SPECTRAL ANALYSIS OF THE PORPHYRINS INCORPORATION INTO HUMAN BLOOD

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ABSTRACT

The porphyrins seem to be the most effective photosensitizers in photodynamic therapy of cancer. Monomers and dimers of sulfonated and nonsulfonated porphyrins [5,10,15,20-tetra-phenyl-porphyrin, 5,10,15,20-tetra-naphtyl-porphyrin (TNP), 5,10,15,20-tetra-*p*-sulfonato-phenyl-porphyrin (TS_4PP) and 5,10,15,20-tetra-*p*-sulfonato-naphtyl-porphyrin], are studied in this paper by means of different spectral methods (UV-vis, fluorescence and polarization fluorescence). The porphyrins as TNP seem to be the most effective photosensitizer especially in a DMSO:water binary mixture of solvent. Monomer–dimer and *J*-aggregation equilibria and the temperature dependence of TS_4PP spectra are also studied in this paper. The incorporation of the forms of these porphyrins into blood cells is studied by means of the cytofluorimetric method. © *1999 Society of Photo-Optical Instrumentation Engineers.* [S1083-3668(99)01003-5]

Keywords porphyrins; aggregation; photodynamic therapy.

1 INTRODUCTION

Photodynamic therapy (PDT) of cancer is a promising method for destruction of tumor tissues. It is based on the photochemical properties of certain sensitizers, which can be accumulated in significant amounts and retained for prolonged periods of time by tumor tissues; tumor necrosis can be obtained by irradiation of the neoplastic area with light corresponding to the longest wavelength absorption band of porphyrin.[1] The investigations to identify better tumor photosensitizers are mainly directed at the synthesis of new porphyrins with absorption properties in the far red spectral region.[2] Sulfonated porphyrins are presently under intense investigation because they are highly water-soluble, nontoxic and absorb strongly chemically useful red light.[3] The photophysical properties of the porphyrins used in PDT change drastically upon aggregation. Anionic porphyrins such as the class of sulphonated meso-tetraphenyl porphyrins, TS_nPP, $n = 1–4$, have been shown to accumulate preferentially either in the cytoplasmatic membranes or in the lysosomes.[4,5] The photophysical properties of the porphyrins (chemical structure, aggregation states, etc.) are considered to be determinants for localization and photodynamic activity.[6]

The dependence of the spectral behavior of porphyrins on medium and concentration has been shown to be associated with the phenomena of aggregation.[7] Aggregation of the porphyrins often occurs in aqueous media, almost causing a reduction of the overall photosensitizer efficiency.[8] The ability of porphyrins to form aggregates is a well-known aspect of this class of compounds.[9,10]

The porphyrins with negatively charged peripheral groups can exhibit aggregation, whereas the porphyrins with positively charged peripheral groups show no indication of aggregation.[11] The cell experiments indicate the occurrence of ‘‘folded’’-type dimers or aggregates in biological membranes. In water or phosphate buffered saline (PBS) solutions, the dimers of anionic porphyrins can have a ‘‘face-to-face’’ (sandwich) or an ‘‘end-to-end’’ (linear) configuration.[12] Because the hydrophobic porphyrins [5,10,15,20-tetra-phenyl-porphyrin (TPP) and 5,10,15,20-tetra-naphtyl-porphyrin (TNP)] do not solubilize in aqueous media, in PDT applications, these porphyrins were introduced in a polar aprotic solvent like dimethyl sulphoxide (DMSO) and in its binary mixtures with water. The sulfonated porphyrins [5,10,15,20-tetra-*p*-sulfonato-phenyl-porphyrin (TS_4PP) and 5,10,15,20-tetra-*p*-sulfonato-naphtyl-porphyrin (TS_4NP)] are easily solubilized in phosphate buffer (PBS), in DMSO, or in the binary mixture DMSO: water. Monomers and dimers TS_4PP are evaluated in this paper by means of different spectral methods (UV-vis, fluorescence and polarization fluorescence). These aspects were evaluated only at TS_4PP and not at TS_4NP because at the last porphyrin a significant aggregation effect was not observed. By means of the temperature dependence of the absorption measurements combined with the fluores-

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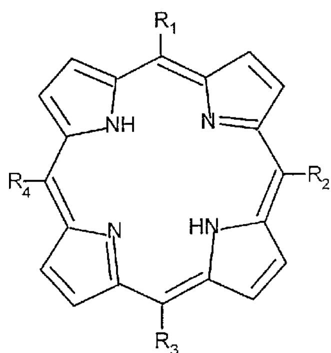

Fig. 1 The structure of the porphyrins.

cence results, we evaluated the possibility of *J*-aggregates generation. By means of flow-cytometry measurements combined with absorption and fluorescence results we tried to evaluate the role of aggregation processes in the incorporation and penetration of such porphyrins into the blood cells. The data on the penetration of the porphyrins into the blood cells are important to study for photodynamic therapy.

2 Experimental Part

2.1 Materials

The nonsulfonated porphyrins (TPP and TNP) and sulfonated porphyrins (TS_4PP and TS_4NP) were prepared and purified as in the literature.[13,14] The chemical structures of the porphyrins are shown in Figure 1 and Table 1. For these porphyrins studied, the solutions were prepared in PBS solutions for TS_4PP and TS_4NP, in DMSO or in DMSO:water binary mixture, and only in DMSO:water for TPP and TNP. The cells incubation was achieved on heparinized blood samples, as was reported elsewhere.[15] Whole heparanized blood samples remaining after routine analysis were incubated with porphyrins by means of a lysing reagent (Ortho Diagnostic, USA).

