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Quantum yields of singlet oxygen of metal complexes of meso-tetrakis(sulphonatophenyl)porphine

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Abstract

The iodide method and the method of bleaching of *p*-nitroso-*N,N'*-dimethylaniline (RNO) were used to measure the quantum yields of singlet oxygen from the tetrasodium salt of meso-tetrakis(4-sulphonatophenyl) porphine (H2TPPS4) and its water-soluble metal complexes with divalent metals, Ag(II)TPPS4, Cd(II)TPPS4, Co(II)TPPS4, Cu(II)TPPS4, Mg(II)TPPS4, Ni(II)TPPS4, Pd(II)TPPS4, Pt(II)TPPS4 and Zn(II)TPPS4.

Complexes of metals that have completely full or completely empty d-orbitals (Cd(II), Mg(II) and Zn(II)) are characterized by high quantum yields of singlet oxygen and are more efficient photosensitizers than the ligand alone.

Comparison of the quantum yields of $^1O_2$ obtained by the two methods confirmed the assumption that the iodide method yields identical values to the standard method of bleaching of RNO. However, the sensitivity of the iodide method is ten times higher. © 1997 Elsevier Science S.A.

Keywords: Singlet oxygen; Photodynamic reaction; Metalloporphyrins

1. Introduction

The mechanism of photodynamic reactions in solution includes the formation of an excited triplet state of the dye-sensitizer and direct transfer of energy from the triplet excited photosensitizer to triplet oxygen dissolved in the solvent (collision energy transfer) with the formation of highly oxidative and cytotoxic singlet oxygen [1].

Considerable interest in the photodynamic effect, especially in the last few years, has been generated by the works of Dougherty et al. [2] and Kessel [3] on the means of utilizing the photodynamic effect, the fluorescence of photosensitizers and the cytotoxicity of $^1O_2$ in the localization and photodynamic therapy of malignant tumours. At the present time, photodynamic photosensitizers are also being tested for possible use in treating atherosclerosis [4]. A further possible use of photodynamic reactions or the cytotoxicity of singlet oxygen for microorganisms lies in the area of the development of new insecticides [5] and herbicides [6].

At the present time, there are several methods of determining the quantum yield of $^1O_2$ for photosensitizers in aqueous media at physiological pH values. One of the most frequently used indirect chemical methods is based on the bleaching reaction of water-soluble *p*-nitroso-*N,N'*-dimethylaniline (RNO) with $^1O_2$ in the presence of imidazole or histidine [7]. The degree of bleaching of the yellow RNO dye is directly proportional to the overall production of $^1O_2$ generated in the photodynamic reaction and can be followed spectrophotometrically as a decrease in the absorbance of the dye at 440 nm. The presence of imidazole or histidine is essential for the determination of $^1O_2$. In the absence of these substances, RNO remains to a certain degree resistant to the action of $^1O_2$. The reaction of $^1O_2$ with imidazole (histidine) results in the formation of a transient trans-annular peroxide intermediate that is able to undergo the subsequent bleaching reaction with RNO.

One of the advantages of the method is the possibility of using it in aqueous, neutral, air-saturated solutions. Thus, this method has found extensive use especially in monitoring the production of $^1O_2$ in biological systems. One of the disadvantages of the method is the complexity of the system. The actual bleaching of RNO is a secondary reaction. Imidazole or histidine, which act as oxygen transmitters, can also quench the triplet excited states of the photosensitizer and thus compete with the oxygen. A further inadequacy is the fact that the absorption bands of the photosensitizer can overlap the absorption bands of RNO, thus limiting the range of

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excitation wavelengths, as the photosensitizer molecules are shielded by the RNO molecules during the irradiation. In addition, it is difficult to assess the decrease in the absorbance by RNO because of overlap of the absorption bands of RNO with the bands of the photosensitizer (e.g. similar as for porphyrin photosensitizers).

For these reasons, the iodide method was developed in our laboratory, based on the reaction of $^1O_2$ (produced in the photodynamic reaction) with I^- in the presence of $(NH_4)_2MoO_4$ as a catalyst. The reaction product is I_3^-, the amount of which (followed spectrophotometrically at $\lambda = 351$ nm) is directly proportional to the generated $^1O_2$ [8]. The method is fast, cheap and very sensitive. In addition, it has the advantage that it does not measure a decrease in the absorbance, as in bleaching methods, but rather an increase in the absorbance. This permits the use of excess acceptor of singlet oxygen (I^-). The newly formed I_3^- band does not extend into the absorption bands of the photosensitizer in the visible region and thus its shielding effect is not apparent.

