CATRINA (2016), 15 (1): 69-76

New probe of lanthanide complex for detection some of nucleobase, nucleosides and nucleotides, by the luminescent Tb (III) - 8-allyl-2-oxo-2 H-chromene-3-carbaldehyde probes

Hassan A. Azab[1], Gasser M. Khairy [1, 2]*, N. Abd El-Ghany, Marwa. A.Ahmed[3]
[1]Chemistry Department, Faculty of Science, Suez Canal University, 41522 Ismailia, Egypt.
[2]Chemistry Department, Faculty of Science and Arts, Aljouf University, P.O. Box # 2014, Skaka-41421, Kingdom of Saudi Arabia.
[3]Chemistry Department, Faculty of Science, Suez Canal University, El-Arish, Egypt.

ABSTRACT

The luminescence arising from lanthanide cations offers several advantages over organic fluorescent molecules sharp, distinctive emission bands allow for easy resolution between multiple lanthanide signals; long emission lifetimes (μs–ms) make them excellent candidates for time resolved measurements and high resistance to photo bleaching allow for long or repeated experiments. A developed method is presented for determination of nucleobase, nucleosides and nucleotides using the effect of quenching of fluorescence of the easily accessible terbium (III) - 8-allyl-2-oxo-2H-chromene-3-carbaldehyde (AOCC) complex in 1:2 metals: ligand ratio. The interaction of terbium (III) - 8-allyl-2-oxo-2H-chromene-3-carbaldehyde (AOCC) complex with nucleosides (NS) (cytidine, inosine), nucleobase (NB) (adenine), nucleotides (NT) (5′-GMP), (5′-IMP), (5′- ATP), (5′-CMP) and (5′-ADP) has been studied using normal luminescence techniques. The linear ranges for determination of the selected biomolecules are 1.6–36.6 μ mol.dm^{-3}, and with limit of detection were in the range of 0.906- 8.28μ mol.dm^{-3}.

Keywords: Fluorescence, Nucleotides, Nucleobases, Nucleosides, Lanthanide complexes, time-resolved.

INTRODUCTION

Terbium and Europium complexes emitting green and red luminescence, respectively, have attracted more attention, due to this RE(III) ions have unique luminescence properties such as hypersensitivity to the coordination environment, long luminescence lifetime millisecond range ,and narrow band width. (Azab, *et al.*, 2015(a); Azab, *et al.,* 2015(b)) Unlike d-transition metal ion complexes for which the electronic excited states are strongly coupled to the environment via the ligand field providing an efficient de excitation mechanism, in the case of lanthanide complexes the coupling between f-excited electronic states and the environment is very small. The absorption coefficients of lanthanide ions, however, are very small. In an effort to obtain high-efficient luminescence, an organic ligand has been introduced as a sensitizer to complexes of these metals (antenna effect).

(Lis et *al.,* 2002) During last year's Chemistry of coordination compounds with biologically active biomolecule has received much attention. Coumarin and its derivatives are important compounds due to their presence in naturally occurring products and their wide range applications in agrochemicals, drugs and pharmaceuticals. (Cacic, *et al.,* 2006; Egan *et al*,.1990; Hoult and Paya 1996; Karalı *et al.,* 2002; Nawrot *et al.,* 2006) such as anticancer, (Devji *et al.,* 2011; Reddy *et al.,* 2011) anti-HIV, (Hai *et al.,* 2010) anti-tuberculosis (Manvar *et al.,* 2011) anti-influenza,(Jiann-Yih *et al.,* 2010) anti-Alzheimer, (Anand and Singh 2012; Piazzi *et al.,* 2008) anti-inflammatory, (Lin *et al.,* 2006), anti-viral, (Gonzalesb and Rodriguezb 2003) and antimicrobial agents. Shi and Zhou 2011 state that the binding of a metal to the coumarin moiety retains or even enhances its biological activity. (Karaliota *et al.* 2001; Kostova *et al.,* 2001) Regarding their high fluorescence ability, they are widely used as fluorescent probes in biology and medicine. (Haugland 2002; Geen *et al.,* 1996) Studies of the interaction between Rare earth metal complexes and DNA have been a spot of interest in bio inorganic chemistry, mostly since the discovery of cisplatin and their analogue compound.

These studies have been useful for the development and the comprehension of the activity of new chemotherapeutic agents designed for the treatment of numerous diseases. (Facchin *et al.,* 2009; Min *et al.,* 2011; Navarro *et al.,* 2003) Uric acid is the end product of purine metabolism and the increase of serum uric acid level causes gout and hyperuricemia (Benedict *et al.,* 1949; Berger *et al.,* 1962). Patients with gout tend to consume high amounts of giblets or meat that contain high levels of purine (Harris *et al.,* 1999; Gibson *et al.,* 1983).

