Kinetic and Spectroscopic Behaviour of Proflavin Transients Studied by Pulse Radiolysis

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The semireduced transients of proflavin were studied in airfree acid aqueous solutions by a combined pulse radiolysis-computer optimization procedure. The main product, semiquinone (C-radical; 75%), formed with $k = 6.4 \times 10^9\,\mathrm{dm^3 mol^{-1} s^{-1}}$, decays by dimerisation ($2k = 2 \times 10^9\,\mathrm{dm^3 mol^{-1} s^{-1}}$) and dismutation ($2k = 0.5 \times 10^9\,\mathrm{dm^3 mol^{-1} s^{-1}}$). It possesses several absorption bands ($\varepsilon_{270} = 1900$, $\varepsilon_{360} = 1530$, $\varepsilon_{460} = 2250$ and $\varepsilon_{530} = 200\,\mathrm{m^2 mol^{-1}}$). The second transient, R·-species (N-radical; 16.5%), is produced with $k = 1.4 \times 10^9\,\mathrm{dm^3 mol^{-1} s^{-1}}$ and decays preferentially by transformation into semiquinone with $k = 2 \times 10^5\,\mathrm{s^{-1}}$; its absorption bands are: $\varepsilon_{295} = 2300$, $\varepsilon_{380} = 1000$, $\varepsilon_{440} = 5000$ and $\varepsilon_{680} = 530\,\mathrm{m^2 mol^{-1}}$. Finally H-adducts on various positions of the aromatic ring (8.5%) are arising with $k = 0.7 \times 10^9\,\mathrm{dm^3 mol^{-1} s^{-1}}$, decay according to a pseudo first order reaction, $k = 1.1 \times 10^4\,\mathrm{s^{-1}}$, and have absorption bands at $\varepsilon_{270} = 2100$ and $\varepsilon_{435} = 1580\,\mathrm{m^2 mol^{-1}}$. The obtained results are of particular interest for the photochemical and photoelectrochemical solar energy utilization.

1. Introduction

Intensive photochemical investigations have been performed on proflavin (PFH^+, 3,6-diamino-acridine), which is frequently used as a photosensitizer in various devices for solar energy utilization [1–7]. These papers concern either its role in the photoproduction of hydrogen from water by light or the fate of its transients appearing in these processes. In addition to this, pulse radiolysis experiments were also carried out in order to obtain information of the decay kinetics and absorption spectra of its electron adducts [8].

Since proflavin semiquinone is the most important intermediate arising in the photochemical and photoelectrochemical systems as well as by laser and common flash photolysis, its kinetic and spectroscopic behaviour is of special interest. Pulse radiolysis offers the possibility to produce it selectively, without disturbing absorptions of triplets, electron donors and their inevitable intermediates. However, manifold processes (dye bleaching, formation of various species) can also occur simultaneously, resulting in transients with rather similar kinetic and spectroscopic data. Hence, a pulse radiolysis technique combined with a special computation procedure was designed, which enables the individual analysis of species with such superimposed characteristics [9, 10]. This method has been already successfully applied for studying of the transients produced by the multisite reactions of H-atoms with methylene blue [11, 12], thionine [12, 13], acridine orange [14] and methyl viologen [15]. Therefore, it was now employed for elucidation of the rather complex kinetic and spectroscopic behaviour of proflavin intermediates, produced by pulse radiolysis in aqueous solution by H-attack on the PFH^+-molecule.

2. Experimental

2.1. *Pulse Radiolysis and Dosimetry*

The pulse radiolysis experiments were performed with a 3 MeV Van de Graaff accelerator (type K, High Voltage, Eng., Burlington, USA) using electron pulses of 0.4 μs duration (applied dose: $1-4\,\mathrm{J\,kg^{-1}}$ per pulse)*. Details of this facility were given elsewhere [16, 17]. The absorbed dose in the samples was determined by modified Fricke dosimeter [18] and the transient absorption spectra were normalized for a dose of $10\,\mathrm{J\,kg^{-1}}$ (1 krad). The solutions were irradiated at room temperature and exchanged after each pulse by means of a remote-controlled valve.

