Research Article

Analytical studies of the interaction of Tb (III)-2-{[(4-methoxy benzoyl) oxy]} methyl benzoic acid binary complex with nucleosides

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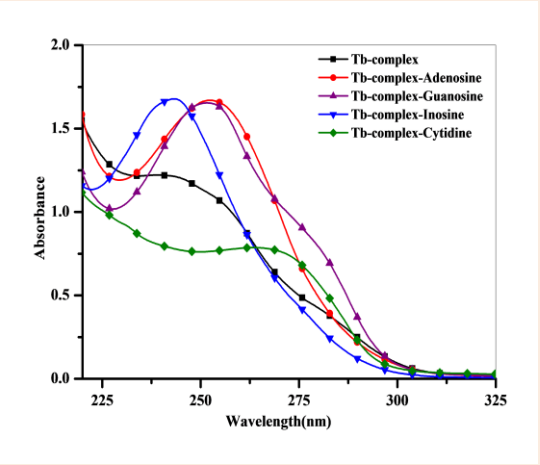
Abstract

The interaction of Tb (III)-2-{[(4-methoxy benzoyl) oxy]} methyl benzoic acid binary complex with nucleosides (Adenosine,Cytidine, Guanosine and Inosine)was investigated using UV and fluorescence methods. The reaction of Tb-complex with cytidine, guanosine and adenosine is accompanied by shift to longer wavelength in the absorption band, while there is a blue shift in the absorption band with an enhancement in the molar absorptivity upon the reaction with inosine. The fluorescence intensity of Tb(III)-2-{[(4-methoxy benzoyl) oxy]} methyl benzoic acid binary complex at λ= 545 nm ($^5D_4 \rightarrow {}^7F_5$) was decreased with the addition of the nucleoside molecule following the order: Cytidine > Inosine> Guanosine> Adenosine.

Keywords: Tb, Adenosine, Nucleosides, Fluorescence, UV



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Introduction

Lanthanide trivalent cations have excellent photo-luminscent properties usable in a high range of different applications. The ions poor ability to absorb light makes it necessary to dress them up with an organic ligand as a skin in the form of a complex [1]. The complexes have become increasingly significant in the last few years due to the wide variety of potential applications in many important areas of chemistry, [2-4] biology, [5, 6] medicine [7, 8] and imaging [9-12]. The design of the organic part of such complexes is thus paramount in achieving the required circumstances for the complex to be efficiently luminescent. Luminescent materials, especially lanthanides, are applied in many devices of importance [13]. The interest in the photophysical properties of lanthanide complexes which act as optical centers in luminescent hybrid materials has grown considerably since Lehn [14] asserted that such complexes could be seen as light-harvesting supramolecular devices. Particularly, the design of efficient lanthanide complexes has drawn the attention of many research groups, focusing on the diverse classes of ligands, β-diketones, heterobiarly ligands, etc.

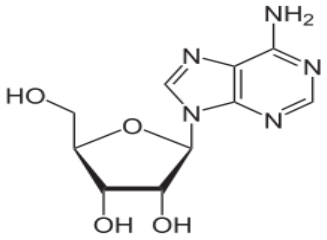
Nucleosides play an important role as metabolic precursors in nucleic acid synthesis being crucial for the control of cell and tissue growth. In addition, nucleosides and nucleotides are also key determinants of energy metabolism (ATP, GTP), ligands of purine receptors (adenosine, inosine) or transducers of endocrine signals (cAMP, cGMP), thereby modulating a wide range of cellular events. Moreover, many nucleoside analogues are currently used in anticancer and antiviral therapies, thus highlighting the pharmacological role these molecules can also play in disease [15]. Several nucleoside-derivatives are currently used in antiviral and antitumor therapies. These compounds show slight structural modifications with respect to natural nucleosides and retain most of the metabolic properties of parent compounds. Both pyrimidine and purine nucleoside analogs are currently used clinically as anticancer drugs [16].

Inosine is a nucleoside that is formed when hypoxanthine is attached to a ribose ring (also known as a ribofuranose) via a β-N_9-glycosidic bond.Inosine is commonly found in tRNAs and is essential for proper translation of the genetic code in wobble base pairs.Knowledge of inosine metabolism has led to advances in immunotherapy in recent decades. Inosine monophosphate is oxidised by the enzyme inosine monophosphate dehydrogenase, yielding xanthosine monophosphate, a key precursor in purine metabolism. Adenosine is a purinenucleoside comprising a molecule of adenine attached to a ribose sugar molecule (ribofuranose) moiety via a β-N_9-glycosidic bond[17].Adenosine plays an important role in biochemical processes, such as energy transferase adenosine triphosphate (ATP) and adenosine diphosphate (ADP) as well as in signal transduction as cyclic adenosine monophosphate (cAMP). It is also an inhibitory neurotransmitter, believed to play a role in promoting sleep and suppressing arousal, with levels increasing with each hour an organism is awake[18-20]. Cytidine is a nucleosidemolecule that is formed when cytosine is attached to a ribose ring (also known as a ribofuranose) via a β-N_1-glycosidic bond. Cytidine is a component of RNA.Guanosine is a purinenucleoside comprising guanine attached to a ribose (ribofuranose) ring via a β-N_9-glycosidic bond. Guanosine can be phosphorylated to become guanosine monophosphate (GMP), cyclic guanosine monophosphate (cGMP), guanosinediphosphate (GDP), and guanosine triphosphate (GTP). These forms play important roles in various biochemical processes such as synthesis of nucleic acids and proteins, photosynthesis, muscle contraction and intracellular signal transduction (cGMP). The present work is focuses on the ability to use the lanthanide metal complexes of 2-{[(4-methoxy benzoyl) oxy]} methyl benzoic acid as probes for nucleosides.