At the present time, extensive research is being carried out on the preparation of new, effective photosensitizers. Of the porphyrins, with excellent photophysical parameters, a number of studies have been devoted to meso-tetra(4-sulphonatophenyl) porphine (H2TPPS4) [9], which has also been used in human medicine [10]. However, less is known of the photophysical properties of metal complexes with H2TPPS4.

A study was carried out in our laboratories on some of the photophysical properties—the lifetimes and quantum yields of excited triplet states, and bimolecular rate constants for quenching of the triplet states with oxygen—of some water-soluble complexes, Ag(II)TPPS4, Cd(II)TPPS4, Co(II)-TPPS4, Cu(II)TPPS4, Mg(II)TPPS4, Ni(II)TPPS4, Pd(II)TPPS4, Pt(II)TPPS4, and Zn(II)TPPS4 [11].

This work was carried out in order to determine the quantum yields of singlet oxygen by the iodide method and the method of bleaching of *p*-nitroso-*N,N'*-dimethylaniline for the above mentioned substances. Comparison of the quantum yields of $^1O_2$ of the two methods was also used to test the iodide method.

2. Experimental details

The amount of $^1O_2$ produced was found in two ways:

1. By the iodide method, based on the reaction of $^1O_2$ (produced by the photodynamic reaction) with I^- in the presence of $(NH_4)_2MoO_4$ as catalyst. The reaction product is I_3^-, produced in an amount that is directly proportional to the generated $^1O_2$.
2. By the standard method based on the bleaching reaction of *p*-nitroso-*N,N'*-dimethylaniline.

2.1. Composition of the detection solution

2.1.1. Iodide reagent

0.12 M KI, 10 mM $(NH_4)_2MoO_4$ in 0.05 M sodium–potassium phosphate buffer (pH = 6.2)

2.1.2. RNO reagent

50 μM of *p*-nitroso-*N,N'*-dimethylaniline, 10 mM of imidazole in 0.05 M sodium–potassium phosphate buffer (pH = 7.5).

2.2. Measuring conditions

The tested metal porphyrin was dissolved in the given detection reagent (iodide reagent or RNO) so that the absorbance at the excitation wavelength did not exceed the value 0.1, to avoid the shielding effect. A solution with a volume of 2 ml was poured into a 1 × 1 × 4 cm quartz cuvette with a stirrer, the cuvette was closed, placed in a thermostated metal block ($T = 25$ °C) on an optical bench and irradiated with a 5 mW green He–Ne laser ($\lambda = 543$ nm). The solution was stirred during the irradiation from above by a rotating magnet. The absorbance of the detection solution was measured one minute after completion of the irradiation (the absorbance of the triiodide band or the decrease in absorbance by RNO was recorded). The blank solution was a solution with the same composition stored in the dark.

2.3. Preparation of photosensitizers

The tetrasodium salt of meso(4-sulphonatophenyl)-porphine (H2TPPS4) and its soluble complexes Ag(II)-TPPS4, Cd(II)TPPS4, Co(II)TPPS4, Cu(II)TPPS4, Mg(II)TPPS4, Ni(II)TPPS4, Pd(II)TPPS4, Pt(II)TPPS4, and Zn(II)TPPS4 were prepared and purified in the manner described previously [11].

3. Results and discussion

3.1. Measuring the quantum yield of $^1O_2$ using the iodide method for various photosensitizers

The measurement was carried out using the method of randomly selected absorbance of the photosensitizer ($A = 0.1$).

The following relationship can be derived from the theory of photooxidation of the substrate (here I^-) by $^1O_2$ [12]:

$$f_{ox} = \frac{r_{ox}}{I_a} = \frac{f_D k_r [I^-]}{k_d + k_q [I^-]} = f_D p \tag{1}$$

where f_{ox} is the quantum yield of oxidation of I^-, r_{ox} is the rate of oxidation of I^-, I_a is the intensity of light absorbed by the irradiated solution, k_r is the bimolecular rate constant of the chemical reaction of I^- with $^1O_2$, k_d is the rate constant of quenching of $^1O_2$ by the solvent, k_q is the overall bimolecular rate constant of quenching of $^1O_2$ by I^-, f_D is the quantum yield of $^1O_2$, p is the fraction of $^1O_2$ reacting chemically with I^-.

