

Photophysical studies of tin(IV)-protoporphyrin: Potential phototoxicity of a chemotherapeutic agent proposed for the prevention of neonatal jaundice

(bilirubin/laser flash photolysis/phototherapy/pulse radiolysis/singlet oxygen)

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ABSTRACT The strongly light-absorbing metalloporphyrin tin(IV)-protoporphyrin IX (SnPP) is currently being considered as a chemotherapeutic agent for preventing severe hyperbilirubinemia in newborns, a condition usually treated by phototherapy with visible light. To assess the potential phototoxicity of SnPP we studied the photophysics of the drug in aqueous and nonaqueous solutions using laser flash photolysis and pulse radiolysis. Quantum yields for formation of triplet-state excited SnPP were measured, along with triplet lifetimes and extinction coefficients. In addition, we measured quantum yields for the SnPP-photosensitized formation of singlet oxygen in MeO²H and in ²H₂O containing cetyltrimethylammonium bromide, using a time-resolved luminescence technique. Quantum yields for formation of triplet SnPP from monomeric ground-state SnPP are high (≈ 0.6 – 0.8), and triplet lifetimes are long (≈ 0.1 – 0.2 ms). Efficient quenching of triplet SnPP by molecular oxygen was seen with rate constants $>10^9$ M⁻¹s⁻¹. SnPP-photosensitized formation of singlet oxygen in aqueous and nonaqueous solvents was confirmed by the detection of the characteristic luminescence at 1270 nm ($\phi_{\Delta} = 0.58$ in MeO²H). The photophysical parameters and singlet oxygen-sensitizing efficiency of SnPP are similar to those reported for hematoporphyrin and other metal-free porphyrins known to be phototoxic to humans. These observations suggest that cutaneous photosensitivity arising from singlet-oxygen damage is likely to be an undesirable side-effect of SnPP therapy.

Tin-protoporphyrin (SnPP; Fig. 1) is a synthetic metalloporphyrin that acts as both an inducer and competitive inhibitor of heme oxygenase [heme, hydrogen-donor: oxygen reductase (α -methene-oxidizing, hydroxylating), EC 1.14.99.3], the first of two enzymes in the metabolic path from heme to bilirubin (1). SnPP and its reduced derivative tin-mesoporphyrin inhibit bilirubin formation in mammals, and both compounds have been proposed as therapeutic agents for the prevention of hyperbilirubinemia in newborns (2–5). Animal studies indicate that the drugs remain in the body for several days after administration and are effective in small doses (3–5, 6).

Many porphyrins and metalloporphyrins are efficient photosensitizers that can cause severe phototoxicity if present in light-exposed tissues *in vivo* (7). The ability of metalloporphyrins to cause photodynamic damage depends on their photophysical properties and excited-state chemistry. In general, porphyrins and metalloporphyrins that are photodynamically active have high quantum yields for formation of triplet excited states, long triplet lifetimes, and high quantum yields for sensitizing the formation of singlet oxygen. Such parameters have not been reported, to our knowledge, for

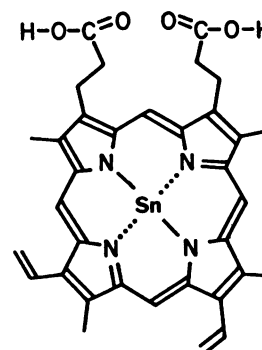


FIG. 1. Chemical structure of SnPP (dication).

SnPP or any other closely related tin-porphyrin, and the influence of tin chelation on porphyrin photochemistry and photobiology has not been well studied. The proposed use of SnPP in a group of patients who are often exposed to intense light, either environmentally (8) or therapeutically (9), and current interest in the development of porphyrins suitable for photochemotherapy of cancer (10), prompted this study of SnPP photochemistry. We find that SnPP is an efficient photosensitizer of singlet-oxygen formation, with photophysical properties that are similar to those of other porphyrins known to cause photosensitivity in humans.