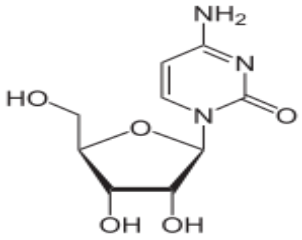
Experimental

Materials and Solutions

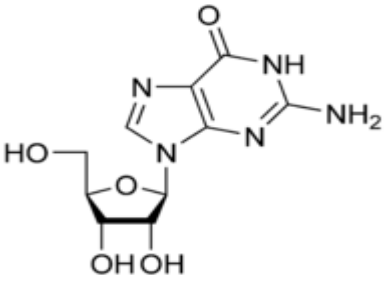
Chemical structures of adenosine, cytidine, guanosine and inosine can be represented as follows:



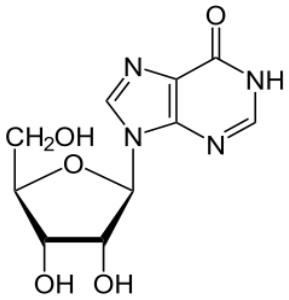
Adenosine



Cytidine



Guanosine

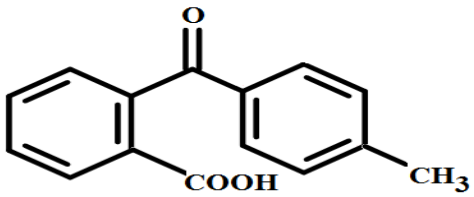


Inosine

All materials employed in the present investigation were of A.R.grade products. [9-β-D-ribfuranosyl-9H-purine-6-amine)(9-β-D-ribfuranosyl-adenine]$C_{10}H_{13}N_5O_4$**(Adenosine)**, [4-amino-1-β-D-ribofuranosyle-2-(1H)-pyrimidine] $C_9H_{13}N_3O_5$**(Cytidine)**,[2-amino-1, 9-dihydro-9- β-D-ribfuranosyl-6H-purine-6-one)(9- β-D-ribfuranosyl-

guanine]$C_{10}H_{13}N_5O_5$**(Guanosine)**, (1, 9- dihydro-9- β-D-ribfuranosyl-6H-purine-6-one)(Hypoxanthine-9-D-ribofuranoside)$C_{10}H_{12}N_4O_5$**(Inosine)**, were purchased from Sigma chemical Co. and were used without purification.

2-{[(4-methoxy benzoyl) oxy]} methyl benzoic acid was purchased from Sigma chemical Co. and was used without purification. The structure of the ligand is shown as follows:



2-{[(4-methoxy benzoyl) oxy]} methyl benzoic acid

Preparation of buffer solutions

Tris (also known as THAM) is an abbreviation of the organic compound known as tris(hydroxymethyl)aminomethane. Tris is extensively used in biochemistry and molecular biology[21].A common form of tris is tris-HCl, which is the acid salt. When titrated to when pH = pKa with the corresponding counterion (OH- for tris-HCl, H+ for tris base) they have equivalent concentrations. However, the molecular weights are different and must be correctly accounted for in order to arrive at the expected buffer strength.Tris has a pKa of 8.1 so This buffer is made at pH 7 to pH 9. This buffer can be prepared at any concentration and any volume and use it to prevent large swings in the pH of a solution.

Procedure for spectrophotometric measurements

The UV and visible spectra of the solutions of the binary and ternary metal complexes were scanned on a Shimadzu-UV Probe spectrophotometer model (Shimadzu-UV Probe Version 2.33 UV-Visible automatic recoding spectrophotometer with 1.0 cm quartz cell). The solutions studied were prepared according to the following procedure:

(a) 5×10^{-5} $molL^{-1}$ Tb(III) + 1.5×10^{-4} $molL^{-1}$ Ligand (1:3) Molar concentration ratio.
(b) 5×10^{-5} $molL^{-1}$ Tb(III) +1.5×10^{-4} $molL^{-1}$ Ligand + 5×10^{-5} $molL^{-1}$ nucleoside (1:3:1) Molar concentration ratio.

Fluorescence measurements

A JASCO-FP 6300 spectrofluorometer with 1.0 cm quartz cell was used for the emission and spectral measurements. All pH measurements were made using a combined glass electrode connected to the digital pH-meter CG 808 (Schott Gerate, Germany). The pH was adjusted to the required value using tris-HCl buffer solution.
A micropipette was used to transfer the analyte solution to the cell throughout the experimental work. The solutions were scanned from 200 to 500 nm. All measurements were performed at room temperature.

Results and Discussion

UV measurements

The interaction of Tb-2-{[(4-methoxy benzoyl) oxy]} methyl benzoic acid complex with nucleosides is shown in figure 1,where the UV spectra for the binary complex exhibits one absorption peak at 249 nm. There is a shift to longer wavelength in the absorption band upon reaction with cytidine, guanosine and adenosine, while there is a shift

to lower wavelength with an enhancement in the molar absorptivity upon reaction with inosine, these data are collected in Table 1.