Although there have been many reports investigating the effects of food on purine metabolism and serum uric acid levels (Faller and Irving 1982; Fumeron *et al.,* 1991; Gibson *et al.,* 1983; Guohua *et al.,* 1989; Waslien *et al.,* 1968), few reports (Yamaoka *et al.,* 2010) have examined the amounts of individual purine nucleosides,

* *Corresponding author: marwaaa_3@yahool.com*

nucleotides, bases and nucleic acids in food. Because purine nucleotides, nucleosides and bases exert different effects on serum uric acid levels (Clifford *et al.,* 1976) it is important to examine the levels of individual nucleosides, nucleotides, and bases in various foods in order to investigate the effects of purine rich foods on the elevation of serum uric acid levels. Several methods have been reported for the determination of nucleoside and nucleotide concentrations in cells (Qian and Yung 2004; Kalász *et al.,* 2010) , biological samples (Krstulović 1982; Klawitter *et al.,* 2007) or milk (Gill and Indyk 2007) such as; high performance liquid chromatography (HPLC) (Krstulović 1982), liquid chromatography–mass spectrometry (LC–MS) (Cordell *et al*., 2008; Qian and Yung 2004; Zongwei and Yung 2002; Klawitter *et al.,* 2007) and LC/MS/MS (Ansermot *et al.,* 2009; Vela *et al.,* 2007; Kalász *et al.,* 2010) used for such measurements.

Due to these draw back a new probe of lanthanide complex for detection some of nucleobase, nucleosides and nucleotides developed. So the present work, study the interaction of terbium (III)–8-allyl-2-oxo-2H-chromene-3-carbaldehyde (AOCC) complex with some of nucleobase, nucleosides and nucleotides using absorption and luminescence spectroscopy.

MATERIALS AND METHODS

Chemicals

All materials used in the present investigation were of analytical reagent grade. The materials were purchased from Sigma. They are as follows:TbCl3·6H2O, adenine, inosine, cytidine, guanosine 5′monophosphate disodium salt hydrate (5′-GMP), inosine 5′- monophosphate disodium salt hydrate (5′-IMP), adenosine 5′-triphosphate disodium salt hydrate (5′-ATP), and cytidine 5′-monophosphate disodium salt (5′-CMP) adenosine 5′-diphosphate monosodium salt dehydrate, uric acid, ascorbic acid, urea, glucose . 8-allyl-2-oxo-2H-chromene-3-carbaldehyde (AOCC) was from Merck. All solvents used are of analytical grade quality.

Stock solutions

The terbium stock solution was prepared by dissolving 37.3 mg of TbCl3·6H2O in 100 ml of aqua media (Hepes, Mops, Mopso, Tapso, or Tris) to give a final concentration of 1.00 x 10^{-3} mol.dm^{-3}. The concentration of the stock Tb (III) solution has been checked by titration with EDTA titration using xylenol orange as indicator. A stock solution of 8-allyl-2-oxo-2H-chromene-3-carbaldehyde was prepared by dissolving 21.42 mg of solid ligand in 100 ml of ethanol to give a final concentration of 1.00 x 10^{-3} mol.dm^{-3}. The stock solutions of adenine, cytidine and inosine were prepared by dissolving 13.51, 24.3 and 26.8 mg, respectively in 100 ml deionized water to give a final concentration of 1.00 x 10^{-3} mol/L. For (5′-GMP), (5′-IMP), (5′-ATP), (5′-CMP) and (5′-ADP) were prepared by dissolving 40, 39, 55, 36.7 and 48.5 mg, respectively in 100 ml deionized water to give a final concentration of 1.00 x 10^{-3} mol.dm^{-3}.

Methods

A 10 ml solution containing appropriate concentration of 1.00 x 10^{-5} mol.dm^{-3}$TbCl_3.6H_2O$ and 2.00 x 10^{-5} mol.dm^{-3} AOCC was added to 1.00 x 10^{-3} mol.dm^{-3} nucleobase, nucleosides or nucleotides solutions and mixed using a magnetic stirrer for about ten minutes.

The fluorescent intensity of the solution was recorded at excitation wavelength of 350 nm by using A JASCO-FP6300 spectrofluorometer and kept at 25±1 °C for 10 min. Luminescence intensity was measured in a 1 cm quartz cell at an excitation wavelength of 350 nm and an emission wavelength of 545 nm.

Instrument

Emission and spectral measurements of the interaction of the Tb (III)-AOCC with nucleotides, nucleosides, and nucleobase were carried out using a JASCO-FP6300 spectrofluorometer with 1 cm quartz cell. pH measurements were carried out on (pH-220L) pH-meter.

RESULTS

Steady-state luminescence spectroscopy

The excitation spectra and emission spectra of Tb (III) complex in aqua media at room temperature are measured. According to the spectra of complex that are reported in figure (1), it is clearly observed that the complex has excellent luminescent property. In the spectra of Tb(III) complex, the wide excitation band, from about 300~400 nm, and the strong emission intensities indicate that the ligand is a good organic ligand to absorb energy and transfer them to Tb(III) ion, emitting the characteristic fluorescence of Tb(III) ion, where, the emission spectra show four emission peaks under the excitation of 350 nm: 480 nm($^5D_4$–$^7F_6$), 545 nm ($^5D_4$–$^7F_5$), 590 nm($^5D_4$–$^7F_4$), 625 nm($^5D_4$–$^7F_3$), attributed to be the characteristic emission for the $^5D_4$ ^7F_J transition (J=6–3) of the Tb(III) ion. Among the $^5D_4$–$^7F_5$ transition exhibits the strongest green emission, and $^5D_4$–$^7F_6$ transition shows the second strongest emission. The complex shows a good antenna effect for Tb(III) luminescence, it means the triplet energy level of the ligand matched as well with the 4f ($^5D_4$) excited state energy level of Tb (III).

Effect of pH

The luminescence intensity of Tb (III) -AOCC complex is strongly dependent on pH. AOCC is β-diketones with aromatic substituent exhibits keto-enol tautomerism.

