

# Fluorimetric and molecular mechanics study of the inclusion complex of 2-quinoxaliny-phenoxathiin with $\beta$ -cyclodextrin

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**Abstract** The complex formed by the inclusion of the polarity-sensitive fluorescent probe 2-[2'-quinoxaliny]-phenoxathiin (QP) into  $\beta$ -cyclodextrin ( $\beta$ -CD) was investigated by steady-state fluorescence spectroscopy in order to confirm the previously stated intramolecular charge transfer nature of the first excited singlet state of QP. A decrease in the emission intensity in the presence of  $\beta$ -CD was observed and explained on this basis. The 1:1 stoichiometry of the inclusion complex and its association constant of  $2,223 \text{ M}^{-1}$  were computed. The QP- $\beta$ -CD complex was further studied by molecular mechanics (MM+ force field), in order to determine its structure and the type of interactions between QP and  $\beta$ -CD. All possible ways QP could penetrate the  $\beta$ -CD cavity were considered and several structures were generated and optimized. The interaction, binding (van der Waals and electrostatic contributions) and perturbation energies were also calculated. The results have showed that the  $\beta$ -CD cavity incorporates the central part of QP and that complexation is mainly due to van der Waals host-guest interactions.

**Keywords** Cyclodextrin · Inclusion complex · Intramolecular charge transfer · Fluorescence · Molecular modeling · 2-Quinoxaliny-phenoxathiin

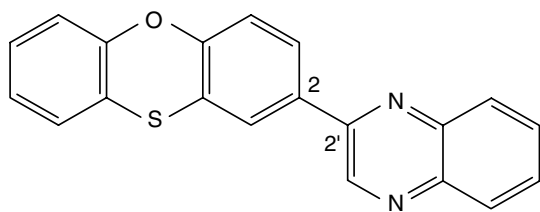
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## Introduction

During the study of new fluorescence probes for proteins, we have found that some phenoxathiin derivatives have the required properties to be used for this purpose, i.e. position of the emission maxima at larger wavelengths than proteins and significant quantum yields [1–3]. As one of the requirements for this application is also a sensitivity of the emission properties to the change of the local polarity, a new phenoxathiin derivative, 2-[2'-quinoxaliny]-phenoxathiin (QP) (Fig. 1) seems to be a potent candidate. The fluorescence spectra in solvents of different polarities evidenced a significant bathochromic shift of the emission band in polar solvents, associated with the increase of the excited state dipole moment. These results, assisted by semiempirical calculations on both the ground and excited state, were rationalized in terms of an intramolecular charge transfer (ICT) character of the first excited singlet state (to be published). However, the lower fluorescence quantum yield of QP (~0.05) compared to other phenoxathiin derivatives and the presence of the single bond between its two constituting fragments made us wonder about the possibility of occurrence of a twisted intramolecular charge transfer (TICT) excited state.

In order to gain more information on the behavior of this compound, the complexation with cyclodextrins was performed. CDs are cyclic oligosaccharides, toroidally shaped, consisting of 6, 7 or 8 glucopyranose units joined by  $\alpha$ -(1,4)-linkages and called  $\alpha$ -,  $\beta$ - and  $\gamma$ -CD respectively [4]. They have hydrophilic outer surface and a relatively hydrophobic cavity, that provides microenvironments very similar to the biological ones [5–7]. The purpose of our study is therefore



**Fig. 1** Molecular structure of 2-[2'-quinoxaliny]-phenoxathiin (QP)

twofold; firstly, to test the sensitivity of our compound to the inclusion process as a potential fluorescence probe and, secondly, to get new experimental support on the ICT or TICT character of the first excited singlet state. Since inclusion of non-rigid molecules such as QP usually prevents the geometrical changes occurring in excited state by forcing the guest to adopt a constrained conformation, an enhancement of the emission will be expected in case of a TICT, or a decrease of the fluorescence in case of an ICT excited state [8–13]. The steady state fluorescence experiments allow for the estimation of the association constant and the stoichiometry of the inclusion complex. From the theoretical point of view, we were interested in the most probable structure of the complex and the relative contribution of the van der Waals and electrostatic terms to the binding energy.