Under conditions of constant source output and given photosensitizer absorbance at the excitation wavelength

($\lambda = 543$ nm), the change in the absorbance of the triiodide band ($A(I_3^-)$) in time is proportional to the rate of oxidation of I^- (r_{ox}). It follows from the Beer–Lambert law that the expression $1 - 10^{-A}$ (where A is the absorbance of the photosensitizer at the excitation wavelength) is directly proportional to the fraction of the radiation absorbed by the photosensitizer. The slope B of the plot of the absorbance of the I_3^- band at 351 nm against the irradiation time is proportional to the rate of oxidation of I^- or of the formation of the product I_3^-. The slope of the dependence of slope B on the expression $1 - 10^{-A}$, denoted as k, is then directly proportional to the relative quantum yield of the oxidation f_{ox} and thus also to f_D.

These relations were used to compare the relative quantum yields of the test photosensitizers. The procedure is demonstrated for the well-known photodynamic photosensitizer H2TPPS4 in Figs. 1 and 2. Fig. 1 depicts the dependence of the absorbance of I_3^- on the irradiation period for variously concentrated solutions of photosensitizer in the iodide reagent, expressed by their absorbance at the excitation wavelength. The absorbance of the photosensitizer at the excitation wavelength ($\lambda = 543$ nm) was randomly selected so that its value was less than or equal to 0.1 (elimination of shielding effects). A regression straight line was fitted through the experimental points for each concentration (expressed by the absorbance at $\lambda = 543$ nm). The slope B, its standard deviation SD and correlation coefficient R were calculated for each such straight line.

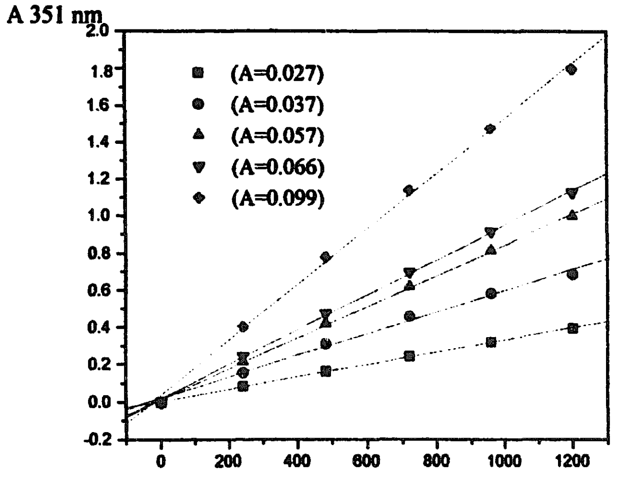

Fig. 1. Dependence of the absorbance of the triiodide band on the irradiation time for variously concentrated solutions of H2TPPS4 in iodide reagent (A is the absorbance of H2TPPS4 at $\lambda = 543$ nm).

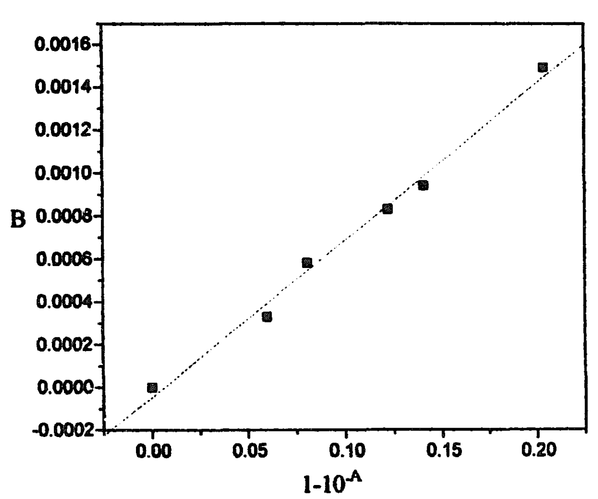

Fig. 2. Relationship between the slope B and the fraction of light ($1 - 10^{-A}$) absorbed by the photosensitizer for variously concentrated solutions of H2TPPS4 in iodide reagent.

Slope B was found from the experimental data on the linear section of the plot of the increase in the absorbance of I_3^- against the irradiation time so that the correlation coefficient R of linear regression was greater than 0.99. The determined slope of the increase in the absorbance of the triiodide band was plotted in a graph in against $1 - 10^{-A}$ and the values were again fitted by a straight line using linear regression. This line is depicted in Fig. 2. The dependence is once again linear and passes through the origin. A non-linear dependence would indicate a shielding effect or the presence of more complex photooxidation mechanisms.