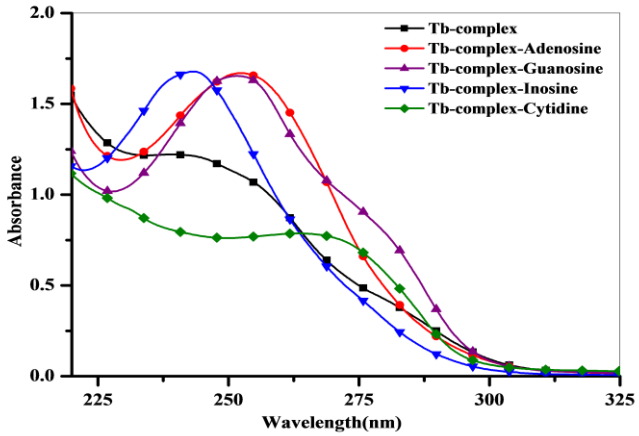


Figure 1 UV-Visspectra for Tb-2-{[(4-methoxy benzoyl) oxy]} methyl benzoic acid complex with nucleosides under study in Tris-HCl buffer pH 7.0

Table 1 Characteristic absorption data for interaction ofTb(III)-2-{[(4-methoxy benzoyl) oxy] } methyl benzoic acid with different Nucleosides in Tris-HCl buffer pH 7.0 and at 25^{o}C.[Tb(III)] =5x10^{-5} molL^{-1}, [2-{[(4-methoxy benzoyl) oxy]} methyl benzoic acid] = 1.5 x10^{-4}molL^{-1}, [Nucleoside] = 5x10^{-5} molL^{-1}

Compound	λ_{max}(nm)	Absorbance
Tb(III)-2-{[(4-methoxy benzoyl) oxy] } methyl benzoic acid complex	249	1.15
Tb(III)-complex-Cytidine	277	0.645
Tb(III)-complex-Guanosine	252	1.66
Tb(III)-complex-Adenosine	254	1.67
Tb(III)-complex-Inosine	243	1.68

The characteristic spectrum for the interaction of Tb(III)-binary complex with nucleosides (Adenosine, Cytidine, Guanosine and Inosine) in a concentration of 5x10^{-5}mol L^{-1}is shown in Figure2. There are four types of transitions obtained at λ_1 = 490 nm, λ_2 = 545 nm, λ_3 = 585 nm and λ_4 = 620 nm, which may be attributed to $^5D_4 \rightarrow {}^7F_6$, $^5D_4 \rightarrow {}^7F_5$, $^5D_4 \rightarrow {}^7F_4$and $^5D_4 \rightarrow {}^7F_3$ transitions[22] Focusing the variation of the peak intensity at λ = 545nm, it is clearly observed that the interaction of the nucleoside molecule with binary complex is accompanied by decreasing of the peak intensity following the order:

Cytidine > Inosine > Guanosine > Adenosine

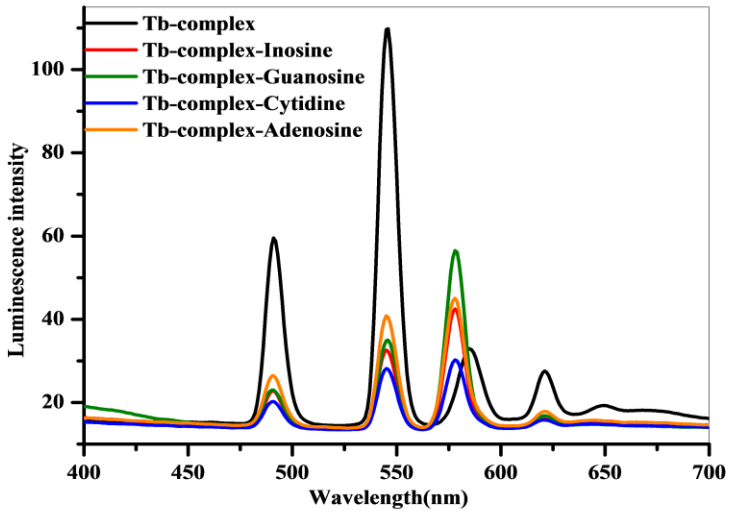


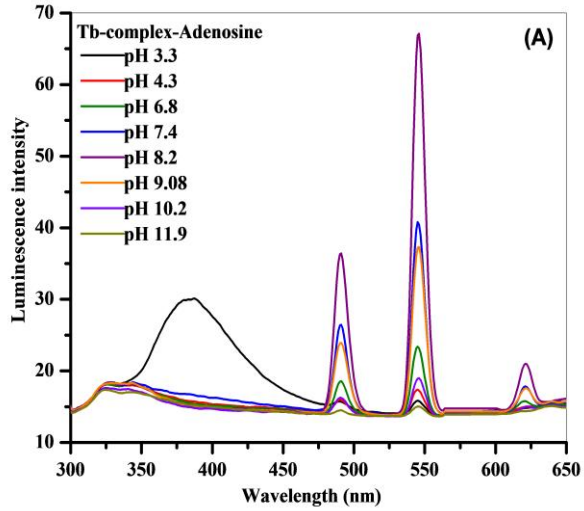
Figure 2 Effect of interaction of different nucleosides on the characteristic emission bands for Tb(III)-2-{[(4-methoxy benzoyl) oxy] } methyl benzoic acid complex ,λ_{ex}=288 nm, λ_{em} =545 nm in Tris-HCl buffer pH 7.0 at 25 ^{o}C.

