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Cation binding characteristics of tetrandrine studied by UV-Vis absorption and fluorescence spectroscopies

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Abstract

The binding of monovalent (Li^+, Na^+, K^+) and bivalent (Mg^{2+}, Ca^{2+}) metal cations to a macrocyclic alkaloid tetrandrine has been studied by UV-Vis absorption and fluorescence spectroscopies. The calculated binding constants are between 3654 and 282 613 $dm^3\ mol^{-1}$ and the corresponding free energies from 4.777 to 7.308 kcal mol^{-1}. The effect of the solvent on the complexation strength as well as the role of the counter-ion are discussed. Small changes are observed in the UV-Vis spectrum after complexation in respect to free ligand. The fluorescence response increases significantly in intensity and a blue shift appears by adding of calcium perchlorate to tetrandrine (6,6′,7,12-tetramethoxy-2,2′-dimethyl—berbaman; TET) as a consequence of conformational restriction induced by cation binding.

Keywords: Ionic recognition; UV-Vis absorption; Fluorescence; Tetrandrine

1. Introduction

Tetrandrine (6,6′,7,12-tetramethoxy-2,2′-dimethyl—berbaman; TET) is a bis-benzylisoquinoline derivative alkaloid, known as, was isolated from the root of Stephania tetrandrae S. Moore. It is used as traditional drug in oriental countries [1]. TET has various biological activities: cardiovascular, anti-inflammatory and as anti-tumour agent. These activities are related to its calcium antagonist properties and they have been studied in previous papers [2–6]. The mechanism of action is not completely understood. We have recently, showed that TET forms a complex with the calcium ion and thus it may interfere in the calcium cycle [7,8].

TET a natural cyclophane-like structure shows a dimer of two benzylisoquinoline subunits condensed in a head to head, tail to tail fashion by two ether bridges, forming a central cavity which may accommodate cationic species (Fig. 1). RMN and X-ray studies of TET have demonstrated that the structure has similar conformations both in solution and in solid state [9,10].

The driving forces of the molecular complex assembly are: hydrogen bonding, ion pairing, van der Waals interactions, metal–ligand binding, solvent reorganization, partial covalent bonds and noncovalent cation-pi type interactions [11]. Studies of ligand binding to alkaline, alkaline-earth and trivalent cations are reported due to potential application in analysis and separation of metal ions, in biology and other fields [12,13].

In this paper we characterize the binding properties of TET as host, towards alkaline and alkaline-earth metals, engaged in a molecular recognition process. This behavior can be related to the three-dimensional characteristics of such relatively rigid structure that can adapt only a small number of ions, thus the complexation being specific [14].

2. Experimental

TET purum (98%), $LiClO_4$, $NaClO_4{\cdot}H_2O$ (99%), $Ca(ClO_4)_2{\cdot}4H_2O$ were purchased from commercial suppliers. Metallic picrates of sodium (NaPic), potassium (KPic), calcium ($CaPic_2$) and magnesium ($MgPic_2$) were prepared in our laboratory [8]. The solvents tetrahidrofurane (THF) and acetonitrile (ACN) were of HPLC grade and used without further purification. Other used reagents like sodium

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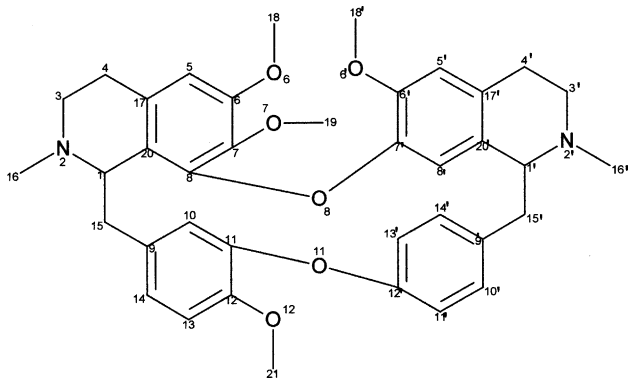

Fig. 1. Molecular structure of TET and the numbering scheme.

carbonate, potassium carbonate, magnesium carbonate and picric acid were of the highest purity commercially available.