MATERIALS AND METHODS

Materials. Tin(IV)-protoporphyrin IX dichloride (Porphyrin Products, Logan, UT), hematoporphyrin dihydrochloride (Sigma), anthracene (microanalytical grade, BDH), biphenyl (Kodak), Triton X-100 (Merck), cetyltrimethylammonium bromide [CetMe₃NBr (Aldrich)], ²H₂O (Sigma), and MeO²H (Sigma) were used as supplied. Deuterated solvents were >99.5 atom %. Acetone was purified by refluxing with KMnO₄, drying over K₂CO₃, and distilling. Other solvents were spectroscopic grade. Aqueous solutions of SnPP were prepared by dissolving a weighed quantity of the dichloride in a minimum volume of 0.01 M NaOH and diluting this at once with 0.1 M sodium phosphate buffer (pH 7.4). Solutions used for laser flash photolysis studies were 3–10 μ M. Solutions were deoxygenated by bubbling with N₂ [British Oxygen (Manchester, U.K.), white spot grade].

Methods. The laser flash photolysis and pulse-radiolysis equipment has been described (11, 12). With the former, the

347-nm line of a J. K. Lasers System 2000 frequency-doubled ruby laser (pulse width, ≈ 25 ns; energy output to 400 mJ) was used for photoexcitation with a detection system supplied by Applied Photophysics (London). Samples were contained in a $1 \times 1 \times 10$ mm flow cell with an excitation-beam pathlength of 1 mm. Pulse-radiolysis samples were contained in 25-mm optical-pathlength quartz capillary cells. Ground-state spectra were recorded on a Beckman model 24 spectrophotometer.

Triplet lifetimes and triplet-minus-ground-state difference spectra were measured by laser flash photolysis. Difference extinction coefficients for triplet SnPP, required for calculating quantum yields for triplet formation, were obtained by the complete conversion method and also, for acetone solutions, by a pulse-radiolysis energy-transfer method (13). In the complete conversion method, excitation of all ground-state molecules to the triplet manifold is achieved by using a laser pulse of sufficient intensity (13, 14). The triplet difference extinction coefficient can then be calculated from the concentration, the ground-state extinction coefficient, and the triplet-minus-ground-state absorbance (ΔA). This method is valid only for molecules with long triplet lifetimes and short excited-singlet lifetimes relative to the duration of the laser pulse (13). In the pulse-radiolysis method, a standard with a known triplet-minus-ground-state extinction coefficient ($\Delta \epsilon_T$) is used to produce the triplet of interest by energy transfer (13, 15). Ideally, conditions are arranged so that all donor triplets are quenched by the acceptor before the acceptor triplet begins to decay. The extinction coefficient of the acceptor triplet can then be calculated from the measured ΔA values for the donor in the absence of acceptor and for the acceptor in the presence of donor, respectively, if $\Delta \epsilon_T$ for the donor is known. We used biphenyl as standard ($\Delta \epsilon_T = 27,100 \text{ M}^{-1}\text{cm}^{-1}$ at 360 nm) (16, 17). Solutions containing biphenyl (0.1 M) were subjected to radiolysis in the presence and absence of SnPP (0.1 mM), and ΔA values were measured. Corrections were made for decay of triplet biphenyl by pathways other than energy transfer to SnPP and for decay of triplet SnPP during its formation, as described previously (13). These corrections were small ($<20\%$). At both the biphenyl and SnPP triplet maxima, a weak underlying radical absorption was present, which probably arose from free ions known to be formed in significant amounts along with excited states in acetone (18). These solute radicals had long lifetimes compared with those of the biphenyl and SnPP triplets, and their absorbance was subtracted from the triplet absorbances on the assumption that they were formed instantaneously and did not originate from solute triplet states. The radical absorbance amounted to $<20\%$ of the total ΔA observed at the triplet maxima.

Quantum yields for intersystem crossing to the triplet state (ϕ_T) were measured by a comparative flash photolysis method using anthracene in cyclohexane as actinometer (14, 19). Ground-state absorbances of the anthracene and SnPP solutions were adjusted to be equal (≈ 0.2) at the wavelength of excitation (347 nm). Values of 0.71 and $64,700 \text{ M}^{-1}\text{cm}^{-1}$ were used for the triplet quantum yield and extinction coefficient (at 422 nm) of anthracene, respectively (16, 19). Depletion of the ground state was kept $<10\%$.

Rate constants for oxygen quenching of triplet states were determined in air-saturated solutions using literature values for oxygen concentrations (20). The concentration of oxygen in air-saturated aqueous CetMe_3NBr solutions was assumed to be the same as that in water.

