Mathematical model for isosbestic points of porphyrins. Implications in cryotherapy and photodynamic therapy

RODICA-MARIANA ION
Analytical Department
ICECHIM
202 Splaiul Indendentei, 060021 Bucharest
ROMANIA

Abstract: - 5, 10, 15, 20-sulfonatophenyl porphine (TSPP or $H_2TPPS_4^{4-}$) is a water-soluble tetrapyrrolic dye. It has been shown that this porphyrin can self-associate to form J- aggregates depending on the dye concentration, pH and ionic strength. In an acidic medium (pH < 5), the nitrogen atoms of porphyrin can be protonated resulting in a diprotonated form, which shows, in contrast to its neutral form, a near to coplanar conformation of the phenyl rings to the tetrapyrrolic ring. In this paper will be studied the transformation of TSPP from J-aggregates to monomer forms in the temperature range $-10\ ^{O}C$ to $+24\ ^{O}C$, putting into evidence the generated isosbestic points. With a mathematical calculus, will be possible to evaluate the main transitions from the vibrational levels, responsible for singlet oxygen generation and photodynamic activity, especially.

Key-Words: - porphyrin, vibrational levels, isosbestic points,J-aggregates, photodynamic therapy, cryotherapy

1 Introduction

Aggregation of the photosensitizers used in the so called photodynamic (photochemical) therapy of tumors (PDT) has *empirically* been found to play a decisive role for their photodynamic activity [1,2]. The principle of the photodynamic therapy is a very promising modality for the non-invasive treatment of tumors and other diseases.

The efficacy and suitability of photodynamic therapy (PDT) was compared with that of cryotherapy in the treatment of different disease [3]. Lesions were randomized to receive either cryotherapy with liquid nitrogen, or PDT using a portable desktop lamp incorporating a 300 W xenon short arc discharge source. PDT using a non-laser light source and topical 5-ALA appears to be at least as effective as cryotherapy in the treatment of Bowen's disease with fewer adverse effects.

In aggregated form, sensitizers show no or only a negligible photodynamic activity. This is due to the fast radiationless energy relaxation, which precludes energy- and/or charge transfer from the sensitizer to acceptor molecules.

Meso- 5, 10, 15, 20-sulfonatophenyl porphyrin (TSPP) is a water-soluble tetrapyrrolic dye. It has been shown that this porphyrin can self-associate to form H- and J- aggregates depending on the dye concentration, pH and ionic strength [69-72]. In an acidic medium (pH < 5), the nitrogen atoms of can be protonated resulting in a diprotonated form $H_2TPPS_4^{4-} \rightarrow H_4TPPS_4^{2-}$. The diprotonated tetraphenylporphyrin shows, in contrast to its neutral form, a near to coplanar conformation of the phenyl rings to the tetrapyrrolic ring.

In this paper will be studied the transformation from J-aggregates to monomer forms in the temperature range $-10\ ^{O}C$ to $+24\ ^{O}C$. From the isosbestic points generated with a mathematical calculus, will be possible to evaluate the main transitions between the vibrational levels, responsible for singlet oxygen generation and photodynamic activity, especially.

2 Problem Formulation

It is well known that the occurrence of more than two absorbing species was highly unlikely since the equation

$$\sum_{i=j}^{n} \left[\epsilon_i(\lambda_o) - \epsilon_j(\lambda_o) \right] \frac{dC_i}{dC_j} = 0 \qquad (1)$$

must hold at each isosbestic point, requiring in general that all molar absorptivities be equal. Systems which provide constant values of dC_i/dC_j, or derivatives all of which show the same dependence on the variable j, were overlooked.

The occurrence of isosbestic points in closed, temperature dependent systems indicates only one absorbing species are still valid, even for linearly related systems. This is directly shown by development of Cohen's and Fischer's equation [4] in extended version with consideration of time, concentration of a j-th species, and temperature as independent variables.