## Experimental

### Steady-state fluorescence

$\beta$ -CD from Aldrich was used as received. QP was prepared as described elsewhere [14]. Formation of host–guest complex by QP with  $\beta$ -CD was done as follows and was studied by steady-state fluorescence measurements: to a stock solution of  $10^{-4}$  M QP in DMF:water (1:9 v/v) were added aliquots of  $10^{-3}$  M  $\beta$ -CD solution, prepared by adding  $\beta$ -CD powder to the stock solution above. The fluorescence emission spectra were obtained on a FP-6300 Jasco spectrofluorimeter (excitation wavelength 360 nm).

### Estimation of the association constant

The association constant,  $K$ , of the inclusion complex was determined by plotting the fluorescence intensity,  $F$ , as a function of the CD concentration,  $[CD]$ . The experimental data were fitted by non-linear regression analysis, considering the formation of 1:1, 1:2 or a mixture of 1:1 or 1:1 and 1:2 complexes [15]. The best

fit was obtained with eq. 1 describing the formation of 1:1 host–guest complexes:

$$F = \frac{F_0 + K \cdot F_1 \cdot [CD]}{1 + K \cdot C}, \quad (1)$$

where  $F_0$  is the fluorescence intensity in the absence of CD and  $F_1$  is the fluorescence intensity of the complex.

### Computational details

The structure of the QP– $\beta$ -CD inclusion complex was determined using semiempirical (AM1 Hamiltonian) and in vacuo molecular mechanics (MM+ force field) calculations performed with the Hyperchem 5.02 program. The molecular modeling of the host–guest interaction was done in three steps: (i) obtaining of the starting geometries and atomic charges of the free host and guest by AM1 semiempirical calculations (optimization method Eigenvector-Following); the geometry of  $\beta$ -CD was taken from neutron diffraction studies [16]; (ii) generation of several structures for the complex by docking the QP molecule into the  $\beta$ -CD cavity. All the possible ways the guest could penetrate the  $\beta$ -CD cavity were considered, that is with either the phenoxathiin (Px) or quinoxaline (Qx) moiety, through the secondary (model A) or primary (model B) cavity rim. The most stable structure of the complex was searched by modifying the host–guest distance; (iii) full optimization of the most stable structures found. The energies characterizing the inclusion process were then calculated.

The interaction energy between guest and host is defined as the energy difference between the optimized complex and the optimized free guest and free host [2]:

$$E_{\text{interaction}} = E_{\text{complex}} - (E_{\text{QP}} + E_{\beta\text{-CD}})_{\text{free}} \quad (2)$$

The binding energy was estimated as the energy difference between the totally optimized complex and the guest and host “frozen” in their conformations from the complex, according to the relationship:

$$E_{\text{binding}} = E_{\text{complex}} - (E_{\text{QP}} + E_{\beta\text{-CD}})_{\text{frozen in the complex}} \quad (3)$$

In the case of the MM+ force field used, the binding energy represents the sum of the van der Waals and electrostatic energies, allowing for the estimation of their relative contributions [17].

The perturbation energy for each component, host and guest, due to the formation of the complex was defined as the difference in the energy of the totally

optimized component compared to its energy in the complex.