UV-Vis spectra were recorded on a Perkin-Elmer Lambda 2S spectrophotometer. The fluorescence spectra were performed also with a Perkin-Elmer luminescence LS 50B spectrometer.

For this study, we have used two different approaches. Firstly, in the case of metallic picrate complexes with TET we have recorded the UV-Vis spectra of metallic picrates in ACN and THF. Next we recorded the same type of spectra adding increased amounts of TET. Otherwise, in the case of metallic perchlorates we have recorded the UV-Vis and fluorescence spectrum of the free ligand in acetonitrile and with various amounts of metallic perchlorates.

3. Results and discussion

The complexes formed by neutral macrocyclic ligands with metallic ions are of the type cationic [15]. They are extracted in organic solvents as ion pairs with an anion having large molecular weight and lypophilic character [16]. Low polarity solvents (THF) inhibit the dissociation of the ion-pair favoring complex formation. The bigger polarity of acetonitrile (see transition energy $E_T = 46\,\text{kcal mol}^{-1}$, at 25 °C) towards THF ($E_T = 37.4\,\text{kcal mol}^{-1}$ also at 25 °C) permit us to expect lower values of the stability constant K of the studied TET complexes in acetonitrile [17].

3.1. UV-Vis study

Firstly, we used for complexation with TET the metallic picrates of Na^+, K^+, Mg^{2+} and Ca^{2+}. UV-Vis absorption spectra were recorded in the range 300–450 nm for the first three cations and between 320 and 380 nm for the $CaPic_2$. In every case the observed changes in absorbance in presence of increased concentrations of TET have permitted the calculation of corresponding stability constant of the complexes.

The absorbance (A) of a solution 3.23×10^{-5} M of $CaPic_2$ in CH_3CN changes after adding of increased amounts of TET as can be seen in Fig. 2a).

Considering the presence of a 1:1 complex in solution it is obtained a good fit of the experimental points. The interaction between ligand (L) and the metal ion (M) is defined by the equilibrium equation and by stability constant K associated with this complex type

$$\text{L} + \text{M} \rightleftarrows \text{LM}, \qquad K = \frac{[\text{LM}]}{[\text{L}][\text{M}]} \tag{1}$$

Using the areas between 350 and 360 nm (TET does not absorb in this region) from the derived absorbance curves (see Fig. 3a), the following treatment has been developed [18]:

$$[\text{LM}] = -\frac{1}{2}\sqrt{\left(\frac{1}{K} + [\text{L}]_T + [\text{M}]_T\right)^2 - 4[\text{L}]_T[M]_T} + \frac{1}{2}\left(\frac{1}{K} + [\text{L}]_T + [\text{M}]_T\right) \tag{2}$$

where T stands for total involved parameter.

Then, for a given value of K, [LM] is known and the spectral characteristic, ε_{LM} (molar absorptivity) of the complex can be calculated, based on the following relation:

$$A = l\,(\varepsilon_M[\text{M}] + \varepsilon_{LM}[\text{LM}]) \tag{3}$$

The estimation of the ε_{LM} is achieved for each concentration of [L]. The difference over the parameter of concern has to be minimized relative to K. All calculations were performed in Excel with minimization algorithm: Newton–Raphson.

For TET/Ca^{2+} complex the value of K is equal to $18\,983\,\text{dm}^3\,\text{mol}^{-1}$. The complexation was accompanied by a very weak bathochromic shift (∼1 nm) of the picrate ion absorbance from 373 to 374 nm related to the change of a tight ion-pair to a looser ion-pair [19].