The slope of the dependence of B on $1 - 10^{-A}$, denoted as k, is directly proportional to the relative quantum yield of singlet oxygen. In relating the tabulated values of the quantum yield for the standard—H2TPPS4 (0.62 for a neutral aqueous solution saturated with air [12])—to the calculated slope k for H2TPPS4 (0.0073) one obtains the values of the quantum yield for the test substance by direct proportionality. The k values for all the test substances and the calculated quantum yields of $^1O_2$ are given in Table 1.

3.1.1. *Proof of oxidation of I^- by singlet oxygen in the iodide method*

In the use of the iodide method, a study was also made of whether oxidizing species other than $^1O_2$ participate in the oxidation of I^-. Thus, the following tests were carried out for the substances of interest.

3.1.1.1. *The effect of the concentration of D_2O or NaN_3 in the iodide reagent*

Figs. 3 and 4 depict the effect of the content of D_2O or of NaN_3 on slope B for the photosensitizer ZnTPPS4. It can be seen from Fig. 3 that, in contrast with the photoreaction in H_2O medium, the slopes of the increase in the triiodide absorbance band in D_2O are approximately twice as large. This fact is a result of the longer lifetime of the singlet oxygen in D_2O. The lifetime of $^1O_2$ in D_2O (64 μs) is 16 times longer than that in H_2O (4 μs) [12]. The constant of quenching of $^1O_2$ by the solvent (k_d in Eq. (1)) in D_2O (1.73×10^4 s^{-1}) is one order of magnitude lower than that in H_2O (2.84×10^5 s^{-1}) [13].

The marked effect of the concentration of NaN_3 on slope B (corresponding to the relative quantum yield of the oxidation of I^- (f_{ox})) is apparent from Fig. 4. NaN_3 is known to quench $^1O_2$ [14] and thus has an inhibiting effect on the production of I_3^-.

Table 1
Calculated slopes k (iodide method), B (RNO method) and quantum yields of $^1O_2$ obtained by the iodide method ($\Phi_{\Delta\ J}$) and by the RNO method ($F_{\Delta\ RNO}$) (both related to the standard H2TPPS4 = 0.62), estimated quantum yield of triplet state formation (Φ_T), natural lifetime (τ), bimolecular constant for oxygen quenching (k_{O_2}) and fraction of triplet state quenched by oxygen which yield $^1O_2(S_\Delta)$ for all the test substances

Compound	Slope k (s^{-1}) (iodide method) $\times 10^{-5}$	$\Phi_{\Delta\ J}$ related to H2TPPS4 = 0.62	Slope B (s^{-1}) (RNO method) $\times 10^{-5}$	$\Phi_{\Delta\ RNO}$ related to H2TPPS4 = 0.62	Φ_T [b]	τ (μs) [b]	$10^9 k_{O_2}$ [b] ($M^{-1} s^{-1}$)	S_Δ [b]
H2TPPS4	730	0.62	13	0.62	0.76	414	2.0 ± 0.1	0.82
H2TPPS4 [a]			10		0.74	418	1.2 ± 0.2	
ZnTPPS4	868	0.74	17	0.81	0.86	2040	1.3 ± 0.1	0.86
PdTPPS4	580	0.49	10	0.48	0.63	268	2.2 ± 0.1	0.78
PtTPPS4	171	0.06	1.2	0.06	0.40	12.7	2.9 ± 0.9	0.15
MgTPPS4	812	0.69	14	0.67		467 (43%)		0.79
					0.85	2640 (57%)	1.2 ± 0.3	
CdTPPS4 [a]			12		0.88	149	1.4 ± 0.1	
CoTPPS4	0	0	0	0				
NiTPPS4	0	0	0	0				
CuTPPS4	0	0	0	0				
AgTPPS4	0	0	0	0		301	1.8 ± 0.1	

All k and B values have standard deviations SD < 0.00001 and correlation coefficients $R > 0.99$.

[a] Solutions of photosensitizer with adjusted absorbance for the excitation wavelength ($A_{543} = 0.05 \pm 0.002$) in detection agent with pH = 12.

[b] Published data [11].