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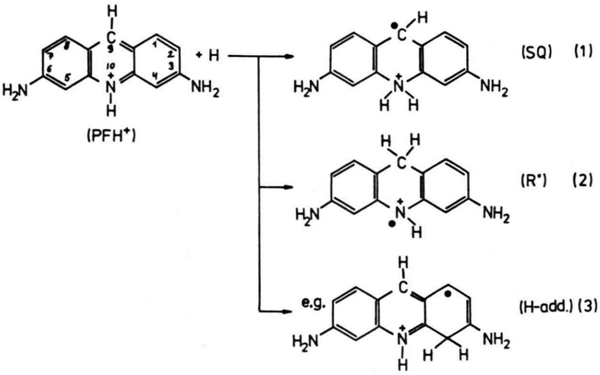
2.2. Preparation of Solutions

Proflavin (EGA Chemie, FRG) was purified in analogy to acridine orange** [14, 19]. All solutions were prepared using at least four times freshly distilled water, containing 5×10^{-6} to 2×10^{-5} mol dm^{-3} PFH$^+$ and 2×10^{-2} mol dm^{-3} t-butanol as OH-scavenger ($k(OH + t\text{-}C_4H_9OH) = 5.5 \times 10^8$ dm^3 mol^{-1} s^{-1} [20]. Most of the experiments were performed at pH = 2.7, which was adjusted by means of high purity $HClO_4$ (E. Merck, FRG). The solutions were deareated by purging with high purity argon for about one hour. Under these conditions the e^-_{aq} are in about 0.1 μs converted into H-atoms ($k = 2.3 \times 10^{10}$ dm^3 mol^{-1} s^{-1}) [21], which subsequently react with the dye molecule.

3. Results

3.1. Spectroscopic and Kinetic Data

The total transient absorption spectrum resulting from the H-attack on PFH$^+$-molecule in acid aqueous media was measured in the range from 250 to 700 nm at various times after the pulse end. The spectra obtained 15 μs and 40 μs after pulse end are presented in Figure 1. For comparison the absorption spectrum of the starting solutions is also given. Since the decay kinetics of the transients, analysed at various wavelengths, showed great differences, it can be concluded that the absorption spectrum represents more than one species. In analogy to previous studies on acridine orange [14], it is to be expected that in this case too, the H-atoms can react with the PFH$^+$-molecules simultaneously on various positions:



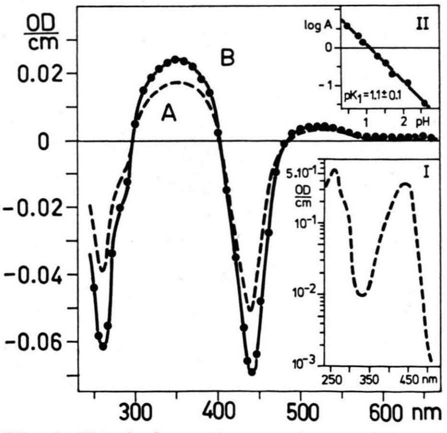


Fig. 1. Total absorption spectrum of transients 15 μs (A) and 40 μs (B) after the pulse end, produced by H-attack on proflavin at pH 2.7. (Solution: 10^{-5} mol dm^{-3} proflavin, 2×10^{-2} mol dm^{-3} t-butanol, airfree). Applied dose: 3 to 5 J kg^{-1} per 0.4 μs pulse; spectrum normalized to 10 J kg^{-1}.

Insert I: Absorption spectrum of 10^{-5} mol dm^{-3} proflavin at pH = 2.7 for comparison.

Insert II: Determination of pK_1 of semiquinone.

As it has been already shown for other dyes [12, 14] there are a number of processes competing with reactions (1), (2) and (3). All these reactions, together with the corresponding rate constants (k-values), so far known, are summarized in Table 1.

Table 1. Reactions (and rate constants) taking place by pulse radiolysis of proflavin (PFH$^+$) in the presence of t-butanol in aqueous solutions.