The value of ΔG_0 was calculated using the equation:

$$-\Delta G_0 = RT \ln K \tag{4}$$

and the obtained value of $5.736\,\text{kcal mol}^{-1}$ at $T = 293\,\text{K}$ ($R = 1.9872\,\text{kcal mol}^{-1}\,\text{K}^{-1}$) is in good agreement with literature data [20].

For the $Ca(ClO_4)_2$ (Fig. 2b) and $MgPic_2$ (Fig. 2c) complexes with TET, by the same strategy of plotting of derived absorbance areas in the region 365–375 nm versus TET molar concentration (Fig. 3b and c) it is obtained for K and $-\Delta G_0$ the values depicted in Table 1. The correlation coefficients of the titration curve fit were as follows: 0.997 for TET/$CaPic_2$, 0.990 for TET/$Ca(ClO_4)_2$ and 0.958 for TET/$MgPic_2$. In the case of complexation of TET/$MgPic_2$ we observed a weak bathochromic shift (5 nm) of the picrate ion absorbance from 323 to 328 nm related to the change of a tight ion-pair to a looser ion-pair.

From Table 1 we may observe that in ACN the Ca^{2+}/TET complex is less stable. Also we may analyze the influence

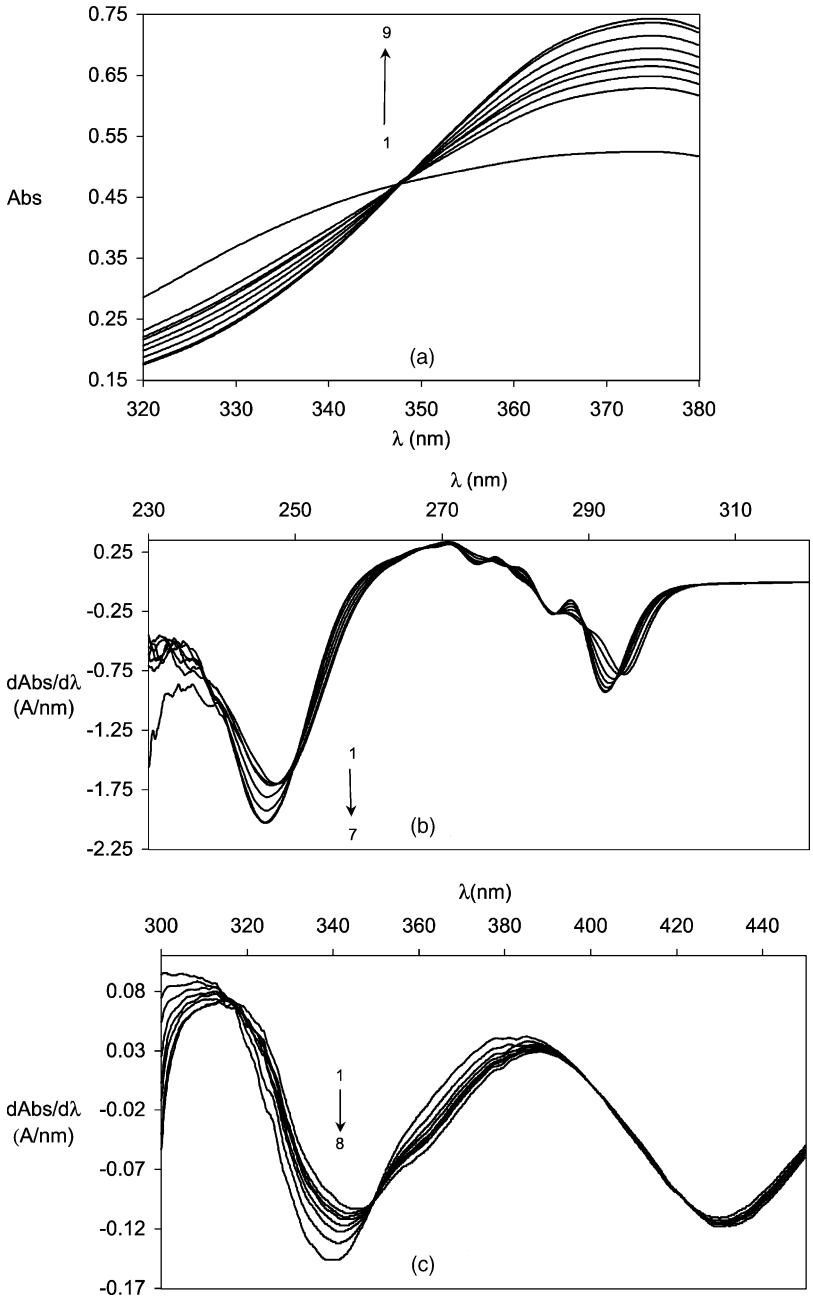

Fig. 2. Titration of: (a) 3.23×10^{-5} M $CaPic_2$ with TET in CAN (1) 0, (2) 2.261×10^{-5} M, (3) 2.907×10^{-5} M, (4) 3.23×10^{-5} M, (5) 3.876×10^{-5} M, (6) 4.522×10^{-5} M, (7) 5.168×10^{-5} M, (8) 5.814×10^{-5} M, (9) 6.46×10^{-5} M; (b) 10^{-4} M TET with $Ca(ClO_4)_2$ in ACN (1) 0, (2) 0.2×10^{-4} M, (3) 0.5×10^{-4} M, (4) 0.9×10^{-4} M, (5) 1.2×10^{-4} M, (6) 1.4×10^{-4} M, (7) 1.8×10^{-4} M; (c) 3.23×10^{-5} M $MgPic_2$ with TET in THF (1) 0, (2) 0.621×10^{-5} M, (3) 1.553×10^{-5} M, (4) 2.795×10^{-5} M, (5) 3.726×10^{-5} M, (6) 4.347×10^{-5} M, (7) 5.589×10^{-5} M, (8) 6.21×10^{-5} M.

of the counter-ion, which is particularly important in the case of the alkaline-earth metal complexes on the formation constant [21]. The picrate anion forms more stable ion-pairs with TET than the perchlorate anion. That may be due to its larger molecular mass and to its more lipophilic character [7].

Fig. 4a shows the derived absorption spectra in THF of 3.23×10^{-5} M NaPic solution in absence (1) and in presence of 6.46×10^{-5} M TET (2) and of 3.23×10^{-5} M KPic in absence (3) and in presence of 6.46×10^{-5} M TET (4). It was considered that adding two equivalents of TET to the picrate solution should produce a significant spectral change.

We can observe that the curves 1 and 2, respectively 3, and 4 are identical with small differences due to diffraction [22]. In the region 300–325 nm where TET starts to absorb the derived spectra are slightly different. These aspects lead to the conclusion that TET selectively complexes the Ca^{2+} and Mg^{2+} ions which are hard acids.