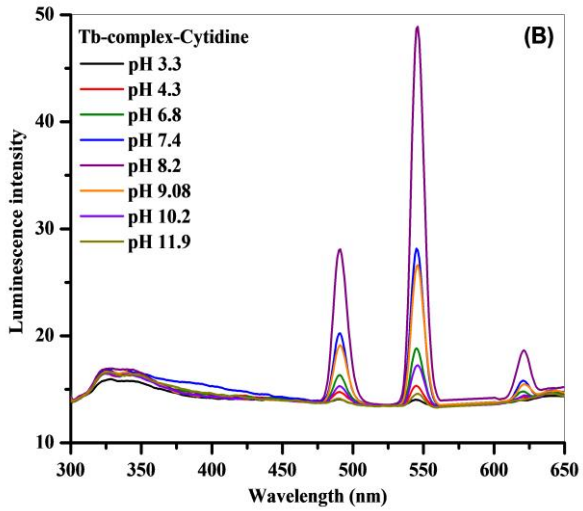
[Tb(III)] = 5x10^{-5} molL^{-1}, [Ligand] = 1.5 x10^{-4}molL^{-1}, [Nucleoside] = 5x10^{-5} molL^{-1}

Effect of pH and the choice of buffer solution

Luminescence intensity ofTb-2-{[(4-methoxy benzoyl) oxy]} methyl benzoic acid complex with nucleosides is strongly dependent on pH (**Figure 3**).

Therefore, we choose pH 7.0 (0.1 M Tris –HCl buffer) which coincide with the physiological pH which is necessary to study the interaction of the complex with bioligands. The effect of pH on the emission intensity of Tb-complex-nucleosides at λ_{em} = 545 nm is indicated in **Figure 4**.





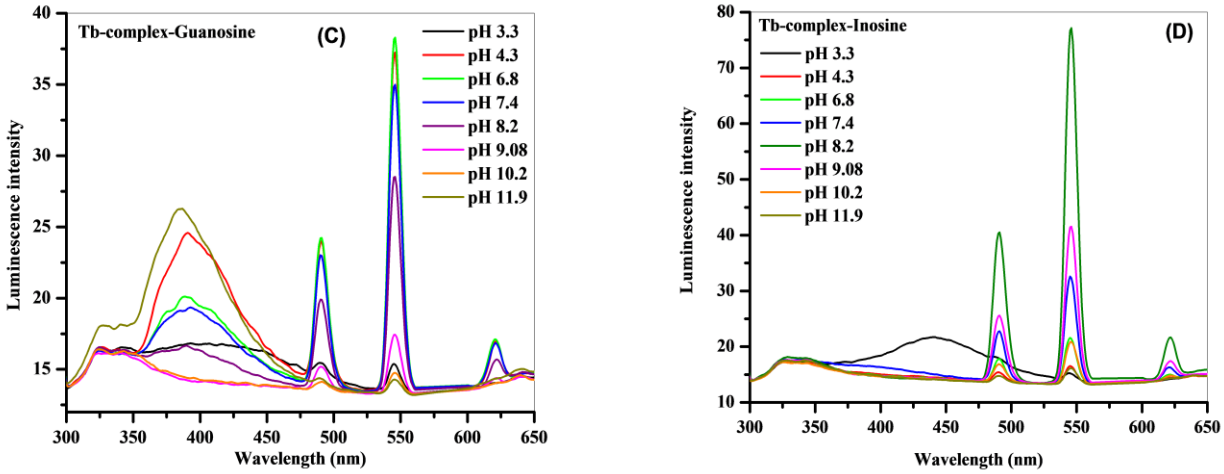


Figure 3 Fluorescence spectra for Tb-2-{[(4-methoxy benzoyl) oxy]} methyl benzoic acid complex with nucleosides under study (A) adenosine, (B) cytidine, (C) guanosine and (D) inosine at different pH