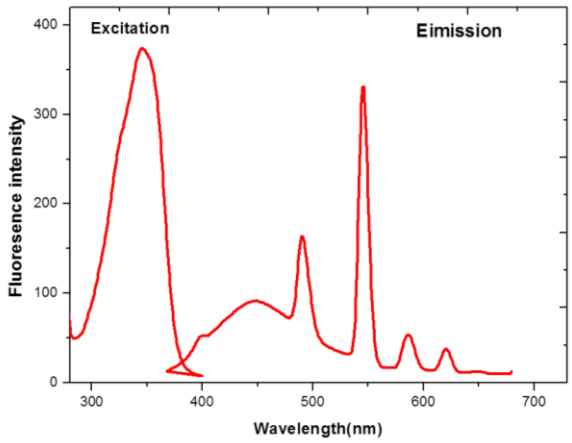

Figure (1): Excitation and emission spectra of Tb (III) $(AOCC)_2$ probe in hepes buffer at pH=7.2 at room temperature.

The effect of pH value on the fluorescence intensity of complexes is shown in figure (2 a and b). The fluorescence intensity is stronger at pH=7 than that in acidic and alkaline aqueous solutions. Obviously, in the acidic aqueous solution the organic ligand AOCC is found in its protonated form, Protonated AOCC ligand; while in the alkaline aqueous solution, the lanthanide ions can coordinate with hydroxyl, which produces precipitation as pH value increases gradually. So they deactivate the process of intra-molecular energy transfer which results in weak fluorescence of Tb (III)–AOCC complex in acidic and alkaline medium (Shakhverdov and Érgashev 1989).

The above two items weaken the chelate ability of the ligands, therefore, the low concentration of the solution results in the decrease of fluorescence intensity of luminescent complexes finally (Yin *et al*., 2000). This is also in accordance with the fact that the ligand will coordinate with Tb (III) more efficiently in its enolic form. Therefore the pH value around 7 was chosen for the further investigations. This value is often referred to as physiological pH in biology and medicine.

Stern–Volmer quenching constant

The calibration plots of the analytes (nucleotides, nucleoside, or nucleobases) assay with Tb-$(AOCC)_2$ probe were obtained in **different buffers at pH 7** ~ 7.2. The fluorescence measurements of the Tb-$(AOCC)_2$ probe with the analytes (nucleotides, nucleoside, or nucleobases) showing quenching of the characteristic peak for Tb(III) at λem = 545 nm at different concentrations as shown in figure.(3). The linear range of the plot is described by the Stern–Volmer relation

$$F_0/F=1+K_{sv}\,[\text{Analyte}] \quad (1).$$

By using concentration range of nucleotides, nucleoside ,or nucleobases from 1.66×10^{-6} to 3.48×10^{-5} $mol.dm^{-3}$ Where F_0 and F are the fluorescence intensities of Tb-$(AOCC)_2$ probe in the absence and presence of analytes, respectively. K_{sv} is Stern–Volmer constant. As the sensitivity of the method is related to the slope, from table (1), it was observed that the larger slope for, adenine, and 5′-GMP is in hepes, for 5′-IMP is in tris. For cytidine, 5′-ATP and 5′-CMP is in mops. For inosine is in tapso. And finally for 5′ADP is in mopso, while tris have larger linear range for 5′-ATP and 5′ADP. Correlation coefficients (r^2), linear ranges, limits of detection (LODs) and, limits of quantification (LOQs) have been determined and collected in table (1).

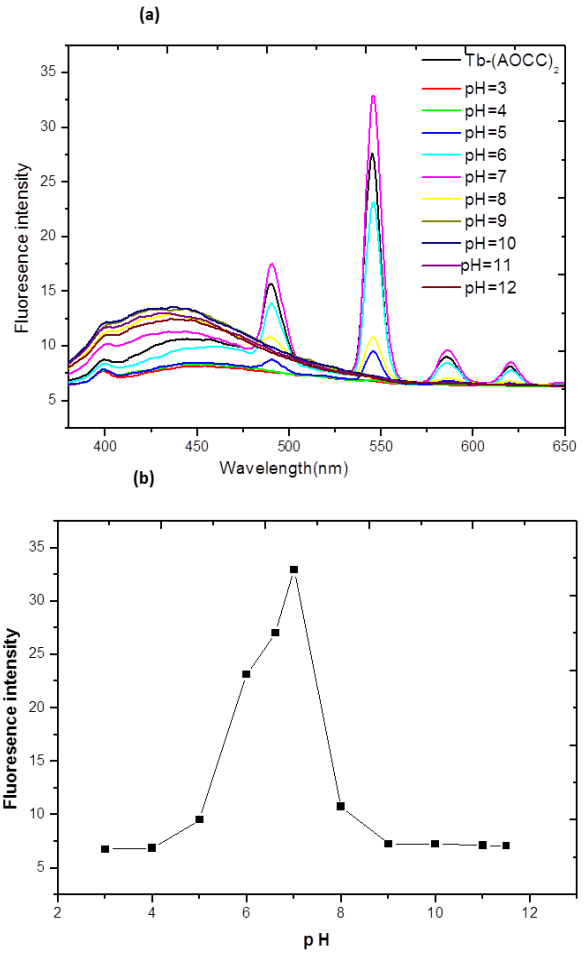

Figure (2): a; effect of pH value on Fluorescence intensity of $1x10^{-5}$ $mol.dm^{-3}$ Tb(III) + $2x10^{-5}$ $mol.dm^{-3}$ AOCC in aqueous solution at λ_{ex}=350nm, **b**; [Tb]/[AOCC] pH plot at λem = 545 nm and λex = 350 nm

CONCLUSION

The study of Tb (III)-$(AOCC)_2$ binary complex with the biomolecules is extended to be measured in aqueous medium in the presence of zwitter ionic buffers(PIPES, HEPES, MOPS, MOPSO) and tris buffer, keeping the pH of the studied solutions to be in the physiological pH range (pH≈7.2-7.6) the data reveal that , HEPES is the suitable for adenine, and 5'-GMP, Tris-buffer for 5'-IMP, MOPS for cytdine, 5'-ATP, 5'- CMP, MOPSO for 5'-ADP.

