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PHOTOACOUSTIC DETECTION OF PHOTOSENSITIZED OXYGENATIONS IN HIGHLY ABSORBING SAMPLES

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Summary. A photoacoustic (PA) study of the relaxation processes of the excited states of photosensitizers in the presence of oxygen and chemical acceptors is described. It has been predicted theoretically that the ratio of PA amplitudes with and without acceptor depends on two parameters α and β, related to the sensitizer and to the acceptor properties, respectively. Experimental studies were carried out on solutions of hematoporphyrin IX and methylene blue. The addition of an acceptor, specific to the singlet oxygen, like tetramethyl ethylene or furfuryl alcohol, appears to decrease significantly the photoacoustic signal amplitude within the band of absorption.

Nowadays, there is a growing interest in the quantitative analysis of singlet oxygen, $O_2(^1\Delta_g)$, production and deactivation mechanisms, as it is generally recognised that tumour destruction in photodynamic therapy is accomplished through the formation of singlet oxygen and its subsequent reactions with cellular substrates [1].

The photoacoustic (PA) and photothermal (PT) methods are known as sensitive and convenient ways to measure the energy absorbed and consequently transformed into heat in condensed samples, as well as to be sensitive to the different types of relaxation processes [2]. In addition to the earlier PA attempts by Malkin and Cahen [3], and Moore et al. [4], we have demonstrated the efficiency of the PA method when applied to the measurements of photophysical properties of molecules which relax through a metastable state [5].

The objective of the present work is to demonstrate the validity of this model for the study of relaxation processes of singlet oxygen, $O_2(^1\Delta_g)$ produced by electronic energy transfer from the triplet state of a sensitizer. The singlet oxygen relaxation pathways have been changed by using different concentrations of chemical acceptors.

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Abbreviations: FAL, furfuryl alcohol; HP, hematoporphyrin IX; MB, methylene blue; PA, photoacoustic; PT, photothermal; TME, tetramethyl ethylene.

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Theoretical model

We have shown earlier [5] that the Γ ratio of PA amplitudes with and without acceptor in the case of non-phosphorescent samples could be presented as:

$$\Gamma = 1 - \frac{\gamma_\Delta \gamma_4 E_4}{E_{ex} - \gamma_f E_f}, \tag{1}$$

where E_{ex} is the absorbed photon energy, γ_f is the quantum yield of fluorescence, E_f is the mean fluorescence photon energy, γ_Δ is the quantum yield of singlet oxygen production of E_Δ energy, γ_4 is the quantum yield of photooxydation of the acceptor and E_4 is the energy of the product. This result can be obtained directly from the following energy balance: PA amplitude ~ Heat production = Absorbed energy (E_{ex}) - Emitted energy ($\gamma_f E_f$) - Stored energy (without acceptor: 0, with acceptor: $\gamma_\Delta \gamma_4 E_4$).

Let us further suppose that singlet oxygen $O_2(^1\Delta_g)$ can relax in the following ways: by physical reaction with the solvent, with a k_d conversion rate; by physical quenching with a substrate with a k_q[C] conversion rate where [C] is the concentration of the substrate; and by chemical reaction with the substrate, with a k_r[C] conversion rate.

Thus, the γ_4 conversion yield of chemical reaction is equal to

$$\gamma_4 = \frac{k_r[C]}{k_d + (k_q + k_r)[C]} \tag{2}$$

and Γ is:

$$\Gamma = 1 - \frac{\gamma_\Delta E_4}{E_{ex} - \gamma_f E_f} \; \frac{k_r[C]}{k_d + (k_q + k_r)[C]} \, . \tag{3}$$

If we suppose a specific and efficient singlet oxygen acceptor ($k_q \ll k_r$), we can finally write the following expression for Γ :

$$\Gamma = 1 - \frac{\gamma_\Delta E_4}{E_{ex} - \gamma_f E_f} \; \frac{[C]}{[C] + k_d / k_r} \tag{4}$$

or

$$\frac{1}{1 - \Gamma} = \alpha \left(1 + \frac{\beta}{[C]} \right), \tag{5}$$

where

$$\alpha = \frac{E_{ex} - \gamma_f E_f}{\gamma_\Delta E_4} \quad \text{and} \quad \beta = k_d / k_r \ .$$

The α parameter can be considered as the inverse of a yield of photochemical reaction, and β is known as the reactivity of the acceptor. These simple relations (4) and (5) between Γ and the concentration of the acceptor can be used for the adjustment of the experimental results obtained.