2.2 Absorption Measurements

Absorption measurements were recorded on a SPECORD M400 Carl Zeiss Jena spectrophotometer. All spectroscopic studies were performed in the porphyrin concentration range 8×10^{-5} to 1×10^{-6} M.

Table 1 The structure of the meso-substituents of the porphyrins.

Substituent	R_1	R_2	R_3	R_4
TPP	C_6H_5	C_6H_5	C_6H_5	C_6H_5
TNP	$C_{10}H_7$	$C_{10}H_7$	$C_{10}H_7$	$C_{10}H_7$
TS_4PP	$C_6H_4–SO_3$	$C_6H_4–SO_3$	$C_6H_4–SO_3$	$C_6H_4–SO_3$
TS_4NP	$C_{10}H_6–SO_3$	$C_{10}H_6–SO_3$	$C_{10}H_6–SO_3$	$C_{10}H_6–SO_3$

2.3 Fluorescence Measurements

The fluorescence spectra were performed with an Aminco–Bowman spectrofluorimeter controlled by an IBM computer and equipped with a polarization accessory. Fluorescence emission and polarization fluorescence were obtained at 550 nm for TS_4PP at the maximum of the Q band II in order to minimize inner filter effects. For fluorescence studies, very dilute solutions were used to avoid spectral distortions due to inner filter effect and emission reabsorption. Temperature measurements were performed in sealed cuvettes controlling the temperature within 0.1 °C.

2.4 Flow-Cytometry Measurements

The three-fluorescence flow cytometer (Cytron Absolute, Ortho) equipped with an argon ion laser was used. The dependence of forward light scattering versus right angle light scattering enables us to distinguish various types of cells. The fluorescence excited at 488 nm was measured through the orange band pass filter (563–607 nm) and through the red filter ($\lambda>620$ nm) in the direction perpendicular to that of the exciting light and the cell stream. The intensity of emission of a given population of the cells was obtained from a specialized program using the gate analysis method.[16] We appreciated the number of stained cells (lymphocytes and granulocytes) incubated with porphyrins either in their monomeric forms or in their dimeric forms.

3 Results and Discussion

One of the main photophysical properties of the porphyrins used as phototherapeutic agents for tumors is based on the extinction of the delocalization of the π electron cloud through the insertion of the conjugated double bonds into the ring system.[17] Because the hydrophobic porphyrins (TPP and TNP) do not solubilize in aqueous media, in PDT applications these porphyrins were introduced in polar aprotic solvent like DMSO and in its binary mixtures with water. It was important to study the absorption spectra of these porphyrins in DMSO and in the mixture between DMSO and water. The investigated porphyrins are in a monomeric state in DMSO (Figure 2). A small amount of DMSO added to water has little effect on the hydrogen bonding of water[18–20] or acts as a "structure breaker" in water.[21]

A large amount of DMSO added to water creates molecular complexes in which water acts as hydrogen-bonded links between DMSO molecules. In a binary mixture of DMSO:water at different concentrations of DMSO the porphyrins change their spectra. Linschitz and Felton[22] reported the reduction of TPP in DMSO, resulting in the generation of a radical anion which could be further reduced to the corresponding dianion. Due to its acid character, the hydrogen atom from the porphyrin

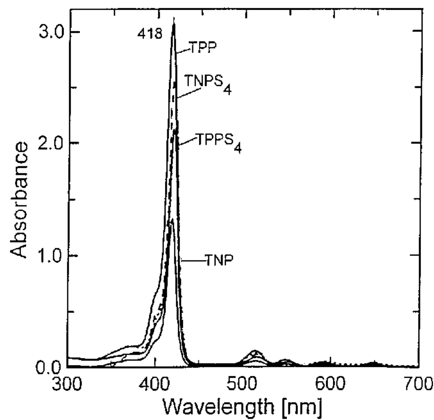

Fig. 2 Absorption spectra of the studied porphyrins.

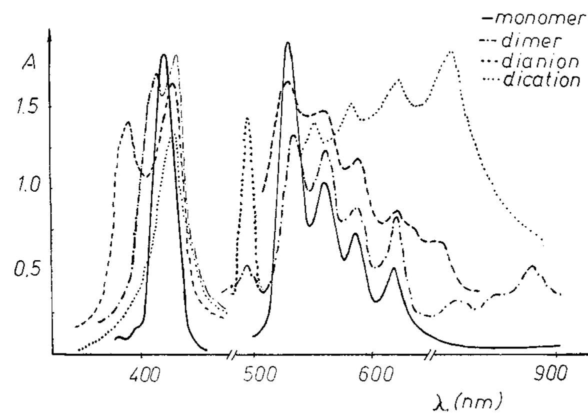

Fig. 4 Absorption spectra of different ionized forms of TNP in DMSO:water.