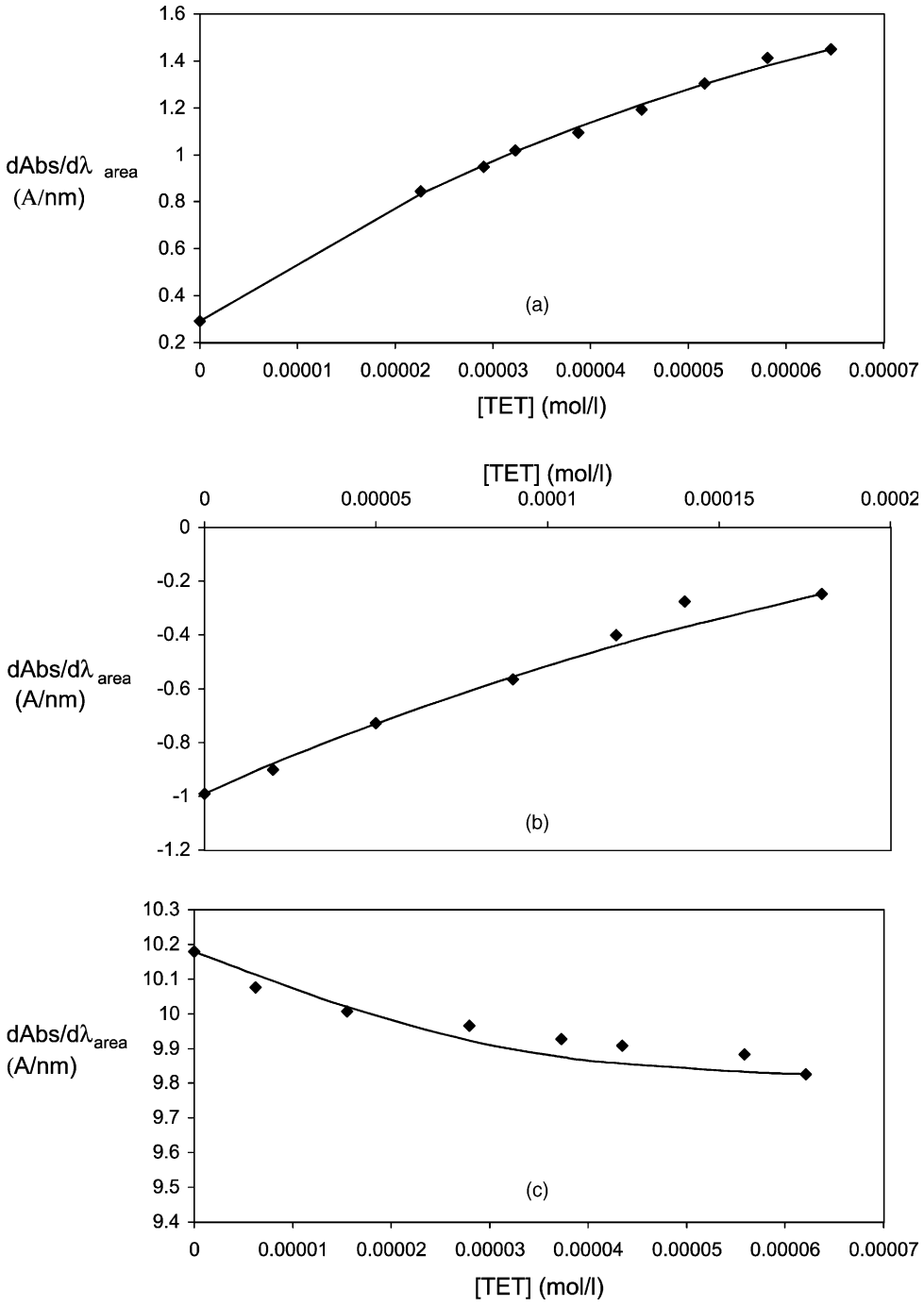

Fig. 3. Fitting curve for: (a) TET/$CaPic_2$ complexation; (b) TET/$Ca(ClO_4)_2$ complexation; (c) TET/$MgPic_2$ complexation.

3.2. Fluorescence spectra

The photophysical properties of the bis-benzyl isoquinoline alkaloids are the object of few researches although they may be interesting as fluorescent chemosensors [23].

The fluorescence spectra for titration of TET with $Ca(ClO_4)_2$ are shown in Fig. 5a), $\lambda_{ex} = 259 \pm 1$ nm (for every solution the excitation wavelength was equal to the absorption maximum wavelength), excitation slit equal to emission slit of 2.5 nm. Upon addition of Ca^{2+}, a hipsochromic shift (~6 nm) is observed, accompanied by a 1.5-fold fluorescence enhancement. The titration was carried out by sequentially adding aliquots of metal solution, via micropipette, to TET solution. The solutions were equilibrated by stirring prior to acquiring fluorescence spectra. The fact that the isosbestic point is not well defined may be due to a small deplacement in the acid–base equilibrium of TET tertiary amine functional groups.