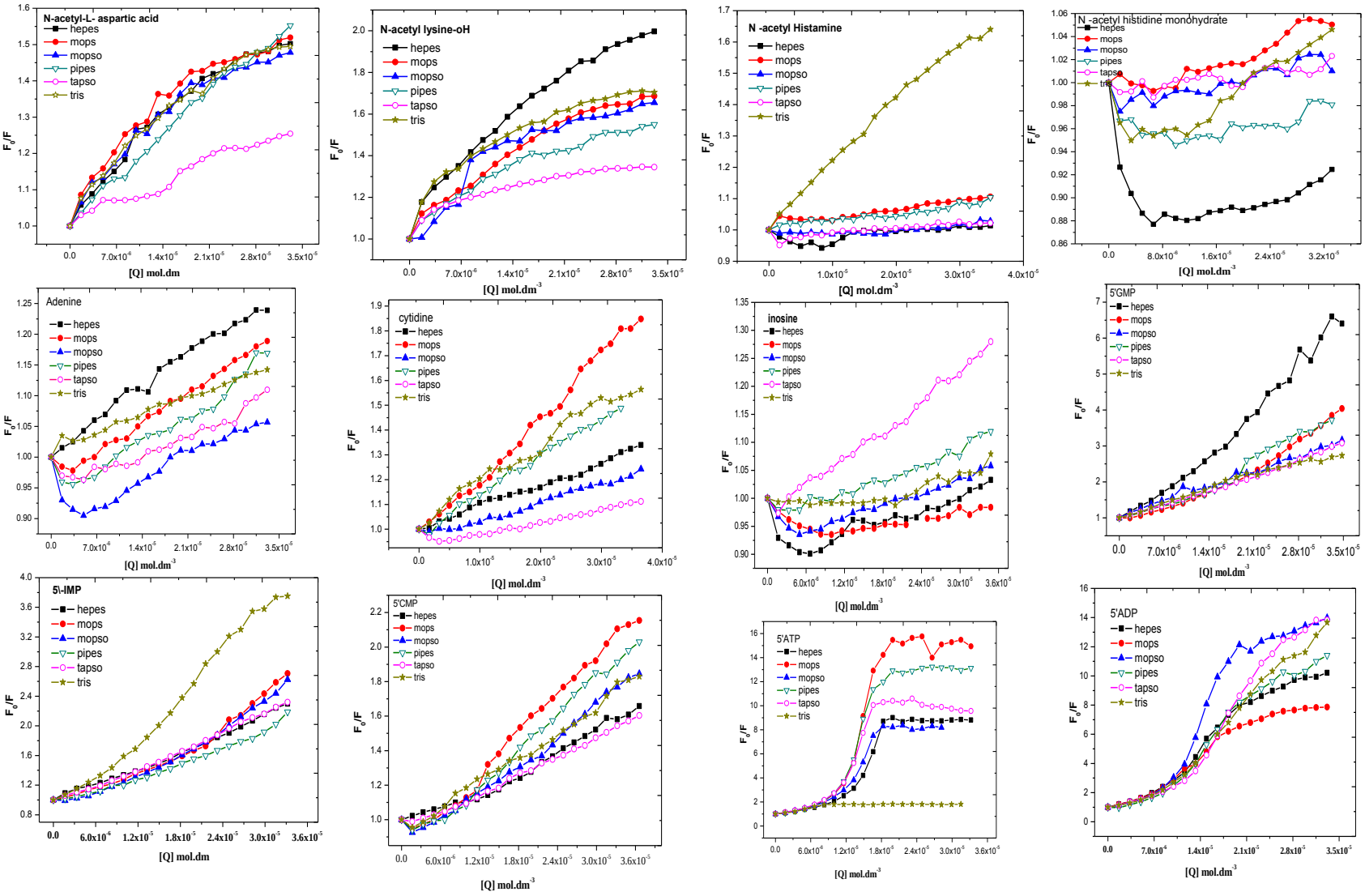

Figure (3): calibration curve of Tb (III)-$(AOCC)_2$ probe with nucleotides ,nucleobases, or N-acetyl amino acids in different buffer

Table1: Linear range, regression equation, correlation coefficients, RSDs and LODs for determination of some N-acetylamino acids, nucleobases and nucleotides in fluorescence with containing 5 µmol.dm^{-3} of Tb(III)-$(AOCC)_2$ probe different buffer.