At low acceptor concentrations when $[C] << \beta$, Γ is approximately equal to 1, i.e. there is no difference between the PA amplitudes from the sample with and without acceptor. At high acceptor concentrations when $[C] >> \beta$, Γ tends to become a constant at the level of $\Gamma = 1-1/\alpha$, and further addition of the acceptor doesn't change the ratio of PA amplitudes.

Methods

The PA spectrometer with a microphone detection was used in the present study. It had been slightly modified in comparison to that reported earlier [5]. Experimental tests of the effect of an acceptor concentration on Γ were done at a 40 Hz modulation frequency.

The following dyes were studied: hematoporphyrin IX (HP) and methylene blue (MB). All dyes were dissolved to 2.5 or 5.0 mM in ethanol without any additional purification. The $O_2(^1\Delta_g)$ - specific acceptors, e.g. tetramethyl ethylene (TME) and freshly distilled furfuryl alcohol (FAL), were added at concentrations ranging from 5 mM up to 400 mM.

Experimental results and discussion

Figure 1 shows the PA spectra of HP in ethanol without acceptor and in the presence of TME at three different concentrations. Decrease in PA amplitude along with acceptor concentration increase occurs only in the range of maximum absorption (350-450 µm). In other regions the shapes of PA spectra are not affected by acceptors to the precision of our experiments. The corresponding dependence of Γ vs wavelength has a minimum at the line of absorption.

These variations in PA amplitudes led us to conduct measurement of the dependence of Γ on the concentration of acceptor. Figure 2 shows the experimental results obtained for solutions of HP in ethanol. A strong decrease in Γ was obtained as the acceptor concentration increased from 5 mM to 400 mM, and when air was used as a gas filling the PA cell. No changes in Γ were noted when nitrogen filled the cell. It shows that this effect only appears in the presence of a significant oxygen concentration.

These variations of the ratio of PA amplitudes are more rapid at relatively low concentrations of acceptor. At high concentrations, Γ tends to become a constant, as predicted by the theoretical model (equation 4). Similar results have been obtained for both the solutions of HP and MB.

Figure 3 shows the linear variations of $1/(1-\Gamma)$ versus $1/[C]$ as predicted by equation (5). The linear regression gives the α intercept and the slope to intercept ratio β. It can be clearly seen from Figure 3 that the experimental results are in good agreement with the theory for all the

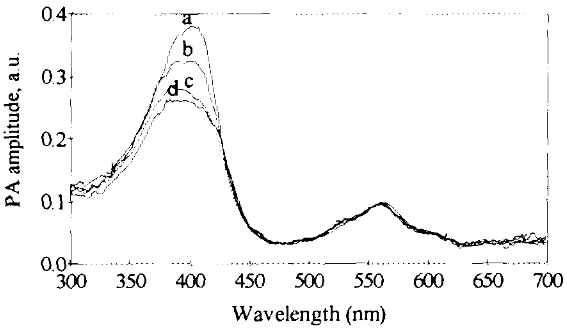

Figure 1. PA spectra of HP in ethanol without acceptor (a), and in the presence of 10 mM TME (b), 50 mM TME (c) and 100 mM TME (d).

samples used. The results of the adjustments to the theoretical model by linear regression of the experimental results obtained are presented in Table 1.

Let us note that the difference between the values of α found for the solutions of HP+TME is due to the variation of the sensitizer concentration. There are no significant changes in α, and correspondingly in the intercepts in Figure 3, if one compares these values adjusted for the same concentration of HP with different acceptors. This result allows us to suppose that the α parameter only depends on the properties of the sensitizer and is independent of the type of the acceptor, in agreement with our model.

In contrast to the α parameter, the second $\beta = k_d / k_r$ parameter only depends on the type of acceptor used, as can be seen from the results presented in Table 1 for the solutions of HP with TME and FAL. It is independent of the sensitizer.