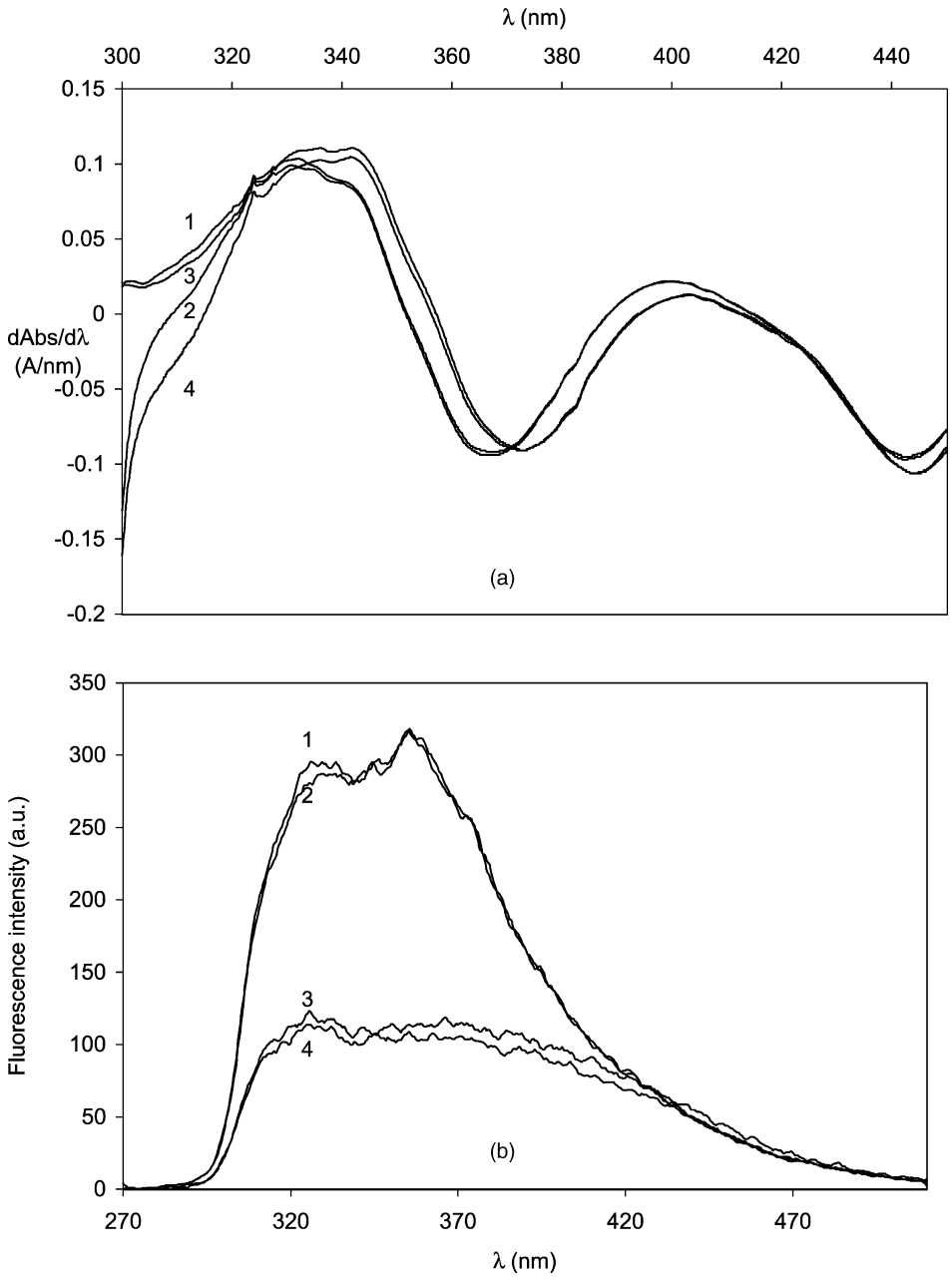

Fig. 4. (a) Derived absorption spectra in THF: 3.23×10^{-5} M NaPic in absence (1) and in presence of 6.46×10^{-5} M TET (2), 3.23×10^{-5} M KPic in absence (3) and in presence of 6.46×10^{-5} M TET (4) and (b) fluorescence spectra in CH_3CN: 10^{-4} M TET in absence (1) and in presence of 2.09×10^{-5} M $LiClO_4$ (2), 10^{-4} M TET in absence (3), and in presence of 20×10^{-4} M $NaClO_4$ (4).

Table 1
Stability constants K and free energies $-\Delta G_0$ of the studied complexes

Metal	Counter ion	Solvent	Method	K (mol l^{-1})	$-\Delta G_0$ (kcal mol^{-1})
Ca^{2+}	Pic	CH_3CN	UV-Vis	18983	5.736
		THF[a]	UV-Vis	20277	5.820
	ClO_4^-	CH_3CN	UV-Vis	3654	4.777
			Fluorescence	4225	4.861
Mg^{2+}	Pic	THF	UV-Vis	282613	7.308

[a] Data published in [8].

The blue shift and the increase in fluorescence intensity are attributed to Ca^{2+} binding to TET which result in a more rigid structure of the ligand after complexation [24–26]. The fluorescence enhancement may be due to the suppression of the intramolecular photoinduced electron transfer (PET) from the oxygen lone pairs or the metal binding alters the rate of one or more relaxation process from the excited state: radiative decay, internal conversion (IC) or intersystem crossing (ISC) [27,28].

Fig. 5b shows the plot of the fluorescence intensity values at $\lambda_{max} = 323.84$ nm versus $[Ca(ClO_4)_2]$. Using the