	Regression equation	r^2	RSD (%,n=5)	LOD (µmol.dm^{-3})	LOQ (µmol.dm^{-3})	Linear Range (µmol.dm^{-3})
HEPES	F_0/F=1.01+ 7.08 [Q] mmol.dm^{-3}	0.982	0.004	2.007	6.693	
MOPS	F_0/F=0.95+ 7.01 [Q] mmol.dm^{-3}	0.996	0.002	0.906	3.020	3.02- 33.2
MOPSO	F_0/F=0.88+ 5.66 [Q] mmol.dm^{-3}	0.973	0.004	2.509	8.366	
PIPES	F_0/F=0.92+ 7.00 [Q] mmol.dm^{-3}	0.967	0.006	2.795	9.316	
TAPSO	F_0/F=0.93+ 4.72 [Q] mmol.dm^{-3}	0.956	0.005	3.2303	10.76	
TRIS	F_0/F=1.01+ 3.97 [Q] mmol.dm^{-3}	0.991	0.001	1.447	4.824	
HEPES	F_0/F=1.00+ 8.94 [Q] mmol.dm^{-3}	0.988	0.004	1.441	4.804	
MOPS	F_0/F=0.96 +24.5 [Q] mmol.dm^{-3}	0.992	0.009	1.202	4.008	
MOPSO	F_0/F=0.96+ 7.09 [Q] mmol.dm^{-3}	0.978	0.004	2.025	6.752	4.00- 36.5
PIPES	F_0/F=0.98+ 15.4 [Q] mmol.dm^{-3}	0.993	0.005	1.035	3.451	
TAPSO	F_0/F=0.94+ 4.35 [Q] mmol.dm^{-3}	0.912	0.006	4.205	14.01	
TRIS	F_0/F=1.03+15.58[Q] mmol.dm^{-3}	0.976	0.01	2.109	7.031	
HEPES	F_0/F=0.88+ 4.01 [Q] mmol.dm^{-3}	0.938	0.005	3.943	13.14	
MOPS	F_0/F=0.92+ 1.54 [Q] mmol.dm^{-3}	0.820	0.003	7.389	24.63	
MOPSO	F_0/F=0.91+ 3.92 [Q] mmol.dm^{-3}	0.986	0.002	1.781	5.938	5.8- 34.8
PIPES	F_0/F=0.95+ 4.39 [Q] mmol.dm^{-3}	0.958	0.004	3.225	10.75	
TAPSO	F_0/F=0.96+ 8.62 [Q] mmol.dm^{-3}	0.988	0.0048	1.669	5.566	
TRIS	F_0/F=0.96+ 2.57[Q] mmol.dm^{-3}	0.774	0.0071	8.284	27.61	
HEPES	F_0/F=0.58+165.7 [Q] mmol.dm^{-3}	0.979	0.5833	1.917	6.391	
MOPS	F_0/F=0.62+ 88.6[Q] mmol.dm^{-3}	0.960	0.1059	2.700	9.000	
MOPSO	F_0/F=1.06+ 59.0 [Q] mmol.dm^{-3}	0.987	0.0798	1.472	4.909	3.2- 34.8
PIPES	F_0/F=0.71+ 1.37 [Q] mmol.dm^{-3}	0.961	0.0289	2.612	8.709	
TAPSO	F_0/F=0.93+ 59.4 [Q] mmol.dm^{-3}	0.994	0.0777	0.9754	3.251	
TRIS	F_0/F=1.06+51.7 [Q] mmol.dm^{-3}	0.980	0.0193	1.867	6.224	
HEPES	F_0/F=-8.8+ 925[Q] mmol.dm^{-3}	0.920	0.0322	6.595	21.98	11-18.3
MOPS	F_0/F=-16.9+ 1735 [Q] mmol.dm^{-3}	0.963	2.5361	4.384	14.61	8.3-18.3
MOPSO	F_0/F=-4.36+ 676 [Q] mmol.dm^{-3}	0.933	1.0021	4.443	14.81	8.3-18.3
PIPES	F_0/F=-8.25+ 1119 [Q] mmol.dm^{-3}	0.943	1.5274	4.094	13.64	8.3-18.3
TAPSO	F_0/F=-6.32+926.4 [Q] mmol.dm^{-3}	0.947	1.2190	3.947	13.15	8.3-18.3
TRIS	F_0/F=0.96+ 89.44 [Q] mmol.dm^{-3}	0.963	0.0426	1.429	4.76	1.6-9.96
HEPES	F_0/F=0.94+ 18.79 [Q] mmol.dm^{-3}	0.979	0.0138	2.212	7.37	
MOPS	F_0/F=0.84 +36.59 [Q] mmol.dm^{-3}	0.981	0.0207	1.704	5.68	
MOPSO	F_0/F=0.86+ 26.3 [Q] mmol.dm^{-3}	0.991	0.0171	1.952	6.50	1.6 -36.6
PIPES	F_0/F=0.83+ 32.1 [Q] mmol.dm^{-3}	0.987	0.0203	1.894	6.314	
TAPSO	F_0/F=0.94+ 17.63 [Q] mmol.dm^{-3}	0.974	0.0072	1.233	4.110	
TRIS	F_0/F=0.92+24.30[Q] mmol.dm^{-3}	0.984	0.0121	1.503	5.011	
HEPES	F_0/F=0.97+ 38.3 [Q] mmol.dm^{-3}	0.990	0.0162	1.272	4.242	
MOPS	F_0/F=0.82+ 50.3 [Q] mmol.dm^{-3}	0.946	0.0520	3.102	10.34	
MOPSO	F_0/F=0.79+49.25 [Q] mmol.dm^{-3}	0.966	0.0396	2.415	8.052	1.6 -32.2
PIPES	F_0/F=0.90+ 33.8 [Q] mmol.dm^{-3}	0.976	0.0226	2.001	6.673	
TAPSO	F_0/F=0.93+ 40.7 [Q] mmol.dm^{-3}	0.994	0.0131	0.969	3.232	
TRIS	F_0/F=0.76+ 92.8 [Q] mmol.dm^{-3}	0.984	0.0507	1.638	5.461	
HEPES	F_0/F=-0.56+390.5[Q] mmol.dm^{-3}	0.966	0.3929	3.018	10.06	6.64 -28.2
MOPS	F_0/F=0.29+ 288.4 [Q] mmol.dm^{-3}	0.979	0.2802	2.917	9.725	4.98 -28.2
MOPSO	F_0/F=-4.90+ 854.9 [Q] mmol.dm^{-3}	0.970	0.7818	2.743	9.144	6.64 -19.6
PIPES	F_0/F=-1.89+ 476.4 [Q] mmol.dm^{-3}	0.991	0.3531	2.223	7.412	6.64 -26.6
TAPSO	F_0/F=-3.39+ 592.1 [Q] mmol.dm^{-3}	0.963	0.5242	2.655	8.853	6.64 -26.6
TRIS	F_0/F=-1.69+ 467.0 [Q] mmol.dm^{-3}	0.960	0.2502	1.607	5.356	6.64 -32.2

