UV-VIS SPECTRAL STUDY OF SOME CALCIUM CHANEL BLOCKERS SUPERMOLECULAR COMPLEXES

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abstract: The supermolecular complexes formation of fangchinoline and berbamine with calcium ions was studied by UV-VIS spectroscopy and titration method. The berbamine complex with calcium perchlorate has the stability constant K = 21434 mol^{-1}. In the case of fangchinoline the observed changes in the spectra could not be rationalized in terms of formation of a 1:1 complex.

Introduction

The calcium channel blockers represent a group of drugs with heterogeneous chemical structure which have as principal property the inhibition of intracellular Ca^{2+} penetration through the membrane channels [1].

The bisbenzylisoquinoline alkaloids represent one of the most important class of isoquinoline alkaloids with more than 400 dimers belonging to this group [2]. The most important member of bisbenzylosoquinoline alkaloids family, tetrandrine (**TET**) is extracted from the roots of *Stephania tetrandrae* S. Moore. Using marked ligands it has been demonstrated [3] that **TET** is a calcium channel blocker. The hypotensive activity of fangchinoline (**FC**), **TET** and its synthetic derivatives lead to the conclusion that derivatives substituted at the position 7-O with different types of alkyl group have variable degree of hypotensive effect [4,5] (s. **Fig. 1**). Berbamine (**BER**) is less active (IC_{50} = 200 μmol/l) than **TET** and hernadezine (both have IC_{50} = 25 μmol/l) in inhibition of Ca^{2+} penetration, activated by TSG [6]. This structure–biological activity relationships study permitted the conclusion that the group $-OCH_3$ of the aromatic cycle of **TET**, which is one of the structural peculiarities in rapport with **BER**, determines the dual pharmacologic effect of **TET**.

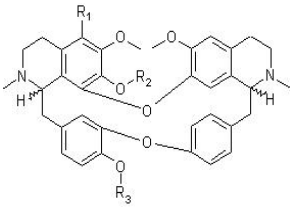

Fig. 1 ***TET****: R_1= -H, R_2= $-CH_3$, R_3= $-CH_3$,* ***BER****: R_1= -H, R_2= $-CH_3$, R_3= -H,* ***FAN****: R_1= -H, R_2= -H, R_3= $-CH_3$.*

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A direct interaction with proteins (the calcium pore) and an interaction of **TET** with calcium and magnesium ions were observed [7,8].

The aim of this paper is the UV-spectral study of the interaction between fanghinoline, respectively berbamine with calcium ions.

Experimental part

Materials

Fangchinoline and berbamine were a generous gift of Professor C.Y. Kwan, Department of Medicine, McMaster University, Hamilton, Ontario, Canada. The explored domain of concentrations for **FC** was comprised between $0.2 \cdot 10^{-5}$ M – $6.46 \cdot 10^{-5}$ M and for **BER** $0.53 \cdot 10^{-5}$ M – $4.1776 \cdot 10^{-5}$ M. $CaPic_2$ concentration was $2 \cdot 10^{-5}$ M. The solvents tetrahidrofuran (for picrate and **FC**), acetonitrile for the system (perchlorate, **BER**) were from SDS, France.

Apparatus and method

UV-VIS spectra were carried out with a Perkin Elmer Lambda 2S spectrometer in the range 310-770 nm. The first derivative $dA/d\lambda = f(\lambda)$ of the obtained spectra was calculated because improves the accuracy of determination of interest band area. We adopted the following mode of operation (specific for a titration procedure): for the study of alkaloid complexes formation with metallic picrates (Ca^{2+}/Mg^{2+}) we firstly carried-out the UV-VIS spectra of metallic picrates in acetonitrile/tetrahydrofuran. After that we carried out the same spectra adding increased quantities of **FC** (see Fig. 2). In the case of complexes with calcium perchlorate we carried out the UV-Vis spectra of free ligand (**BER**) in acetonitrile and with different quantities of calcium perchlorate (see Fig. 3).