Using the values obtained for the α parameters we have found the product $\gamma_\Delta E_4$ for HP to be equal to 0.89 eV (5 mM) and 0.54 eV (2.5 mM), using the published value $\gamma_f E_f = 0.17$ eV, [6]. For MB the value of 0.67 eV (5 mM with TME) was obtained for $\gamma_\Delta E_4$, when the sample was supposed to be non-fluorescent.

From the measured β and known values of k_r, $1.2 \cdot 10^8\ M^{-1}s^{-1}$ for FAL [7] and $2.4 \cdot 10^7\ M^{-1}s^{-1}$ (in methanol) for TME, the k_d conversion yield can be calculated. The results obtained, i.e.

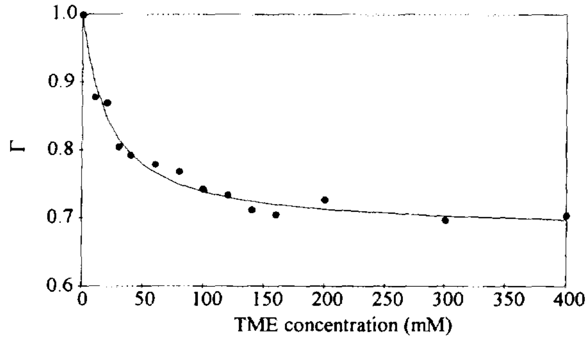

Figure 2. Dependence of Γ on the concentration of acceptor (TME) for HP in ethanol at 400 nm (points) and corresponding result of the adjustments to the theoretical model (line).

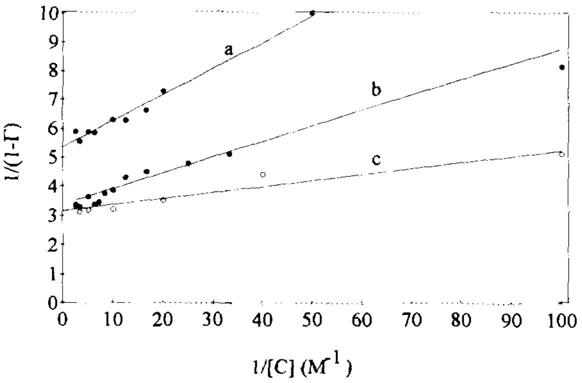

Figure 3. Plot of the photoacoustic number 1/(1-Γ) versus 1/[C] for the solutions of Hp in ethanol: HP 2.5 mM + TME (a), HP 5.0 mM + TME (b) and HP 5.0 mM + FAL (c) - experimental results (points) and linear regression fits (lines). Parameters determined are shown in Table 1.

$(3.9\pm0.5)\cdot10^5$ s^{-1} (for TME) and $(8 \pm 2)\cdot10^5$ s^{-1} (FAL), correspond to the lifetimes of singlet oxygen in ethanol equal to 2.5 μs (TME) and 1.2 μs (FAL), which are much shorter than the expected value (15 μs).

The variations of $\gamma_\Delta E_4$ with the concentration of HP can be interpreted as an oxygen consumption and diffusion effect [8]. In any case all the light is absorbed in our sample and oxygen consumption arises in a depth whose thickness decreases when the sensitizer concentration is increased. The diffusion of oxygen from surrounding gas or from deeper liquid layers is therefore more efficient when the outermost layer thickness decreases. This fact can be checked out by varying the diffusion time which is controlled by the modulation frequency of light. The discrepancy between the measured β values and the corresponding singlet oxygen lifetime and published data can be related to the high sensitizer concentrations used in our experiments and to singlet oxygen quenching.

Preliminary results presented in this study, demonstrate the effect of singlet oxygen deactivation by chemical acceptors on the PA signal produced in solutions of various photosensitizers. Further development of the theoretical model as well as experimental studies

Table 1. Results (standard deviations) of the adjustments by linear regression of the experimental results obtained for the solutions of HP in ethanol

Experimental conditions	Acceptor	α	β, mM
HP 2.5 mM	TME	5.34 (0.18)	17 (2)
HP 5.0 mM	TME	3.36 (0.28)	15 (2)
HP 5.0 mM	FAL	3.16 (0.22)	6.6 (1.6)

to take into account oxygen consumption and diffusion, which are of the upmost importance for the photodynamic therapy response of tumours, are now in progress.

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