macrocycle can be eliminated and the porphyrins can form hydrogen bonds with proton donor solvents[23] like DMSO. Between the ratio 100:0 to 80:20 DMSO:water, water can intercalate between two DMSO molecules. The whole aggregate is bonded with the porphyrin molecule (by means of the NH or N atoms).[24] The proton from inside the porphyrin macrocycle can leave the macrocycle and the porphyrin becomes an anion form with a strong electronic density at the methine carbon position.[25] In such conditions, the anionic form of the porphyrin could aggregate in good agreement with the literature data.[26] The dimerization processes are valuable for these conditions between 75%:25% and 50%:50% DMSO–water. Also, in water (even for low content of water) DMSO hydrolyzes to form methane and methyl–sulfonic acid evidenced by means of the IR spectrum (Figure 3). At 50% DMSO in water, due to these acid forms generated in our system the dimeric forms start to the break, yielding to one neutral porphyrin form and after that to one acidic form until there is a 33%:67% DMSO:water ratio. At a 25%:75% ratio in the system studied there are only monomeric and dimeric forms of porphyrin (this being the apparent concentration of

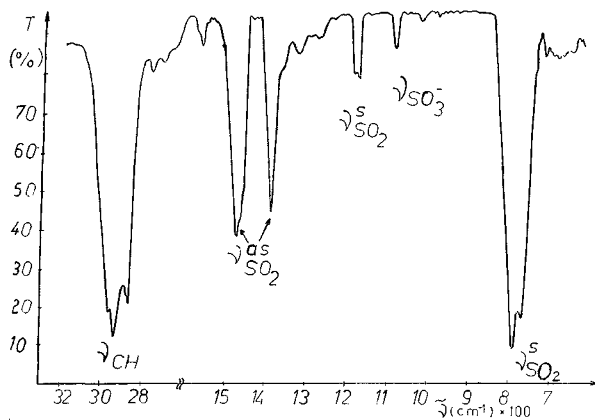

Fig. 3 IR spectrum of methyl–sulphonic acid.

DMSO). Even the DMSO has a breaking effect on the dimer and acts like an axial ligand.[27]

At the noncytotoxic concentration of this solvent in cellular systems (0.5%) the porphyrins exist in a mixture of dimer and monomer forms. As a conclusion, as a result of the addition of water in the case of nonsufonated porphyrins, the changes characteristic for the aggregation of dye are seen. The Soret band became broader and the Q_X band is shifted from 515 to 518 nm. It is characteristic for the formation of large oligomers, because in a case of dimerization the coefficient is increasing[28] not decreasing as we observed in our case. These results will be studied subsequently. For sulfonated porphyrins the situation is different, because in our system *p*H changes and we must study the ionization processes for these porphyrins.[29] By decreasing the DMSO concentrations in the binary mixture with water from 100% to 0.5%, the porphyrins pass from neutral form to anionic form (75% DMSO) and dimeric form (67% DMSO), to neutral form again (at 50% DMSO) to a monocationic (37% DMSO) and dicationic form (25%–0.5% DMSO) (Figure 4). At 0.5% DMSO at TS_4PP the spectra are as superpositions of free base monomeric form and bication forms, which subsequently yield to dimeric forms ($C<2.5\times10^{-3}$ M) and to J aggregates ($C>2.5\times10^{-3}$ M), where C is concentration.[30] We expected that because TNP had shown more interesting properties and high efficiency in different biomedical applications, the new one—TS_4NP—to present similar properties, including water solubility. But the results were not at all spectacular. The TS_4NP absorption spectrum seems to be identical to that of TS_4PP. For the TS_4PP dimer, a red shift and a distinct splitting of Soret band could be observed in two absorption bands (414 and 434 nm), at a constant Q band at 552 nm (Figure 5).[31] For TS_4NP, at Soret region, there is also a redshift of this band at a constant Q band and a reduced splitting of this band upon dimerization.[31] A broad absorption between 600 and 700 nm is characteristic of closely spaced porphyrin dimers as in the case of TS_4PP.

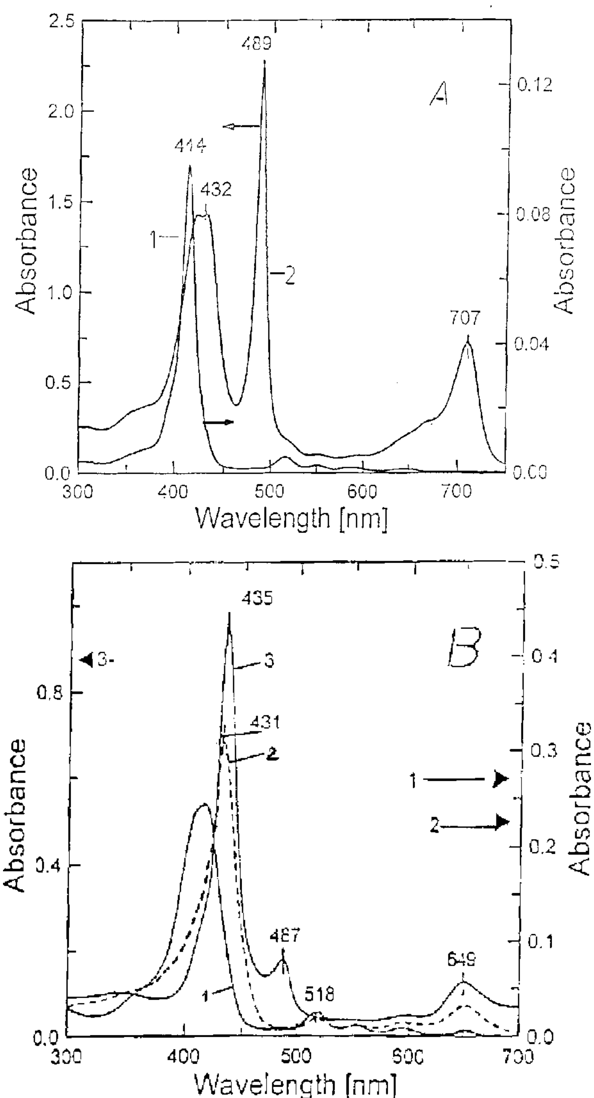

Fig. 5 Absorption spectra of porphyrins TS_4PP (5×10^{-4} M) (A) and TS_4NP (2.5×10^{-5} M) (B) in 0.5% DMSO+99.5% water.