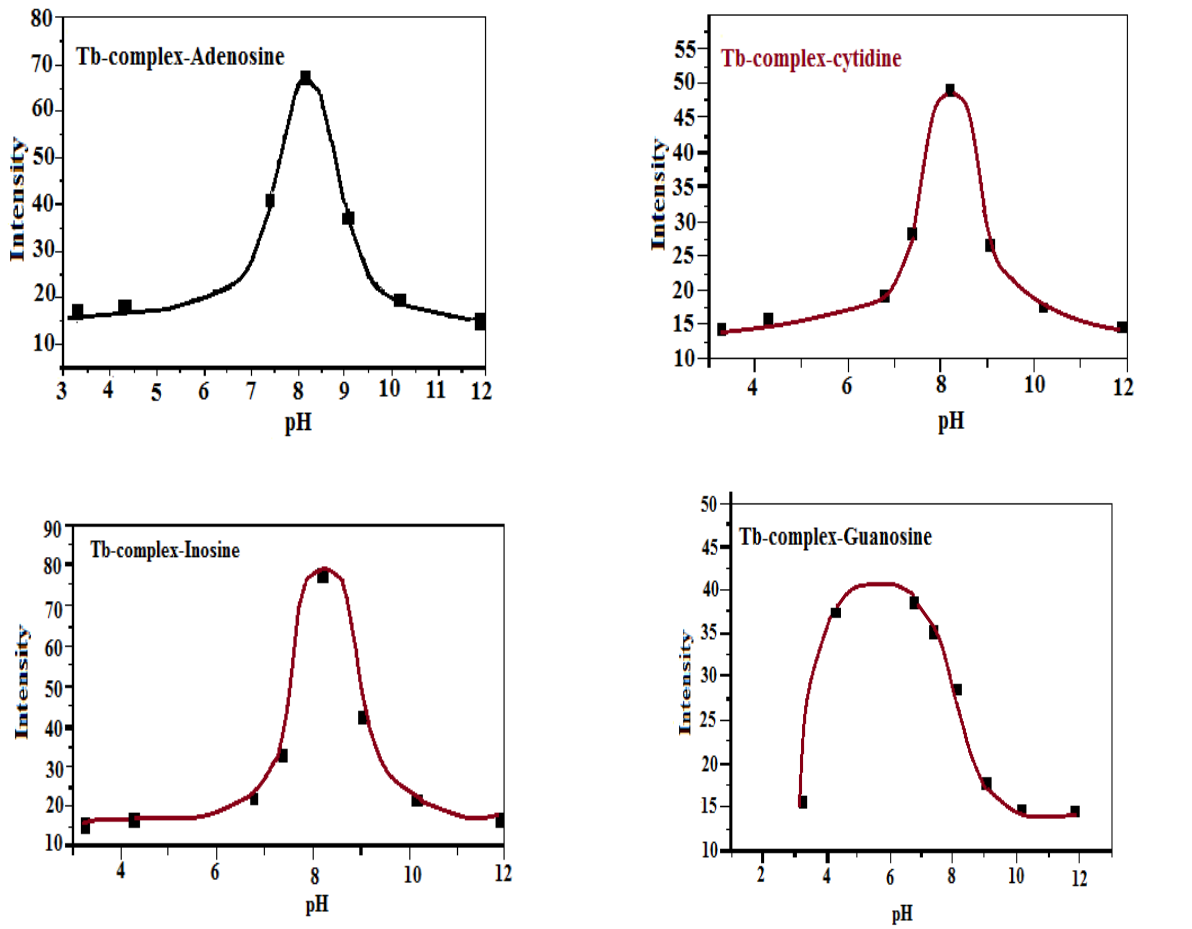


Figure 4 Effect of pH on the emission intensity of Tb-complex-nucleosides at λ_{em} = 545 nm at 25°C

Effect of nucleosides on the fluorescence intensity of Tb(III)-2-{[(4-methoxy benzoyl)oxy] } methyl benzoic acid binary complex

The effect of nucleosides concentration on the luminescence intensity on the Tb (III)-2-{[(4-methoxy benzoyl) oxy]} methyl benzoic acid binary complex under optimum conditions was investigated in the concentration range between(8×10^{-5}- 2.5×10^{-4} $molL^{-1}$) as shown in Fig.5.The fluorescence intensity of Tb-complex decreased regularly with gradual increase in the concentration of nucleosides. Fluorescence quenching can be induced by a variety of molecular interactions of a fluorophore with quencher molecule, including ground-state complex formation, energy transfer, molecular rearrangement, excited-state reactions, and collisional quenching [23]. The mechanisms of fluorescence quenching are usually classified as either dynamic quenching or static quenching [24]. Linear calibration plots were established (Fig. 6). The regression data and LODs (3σ/slope) are summarized in Table 2. As can be seen from the data, the regression parameters for calibration curves exhibit linearity, sensitivity and detection limit in the given concentration range.

