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# The excited-states quenching of resazurin and resorufin by *p*-benzoquinones in polar solvents

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**Abstract**

The quenching of the excited singlet and triplet states of resazurin and resorufin by *p*-benzoquinones was investigated in methanol and in aqueous solution at pH 10. Fluorescence lifetimes were determined for the dyes and from the fluorescence quenching experiments rate constants were obtained for the singlet state quenching in MeOH. The rate constants were nearly diffusion controlled. Triplet state processes and transient absorption spectra were investigated by laser flash photolysis. Triplet quenching rate constants were obtained from the decay of the triplet at 825 nm for resazurin and at 700 nm for resorufin as a function of the quinone concentration. In the case of resazurin, the triplet quenching rate constants were lower than those of the singlet state. For resorufin, the values of the singlet and triplet were in the same order. From the transient absorption spectrum determinations it was concluded that the quenching proceeded by an electron-transfer reaction from the dye excited-states to the quinones. The results suggest that these dyes may function as very efficient photochemical-reductants.

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*Keywords:* Resazurin; Resorufin; Benzoquinones; Excited-states; Photochemical-reductants; Electron-transfer

## 1. Introduction

Dyes are used quite frequently as probes to analyze the behavior of various types of systems [1], especially those having microheterogeneous regions. Additionally, various biochemical and industrial properties can be determined using dyes by utilizing the color changes of the compounds upon oxidation or reduction.

Resazurin is a phenoxazin-3-one dye widely used for testing various biological materials such as biochemical antioxidants [2]. Other example of its use is the "resazurin test"; in this test the reduction of the resazurin to resorufin (Scheme 1) depends on the ability of metabolically active spermatozoa. This reduction is manifested by a visible change in color from blue to pink, and this color change correlates significantly with concentration of motile spermatozoa [3]. Resorufin has also been used as a probe molecule to study the re-orientation of solvent molecules, and has shown an interesting chemistry and photochemistry in protic solvents which strongly depends on temperature, viscosity and structure of the solvent [4–6].

We have previously reported the photoreduction of dyes (flavines, safranine, resazurin and resorufin) by amines [7–11]. Many of the above mentioned dyes have a potential for use as polymerization photoinitiators due to high absorption coefficients in the visible region. It was found that a clean photodeoxygenation to resorufin occurs from the quenching of the triplet state of the dye [10]. This photodeoxygenation is dependent on the amine structure and is efficient only in the presence of tertiary aliphatic amines [11].

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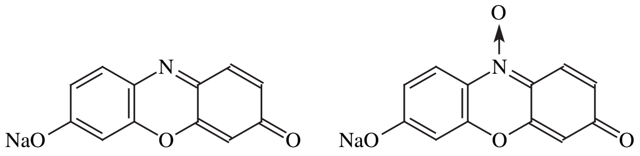

RESORUFIN RESAZURIN

Scheme 1.

The photoinduced deoxygenation of N-oxides has been reported previously for other molecules [12] and was found to proceed on irradiation of N-oxide alone or in the presence of suitable electron donors such as amines [13,14]. But in most cases the reaction is accompanied by side reactions and rearrangements of the N-oxide [15]. More recently, we found that the related dyes resazurin and resorufin, produce radicals in the presence of triethanolamine (TEOHA) that are efficient photoinitiators of aqueous acrylamide polymerization [16].

The one electron photooxidation of dyes has received less attention [17,18]. However, the quenching of excited-states of dyes by quinones is of interest due to the important role of the quinone moiety as an electron acceptor in photobiological process [19]. In this communication we present data on the interaction of the excited-states of the phenoxazin-3-one dyes resazurin and resorufin with *p*-benzoquinones as electron acceptors in alkaline water and methanol. Water was selected due to biological and environmental considerations while the organic solvent methanol was employed for comparative purposes. The quenching of singlet and triplet states was investigated and rate constants were determined. The nature of the quenching process was investigated by laser flash photolysis.

## 2. Experimental procedures

### 2.1. Chemicals

Resazurin and resorufin were obtained from Aldrich and used as supplied. The quinones, *p*-benzoquinone (BQ), methyl-*p*-benzoquinone (MQ), 2,5-dimethyl-*p*-benzoquinone (DMQ), 1-chloro-*p*-benzoquinone (ClQ) and duroquinone (tetramethyl-*p*-benzoquinone) (DQ) were obtained from various commercial sources. They were purified by recrystallization and/or sublimation when necessary. The solvent methanol (MeOH) was Sintorgan HPLC grade. Water was purified through a Millipore Milli-Q system.

### 2.2. Measurements

In water, both dyes are fluorescent only in their anionic forms at pH above 7.0. For their determination in aqueous solution the pH was adjusted by addition of NaOH (Aldrich, 99.9%) at $10 \pm 0.1$. In all cases the temperature was constant at $25 \pm 1$ °C.

Absorption spectra were determined on a Hewlett–Packard 6453E diode array spectrophotometer.

Steady-state fluorescence measurements were made using a Spex Spectrofluorimeter. Fluorescence quantum yields were determined relative to cresyl violet in methanol [20]. Fluorescence lifetime measurements were performed with an Edinburgh Instruments OB 900 time-correlated single-photon counting fluorometer. The singlet quenching rate constants were measured following the decrease of the lifetime by the quinone addition.

Transient absorption measurements were made using a laser flash photolysis equipment. The solutions were purged with argon for 30 min before their use. A Spectron SL400 Nd:YAG laser generating 532 nm laser pulses (~18 ns pulse width) was used for sample excitation. The laser beam was defocused in order to cover all the path length (10 mm) of the analyzing beam from a 150 W Xe lamp. The experiments were performed with rectangular cells with right angle geometry. The detection system comprises a PTI monochromator coupled to a Hamamatzu R666 PM tube. The signals were initially captured by an HP54504 digitizing oscilloscope where they were averaged and then transferred to a computer for storage and analysis. Measurements were performed in samples subjected to a continuous bubbling with high purity argon. For the measurement of the quenching rate constants a concentrate quinone solution was prepared in MeOH and a few microliters were added to solution in water.

Quantum yields were determined by actimonetic method using zinc tetraphenylporphyrin (ZnTPP) as reference. Values of $7.3 \times 10^4\ \mathrm{M^{-1}\,cm^{-1}}$ and 0.83 were used for $\varepsilon_\mathrm{T}$ and $\phi_\mathrm{T}$ of ZnTPP, respectively [21].

The molar absorption coefficients of triplet dyes were determined by the total depletion method [22] (Table 1). In both cases the negative absorption of the difference transient spectra matched the ground-state band. This is consistent with the lack of photoproduct formation under our conditions of laser experiments.

The voltammetric characterizations of the redox processes for all compounds were done with a potentiostat–galvanostat Autolab (Electrochemical Instruments), using a Pt disc as working electrode, a saturated calomel electrode as reference, and a large area Pt counter electrode, in a conventional three compartment Pyrex cell. Studies were carried out in ultrapure water (LABCONCO system) pH of 10 (NaOH; 0.1 M $NaClO_4$), and in methanol (0.1 M tetrabutylammonium hexafluorophosphate (TBAHFP, Aldrich) as supporting

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