Some of the absorption bands from the red spectral region of the dimers are redshifted and are absent in the spectra of porphyrin monomers, and under these conditions we think that the dimers have a cofacial oblique structure with larger and more flexible space between the rings, like in μ-oxo-porphyrin complexes, in good agreement with the literature.[30,32,33]

Although some dimeric porphyrin derivatives appear to display optimal tumor-localizing activity,[34] aggregation decreases the probability of porphyrin photoexcitation[35] and hence of singlet oxygen formation if face-to-face clusters are formed. The measured shifts of the specific bands are consistent with the sandwich type geometry of the porphyrin dimer.[36] At room temperature the dimerization constant of TS_4PP in PBS is found to be $K_d = 3.42\times10^4$ M^{-1}. Figure 6 presents the absorption spectra of TS_4PP varying with temperature. A

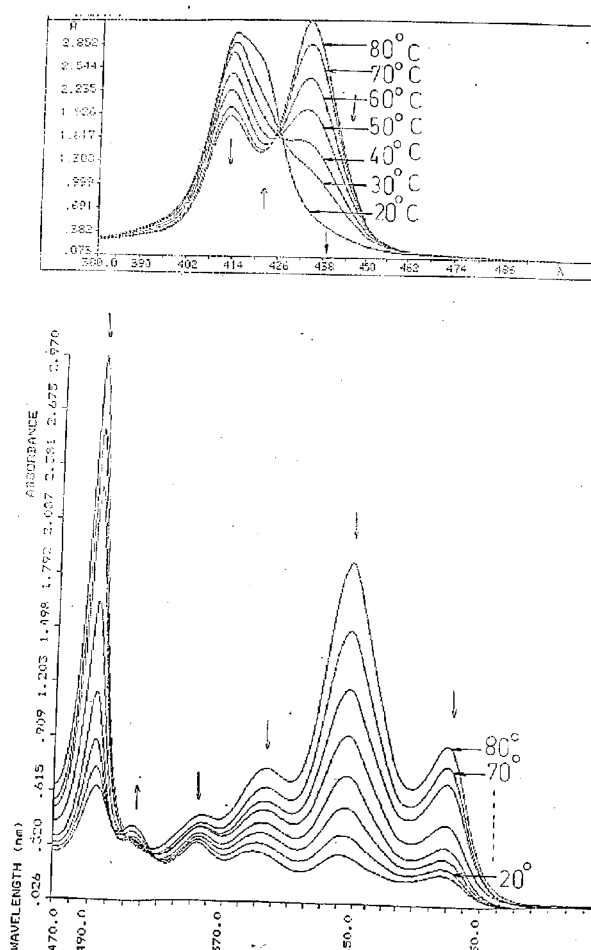

Fig. 6 The variation of the absorption spectrum of TS_4PP with temperature.

change of the spectrum into the monomer one is obvious. The temperature dependence of the dimerization constants of TS_4PP is given in Table 2. For the TS_4PP dimer, a redshift, a distinct splitting of the Soret band, and a hypsochromic shift of the other Q bands could be observed.

For large aggregates [at very high concentration ($C > 2.5\times10^{-3}$ M) and at acidic pH 1.5 observed at a DMSO:water ratio of 0.5%–99.5%], the molar absorbtivity decreases, the Soret band is blueshifted,

Table 2 The temperature dependence of the dimerization constant of TS_4PP.

T (K)	K_d ($\times10^4$ M^{-1})
333	1.1781
323	1.5246
313	2.0329
303	1.9965
293	3.4230

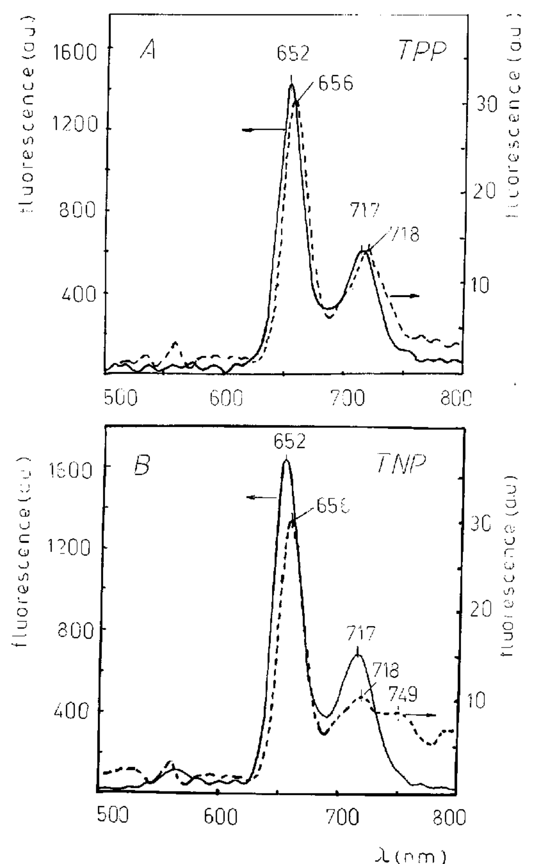

Fig. 7 Fluorescence spectra of TPP (A) and TNP (B) at λ_{exc} = 488 nm in DMSO (———) and in 0.5% DMSO+99.5 % water (- - - -).