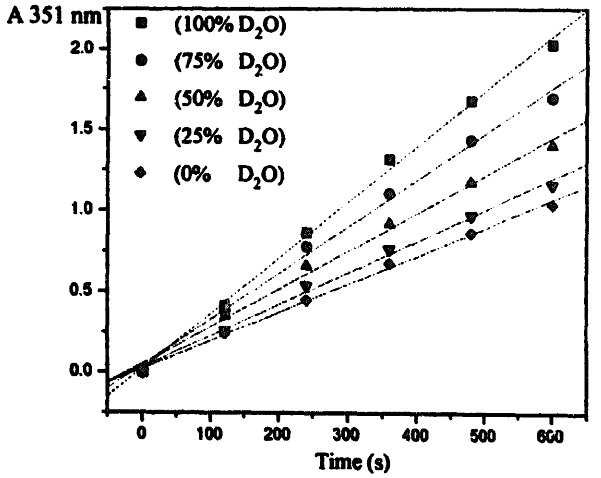

Fig. 3. Dependence of the absorbance of I_3^- on the time of irradiation of a solution of ZnTPPS4 ($A_{543} = 0.1$) in iodide reagent with various concentrations of D_2O.

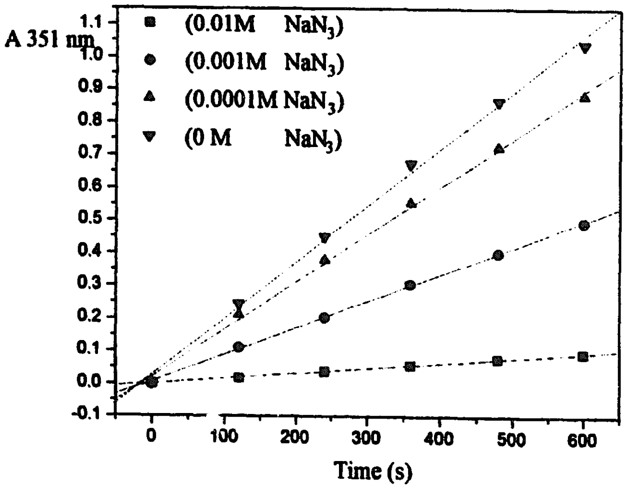

Fig. 4. Dependence of the absorbance of I_3^- on the time of irradiation of a solution of ZnTPPS4 ($A_{543} = 0.1$) in iodide reagent with various concentrations of NaN_3.

Similar trends of the effect of D_2O and NaN_3 were also observed for the other tested photosensitizers.

3.1.1.2. Irradiation of the photosensitizer in the detection reagent without KI

Any production of O_2^- or of H_2O_2 (based on "Type I" photoreaction) [15] was found by irradiating the test solution in the absence of KI in the detection solution. KI was added after completion of the irradiation. If these oxo-species are formed, the accumulation of the final product H_2O_2 appears as a growth in the triiodide absorption band. The short-lived species $^1O_2$ disappears immediately after the end of the irradiation and thus can produce a similar reaction only if peroxidation of the actual photosensitizer occurs. In no case was photoproduction of these oxo-species found for the test substances.

3.2. Measurement of the quantum yield of $^1O_2$ using the method of bleaching of p-nitroso-N,N′-dimethylaniline for various photosensitizers

The method of bleaching of *p*-nitroso-*N*,*N*′-dimethylaniline [7] was employed to determine the quantum yields of singlet oxygen in a medium of 0.05 mol dm^{-3} phosphate buffer (pH = 7.5) for the test substances with the exception of CdTPPS4. Because of the demetallization of CdTPPS4 in neutral medium, this complex was measured in detection agent with pH = 12 (Table 1).

The changes in the absorbance A of *p*-nitroso-*N,N'*-dimethylaniline with the irradiation time with an He–Ne laser ($\lambda = 543$ nm) were measured in photosensitizer solutions at pH = 7.5 or 12. The measurement was carried out with photosensitizer solutions with identical absorbance ($A_{543} = 0.1$) at the excitation wavelength. The determined absorbance values were the average of five measurements.

The slopes B of the plots of $-\ln A$ against the irradiation time, their standard deviation SD and correlation coefficients R were found by linear regression. The calculated slopes B are proportional to the relative quantum yields of $^1O_2$. Thus, comparison of the quantum yield value for the standard—H2TPPS4 (0.62 for a neutral aqueous solution saturated with air [12]) with the calculated slope B for H2TPPS4 (0.00013) allows one to obtain the values of the quantum yield for the test substance by direct proportionality (see Table 1).