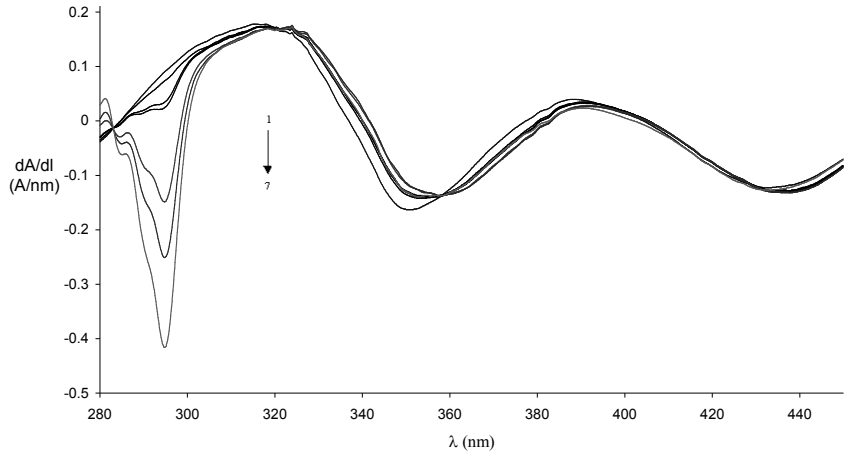

Fig. 2 *Titration of [$CaPic_2$] = $3.23 \cdot 10^{-5}$ M with **FC*** 1) 0, 2) $0.1615 \cdot 10^{-5}$M, 3) $0.323 \cdot 10^{-5}$M, 4) $0.646 \cdot 10^{-5}$M, 5) $2.907 \cdot 10^{-5}$M, 6) $4.3067 \cdot 10^{-5}$M, 7)$6.46 \cdot 10^{-5}$M

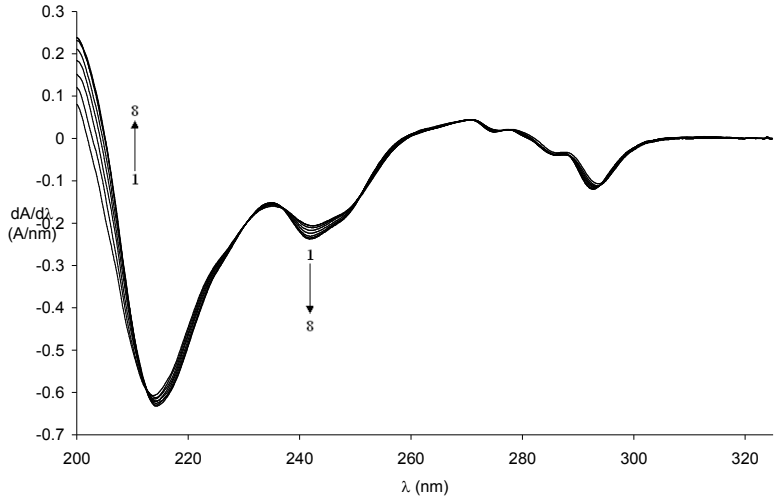

Fig. 3 *Titration of [**BER**] = 2·10^{-5} M with Ca(ClO$_4$)$_2$ in acetonitrile* (1) 0, (2) 0.532·10^{-5} M, (3) 1.061·10^{-5} M, (4) 1.5874·10^{-5} M, (5) 2.1108·10^{-5} M, (6) 3.1496·10^{-5} M, (7) 3.665·10^{-5} M, (8) 4.1776·10^{-5} M.