No.	Reaction	Rate constant (dm^3 mol^{-1} s^{-1})
1	$PFH^+ + H \rightarrow SQ$	$k_1 = ?$
2	$\rightarrow R\cdot$	$k_2 = ?$
3	$\rightarrow$ H-add.	$k_3 = ?$
4	$PFH^+ + OH \rightarrow \dot{P}FH^+ OH$	$k_4 = 8.0 \times 10^9$
5	$PFH^+ + e^-_{aq} \rightarrow SQ$	$k_5 = 2.5 \times 10^{10}$
6	$2\,SQ \rightarrow$ Disprop. Products	$2k_6 = ?$
7	$\rightarrow$ Dimer	$2k_7 = ?$
8	$R\cdot \rightarrow SQ$	$k'_8 = ?$
9	H-add. $\rightarrow$ Products	$k'_9 = ?$
10	$H + H \rightarrow H_2$	$2k_{10} = 2.3 \times 10^{10}$
11	$OH + OH \rightarrow H_2O_2$	$2k_{11} = 1.2 \times 10^{10}$
12	$H + OH \rightarrow H_2O$	$k_{12} = 2.0 \times 10^{10}$
13	$e^-_{aq} + OH \rightarrow OH^-$	$k_{13} = 3.0 \times 10^{10}$
14	$e^-_{aq} + e^-_{aq} \rightarrow H_2 + 2\,OH^-$	$2k_{14} = 1.2 \times 10^{10}$
15	$e^-_{aq} + H \rightarrow H_2 + OH^-$	$k_{15} = 2.5 \times 10^{10}$
16	$H^+ + e^-_{aq} \rightarrow H$	$k_{16} = 2.3 \times 10^{10}$
17	$t{-}C_4H_9OH + H \rightarrow t{-}\dot{C}_4H_8OH + H_2$	$k_{17} = 3.0 \times 10^5$
18	$t{-}C_4H_9OH + OH \rightarrow$ $\rightarrow t{-}\dot{C}_4H_8OH + H_2O$	$k_{18} = 5.5 \times 10^8$
19	$2t{-}\dot{C}_4H_8OH \rightarrow (C_4H_8OH)_2$	$2k_{19} = 1.4 \times 10^9$
20	$t{-}\dot{C}_4H_8OH + PFH^+ \rightarrow SQ +$ Products	$k_{20} = 5.0 \times 10^6$
21	$PFH^+ OH \rightarrow$ Products	$k'_{21} = 5.0 \times 10^2$

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Applying the previously described combined pulse radiolysis-simulation computation method [9, 10], it was possible to resolve the superimposed kinetics of reactions (1) to (3) and to determine the unknown specific molar extinction coefficients (ε) for the corresponding transients.

For illustration the change of the OD/cm as a function of time at 320 nm is given in Figure 2. It can be seen that in addition to the dye bleaching also two other processes are taking place, namely the formation of semiquinone and as a result of its decay, the appearance of dimers.

The kinetics of the transients become even more complicated at 300 nm as shown in Fig. 3, where simultaneous production of semiquinone and R$^\cdot$-species, as well as dye bleaching occur. The dimer formation is also observable at this wavelength. It might be pointed out that in all cases the measured and computed total absorptions showed a satisfactory consistency.

The rate constants for formation and decay of the transients (SQ, R$^\cdot$, H-add.), as well as their characteristic molar extinction coefficients ($\varepsilon_{\max}$) were determined and are presented in Table 2.

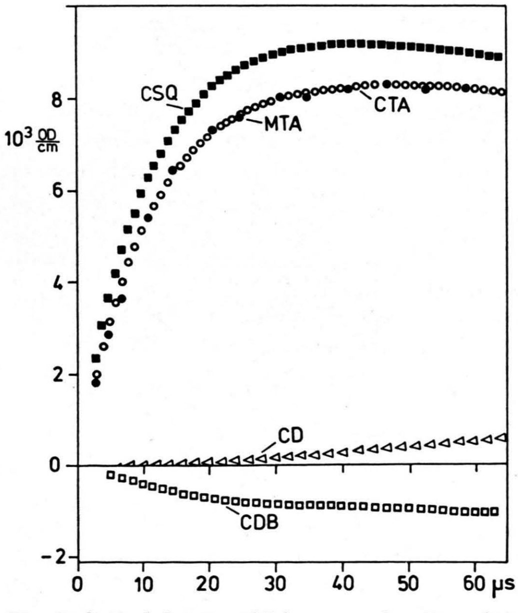


Fig. 2. Optical density (OD/cm) as a function of time (μs) at 320 nm for computed formation of: semiquinone (CSQ, ■), dimer (CD, ◁) and dye bleaching (CDB, □) in comparison to measured (MTA, ●) and computed (CTA, ○) total absorption. (Solution: 10^{-5} mol dm^{-3} proflavin, 2×10^{-2} mol dm^{-3} t-butanol, pH 2.7, saturated with argon; 5.3 J kg^{-1} per 0.4 μs pulse.)