Measurements were carried out for CdTPPS4 in detection agent with pH = 12. Its efficiency was compared with the uncomplexed ligand H2TPPS4 measured under the same conditions. The relative quantum yield of CdTPPS4 compared to H2TPPS4 is given by the ratio of their slopes B: 0.00012/0.00010 = 1/0.83. The value of the quantum yield of $^1O_2$ for H2TPPS4 at pH = 12 is not given in the literature. Thus, the (apparently quite high) quantum yield of $^1O_2$ for CdTPPS4 could not be found by recalculation.

3.3. Comparison of the quantum yields of $^1O_2$ obtained by the iodide method and the method of bleaching of p-nitroso-N,N'-dimethylaniline

Both of the above methods of determination of $^1O_2$ indicated that, of the total of ten water-soluble substances (H2TPPS4 and its complexes), six are photodynamically active. The following order of photodynamic efficiency was found for H2TPPS4 and its complexes: (CdTPPS4), ZnTPPS4 > MgTPPS4 > H2TPPS4 > PdTPPS4 > PdTPPS4 > the other photodynamically inactive complexes (i.e. CoTPPS4, NiTPPS4, CuTPPS4, AgTPPS4).

The use of CdTPPS4 for the photodynamic reaction is limited. The unstable CdTPPS4 complex is highly photodynamically active, but requires an alkaline medium.

The value of the quantum yield of $^1O_2$ is given in the literature for ZnTPPS4 (0.77) and for PdTPPS4 (1) [12,16]. The quantum yield value for $^1O_2$ for ZnTPPS4 is in good agreement with the values found here using the iodide method (0.74) and the method of bleaching of *p*-nitroso-*N,N'*-dimethylaniline (0.81). In contrast, the given value of the quantum yield of $^1O_2$ was not confirmed for PdTPPS4; the two methods used to determine $^1O_2$ independently yielded a value about half as large (0.49 and 0.48, respectively). However, the quantum yields of $^1O_2$ measured here correspond better to the measured lifetimes *and triplet yield* of the triplet states of PdTPPS4. It remains unclear why the formation of $^1O_2$ was not detected for AgTPPS4, in spite of the fact that its triplet excited state was found to have a relatively long lifetime. This is probably because of the low quantum yield of this state [11].

The experimentally determined quantum yields of singlet oxygen [17] can be written as

$$\Phi_\Delta = \Phi_T P_{O_2} S_\Delta \tag{2}$$

where Φ_T is the quantum yield for formation of the triplet excited state, P_{O_2} is the fraction of triplet states quenched by O_2 and S_Δ is the probability that quenching of the triplet state by O_2 results in production of $^1\Delta_2$.

P_{O_2} can be calculated as:

$$P_{O_2} = k_{O_2}[O_2] / k_{O_2}[O_2] + k_d \tag{3}$$

where k_{O_2} is the bimolecular constant for quenching of the triplet by oxygen, $[O_2]$ is the concentration of O_2 dissolved in solution and k_d is the constant of quenching of triplet states (in the absence of oxygen).

Because of the high bimolecular quenching constant k_{O_2} (of the order $10^{-9}\ M^{-1}\ s^{-1}$) and long natural triplet state lifetime in deoxygenated solutions ($\tau = k_d^{-1}$) for all tested compounds the corresponding P_{O_2} values are close to unity.

The quantum yields of $^1O_2$ determined by the iodide method and the method of bleaching of *p*-nitroso-*N,N'*-dimethylaniline are practically identical and have a similar trend to the measured lifetimes of triplet states (τ), triplet yields (Φ_T) and S_Δ (see Table 1).

4. Conclusions

Complexes of metals that have completely full or completely empty d-orbitals (Cd(II), Mg(II) and Zn(II)) are characterized by high quantum yields of singlet oxygen and thus seem to be more efficient photosensitizers than the ligand alone.

Comparison of the quantum yields of $^1O_2$ found by the two methods simultaneously confirmed that the iodide method yields identical values to the standard method of bleaching of *p*-nitroso-*N,N'*-dimethylaniline. However, the sensitivity of the iodide method is tenfold higher. This follows from comparison of the studied absorbance changes for the two detection systems for identical irradiation times, using an identical photosensitizer.

Acknowledgements

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