REFERENCES

ANAND, P., B. SINGH, AND N. SINGH. **2012**. A review on coumarins as acetylcholinesterase inhibitors for Alzheimer's disease.. Bioorganic & medicinal chemistry **20**(3): 1175-1180.

ANSERMOT, N., S. RUDAZ, M. BRAWAND-AMEY, S. FLEURY-SOUVERAIN, J. VEUTHEY, AND C.B. EAP. **2009**. Validation and long-term evaluation of a modified on-line chiral analytical method for therapeutic drug monitoring of (R, S)-methadone in clinical samples. Journal of Chromatography B 877, no. **23:** 2301-2307.

AZAB, H.A., A.S. ORABI, AND A.M. ABBAS. **2015 (a)**. New probe for fluorescence detection of Azinphous ethyl, Malathion and Heptachlor pesticides. Journal of Luminescence **160**: 181-187.

AZAB, H.A., Z.M. ANWAR, M.A. RIZK, M. KH GASSER, AND M. H. EL-ASFOURY. **2015. (b).** Determination of organophosphorus pesticides in water samples by using a new sensitive luminescent probe of Eu (III) complex. Journal of Luminescence **157**: 371-382.

BENEDICT, D.J., H. . FORSHAM, AND J.R. DEW STETTEN. **1949**. The metabolism of uric acid in the normal and gouty human studied with the aid of isotopic uric acid." Journal of Biological Chemistry **181**: 183-193.

BERGER, L, AND A.B. GUTMAN. **1962**. Renal function in gout: II. Effect of uric acid loading on renal excretion of uric acid. The American journal of medicine **33**(6): 829-844.

CACIC, M., M. TRKOVNIK, F. CACIC, AND E.HAS-SCHON. 2006. Synthesis and Antimicrobial Activity of Some Derivatives on the Basis (7-hydroxy-2-oxo-2H-chromen-4-yl)-acetic Acid Hydrazide. Molecules **11**(2): 134-147.

CLIFFORD, A.J., J.A. RIUMALLO, V.R. YOUNG, AND N.S. SCRIMSHAW. **1976**. Effect of oral purines on serum and urinary uric acid of normal, hyperuricemic and gouty humans. journal of nutrition **106**: 428-434.

CORDELL, R.L., J. H. STEPHEN, C. A. ORTORI, AND D.A. BARRETT. **2008**. Quantitative profiling of nucleotides and related phosphate-containing metabolites in cultured mammalian cells by liquid chromatography tandem electrospray mass spectrometry. Journal of Chromatography B **871**(1): 115-124.

DEVJI, T., C. REDDY, C. WOO, S. AWALE, S. KADOTA, AND D. CARRICO-MONIZ. **2011**. Pancreatic anticancer activity of a novel geranylgeranylated coumarin derivative. Bioorganic & medicinal chemistry letters **21** (19): 5770-5773.

EGAN, DENISE, R. O'KENNEDY, E. MORAN, D. COX, E. PROSSER, AND R. DOUGLAS THORNES. **1990**. "The pharmacology, metabolism, analysis, and applications of coumarin and coumarin-related compounds." Drug metabolism reviews **22**(5): 503-529.

FACCHIN, G., EDUARDO KREMER, A.B. DANIEL, B.E. SUSANA, J.C., ANTONIO, AND H.T. MARÍA**.** **2009**. Interaction of Cu-dipeptide complexes with Calf Thymus DNA and antiproliferative activity of [Cu (ala-phe)] in osteosarcoma-derived cells. Polyhedron **28**(12): 2329-2334.

FALLER, J., AND H.F. IRVING. **1982**. Ethanol-induced hyperuricemia: evidence for increased urate production by activation of adenine nucleotide turnover. New England Journal of Medicine **307**(26): 1598-1602.

FUMERON, F., L. BRIGANT, H. PARRA, J.BARD, F. JEAN-CHARLES, AND A.MARIAN. **1991**. Lowering of HDL2-cholesterol and lipoprotein AI particle levels by increasing the ratio of polyunsaturated to saturated fatty acids. The American journal of clinical nutrition **53**(3): 655-659.