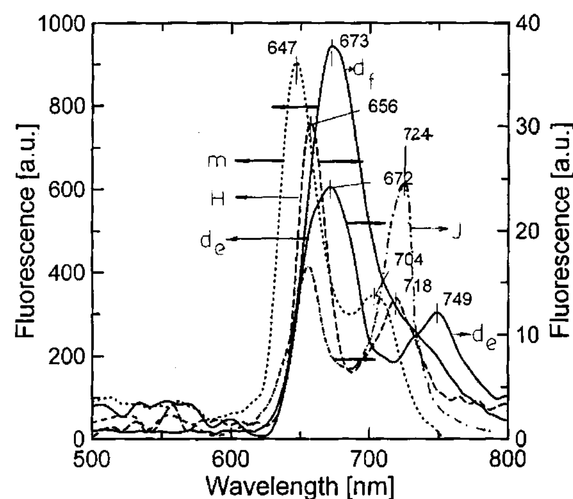

Fig. 8 The fluorescence spectra of different forms of TS_4PP: (···) monomer; (— — —) *H* aggregate; (—) dimer (face-to-face configuration) d_f; (———) dimer (end-to-end configuration) d_e; (– · – ·) *J* aggregate.

and the *Q* bands are strongly redshifted. Could we presume that *J* aggregates could be formed in this aggregation process?

The formation of a *J* aggregate is clearly identified by a relatively large (2000 cm^{-1}) bathochromic shift, a band narrowing and an increase in extinction in the absorption spectrum compared with the case for unassociated monomers.[30] Determining the temperature dependence of the dimerization constant could be observed in the transformation of the absorption spectra of the dimer to that of pure monomer with increasing temperature similar to the reversible dissociation of *J* aggregates upon heating.[37] Because the new aggregated species reformed the monomer species, it could be presumed that these new species could be *J* aggregates (only *J* aggregates dissociate into monomers because the thermal energy by heating is distributed equally to all of the *J* aggregates).[38] All these results were completed with the fluorescence experiments.

Porphyrins readily aggregate in aqueous solutions as well as in buffer solutions and in cells.[39] TPP and TNP in DMSO present fluorescence spectra with two emission bands (Figure 7), while in the binary mixture DMSO:water, at TPP a redshift of the entire emission spectrum and in the case of TNP only a new emission band at longer wave length (749 nm) could be observed.

The sulfonated porphyrins exhibit the same emission characteristics but with different intensities. The fluorescence spectrum of the monomer of TS_4PP in DMSO shows two peaks: at 650 and 700 nm (Figure 8). The fluorescence of the concentrated TS_4PP solution is highly quenched, which brings us to the conclusion that the TS_4PP aggregate is weakly fluorescent. The fluorescence properties are drastically reduced upon dimer and higher aggregate formation, due to the hydrophobic and π–π interactions which occur in the porphyrin aggregation processes.

All values were obtained for 488 nm excitation. The intensities of the fluorescence of the same pigments in 0.5% DMSO in water are 6–10 times lower than in 100% DMSO. It shows again that the dyes (TPP, TNP, TS_4PP) in aqueous DMSO solvent (0.5% DMSO–99.5% water) are to a high degree aggregated. The shapes of long wavelength bands and the ratios of both band intensities are changed. A shoulder at longer wavelength appears in the case of high concentration, which suggests that this form (*J* aggregate) is weakly fluorescent. End-to-end planar configurations (attributed to *J* aggregates) exhibit emission properties comparable with those of a monomer; even recent studies predict a concerted structure: face-to-face (d_f) and end-to-end structure (d_e).[40] So, under these conditions, we could presume that all these porphyrins in DMSO: water binary mixtures exist like a sandwich and a linear structure mixture. Because the first fluorescence band of TS_4PP is almost identical to the fourth absorption band of this porphyrin, we come to another conclusion about the *J*-aggregation generation.[41]

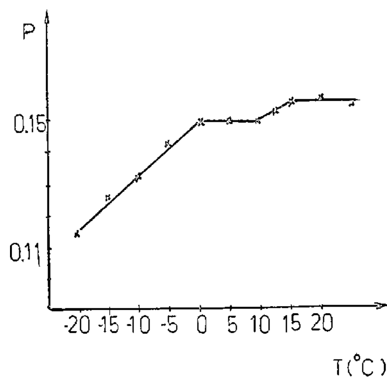

Fig. 9 The dependence of the polarization vs the temperature at TS_4PP.

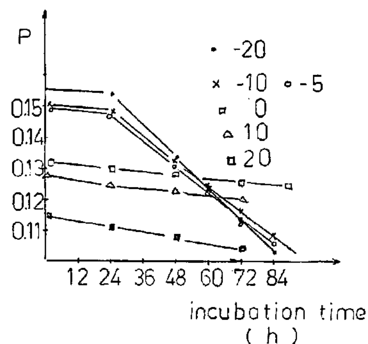

Fig. 10 The variation of the polarization vs the incubation time.