Singlet-oxygen production was measured by monitoring the $^1\Delta_g \rightarrow ^3\Sigma_g$ phosphorescence emission at 1270 nm from air or oxygen-saturated solutions after laser excitation (21–23) using an apparatus described previously (24). Initial singlet-oxygen emission intensities were calculated by extrapolating the first-order singlet-oxygen decay curve ($\tau = 28 \text{ } \mu\text{s}$ in

MeO^2H) back to the midpoint time of the laser pulse. Quantum yields for singlet-oxygen formation (ϕ_Δ) were determined by a comparative method using oxygen-saturated solutions and hematoporphyrin as a standard (24, 25). A value of $\phi_\Delta = 0.53$ was assumed for hematoporphyrin in MeO^2H . This value was based on measured values of $\phi_\Delta = 0.53$ in EtOH and EtO^2H (by a thermal lensing technique) (25) and $\phi_\Delta = 0.58$ and 0.52 in MeO^2H (by diphenylisobenzofuran quenching and time-resolved luminescence measurements relative to erythrosin, respectively) (26).

Quantum yields, triplet lifetimes, and extinction coefficients reported are averages of at least three determinations.

RESULTS

The photophysical properties of a porphyrin depend on its molecular environment and state of aggregation (7). Like related porphyrins and metalloporphyrins, SnPP forms aggregates in weakly basic aqueous buffer (27). Monomeric SnPP shows an intense Soret absorption peak near 410 nm with a relatively weak shoulder near 390 nm. Dimerization, or higher aggregation, causes a decrease in the intensity of the 410-nm band and emergence of the 390-nm shoulder as a clear peak (27). The ground-state absorption spectrum of SnPP in MeOH at the concentrations used in these studies (Fig. 2) showed an intense sharp Soret peak ($\lambda_{\text{max}} = 408 \text{ nm}$; $\epsilon = 2.3 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$) and two sharp "Q" bands in the visible ($\lambda_{\text{max}} = 542$ and 580 nm ; $\epsilon = 1.5 \times 10^4$ and $1.2 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$, respectively) indicating a monomeric (or predominantly monomeric) solute. Similarly, solutions in acetone appeared monomeric ($\lambda_{\text{max}} = 416, 546, 583 \text{ nm}$). Dilute solutions of SnPP in water at high pH (10.8) contained mainly monomer, as previously reported (27), whereas solutions in buffer at pH 7.4 showed spectroscopic evidence of extensive dimerization. Disaggregation to monomer was readily effected by adding Triton X-100 or CetMe_3NBr and allowing the solutions to reach thermodynamic equilibrium in the dark.

Laser flash photolysis of N_2 -flushed solutions of SnPP in MeOH, acetone, or water at 347 nm generated a short-lived transient. This transient decayed quantitatively to the ground state, except at very high laser intensities when there was some irreversible loss of SnPP. The transient was identified as the lowest triplet state on the basis of its absorption spectrum, lifetime, and quenching by oxygen. A similar transient was also obtained on pulse radiolysis of SnPP in acetone in the presence of the triplet sensitizer biphenyl, confirming its identification as triplet SnPP. The absorbance

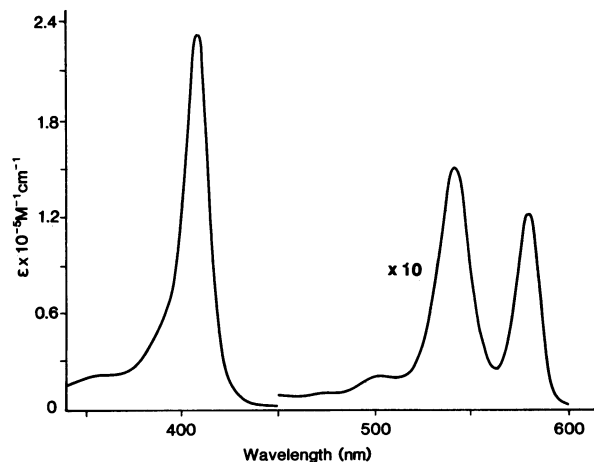


FIG. 2. Absorption spectrum of ground-state SnPP (dichloride) ($6.7 \text{ } \mu\text{M}$) in MeOH. Ordinate values $>450 \text{ nm}$ have been multiplied by 10.