GEEN, G.R., J.M. EVANS, AND A.K. VONG. **1996**. Pyrans and their benzo derivatives: applications." Comprehensive heterocyclic chemistry II. Pergamon, Oxford 469-500.

GIBSON, T., A.V. RODGERS, H.A. SIMMONDS, E. TODD, AND V. MEILTON. **1983**. A controlled study of diet in patients with gout. Annals of the rheumatic diseases **42**(2): 123-127.

GILL, B.D., AND H. E. INDYK. **2007**. Determination of nucleotides and nucleosides in milks and pediatric formulas: a review. Journal of AOAC International **90**(5): 1354-1364.

GONZALESB, S.P., AND J.C. RODRIGUEZB. **2003**. Synthesis of collinin, an antiviral coumarin. Austuralian journal of chemistry **56**: 59-60.

GUOHUA, C., R.M. RUSSELL, N. LISCHNER, AND R.L. PRIOR. **1998**. Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women." *The* Journal of nutrition **128**(12): 2383-2390.

HAI, X., , L. XIAOFAN, Z. PURONG, L. LIU, C. HAN, H. JINPING, L. ZIJIE. **2010**. Highly suppressing wild-type HIV-1 and Y181C mutant HIV-1 strains by 10-chloromethyl-11-demethyl-12-oxo-calanolide A with druggable profile. Journal of medicinal chemistry **53**(3): 1397-1401.

HARRIS, M.D., L.B. SIEGEL, AND J.A. ALLOWAY. **1999**. Gout and hyperuricemia. American family physician **59**(4): 925-934.

HAUGLAND, R.P. **2002**. Handbook of fluorescent probes and research products. Molecular Probes.

HOULT, J.R.S., AND M. PAYA. **1996**. Pharmacological and biochemical actions of simple coumarins: natural products with therapeutic potential. General Pharmacology: The Vascular System **27**(4):713-722.

JIANN-YIH, Y., M.S. COUMAR, J. HORNG, H. SHIAO, F. KUO, H. LEE, AND C. CHEN. **2010**. Anti-Influenza Drug Discovery: Structure− Activity Relationship and Mechanistic Insight into Novel Angelicin Derivatives. Journal of medicinal chemistry **53**(4): 1519-1533.

KALÁSZ, H., M.Y. HASAN, E. ADEGHATE, K. TEKES, AND A. ADEM. **2010**. Chromatographic separation of antiviral/anticancer nucleoside reverse transcriptase inhibitor drugs. Analytical and bioanalytical chemistry **398**(1): 295-312.

KARALI, N., A. KOCABALKANLI, A. GÜRSOY, AND Ö. ATEŞ. **2002**. Synthesis and antitubercular activity of 4-(3-coumarinyl)-3-cyclohexyl-4-thiazolin-2-one benzylidenehydrazones. Il Farmaco **57**(7): 589-593.

KARALIOTA, A., O. KRETSI, AND C. TZOUGRAKI. **2001**. Synthesis and characterization of a binuclear coumarin-3-carboxylate copper (II)

complex. Journal of inorganic biochemistry **84**(1):33-37.

KLAWITTER, J., V. SCHMITZ, J. KLAWITTER, D. LEIBFRITZ, AND U. CHRISTIANS. **2007**. "Development and validation of an assay for the quantification of 11 nucleotides using LC/LC–electrospray ionization–MS. Analytical biochemistry **365**(2): 230-239.

KOSTOVA, I., I. MANOLOV, I. NICOLOVA, S. KONSTANTINOV, AND M. KARAIVANOVA. **2001**. New lanthanide complexes of 4-methyl-7-hydroxycoumarin and their pharmacological activity. European journal of medicinal chemistry **36**(4): 339-347.

KRSTULOVIĆ, A.M. **1982**. Investigations of catecholamine metabolism using high-performance liquid chromatography: analytical methodology and clinical applications. Journal of Chromatography B: Biomedical Sciences and Applications **229** (1): 1-34.

LIN, CHUN-MAO, SHENG-TUNG HUANG, FU-WEI LEE, HSIEN-SAW KUO, AND MEI-HSIANG LIN. **2006**. 6-Acyl-4-aryl/alkyl-5, 7-dihydroxycoumarins as anti-inflammatory agents. Bioorganic & medicinal chemistry **14**(13): 4402-4409.

LIS, S., M. ELBANOWSKI, B. MAKOWSKA, AND Z. HNATEJKO. **2002.** Energy transfer in solution of lanthanide complexes. Journal of Photochemistry and Photobiology A: Chemistry **150** (1): 233-247.

MANVAR, A., B. ABHAY, R .ASHISH, P. JIGNESH, V. VIPUL, D. NARSHIH, R. KENA, AND S. ANAMIK. **2011**. Diversity oriented design of various hydrazides and their in vitro evaluation against Mycobacterium tuberculosis H37 Rv strains. Bioorganic & medicinal chemistry letters **21**(16):4728-4731.

MIN, XU., M. ZHAO-RONG, L. C. HUANG, FENG-JUAN, AND Z. ZENG. **2011**. Spectroscopic studies on the interaction between Pr (III) complex of an ofloxacin derivative and bovine serum albumin or DNA. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy **78**(1): 503-511.

NAVARRO, E.M.., E.J. CISNEROS-FAJARDO, A. SIERRALTA, , M. FERNÁNDEZ-MESTRE, P. SILVA, D. ARRIECHE, AND MARCHÁN. **2003**. Design of copper DNA intercalators with leishmanicidal activity., JBIC Journal of Biological Inorganic Chemistry **8**: 401–408.