Monomers and dimers have been shown to exhibit various degrees of polarization. In cell membranes, owing to either migration into areas of higher viscosity or binding to protein molecules, dimers and monomers can also exhibit rather pronounced polarization values.[42] Also, by aggregation, there is a rather pronounced polarization value.

The changes in polarization degree of TS_4PP as a function of temperature have been studied in the porphyrin concentration range 0.125×10^{-5} M. The temperature-dependent polarization fluorescence plots of TS_4PP showed typical phase transitions of this porphyrin. It can be observed that the changes in polarization fluorescence ($\lambda_{exc}=550$ nm) take place at increasing temperature (Figure 9). At this λ_{exc}, we can observe two transitions which appear in the polarization plots: the first transition has the critical temperature $T_c=0$ °C, and the second has $T_c=10$ °C ($\lambda_{em}=650$ nm). All these results are proof of increasing aggregation of the TS_4PP with decreasing temperature. We presumed that at temperature values up to 10 °C in the solution the porphyrin could exist as *J* aggregates and "sandwich" dimer mixtures. But because the dissociation processes of this porphyrin do not take place at a temperature lower than 5 °C, we concluded that the *J* aggregates exist only up to 5 °C (in solid matrice) (−20–5 °C), after that existing only in dimeric form (5–10 °C). After 10 °C in the solution, the fluorescent monomeric form ($c=1\times10^{-7}$ to 2×10^{-6} M) exists. At temperatures higher than 10 °C, in the solution the concentration of the monomer will increase while all aggregated forms will decrease for this concentration area. Figure 10 shows the temperature dependence of the fluorescence polarization degree *P* for various experimental conditions like incubation time. As we know, at *P* 650 nm for $\lambda_{exc}=550$ nm there is a pronounced decrease of the polarization at increasing temperature due to the deaggregation processes, in agreement with the literature data.[43] After prolonged incubation in cells a tendency of monomers to aggregate was observed. This aggregation could be attributed to the partition properties change of porphyrin, which causes the concentration of the porphyrin in specific membrane sites. Structural constraints caused by the possible overcrowding of this porphyrin molecule in a small space show that this porphyrin could interact with very polar regions in close contact with the solvent.

Incorporation of all these porphyrins into human blood cells was analyzed by flow-cytometry measurements. The fluorescence of lymphocytes and granulocytes was analyzed separately. The number of stained cells of a given type was obtained from the percent of gated cells exhibiting observed fluorescence. The different ratio of "mean fluorescence intensities" of the same cells stained suggests that the aggregation of the dye is different or perhaps the interaction of these dyes with the cell membrane is different.[44,45] When the easily penetrating form occurs in a low concentration, then the number of stained cells is low, but the fluorescence yield is rather high, which is characteristic of the strong fluorescence monomeric form of the dye. From these histograms, it can be assumed that aggregated forms are better penetrating membranes but once in the membranes, the dye is deaggregated by interaction with lipids, therefore exhibiting similar efficiency of fluorescence in the incorporated monomeric form. In both cases penetration into granulocytes is more efficient than into lymphocytes. After prolonged incubation there is a tendency of monomers to aggregate.[46] As we can see from Table 3, TS_4PP appear to display an optimal tumor-localizing activity. Also, this porphyrin is efficiently incorporated into leucocytes, but in its monomeric free base form.

The penetration of this dye depends on the type of cells. It is higher for the granulocytes than for the lymphocytes. It suggests that in both solvents the same forms of dyes penetrate the cell membrane.

In the case of TPP and TNP the percent of stained fluorescent in aqueous DMSO is much higher in DMSO because in aqueous DMSO the pigments are to a high degree in aggregated forms. This suggests that aggregated forms are better penetrating membranes but in membrane dye is deaggregated by the

Table 3 Incorporation of the dyes into cells in DMSO:water binary mixture.[a]

Dye	Cells	% stained cells	Mean intensity fluorescence	State of dye
TPP	*L*	4.5	82.3	*A*
TPP	*G*	63.8	113.1	*A*
TNP	*L*	4.5	69.0	*A*
TNP	*G*	63.8	92.0	*A*
TS_4PP	*L*	0	91.0	*D*+*A*
TS_4PP	*G*	0.1	77.7	*D*+*A*
TS_4NP	*L*	0	0	*M*+*A*
TS_4NP	*G*	0.1	72.0	*M*+*A*

[a] From Ref. 15: (*L*) lymphocytes; (*G*) granulocytes; (*M*) monomer; (*D*) dimer; (*A*) *J* aggregate.

interaction with lipids that exhibit similar efficiencies of fluorescence to the monomeric form incorporated from DMSO. In both cases, penetration to granulocytes is more efficient than into lymphocytes. TNP is more efficiently penetrated into the cells in aggregated form than in monomeric form.

4 CONCLUSIONS

The nonsulfonated porphyrins—TPP and TNP—in aggregated forms are more efficient in penetration into membrane than nonsulfonated monomers. TNP is more efficiently incorporated into cells when it is in aggregated form than it is in free base. The sulfonated porphyrins as TS_4PP are efficiently incorporated into leukocytes and granulocytes but only in monomeric form. Two kinds of dimers can be identified by absorption and fluorescence spectroscopy, namely face-to-face and end-to-end, and some aggregates form *J* aggregates. The penetration is higher for the granulocytes than for the lymphocytes. The monomer–dimer–*J* aggregates equilibrium of TS_4PP is discussed in this paper in a large temperature range (−20–60 °C). The dimerization constants, absorption spectra, fluorescence and polarization fluorescence are evaluated and discussed.