Table 1. Difference extinction coefficient ($\Delta\epsilon_T$), lifetime (τ), oxygen-quenching rate constant (k_A), and quantum yield (ϕ_T) for formation of triplet SnPP in several solvents

Solvent	$\Delta\epsilon_T (\lambda_{\max}),$ $\text{mM}^{-1}\cdot\text{cm}^{-1}$	$\tau, \mu\text{s}$	$k_A \times 10^{-9},$ $\text{M}^{-1}\cdot\text{s}^{-1}$	ϕ_T
MeOH	43.5	196	1.2	0.83
Acetone	51.8*	154*	1.1	0.60
H ₂ O, pH 10.8†	36.8			0.72
2% Triton X-100/0.1 M PO ₄ buffer, pH 7.4	34.5	95		0.75
1.0 mM CetMe ₃ NBr/0.1 M PO ₄ buffer, pH 7.4	30.0	162	1.2	0.68
0.1 M PO ₄ buffer, pH 7.4	11.2			0.32

*Obtained by pulse radiolysis; all other table data obtained by laser excitation.

†Prepared by dissolving SnPP in a minimal volume of 0.01 M NaOH and diluting to $\approx 10 \mu\text{M}$ with water.

maximum of triplet SnPP was at 440 nm for all solvents listed in Table 1. Photolysis of SnPP in these solvents with increasingly intense laser pulses resulted in plateauing of the ΔA_{440} curve at higher laser intensities (Fig. 3), reflecting complete conversion of ground-state SnPP to the triplet excited-state without photodestruction. Measurements at sufficiently high laser intensities, therefore, allowed determination of triplet-minus-ground-state difference spectra and derivation of difference extinction coefficients for triplet SnPP. A representative difference spectrum is shown in Fig. 4, and $\Delta\epsilon_T$ values at λ_{\max} in several solvents are given in Table 1. The $\Delta\epsilon_T$ values for triplet SnPP in acetone obtained by pulse radiolysis were similar to those obtained by flash photolysis in other solvents in which the compound is monomeric.

The lifetime of monomeric triplet SnPP was ≈ 100 – $200 \mu\text{s}$ in N₂-flushed solutions and somewhat sensitive to the nature of the solvent. Quantum yields for triplet SnPP formation in the same solutions were ≈ 0.6 – 0.8 (Table 1). The difference extinction coefficient and quantum yield for formation of triplet SnPP were much lower in 0.1 M PO₄ buffer (pH 7.4) than in the other solvents, presumably because of aggregation (28, 29). Triplet SnPP was quenched efficiently by oxygen with a rate constant of $\approx 10^9 \text{ M}^{-1}\cdot\text{s}^{-1}$. Oxygen quenching of triplet SnPP in MeO²H and in CetMe₃NBr/²H₂O was accompanied by luminescence at 1270 nm, directly confirming formation of singlet oxygen. The singlet-oxygen luminescence intensities from solutions of SnPP and hematoporphyrin with equal absorbance at 347 nm in air-saturated MeO²H (O₂ concentration, 2.12 mM) were similar over a range of

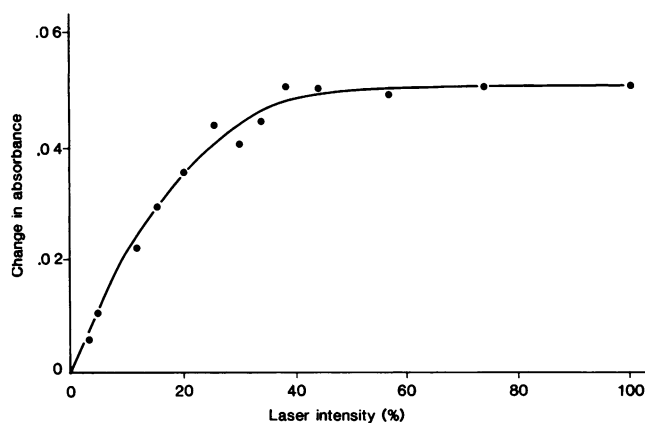


FIG. 3. Laser flash photolysis (347 nm) of SnPP (dichloride) ($1.6 \mu\text{M}$) in MeOH. Effect of laser intensity on the triplet-minus-ground-state absorbance difference (ΔA) at 440 nm. The laser intensity at 100% was $\approx 200 \text{ mJ}$.