NAWROT-MODRANKA, J., N . EWA, AND G. JULITA. **2006.** In vivo antitumor, in vitro antibacterial activity and alkylating properties of phosphorohydrazine derivatives of coumarin and chromone. European journal of medicinal chemistry **41**(11): 1301-1309.

PIAZZI, L., A. CAVALLI, F. COLIZZI, F. BELLUTI, M. BARTOLINI, F. MANCINI, M. RECANATINI, V. ANDRISANO, AND A. RAMPA. **2008**. Multi-target-directed coumarin derivatives: hAChE and BACE1 inhibitors as potential anti-Alzheimer compounds. Bioorganic & medicinal chemistry letters **18**(1): 423-426.

QIAN, T., Z. CAI, AND M. S. YANG**. 2004**. Determination of adenosine nucleotides in cultured cells by ion-pairing liquid chromatography–electrospray ionization mass spectrometry. Analytical biochemistry **325**(1): 77-84.

REDDY, N. S., M.R. MALLIREDDIGARI, S. COSENZA, K. GUMIREDDY, S.C. BELL, E.P. REDDY, AND MV REDDY. **2004**. Synthesis of new coumarin 3-(N-aryl) sulfonamides and their anticancer activity. Bioorganic & medicinal chemistry letters **14**(15): 4093-4097.

SHAKHVERDOV, T. A., AND R. ÉRGASHEV. **1989**. Absorption and fluorescence of complex-compounds of anthracenecarboxylic acid with ions of metals in liquid solutions. teoreticheskaya i eksperimentalnaya khimiya **25**(6): 673-682.

SHI, Y., AND C. ZHOU. **2011**. Synthesis and evaluation of a class of new coumarin triazole derivatives as potential antimicrobial agents. Bioorganic & medicinal chemistry letters **21**(3): 956-960.

VELA, J.E., L.Y. OLSON, A. HUANG, A. FRIDLAND, AND A.S. RAY. **2007**. Simultaneous quantitation of the nucleotide analog adefovir, its phosphorylated anabolites and 2′-deoxyadenosine triphosphate by ion-pairing LC/MS/MS. Journal of Chromatography B **848**(2): 335-343.

WASLIEN, W., I. CAROL, D.H. CALLOWAY, AND S. MARGEN. **1968**. Uric acid production of men fed graded amounts of egg protein and yeast nucleic acid. The American journal of clinical nutrition **21**(9): 892-897.

YAMAOKA, N., K. KANEKO, Y. KUDO, M. AOKI, M. YASUDA, K. MAWATARI, K. NAKAGOMI, Y. YAMADA, AND T. YAMAMOTO. **2010**. Analysis of purine in purine-rich cauliflower. Nucleosides, Nucleotides and Nucleic Acids **29**: 518-521.

YIN XIAN-HONG, Y. MAN-YA, S. HUA-HONG, Y. GUANG, C. XIAO-MING, AND G. LIAN-QUAN. **2000**. Luminescence of terbium chelates with substituted pyridine-2, 6-dicarboxylic acid derivatives by synergistic effect of EDTA . Journal of the Chinese Rare Earth Society 18**(3): 212−215**

ZONGWEI, C., F. SONG, AND M.S. YANG.**2002**. Capillary liquid chromatographic–high-resolution mass spectrometric analysis of ribonucleotides. Journal of Chromatography A **976** (1): 135-143.

الملخص العربي

الوميض الناشئ عن كاتيونات اللانثانيدات يقدم العديد من المزايا من خلال جزيئات الفلورسنت العضوية مثل ان الانبعاثات حادة، و الانبعاثات مميزة تسمح لسهولة التفرقة بين الإشارات اللانثانيدات المتعددة؛ عمر الانبعاثات طويلة (ميكرو ثانية مللي) ادي الي جعلها المرشحة بأمتياز لقياس فترة الانحلال : ومقاومة عالية للتبيض الصورة تسمح للتجارب طويلة أو متكررة. و الطريقه المقدمه استخدمت الانخفاض في الانبعاث متراكب أيون التربيوم-مشتق الكومارين ٨- أليل-٢-أوكسو-٢-هيدروجين-كرومين-٣-كربالدهايد (AOCC) ذات فترة عمر النصف الكبيرة وتمة دراسة تفاعل المتراكب الثنائي (ليجاند:المعدن) بنسبة تركيبية ١:٢ للكشف عن القواعد النووية، النيوكليوسيدات والنيوكليوتيدات. وتم دراسة تفاعل التربيوم-مشتق الكومارين ٨- أليل-٢-أوكسو-٢-هيدروجين-كرومين-٣-كربالدهايد (AOCC)مع النيوكليوسيدات(ادينين ، سيتدين)، القواعد النووية (اينوسين)، النيوكليوتيدات(٥/-جوانسين أحادي فوسفات،٥/اينوسين أحادي فوسفات،٥/ادينوسين ثلاثي فوسفات،٥/- سيتدين أحادي فوسفات ،و/ادينوسين ثنائي فوسفات)باستخدام تقنيات الانبعاث العادي. تفاعل وقد تم الحصول علي علاقة خطية مع التركيز للجزيئات الحيوية في مدي من ١,٦الي ٣٦,٦ ميكرومول ديسمتر-٣وحد التركيز في المدي من ٠,٩٠٦ الي ٨,٢٨ ميكرو مول ديسيميتر -٣.