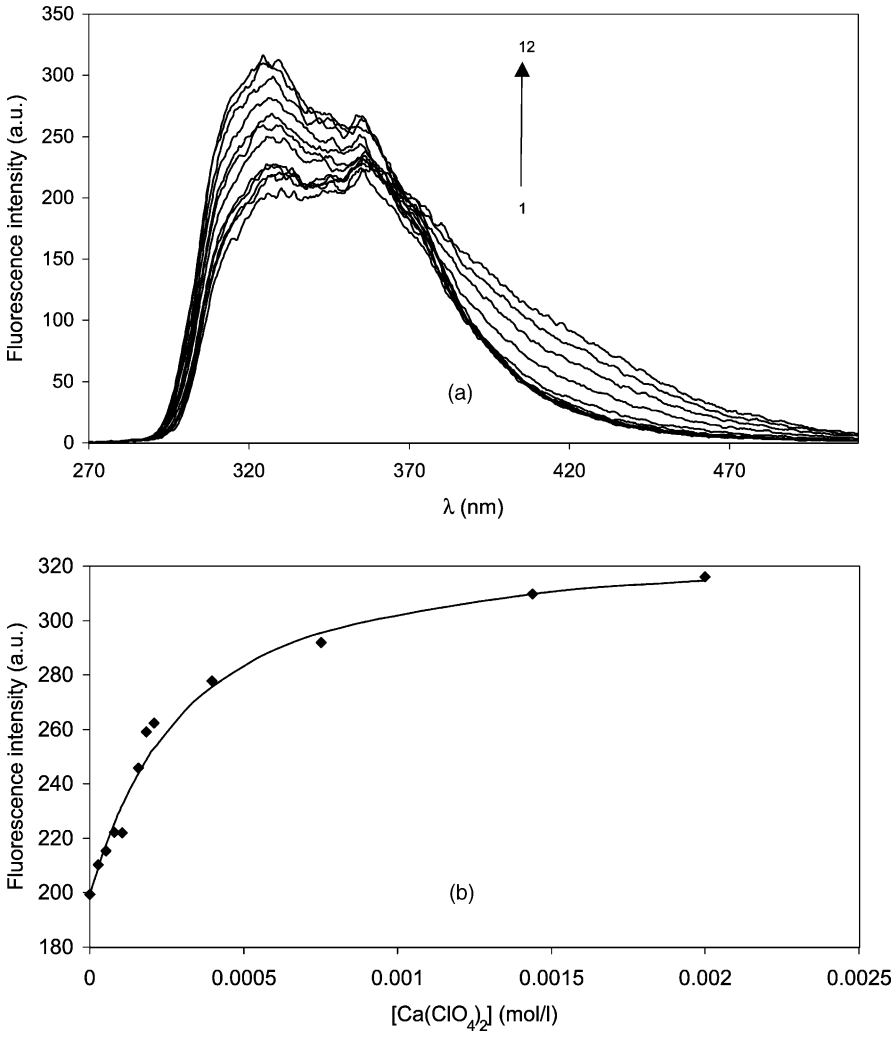

Fig. 5. (a) Fluorescence spectra of titration of 10^{-4} M TET with $Ca(ClO_4)_2$ (1) 0, (2) 0.266×10^{-4} M, (3) 0.5305×10^{-4} M, (4) 0.7937×10^{-4} M, (5) 1.0554×10^{-4} M, (6) 1.5748×10^{-4} M, (7) 1.8325×10^{-4} M, (8) 2.0888×10^{-4} M, (9) 3.9693×10^{-4} M, (10) 7.5216×10^{-4} M, (11) 14.3836×10^{-4} M, (12) 20×10^{-4} M in CH_3CN; (b) fitting curve.

same formalism as for the UV-Vis titration we have obtained the value of $K = 4225\,dm^3\,mol^{-1}$ and $-\Delta G_0 = 4.861\,kcal\,mol^{-1}$. The correlation coefficient was 0.989. The values for equilibrium constant and free energy obtained using both different techniques are close.

In Fig. 4b are given the fluorescence spectra in CH_3CN of 10^{-4} M TET in absence (1) and in presence of 2.09×10^{-5} M $LiClO_4$ (2) ($\lambda_{ex} = 260$ nm, excitation slit equal with emission slit of 2.5 nm) and of 10^{-4} M TET in absence (3) and in presence of 20×10^{-4} M $NaClO_4$ (4) ($\lambda_{ex} = 266$ nm, respectively, 267 nm, excitation slit equal to emission slit of 2.5 nm). There are no modifications in the spectra of the ligand before and after complexation. It is concluded that TET does not complexe the Li^+ and Na^+ ions. Thus, the previous announced results are proved.