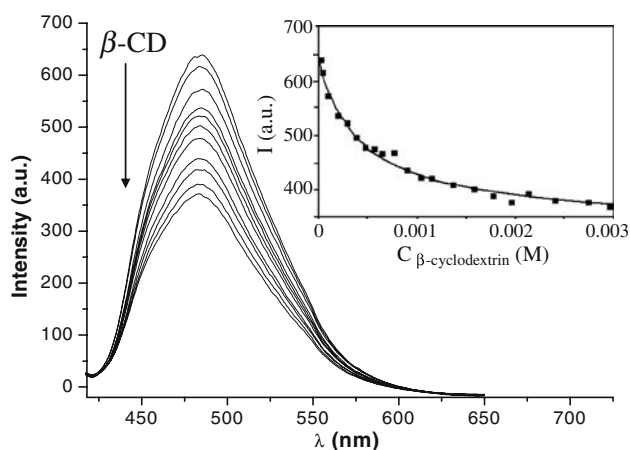
## Results and discussion

### Fluorescence quenching due to inclusion

The fluorescence spectra of QP in the presence of increasing amounts of  $\beta$ -CD are presented in Fig. 2. It can be seen a progressive decrease of the intensity reflecting the complexation process. The quenching of the emission in the presence of  $\beta$ -CD proves that the restricted motion of the guest is not the main factor determining the relative contribution of the radiative and nonradiative deactivation processes in the isolated compound. Since no fluorescence increase was observed upon QP complexation, we can conclude that the first excited singlet state of QP is of ICT nature. A comparison of the position of the emission maxima in water and in the  $\beta$ -CD presence shows that there is no significant change due to the inclusion; the main effect remains that of the water polarity and hydrogen bonding capacity.

### Association constant

The fit based on eq. (1) and revealing the 1:1 stoichiometry of the complex formed in aqueous solution is presented as inset in Fig. 2 and the respective data are listed in Table 1. The goodness of the fit was characterized by the correlation coefficient,  $r^2$ , and by the low values of the standard errors for  $K$  and  $F_I$  around 6% and 1.6% respectively. The calculated association



**Fig. 2** Fluorescence spectra of QP in DMF:water in absence and presence of various concentrations of  $\beta$ -CD; inset: plot of the fluorescence intensity of complexed QP vs. various concentrations of  $\beta$ -CD

**Table 1** Estimated association constants ( $K$ ) and fluorescence intensities ( $F_I$ ) for the 1:1 QP- $\beta$ -CD complex

CD type	$K$ ( $M^{-1}$ )	$F_I$ (a.u.)	$r^2$	$F$ -stat
$\beta$ -CD	$2222.8 \pm 131.0$	$329.8 \pm 5.2$	0.9934	1702.8

constant of  $2,222.8 M^{-1}$  is in agreement with the relative dimensions of QP and of the  $\beta$ -CD cavity and is comparable to those previously obtained for other Px derivatives, ranging between  $1,000$  and  $8,000 M^{-1}$  [2, 18].

### Molecular mechanics calculations

According to the dimensions of the QP molecule ( $15.5 \text{ \AA}$  length,  $4.9 \text{ \AA}$  width), and to the  $\beta$ -CD dimensions [15, 19], the QP molecule is not totally entrapped into the  $\beta$ -CD cavity. QP was firstly approached and introduced in the  $\beta$ -CD cavity from the Qx to the Px moiety, from the secondary (model A) and primary (model B) rim. In both models, the docking of the guest was performed considering a defined guest–host distance (sulphur atom of Px and nitrogen atom of Qx from QP and two oxygen atoms of  $\beta$ -CD rings were chosen as reference), that was restricted to a chosen value, allowing for the total relaxation of all the other internal coordinates the complex; this parameter was varied with a step of  $1 \text{ \AA}$  in the limit  $6$ – $3 \text{ \AA}$  and the lowest energy structures were selected for the total optimization. The optimizations were performed up to a gradient of  $0.05 \text{ kcal}/(\text{ \AA} \text{ mol})$ . The results for the three more stable geometries are given in Table 2.

In both models, the energy of the system decreased significantly when the Px–Qx junction was included in the  $\beta$ -CD cavity, designating this structure of the complex as the most favorable (Fig. 3). The van der Waals interactions have the major contribution to the stability of the complex (80–90%). Inclusion of QP through the secondary rim seems to lead to the most stable complex. However, considering the approximations involved in the calculations and the solvent neglect, the slightly increased binding energy obtained for primary rim inclusion ( $-30.58 \text{ kcal/mol}$ ) is not high enough to exclude this possibility of complex formation.