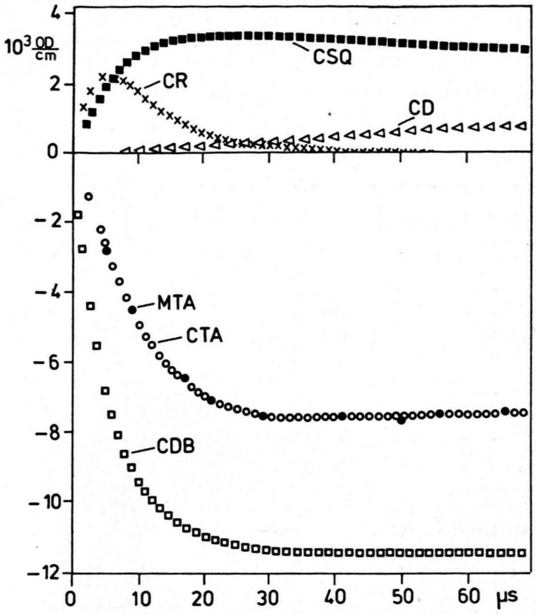


Fig. 3. Optical density (OD/cm) as a function of time (μs) at 300 nm for computed formation of: semiquinone (CSQ, ■), R-species (CR, ×), dimer (CD, ◁) and dye bleaching (CDB, □) in comparison to measured (MTA, ●) and computed (CTA, ○) total absorption. (Solution: 2×10^{-5} mol dm^{-3} proflavin, 4×10^{-2} mol dm^{-3} t-butanol, pH 2.7, saturated with argon; 5.4 J kg^{-1} per 0.4 μs pulse.)

Based on the formation kinetics it has been calculated, that the fraction of H-atoms reacting with PFH$^+$-molecules results in 75% SQ, 8.5% H-adducts and 16.5% R$^\cdot$-species.

Table 2. Molar extinction coefficients (ε) and rate constants (k) for formation and decay of transients generated by multisite H-attack on proflavin (3.6-diamino-acridine) in airfree aqueous solutions.

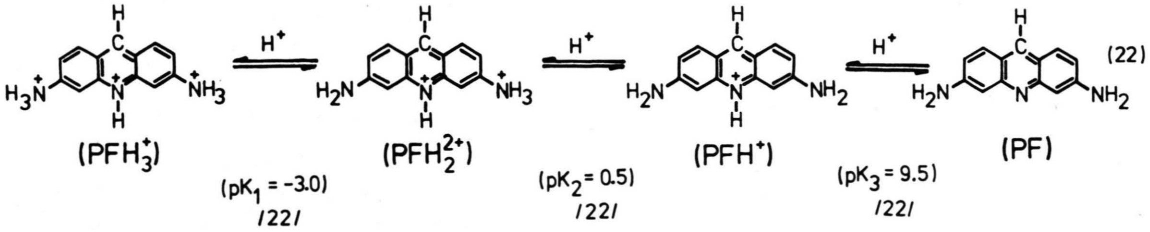
Kinetic and spectroscopic data	Proflavin transients at *p*H 2.7		
	Semiquinone (SQ; 75%)	H-adducts (8.5)%	R$^\cdot$-species (16.5%)
Formation:			
k(H + Dye) (dm^3 mol^{-1} s^{-1})	$(6.4 \pm 0.5) \times 10^9$	$(0.7 \pm 0.2) \times 10^9$	$(1.4 \pm 0.3) \times 10^9$
Decay ($2k$) (dm^3 mol^{-1} s^{-1})	$(2.5 \pm 0.3) \times 10^9$	$(1.1 \pm 0.4) \times 10^4$ *	$(2.0 \pm 0.5) \times 10^5$ * ($\tau/2 \cong$ 3.5 μs)
ε-values at $\lambda_{\max}$ (m^2 mol^{-1})	$\varepsilon_{270} = 1900$ **		$\varepsilon_{295} = 2300$
	$\varepsilon_{360} = 1530$	$\varepsilon_{270} = 2100$ **	$\varepsilon_{380} = 1000$***
	$\varepsilon_{460} = 2250$	$\varepsilon_{435} = 1580$	$\varepsilon_{440} = 5000$
	$\varepsilon_{530} = 200$		$\varepsilon_{680} = 530$

* First or pseudo first order rate constants k (s^{-1}).
** Not at $\lambda_{\max}$.
*** Shoulder