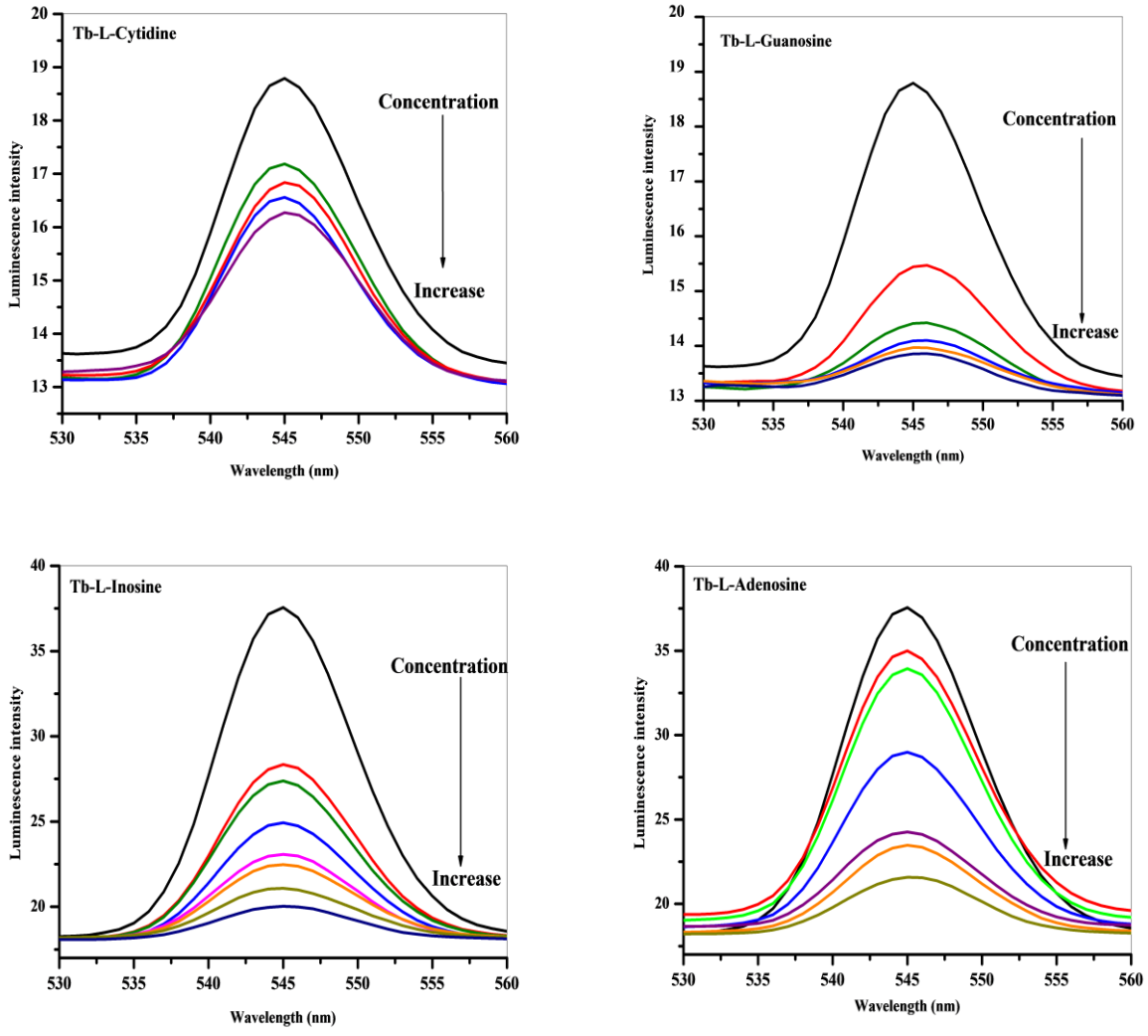


Figure 5 Decrease of emission of Tb-complex at 545 nm in the presence of nucleosides in Tris-HCl buffer pH 7.0