Results and discussions

Considering that in solution is present the complex 1:1, the interaction between ligand (L) and metallic ion (M) is defined by the equilibrium constant K :

$$L + M \Leftrightarrow LM \tag{1}$$

$$K = \frac{[LM]}{[L]\cdot[M]} \tag{2}$$

where

$$[L]_T = [L] + [LM] \tag{3}$$

$$[M]_T = [M] + [LM] \tag{4}$$

On this basis the concentration of complex can be expressed as:

$$[LM] = -\frac{1}{2}\sqrt{\left(\frac{1}{K} + [L]_T + [M]_T\right)^2 - 4[L]_T\cdot[M]_T} + \frac{1}{2}\left(\frac{1}{K} + [L]_T + [M]_T\right) \tag{5}$$

It is evident that [LM] is a function of total concentrations of metal and ligand (given values) and formation constant K which must be determined. After [9,10] solving of this equation suppose the following algorithmic procedure:

Step I: It is proposed an initial value for K which permits calculation of [LM] after eq. (5)

Step II: Absorptivity of complex can be calculated because the other variables from eq. (6) are known:

$$A = l\,(\,\varepsilon_L\cdot[L] + \varepsilon_{LM}\cdot[LM]\,) \tag{6}$$

The computation of absorptivity is realized for each of prepared solutions because [L] changes as the resulted absorption.

Step III: Theoretical absorptivity of complex at a given temperature is uniquely defined. By consequence the dispersion of calculated ε_{LM} values for each solution is an index of its accuracy: Dispersion can be calculated from standard deviation of calculated absorptivities: $\delta(\varepsilon_{LM})$.

Step IV: Because the ε_{LM} values are calculated for a given value of the formation constant, ε_{LM} is directly dependent of K. It is possible to minimize $\delta(\varepsilon_{LM})$ by varying K. When the difference between two standard consecutive deviations is less than the convergence criterion the procedure stops and K is determined. In this mode the formation constant K is calculated very accurately.

The calculations were effected in Excel using Newton-Raphson minimization algorithm.

From analysis of titration realised in case of **FC** with calcium picrate. (s. Fig. 2) the model of complex 1:1, valid in case of **TET**, can not be used probably due to existence of association equilibrium, mediated by hydrogen bonding for **FC**.

Titration of **BER** with calcium perchlorate in acetonitrile revealed the dependence of the absorption bands in the range 240-245 nm of the molar concentration of $Ca(ClO4)_2$ (s. Fig. 4) using the given algorithm. The obtained value for K = 21434 mol^{-1} in this case is higher than that of **TET** [11], results that complex of **BER** with calcium posses a higher stability.

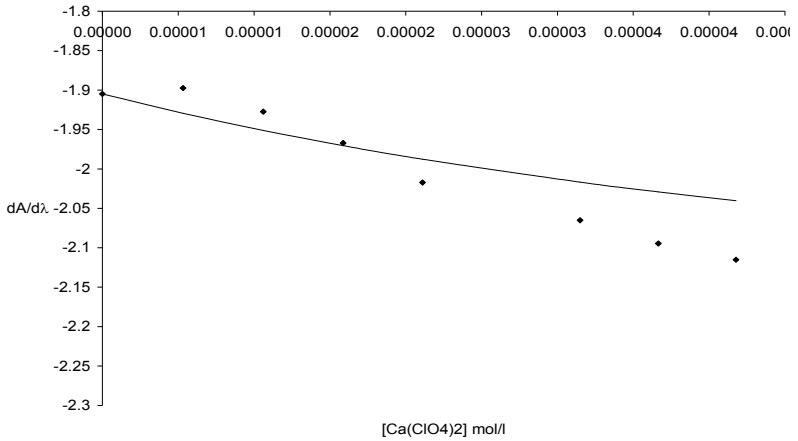

Fig. 4 *Fitting curve of **BER**/$Ca(ClO_4)_2$ complexation*

Conclusions

BER complexates calcium ion, the resulted value of K being in agreement with literature data for cyclophane macrocyclic ligands.

Complexation of **FC** with calcium ion needs systematic study because of its auto-association mediated by hydrogen bonds.

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