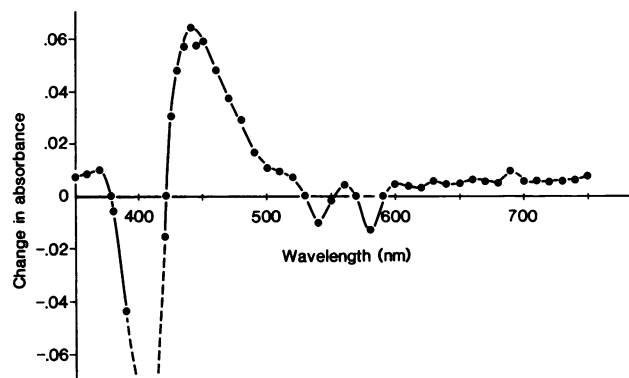


FIG. 4. Triplet-minus-ground-state absorbance difference spectrum of SnPP (dichloride) ($\approx 1 \mu\text{M}$) in MeOH.

laser intensities (Fig. 5). Comparison of the slopes of the two plots gave a value of $\phi_A = 0.58$ for SnPP in MeO²H. The fraction of triplet SnPP states that is quenched by ground-state molecular oxygen in MeO²H (ϕ_A/ϕ_T) is, therefore, 0.7, assuming that ϕ_T is the same for MeOH and MeO²H. Similarly, SnPP gave a linear luminescence versus laser-intensity plot and was found to be a slightly better singlet-oxygen sensitizer than hematoporphyrin in CetMe₃NBr/²H₂O solution at pH 7.4.

DISCUSSION

Although there are a few reports in the literature on the photochemistry of tin-porphyrins, mainly at low temperature, the room-temperature photochemistry of SnPP has been little studied. Our results show that absorption of light by monomeric SnPP results in efficient formation of triplet SnPP in both polar (water, MeO²H) and relatively nonpolar (acetone) solvents. The lifetime of the triplet is solvent sensitive, but not very different (≈ 150 – $200 \mu\text{s}$) in N₂-flushed MeOH, acetone, and CetMe₃NBr/H₂O. These values are close to the 180- μs value estimated by Whitten *et al.* (30) for the lifetime of triplet tin(IV)-octaethylporphyrin in pyridine. For comparison, the lifetimes, at similar concentrations, of triplet Zn-protoporphyrin in EtOH (31) and triplet hematoporphyrin in MeOH (32) are 280 and 78 μs , respectively. In aerated solutions triplet SnPP is quenched rapidly by oxygen giving singlet oxygen, which was detected directly by its characteristic long-wavelength luminescence. Quantum yields for

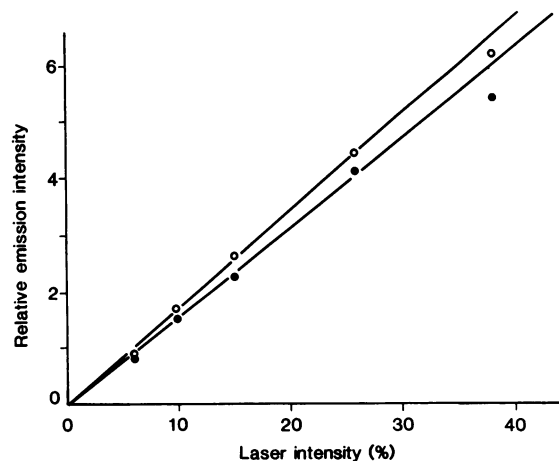


FIG. 5. Formation of singlet-oxygen during laser flash photolysis of SnPP (dichloride) (\circ) and hematoporphyrin dichloride (\bullet) in O₂-saturated MeO²H at 347-nm excitation. The plot shows the observed relative singlet-oxygen $^1\Delta_g \rightarrow ^3\Sigma_g$ emission versus laser intensity. Concentrations of both porphyrins were $\approx 10 \mu\text{M}$, and the 100% energy of the laser pulse was $\approx 200 \text{ mJ}$.