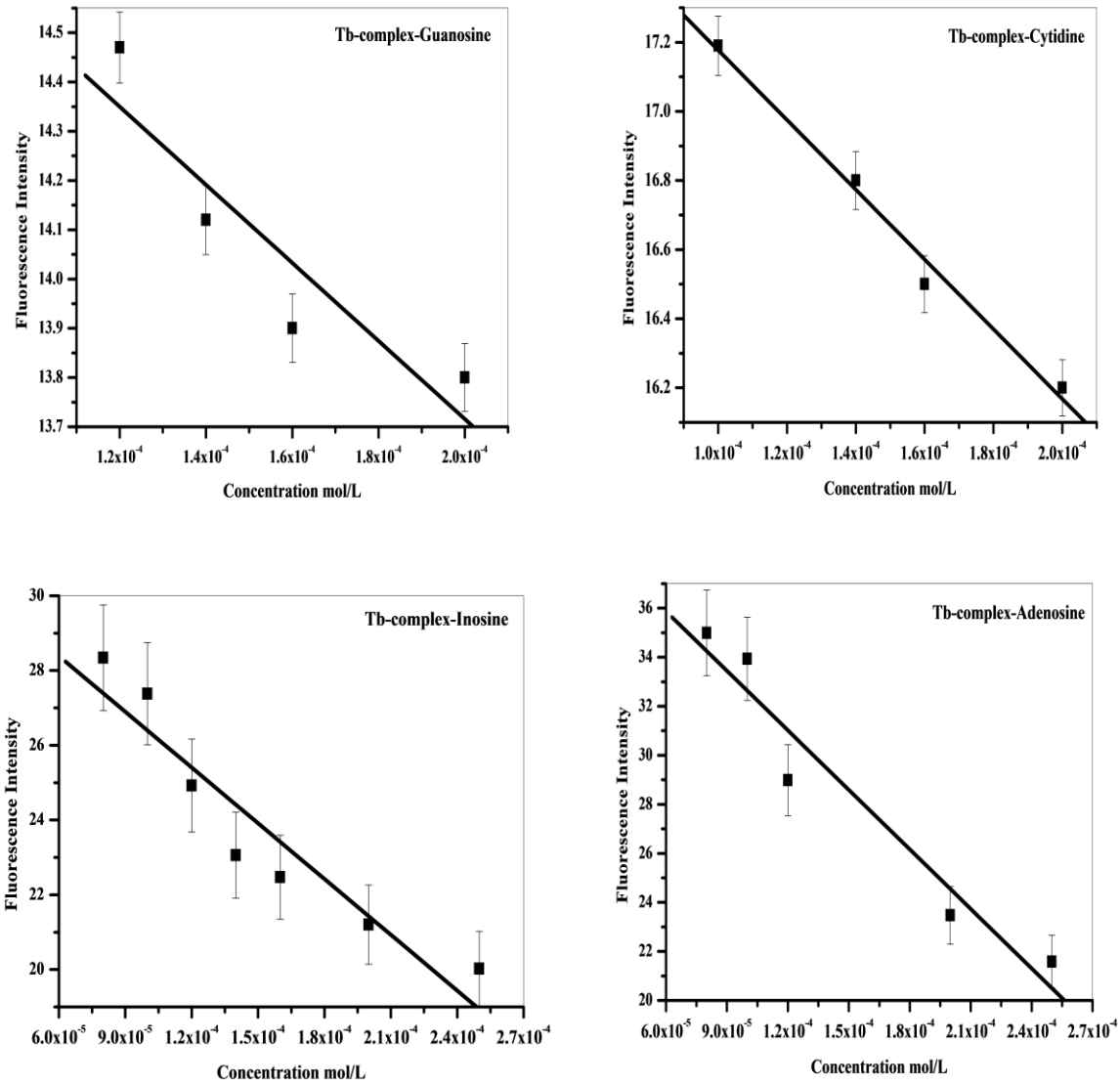


Figure 6 Calibration plots for determination of nucleosides λ= 545 nm by normal luminescence mode

Table 2 Parameters of curves calibrations obtained for the determination of nucleosides

Nucleoside	R	SD(σ)	LOD (mmolL^{-1})
Adenosine	0.9695	1.702	0.063
Cytidine	0.9934	0.0592	0.0175
Inosine	0.9465	1.0978	0.0662
Guanosine	0.9124	0.1487	0.0562

Stern-Volmer quenching constant

Using Stern-Volmer equation [25] ; $\mathbf{F^o/F = 1+K_{SV}\,[Q]}$ the ratio F^o/F increases linearly with the nucleoside concentration and a linear regression equation following Stern-Volmer relation is obtained. K_{SV} is the bimolecular quenching constant and it can be obtained from the slope of regression curve of F^o/F versus [Nucleoside]. The linear fit in Stern-Volmer equation for the interaction of nucleoside with Tb(III)-2-{[(4-methoxy benzoyl) oxy] } methyl benzoic acid binary complex is shown in **Figure 7**.The data for the calibration curve are collected in Table 3.

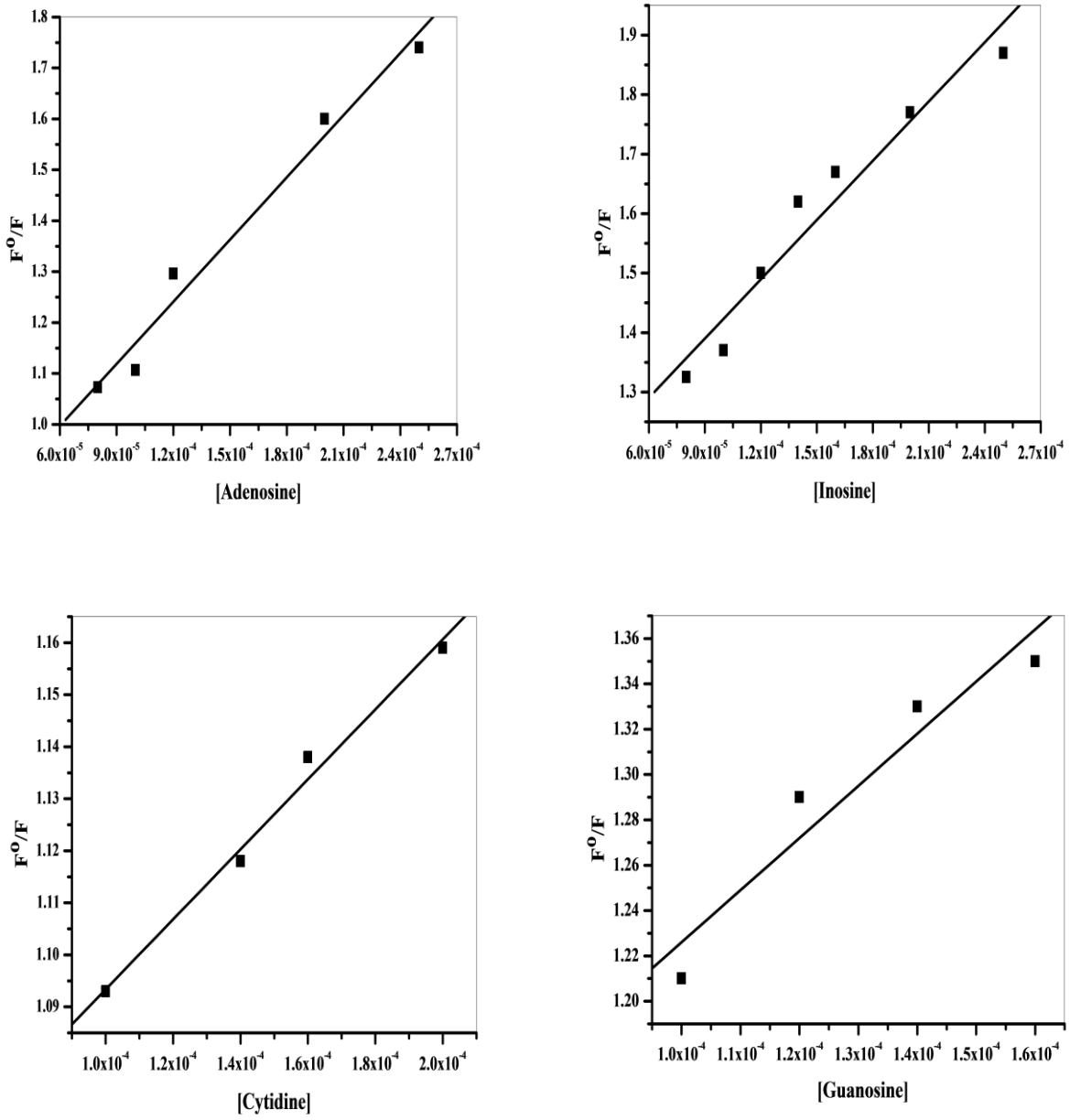


Figure 7 Stern-Volmer plot for the interaction of nucleosides with Tb(III)-2-{[(4-methoxy benzoyl) oxy] } methyl benzoic acid binary complexin Tris-HCl buffer pH7.0 andat 25^{o}C