Temperature variation is not conductive to the formation of conventional isosbestic points because the concentration of the absorbing species changes as the fluid expands and contracts law of mass action, from those which are not. To make this distinction, eq. 1 can be rewritten:

$$A(\lambda, C_i, D_j) = \sum_{i=1}^{n} \epsilon_i(\lambda) C_i + \sum_{j=1}^{m} \epsilon_j(\lambda) D_j \qquad (2)$$

where the various species C_i are mutually dependent but independent of the various species D_j. If temperature is variable, eq. 2 becomes:

$$A(\lambda, C_i, D_j, t) = \sum_{i=1}^{n} \epsilon_i(\lambda, t) C_i(t) + \sum_{j=1}^{m} \epsilon_j(\lambda, t) D_j(t) \qquad (3)$$

The concentrations at a reference temperature are represented by C_i^0 and D_j^o. Since $f(t) = 1/(1 + \alpha t)$ where αt is the coefficient of expansion, eq. 3 can be rewritten:

$$A(\lambda, C_i, D_j, t) = \left(\frac{1}{1 + \alpha t}\right) \times \left[\sum_{i=1}^{n} \epsilon_i(\lambda, t) C_i^0 g_i(t) + \sum_{j=1}^{m} \epsilon_j(\lambda, t) D_j^0 \right] \qquad (4)$$

At an isosbestic point resulting from a temperature change, the derivative of the absorbance with respect to the temperature is zero

$$\frac{dA(\lambda_o, C_i, D_j, t)}{dt} = 0 = \sum_{i=1}^{n} \left[g_i(t) \frac{d\epsilon_i(\lambda_o, t)}{dt} + \epsilon_i(\lambda_o, t) \frac{dg_i(t)}{dt} - \left(\frac{\alpha}{1 + \alpha t}\right) \epsilon_i(\lambda_o, t) g_i(t) \right] C_i^0 + \sum_{j=1}^{m} \left[\frac{d\epsilon_j(\lambda_o, t)}{dt} - \left(\frac{\alpha}{1 + \alpha t}\right) \epsilon_j(\lambda_o, t) \right] D_j^0 \qquad (5)$$

Again the coefficients of C_i^o and D_j^o must be zero in order for eq. (5) to hold with any degree of probability. They therefore give rise to two

differential equations, the solutions of which are either trivial, when

$$\frac{d\epsilon_i(\lambda_o, t)}{dt} = \frac{d\epsilon_j(\lambda_o, t)}{dt} = \epsilon_i(\lambda_o, t) = \epsilon_j(\lambda_o, t) = 0$$

$$\epsilon_j(\lambda_o, t) = \epsilon_j^0(\lambda_o)(1 + \alpha t) \qquad (6)$$

Thus $(n + m)$ equations (10) must hold at every isosbestic point and $S(n + m)$ such equations must hold for a given spectrum [5, 6].

It therefore follows that at temperature-induced isosbestic points there can be no change in the concentration of the species absorbing at the isosbestic point due to being in chemical equilibrium with a non-absorbing species. In addition, the temperature dependence of ϵ_i must be of the form

$$\epsilon_i(\lambda_c, t) = \epsilon_i^o (1 + h(\lambda) t) \qquad (7)$$

The presence of isosbestic points in a set of spectra, generated by changing the composition or temperature, can be used under appropriate circumstances as evidence for the number of independent reaction parameters. However, isosbestic points are also observed if a single type of molecule in solution is studied under varying external parameters such as temperature, pressure or external fields. The appearance of isosbestic points in the temperature-dependent absorption of pure solutes has been investigated by Morrey [5,6]. He discusses two mechanisms which influence the temperature dependence of electronic spectra. One is the variation in concentration due to volume contraction of the solvent which is a linear function of temperature [5]. The more interesting mechanism is the temperature dependence of the extinction coefficient [6].

In this paper, ee show that the isosbestic points of this type correspond exactly to the energy of J,m ← 0,O transitions. m and 0 denote vibrational levels of the electronic states J and 0 respectively. This means that in cases where isosbestic points are observed in the temperature-dependent electronic absorption spectra of pure molecules in solution, it is easy to locate the corresponding vibrational levels in the excited state. This is illustrated by the temperature-dependent spectra of the 5,10, 15, 20-p-tetra-p-sulphonato-phenyl-porphyrin (TSPP), which will be studied in this paper.