triplet production and singlet-oxygen formation were high, and oxygen-quenching rate constants in MeOH and CetMe_3NBr solution were similar to those reported for hematoporphyrin in the same solvents (29, 32). These observations explain why SnPP photosensitizes the destruction of bilirubin (an avid singlet-oxygen acceptor) (33) *in vitro* (34) and *in vivo* in jaundiced rats (A.F.McD., K. H. Pua, and W. Huang, unpublished data). Aggregation of SnPP diminished triplet formation substantially, as shown by the low ϕ_T in pH 7.4 buffer. The lifetime of singlet oxygen in SnPP/MeO²H was found, from the luminescence decay, to be 28 μs . This lifetime is not significantly different from the intrinsic singlet-oxygen lifetime in this solvent (35). These results indicate that SnPP photosensitizes singlet-oxygen formation efficiently in MeOH but does not react rapidly with it. Overall, the photophysical and photosensitizing properties of SnPP are similar to those of structurally related free-base porphyrins, particularly protoporphyrin and hematoporphyrin. Incorporating the closed-shell Sn(IV) into the porphyrin macrocycle has a marked effect on the ground-state optical spectrum and fluorescence spectrum, but this incorporation does not have a dramatic effect on the excited-state photochemistry, even though such diamagnetic complexing may increase the triplet lifetime (36).

Porphyrin-sensitized photodynamic damage is believed to be caused mainly by singlet oxygen, which reacts with many different types of biomolecules (7, 37). Whether singlet oxygen is mutagenic or carcinogenic is presently unclear. Manifestations of porphyrin-sensitized photodynamic damage in humans range from mild erythema, through tissue necrosis, to death (37, 38). These sequelae depend not only on the photochemical properties of the particular porphyrin involved but also on its pharmacokinetics and biological distribution. Other factors also influence the degree of photodamage caused by exogenous porphyrins. These include the dose, route of administration, and state of aggregation of the porphyrin; the time elapsed between administration and light exposure; and the dose and wavelength of the light to which the porphyrin recipient is exposed. The structural and photochemical similarity of SnPP to protoporphyrin and hematoporphyrin, which are known to be potent photodynamic agents in humans, suggests that SnPP is likely to be photodynamically active *in vivo* also—even worth testing as a potential agent for photochemotherapy of cancer. Furthermore, cutaneous photosensitization induced by SnPP might persist for a long time, as with hematoporphyrin (39), because of the slow clearance of SnPP (6). These predictions are supported by reports of dermal photosensitivity in adult patients treated with SnPP (3) and observations of severe skin damage in depilated rats treated with SnPP and broad-spectrum fluorescent light (40). However, the effectiveness of SnPP as a singlet-oxygen sensitizer *in vivo* is unlikely to be the same as that seen in protein-free solvents *in vitro* because of the influence of protein, lipid, or membrane binding. Such binding would not necessarily decrease the lifetime of triplet SnPP or inhibit its quenching by oxygen (31, 41).

Newborn babies are more translucent than adults (42) and have a larger surface/volume ratio. In nurseries and at home, they are often exposed to strong light, particularly if given phototherapy for neonatal jaundice (8, 9, 43). Therefore, newborns are more likely than adults to suffer photodynamic damage if treated with photosensitizers that absorb visible radiation. In view of the potentially hazardous photodynamic effects of SnPP, it would be a sensible precaution to shield babies treated with the drug from light in the 410-, 540-, and 580-nm regions of the spectrum. Babies with jaundice might be partially protected from SnPP-sensitized damage because of bilirubin's antioxidant properties (44) and high reaction rate with singlet oxygen (45). Furthermore, because SnPP

sensitizes bilirubin photodegradation (34), the combined use of phototherapy and SnPP treatment on jaundiced infants would be expected to increase the rate of plasma bilirubin disappearance compared to treatment with either agent alone. However, because of the potential phototoxic hazards, combined treatment would be unwise. Although narrow-waveband phototherapy lights emitting near the absorption maximum of bilirubin at $\approx 450\text{--}460\text{ nm}$ would probably be safe, because of the low absorbance of SnPP in that region, such narrow-waveband lights are not in common use. It would be unfortunate if using SnPP to prevent formation of one toxic compound (bilirubin) inadvertently led to the production of another (singlet oxygen).

Note Added in Proof. A value of 0.8 ms has been reported recently for the triplet lifetime of SnPP in MeOH/H₂O (1:1, vol/vol), along with other photophysical measurements on SnPP and related compounds (46). Results of clinical trials of SnPP also have been published recently (47). Of 12 babies given both SnPP and phototherapy, 2 showed evidence of mild, transient, cutaneous photosensitivity.

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