REFERENCES

1. T. J. Dougherty, *Photosensitizing Compounds: Their Chemistry, Biology and Clinical Use*, G. Blok and S. Harnett, Eds., pp. 1–3, Wiley, Chichester (Ciba Foundation Symposium 146) (1989).
2. R. M. Ion, "The PDT—A photosensitization or a photocatalytic process?" *Prog. Catal.* **1**, 55–70 (1998).
3. K. Berg, "Cellular uptake and photosensitizing effects of porphyrins and phtalocyanines," PhD Thesis, The Norewegian Radium Hospital, p. 22 (1991).
4. A. C. Allison, I. A. Magnus, and M. R. Young, "Role of lysosomes and of cell membranes in photosensitization," *Nature (London)* **209**, 874–878 (1966).
5. F. Slatert and P. A. Riley, "Photosensitization and lysosomal damage," *Nature (London)* **209**, 151–154 (1966).
6. K. Berg, A. Western, J. C. Sommer, and J. Moan, "Intracellular localization of sulfonated meso-tetrephenylporphyrin in a human carcinoma cell line," *Photochem. Photobiol.* **52**, 481–487 (1990).
7. R. J. Abraham, "π–π aggregation in metalloporphyrins causative factors," *J. Chem. Soc. Chem. Commun.* **1978**, 699–700.
8. L. E. Bennett, K. P. Ghigginno, and R. W. Henderson, "Singlet oxygen formation in monomeric and aggregated porphyrin," *J. Photochem. Photobiol., B* **3**, 81–89 (1989).
9. R. F. Pasternack, P. R. Huber, P. Boyd, G. Engasser, L. Francesconi, E. Gibbs, P. Fassella, G. Cerio Ventura, and L. De Hinds, "On the aggregation of meso-substituted water-soluble porphyrins," *J. Am. Chem. Soc.* **94**(13), 4511–4517 (1972).
10. M. Krishnamurthy, J. R. Sutter, and P. Hambright, "Monomer-dimer equilibration of water soluble porphyrins as a function of the coordinated metal ion," *J. Chem. Soc. Chem. Commun.* **1975**, 13–15.
11. G. A. Karns, W. A. Gallagher, and W. B. Elliott, "Dimerization constants of water soluble porphyrins in aqueous alkali," *Bioorg. Chem.* **8**, 69–81 (1979).
12. G. J. Smith, K. P. Ghiggino, L. E. Bennett, and T. L. Nero, "The "*Q*-band" absorption spectra of hematoporphyrin monomer and aggregate in aqueous solution," *Photochem. Photobiol.* **49**, 49–52 (1989).
13. L. Teodorescu, R. M. Ion, E. Mocanu, D. Badica, and H. Culetu, "Porphyrinic sensitizers used in photooxidation reactions," *Rev. Chim. (Bucharest)* **38**(8), 129 (1988).
14. R. M. Ion and L. Teodorescu, "The degradation of the porphyrinic photosensitizers used in the photochemical conversion of the solar energy," *Rev. Chim. (Bucharest)* **41**(2), 129–134 (1990).
15. R. M. Ion, A. Planner, K. Wiktorowicz, and D. Frackowiak, "The incorporation of various porphyrins into human blood cells measured using the flow-cytometry, the absorption and emission spectroscopy," *Acta Biochim. Pol.* **45**(3), 833–845 (1998).
16. N. P. Carter and E. W. Meyer, in *Flow Cytometry: A Practical Approach*, M. G. Ormerod, Ed., pp. 1–28, Oxford University Press, Oxford (1990).
17. R. M. Ion and C. Mandravel, "The effect of meso-substituents on the photodegradation of some porphyrins 1. Spectral and cuantic aspects," *Anal. Univ. Buc.* **V**, 51–589 (1996).
18. G. J. Stanfford, P. C. Scahher, P. S. Leung, G. F. Doebler, G. W. Doebler, G. W. Brady, and E. F. X. Lyden, "Neutron Inelastic scattering and X-ray studies of aqueous solutions of DMSO and dimethylsulphone," *J. Chem. Phys.* **50**, 2140–2143 (1969).
19. D. D. Macdonald, M. D. Smith, and J. B. Hune, "The influence of sulfoxides and sulfones on the temperature of maximum density of water," *Can. J. Chem.* **49**, 2817–2819 (1971).
20. C. Devisser, M. J. Henvesland, L. B. Dunn, and G. J. Somsen, "Some properties of binary aqueous liquid mixtures," *J. Chem. Soc., Faraday Trans.* **74**, 1159–1165 (1978).
21. R. M. Ion, "Spectrophotometric study of the photodegradation reaction of TPP. The binary mixtures of solvents effect," *Rev. Chim. (Bucharest)* **45**(10), 879–881 (1994).
22. R. H. Felton and H. Linschitz, "Polarographic reduction of porphyrins and electron spin resonance of porphyrin anion," *J. Am. Chem. Soc.* **88**, 1113–1116 (1966).
23. C. Y. Lee and G. Levin, "Chemistry and photochemistry of the disodium salts of free-base porphyrin," *J. Phys. Chem.* **83**(24), 3165–3168 (1979).
24. R. M. Ion, "Spectrophotometric study of the photodegradation reaction of TPP. The solvent effect," *Rev. Chim. (Bucharest)* **44**(11), 957–960 (1993).
25. R. M. Ion, "Spectrophotometric study of the photodegradation reaction of tetrarylporphyrins. The meso-substituents effect," *Rev. Chim. (Bucharest)* **44**, 431–434 (1993).
26. H. Schneckenburger, M. H. Gschwend, R. Sailer, A. Ruck, and WS. L. Strauss, "Time-resolved *p*H dependent fluorescence of hydrophilic porphyrins in solutions and in cultivated cells," *J. Photochem. Photobiol. B* (to be published).