Table 3 Stern-volmer constant (K_{sv}) and limit of detection (LOD) obtained for the determination of nucleosides at 25°C

Nucleoside	LOD(mmolL^{-1})	K_{sv}(mol^{-1}L)	R	SD(σ)
Adenosine	0.0377	4.07×10^{3}	0.988	0.05116
Cytidine	0.016	0.673×10^{3}	0.994	0.0036
Inosine	0.046	3.3×10^{3}	0.9727	0.0512
Guanosine	0.027	2.3×10^{3}	0.959	0.021

Conclusion

UV and fluorescence spectrophotometric methods were used to study the interaction of nucleosides with Tb- binary complex based on the quenching of Tb-complex. Stern-Volmer constant (K_{SV})is obtained. Using this method, the decrement intensity is proportional to the amount of nucleoside. Under optimized assay conditions, a linear range of (8×10^{-5} - 2.5×10^{-4} molL^{-1}) is got with low detection limit of 63, 17, 66 and 56 μmol$L^{-1}$for adenosine, cytidine, inosine and guanosine respectively. The regression parameters for calibration curves exhibit linearity, sensitivity and detection limit in the given concentration range.

References

[1] Brunet E., Juanes O., Sedano R., and Rodriguez J. C.- Ubis,Tetrahedron ,2005, 61,6757 .
[2] Buenzli J. C. Acc. Chem. Res. 2006, 39, 53.
[3] Nasso I., Galaup C., Havas F., Tisnes P., Picard C., Laurent S., Vander Elst L. and Muller,Inorg. R. Chem, 2005, 44, 8293.
[4] Dossing A. E., Inorg. J. Chem. 2005, 8, 1425.
[5] Yuan J. and Wang G., Trends Anal.Chem. 2006, 25,490.
[6] Robert J.C., Coord. Chem. Rev. 2014, 1,273.
[7] Arnesano F., Banci L., Bertini I., Capozzi F., Ciofi-Baffoni S., Ciurli S. ,Luchinat C., Mangani S., Rosato A., Turano P., and Viezzoli M. S., Coord. Chem. Rev. 2006, 250, 1419.
[8] Kostova I., Curr. Med. Chem.: Anti-Cancer Agents, 2005, 5,591.
[9] Aime S., GeninattiCrich S., Gianolio E., Giovenzana G. B., Tei L. and Terreno E. Coord. Chem. Rev. 2006, 250, 1562.
[10] Caravan P., Chem. Soc. Rev. 2006, 35,512.
[11] Pandya S., Yu J., and Parker D., Dalton Trans., 2006, 23, 2757.
[12] Picard C., Geum N.I., Nasso I., Mestre B., Tisnes P., Laurent S. R., Muller N., and Vander L., Elst,Bioorg. Med. Chem. Lett. 2006, 16, 5309.
[13] Blasse G., Journal of Alloys and Compounds, 1995, 225,529.
[14] Lehn J. M., " perspectives in supramolecular chemistry-from molecular recognition towards molecular information processing and self-organization", AngewandteChemie International Edition in English, Paris, France, 1990, 29, 1340.
[15] Míriam M-A., Casado F. J., and Marçal P-A., Current Vascular Pharmacology, Nucleoside Transporter Proteins 2009, **7**,426.
[16] Galmarini C. M., Mackey J. R. and Dumontet C., Leukemia 2001, 15,875.
[17] Huang D-W.,Niu, C-G M. ,Zeng G., Ruan M., Biosensors and Bioelectronics 2011, 29,178.

[18] Wu Z. S., Guo M. M., Zhang S. B., Chen C. R., Jiang J. H., Shen G. L. and Yu, R. Q. Anal. Chem. 2007, 79, 2933.
[19] Zhang S. S., Xia J. P., Li X. M., Anal.Chem. 2008, 80 ,8382.
[20] Baraldi P. G. ,Tabrizi M. A., Gessi S., Borea P. A., Chem. Rev. 2008, 108, 238.
[21] Gomori G. " 8 Preparation of Buffers for Use in Enzyme Studies", Methods Enzymology., 1955,1,138.
[22] Cha Y-E Li, X. Ma, D. and Huo, R. European Journal of Inorganic Chemistry. ..2014, 18, 2969.
[23] Nanda R. K., Sarkarand N., Banerjee R. J. Photochem. Photobiol.A Chem., 2007, 192,152.
[24] Currie L. A., Anal. Chem. Acta, 1999, 391, 103.
[25] Lakowicz J. R. " Principles of Fluorescence Spectroscopy" 2nd KluwerAcademic / Plenum Publishers 1999.

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