، ثنائى ميثيل سلفوكسيد، ثنائى ميثيل فورماميد،الاسيتون والاسيتونيتريل).
فى حالة متراكبات أيون الإيروبيوم مع البنزوكومارين فقد أوضحت النتائج أن متراكب أيون الإيروبيوم – بنزوكومارين له قيم إنبعاث واضحة فى الكحول الميثيلى وثنائى ميثيل سلفوكسيد ولذلك يمكن دراسة تغير قيم هذا الإنبعاث مع تفاعل المتراكب والنيوكليوسيدات والنيوكليوتيدات والحامض النووى الديوكسى الريبوزى.
ولذلك فقد تم دراسة قيم ترابط المتراكب الثنائى لأيون الأيروبيوم – بنزوكومارين مع النيوكليوسيدات (الادينوسين،الجوانسين،الاينوسين والسيتدين) و النيكليوتيدات (جوانسين-٥'-أحادى الفوسفات ، اينوسين- ٥'- أحادى الفوسفات وأدينوسين - ٥'- ثلاثى الفوسفات) بحيث وجد أن درجة الترابط تتبع الترتيب جوانسين >اينوسين ≈ السيتدين و 5'-IMP >5'-GMP>5'-ATP بينما لا يحدث ترابط بين المتراكب الثنائى مع جزئ الأدينوسين وقد تم دراسة درجة ترابط متراكب الأيروبيوم مع البنزوكومارين مع الحامض النووى الديوكسى الريبوزى DNA المذاب فى الماء وفى محلول التريس المنظم عند قيم pH=7.4 وقد وجد أن قيمة ترابط المتراكب مع ل DNA فى وجود التريس أعلى منها فى حالة ل DNA فى الماء.