3 Experimental details

3.1. Materials and apparatus

TSPP (Fig. 1) has been prepared according to following procedure:

5 g of 5,10,15,20- tetra-phenyl-porphyrin (TPP) was dissolved by stirring for aprox. 6 hours at room temperature, then poured over 180g of crushed ice. When the ice is melted, the product was isolated by centrifugation and washed twice with 100 ml of water. The product was dissolved in aprox. 250 ml of water keeping the pH above 10 with addition of 10 N NaOH, and the pH then adjusted to 8 with HCl. The solution was filtered and then applied to a C-18 silica column. The column was eluted with a mixture of methanol and water, increasing progressively the methanol content. The final fractions were collected and tested on HPLC, selecting only the TSPP fraction. The preparation and purification procedure is in a good agreement with others literature data [7-10].

J-aggregates were prepared according to the procedure reported in [11] by adding an HCl and/or NaCl solution to a solution of meso-tetrakis(4-sulfonatophenyl)porphine tetrasodium salt, and used within a couple of days.
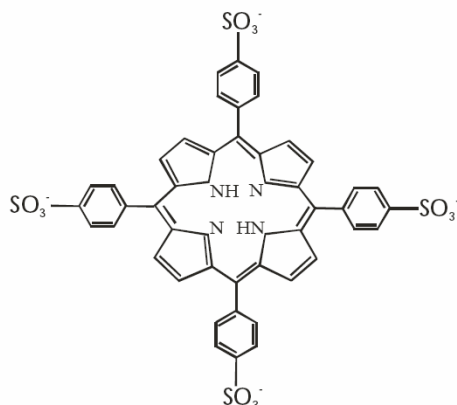

Fig. 1. The structure of TSPP (5,10,15,20-tetra-(4-sulfonatophenyl) porphyrin)

A Carl Zeiss Jena M400 spectrophotometer was used for recording of absorption spectra. All solutions were measured at room temperature in 1 cm or 1 mm pathlength quartz cuvettes. Absorption spectra, were taken for all solutions prepared in water.

4 Results and discussion

TSPP is an anionic porphyrin, a very large disk-shape molecule which possess four negative charges the sulfonate groups from the four corners. In aqueous solutions, at neutral pH, the electronic absorption spectrum of TSPP is typical of free base porphyrins (D_{2h} symmetry) and is characterized by an intense Soret band at arround 420 nm and four Q bands in the 500-700 nm range (the aetio-type spectrum). The absorption spectrum of TSPP at pH 7.5 is characteristic for the free-base porphyrins. It exhibits four Q bands located at 515, 553, 580 and 633 nm and an intense near-UV band (Soret band) with a maximum at around 414 nm. The absorption spectrum changes in acidic environment (pH 3.4) exhibiting a red-shifted and slightly broadened Soret band at 433 nm, a narrow intense band at 490 nm, and two broad weaker bands at 642 and 707 nm. The spectral bands at 433 and 642 nm can be attributed to the diprotonated ionic species, whereas the band at 490 nm (B-band) and at 707 nm (Q-band) are characteristic for J-aggregates [12].

Could be a zwitterionic between the central diacid group of the sulfonic groups, which could be more responsible for J-aggregation. However, it has been proposed that J-aggregates of TSPP tend to form large fractal-like structures or macroaggregates in organic solvents. J-aggregates of some porphyrins are based on the intermolecular interactions of 3-5 Kcal/mol per porphyrin face. Such aggregates have the size of 5-6 nm in solution. The columnar structure formed by porphyrins has a length of 5 to 27 porphyrin unities [13].