27. R. M. Ion, N. D. Totir, and St. Popovici, ''The photodegradation reaction of Ru and Rh porphyrins,'' *Electroanal. Chim. Acta* (submitted).
28. D. Frackowiak, A. Planner, R. M. Ion, and K. Wiktorowicz, ''Incorporation of dyes in resting and stimulated leucocytes,'' in *Near-IR Dyes in High Technology Fields*, S. Daechne, Ed., pp. 87–114, Kluwer, NATO ASI Series (1998).
29. R. M. Ion and G. A. Petre, ''Spectral study of some porphyrins used in the photodynamic therapy of cancer. The ionization processes,'' *St. Cerc. Biotechnol.* **29-30**, 216–222 (1997).
30. R. M. Ion and A. Iosif, ''The role of porphyrin aggregation in the photodynamic therapy of cancer,'' *Gen. Physiol. Biophys.* (accepted for publication).
31. R. M. Ion, ''Spectral studies of tetra(*p*-sulfonato-phenyl) porphyrins used in the photodynamic therapy of cancer,'' *Rom. J. Biophys.* **6**(3–4), 215–220 (1996).
32. J. M. Ribo, J. Crusats, J. A. Farrera, and M. L. Valero, ''Aggregation in water solutions of tetrasodium diprotonated meso-tetrakis(4-sulfonatophenyl)porphyrin,'' *J. Chem. Soc. Chem. Commun.* **1974**, 681–682.
33. R. M. Ion, M. Kocak, and O. Bekaroglu, ''Catalytic decomposition of hydrogen peroxide with crowned-ether phthalocyanines,'' *Prog. Catal.* **2**, 59–71 (1998).
34. D. Kessel, P. Thomson, K. Saatio, and K. D. Nantwi, ''Tumor localization and photosensitization by sulfonated derivatives of tetraphenylporphyrin,'' *Photochem. Photobiol.* **45**, 787–790 (1987).
35. J. Moan, ''Porphyrin-sensitized photodynamic inactivation of cells—a rewiew,'' *Lasers Med. Sci.* **1**, 5–12 (1986).
36. I. I. Chou and A. J. Hallock, ''Absorption study of an aggregated porphyrin under high pressure,'' *J. Chem. Phys.* **107**(22), 9297–9301 (1995).
37. O. Ohno, Y. Kaizu, and H. Kobayashi, ''*J*-aggregate formation of a water-soluble porphyrin in acidic media,'' *J. Chem. Phys.* **99**(5), 4128–4132 (1993).
38. S. Mobius, ''Schreibe aggregates,'' *Adv. Mater.* **7**(5), 437–444 (1995).
39. G. J. Smith, ''The effects of aggregation on the fluorescence and the triplet state yield of hematoporphyrin,'' *Photochem. Photobiol.* **41**, 123–126 (1985).
40. A. Planner, R. M. Ion, K. Wiktorowicz, and D. Frackowiak, ''The incorporation of porphyrins in human leucocytes measured by flow-cytometry, absorption and emission spectroscopy,'' in *Proceedings of the First Internet Conference on Photochem. Photobiol.*, http/www://netsci-journal.com/97v3/intro.htm.
41. N. C. Maiti, S. Mazumdar, and N. Periasamy, ''*J*- and *H*-aggregates of porphyrins with surfactants, stopped-flow and electron microscopy studies,'' *J. Porphyrin Phthalocyan.* **2**, 369–376 (1998).
42. H. K. Seidlitz, K. Stetmaier, J. M. Wessels, and H. Schneckenburger, ''Time-resolved fluorescence polarization, picoseconds kinetics and light-induced reactions of photosensitizing porphyrins,'' *Opt. Eng.* **31**, 1482–1486 (1992).
43. C. F. Paolo, A. L. Frisardi, E. R. Resnik, A. E. M. Schona, and A. M. Del C. Battle, ''Factors influencing fluorescence spectra of free porphyrins,'' *Clin. Chem.* **34**, 757–760 (1980).
44. R. Sanus, C. Kohen, E. Kohen, J. P. Reymann, P. Morlierre, L. Dubbere, and P. M. Tocci, ''Permeation of lysosomal membranes in the course of photosensitization with methylene blue and hematoporphyrin: Study by cellular microspectrofluorimetry,'' *Photochem. Photobiol.* **38**, 71–77 (1983).
45. V. V. Borokov, M. Anikin, K. Wasa, and Y. Sakata, ''Structurally controlled porphyrin-aggregation process on phospholipid membranes,'' *Photochem. Photobiol.* **63**, 477–482 (1996).
46. F. Ricchelli and S. Gobo, ''Porphyrins as fluorescent probes for monitoring of phase transitions of lipid domains in biological membranes, Factors influencing the microenvironment of hematoporphyrin and protoporphyrin in liposomes,'' *J. Photochem. Photobiol. B* **63**, 877–883 (1996).