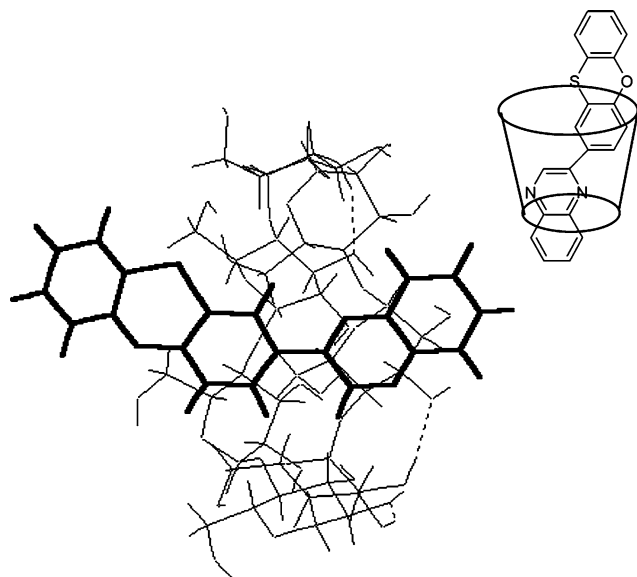
The results obtained when QP was inserted into the cavity from the Px to the Qx fragment (not shown) indicate the same structure of the complex.

## Conclusions

The fluorescence study of the inclusion of QP into  $\beta$ -CD indicated the formation of a binary 1:1 complex

**Table 2** Energy contributions to the total energy of the QP- $\beta$ -CD complex for Px, Qx moieties and Px-Qx junction penetrating the  $\beta$ -CD cavity through the (A) secondary rim and (B) primary rim

Fragment of QP included in CD	$E_{\text{binding}}$ (kcal/mol)	% van der Waals interactions	% electrostatic interactions	$E_{\text{perturbation}}$ (kcal/mol)
Panel (A)				
PX	-25.27	94.02	5.98	3.64
Qx	-26.53	85.56	14.44	2.70
Px-Qx	-33.85	83.90	16.10	4.40
Panel (B)				
PX	-27.57	89.34	10.66	4.52
Qx	-29.85	86.91	13.09	3.34
Px-Qx	-30.49	88.36	11.64	4.03



**Fig. 3** The proposed structure of the QP- $\beta$ -CD complex. The dotted lines represent hydrogen bonds

in aqueous solution. The structure of the QP- $\beta$ -CD complex corresponds to the Px-Qx junction accommodated into the  $\beta$ -CD cavity and the stability of the complex is due to van der Waals forces. The fluorescence intensity of QP decreases upon complexation, confirming the ICT nature of the first singlet excited state of QP.