These changes in the absorption spectra have been assigned to the formation of the J-aggregates, as indicated by the diminution of the absorption at 433 nm [14].
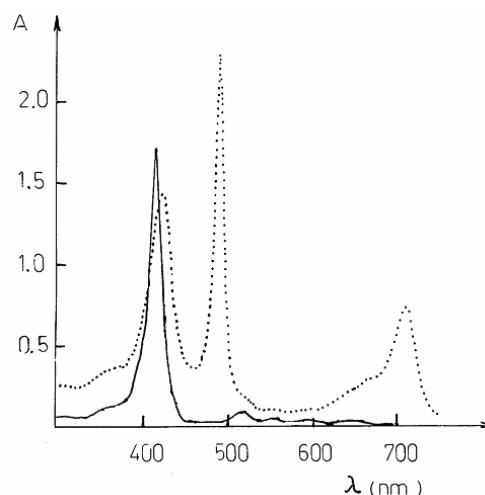

Fig.2. The absorption spectra of TSPP monomer (….) and TSPP J-aggregate (___)

The existence of isosbestic points results from the linear dependence of the absorption spectra [15]. Let

us assume that a pure dye dissolves in an ideal solvent showing no change in refractive index with temperature and no change in volume. The change in shape with temperature can only be caused by the change in population of the vibrational levels of the electronic ground state. The electronic absorption spectrum $S(\lambda,T)$ is thus represented by the following formula:

$$S(\bar{\lambda},T) = B_{01}N\sum_{i=0}\sum_{m=0}\frac{e^{-E_i/kT}}{Q_{vib}(T)}\langle i|m\rangle^2 \quad (8)$$

The index i represents the vibrational levels in the electronic ground state and m the vibrational levels in the electronically excited state. $Q_{vib}(T)$ is the vibrational partition function. The electronic transition moment B_{01} and the number of molecules N in the cross-section of the light path are constant provided that no conformational changes occur. The first term in eqn. (8) represents the vibronic population of the electronic ground state according to the Boltzmann distribution, and the second term describes the Franck-Condon transition probabilities to vibronic levels of the electronically excited state. The condition for an isosbestic point at the position i,

$$S(\lambda_a, T_\alpha) = S(\lambda_a, T_\beta) \quad (9)$$

must hold for any temperatures T_α and T_β. There is always a temperature T_α, at which only the lowest vibrational level no is populated, so that eqn. (9) holds

$$n_0(T_\alpha) = \frac{e^{-E_0/kT_\alpha}}{Q_{vib}(T_\alpha)} \approx N \quad (10)$$

Combining all these equations will be obtained:

$$\left[\sum_{i=0}\sum_{m=0}n_i(T_\beta)\langle i|m\rangle^2\right]_{\lambda_a} \approx N\left[\sum_{m=0}\langle 0|m\rangle^2\right]_{\lambda_a} \quad (11)$$

This is an interesting equation which is satisfied at any isosbestic point observed under the conditions assumed. The sum of all the molecules in the individual levels $n_i(T_\beta)$ is equal to the total number of dye molecules N

$$\sum_{i=0}n_i(T_\beta) = \sum_{i=0}\frac{e^{-E_i/kT_\beta}}{Q_{vib}(T_\beta)} = N \quad (12)$$

Therefore we can write

$$\left[\sum_{i=0}n_i(T_\beta)\left\{\sum_{m=0}(\langle i|m\rangle^2 - \langle 0|m\rangle^2)\right\}\right]_{\lambda_a} \approx 0 \quad (13)$$

This equation holds for any temperature T_β. It can thus only be fulfilled in the trivial case i= 0 and much more interestingly for transitions J,m ← O,0 . From this it follows that the position λ_a of an isosbestic point corresponds to the energy of a J,m ← 0,O transition. As a consequence it can be used to determine the energy of the vibrational level m in the excited state. The spectra can be corrected in cases where volume and/or refractive index change in a continuous way with temperature.

Table 1. The isosbestic points of TSPP in the temperature range (-10 ^{O}C - 24 ^{O}C)

Isosbestic point (nm)	Transition	S_1←S_o l,m←O,o (cm^{-1})	Difference m←O' (cm^{-1})
576	0'←0	17361	0
504	m_1←0	19841	2480
421	m_2←0	23752	6391