The values of the ionic radii of the studied cations $Li^+ = 0.60$ Å, $Na^+ = 0.95$ Å, $K^+ = 1.33$, $Mg^{2+} = 0.65$ Å and $Ca^{2+} = 0.99$ Å are always smaller then the intramolecular cavity of TET [29]. Considering the behavior of TET towards complexation we may conclude that the most important factor in complexation is the ionic charge. Na^+ and Ca^{2+} have almost identical radii. The bigger value for the stability constant of TET/Mg^{2+} complex may be explained by the unusually structure of $MgPic_2$ among of the main group of metallic picrates [30]. Its X-ray structure shows no coordinative interaction between the metal and the picrate anion, the metal being hexahydrated.

4. Conclusions

1. Tetrandrine a natural macrocycle with a cyclophane type structure can be engaged in ionic recognition of Mg^{2+} and Ca^{2+}. Also, it was proved that TET does not form complexes with Li^+, Na^+ and K^+ ions.

2. Following this study, we established the stoichiometry of 1:1 for TET complexes with Mg^{2+} and Ca^{2+}.
3. In low polarity solvent like THF the complexation is stronger than in ACN.
4. By obtained UV-Vis and fluorescence spectroscopic measurements the picrate anion forms more stable complexes with TET than the perchlorate anion.

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