## References

- Ionescu, S., Gavrilu, D., Maior, O., Hillebrand, M.: Excited state properties of some phenoxathiin derivatives. *J. Photochem. Photobiol. A* **124**, 67–73 (1999)
- Oana, M., Tintaru, A., Gavrilu, D., Maior, O., Hillebrand, M.: Spectral study and molecular modeling of the inclusion complexes of  $\beta$ -cyclodextrin with some phenoxathiin derivatives. *J. Phys. Chem.* **106**, 257–263 (2002)
- Tintaru, A., Oana, M., Gavrilu, D., Hillebrand, M.: Experimental and theoretical study of the ground and excited state properties of 3-carboxyphenoxathiin. *Rev. Roum. Chimie* **49**, 317–326 (2004)
- Roberts, E., Dey, J., Warner: Ground and excited-state structural orientation of 2-(2'-hydroxyphenyl)benzazoles in cyclodextrins. *J. Phys. Chem.* **100**, 19681–19686 (1996)
- Torgerson, P.M., Drickamer, G., Weber, G.: Inclusion complexes of poly-beta-cyclodextrin: a model for pressure effects upon ligand-protein complexes. *Biochemistry* **18**, 3079–3083 (1979)
- Harrison, J.C., Eftink, M.R.: Cyclodextrin-adamantanecarboxylate inclusion complexes: A model system for the hydrophobic effect. *Biopolymers* **21**, 1153–1166 (1982)
- Abou-Zied, O.K., Al-Hinai, A.T.: Caging effects on the ground and excited states of 2,2'-Bipyridine-3,3'-diol embedded in cyclodextrins. *J. Phys. Chem. A* **110**, 7835–7840 (2006)
- Grabowsky, Z.R., Rotkiewicz, K., Siemarczuk, A., Cowley, D.J., Baumann, W.: Twisted intramolecular charge transfer states (TICT). A new class of excited states with a full charge separation. *J. Chim.* **3**, 443–454 (1979)
- Rettig, W., Marschner, F.: Molecular conformation and biradicaloid charge transfer states in substituted N-phenylpyrroles. *New J. Chem.* **14**, 816 (1990)
- Rettig, W., Zietz, B.: Do twisting and pyramidalization contribute to the reaction coordinate of charge-transfer formation in DMABN and derivatives? *Chem. Phys. Lett.* **317**, 187–196 (2000)
- Zhan, C.L., Wang, D.Y.: Nonlinear dependence of solvent polarity effects on twisted intramolecular charge-transfer states and linear relation for electronic spectra in a stilbazolium-like dye. *J. Photochem. Photobiol. A* **147**, 93–101 (2002)
- Ionescu, S., Chiorescu, I., Hillebrand, M.: Theoretical study of non-radiative deactivation pathways for some heterocyclic compounds. I. Pyrrolyl-izoxazole derivatives. *J. Mol. Struct. (Theochem)* **630**, 125–133 (2003)
- Ionescu, S., Hillebrand, M.: Theoretical study of the excited states of 3-phenyl and 3-thiophenyl-coumarins. *Chem. Phys.* **293**, 53–64 (2003)
- Nicolae, A., Gavului, D., Maior, O., Drăghici, C.: Phenoxathiin chemistry synthesis based on 2- $\omega$ -bromoacetylphenoxathiin. *South. Braz. J. Chem.* **6**, 33–45 (1998)
- Shen, X., Belletête, M., Durocher, G.: Spectral and photophysical studies of the 1:3 (guest/host) rotaxane-like inclusion complex formed by a 3H-indole and  $\beta$ -cyclodextrin. *J. Phys. Chem. B* **102**, 1877–1883 (1998)
- Betzel, C., Saenger, W., Hingerty, B.E., Brown, G.M.: Topography of cyclodextrin inclusion complexes, part 20. Circular and flip-flop hydrogen bonding in beta.-cyclodextrin

- undecahydrate: a neutron diffraction study. *J. Am. Chem. Soc.* **106**, 7545–7557 (1984)
17. Madrid, J.M., Villafruela, M., Serrano, R., Mendicuti, F.: Experimental thermodynamics and molecular mechanics calculations of inclusion complexes of 9-methyl anthracenate and 1-methyl pyreneate with  $\beta$ -cyclodextrin. *J. Phys. Chem. B* **103**, 4847–4853 (1999)
  18. Tintaru, A., Hillebrand, M., Thevand, A.: NMR study of the inclusion complexes of carboxy-phenoxathiin derivatives with  $\beta$ -cyclodextrin. *J. Incl. Phenom. Macrocycl. Chem.* **45**, 35–40 (2003)
  19. Agbaria, R., Uzan, B., Gill, D.: Fluorescence of 1,6-naphthalenediol with cyclodextrins. *J. Phys. Chem.* **93**, 3855–3859 (1989)