The first isosbestic point corresponds to the 0' ←0 transition for TSPP.
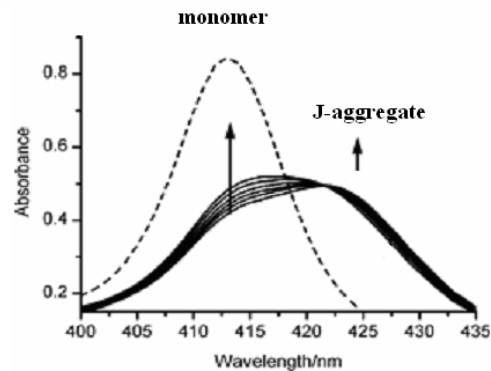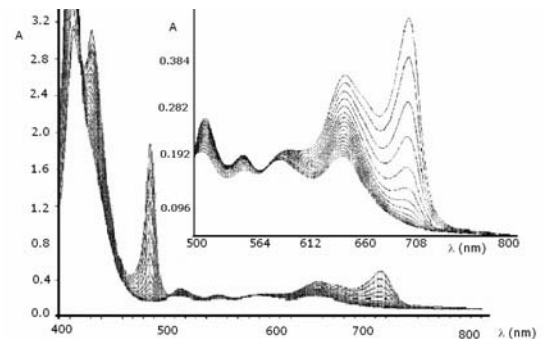

Fig.3. The isosbestic points obtained from TSPP J-aggregate to TSPP monomer in the temperature range –10 ^{O}C – 24 ^{O}C
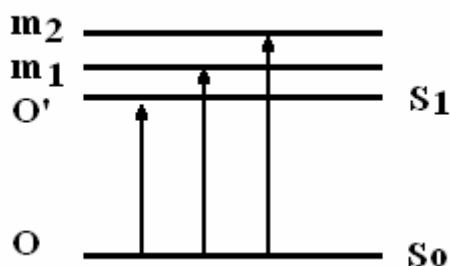
Fig. 4. $S_1 \leftarrow S_o$ transitions at low temperature and vibrational levels of TSPP in the S_1 state.

Water soluble porphyrins are important agents for photodynamic therapy (PDT). The mechanisms by which these sensitizers induce tumor necrosis are very different from that observed in the case of lipophylic sensitizers [16] and it is assumed that they kill neoplastic cells (as K562) indirectly by damaging blood vessels and interrupting the supply of oxygen [17]. Among water soluble porphyrins, TSPP is one of the best tumor localizer.
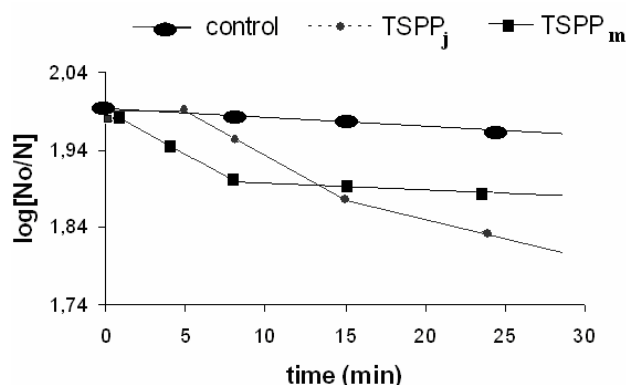

Fig.5. The photodynamic activity of TSPP J and TSPP m on K562 cells, from publication [13].

TSPP porphyrin, was applied topically 4 h before irradiation in the PDT group. Each cell received 125 J/cm^2 at a fluence rate of 70 mW/cm^2. The probability that a cellular lesion cleared was greater with PDT than cryotherapy. But, using TSPP J-aggregate, freezed, the local presence of this porphyrin is longer, avoiding the porphyrin photobleaching and keeping a longer time the singlet oxygen action.
Cryotherapy was associated with ulceration, infection and recurrent disease; no such complications occurred following PDT.

4 Conclusion

In this paper will be studied the transformation of TSPP from J-aggregates to monomer forms in the temperature range −10 ^{O}C to +24 ^{O}C, putting into evidence the generated isosbestic points. With a mathematical calculus, $S_1 \leftarrow S_o$ transitions at low temperature and vibrational levels of TSPP in the S_1 state was possible to be evaluated. Such transitions from the vibrational levels, are responsible for singlet oxygen generation and photodynamic activity, especially.

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