3.2. Determination of pK_1-value of Semiquinone

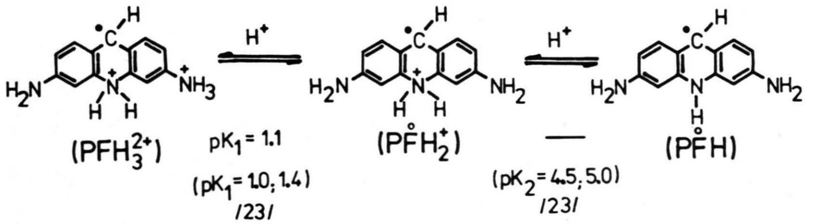
Based on the three amino nitrogens, the ground state of proflavin in aqueous solutions can exist in four different forms (equilibrium set I). The acid-base equilibrium constants (pK-values) given below were reported previously [22]. The acid-base equilibria, together with the corresponding pK-values of the three SQ-forms, which were determined by polarographic analysis [23], are also presented (equilibrium set II):

Equilibrium set I



(22)

Equilibrium set II



(23)

The pK_1-value of SQ was redetermined by following the change of OD/cm at 350 nm as a function of pH by using the Hammett's relation (24).

$$pK = pH - \log \frac{OD_b - OD}{OD - OD_a}. \tag{24}$$

OD_a and OD_b signify the optical densities of the pure acid and basic forms of SQ, whereas OD is the measured one respectively. The plot of the logarithmic expression from Eq. (24) as $\log A$ in dependence of pH (insert II in Fig. 1) resulted a $pK_1 = 1.1 \pm 0.1$.

4. Discussion

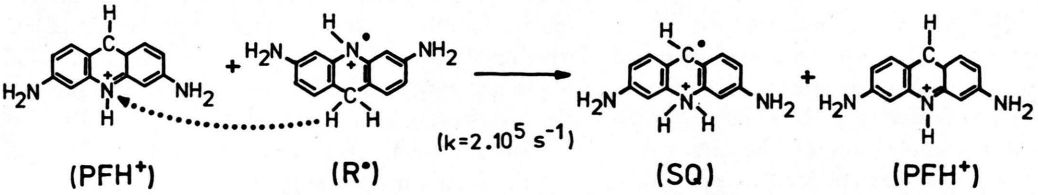
Based on the combined pulse radiolysis-simulation computation procedure it was now shown that the absorption spectrum in Fig. 1 is in fact composed of the absorptions belonging to SQ, $R^{\cdot}$-species and H-adducts. The same observation has been made previously for acridine orange in aqueous acid solutions [14]. The main transient, SQ, exhibits four absorption maxima (Table 2). The absorption bands about 250 nm and 400 nm are strongly superimposed with those of the other two species, whereas the absorption bands about 360 and 530 nm belong practically to SQ only. The disappearance of this transient is established to proceed by dismutation to PFH^+ and 3,6-diamino-acridan (3,6-DAA) as well as by dimerisation to a dimer (3,6,3′,6′-diamino-biacridan) with different k-values:

$$2\,SQ \longrightarrow PFH^+ + 3{,}6\text{-DAA} \quad (2\,k_6 = 0.5 \times 10^9\ dm^3\,mol^{-1}\,s^{-1}) \tag{6}$$

$$\longrightarrow \text{Dimer} \quad (2\,k_7 = 2.0 \times 10^9\ dm^3\,mol^{-1}\,s^{-1}). \tag{7}$$

The 3,6-DAA exhibits a $\varepsilon_{292} = 1585\ m^2\,mol^{-1}$ [24] measured by conventional method in aqueous solution at pH = 4. As a result of our experiments two absorption bands for the dimer were observed ($\varepsilon_{290} = 2700\ m^2\,mol^{-1}$ and $\varepsilon_{430} = 6600\ m^2\,mol^{-1}$).

As illustrated in Fig. 3 the $R^{\cdot}$-species (often assigned as N-radicals) are relatively short-lived ($\tau/2 = 3.5\ \mu s$; Table 2) in comparison to SQ (also denoted as C-radical). This fact was previously considered in the case of acridine orange [25, 26] and later experimentally proved [14]. Special interest has been devoted to the decay of the R$^\cdot$-species. For the disappearance of the N-radicals, produced from acridine in benzene and methanol solutions, Kira and Koizumi [25] have considered two processes, a second order decay and a transformation into SQ under participation of the starting molecule. But, based on their kinetic data, the authors excluded the second process. In the present work, however, for the disappearance of the R$^\cdot$-species from proflavin in acid aqueous medium the transformation into SQ was established to be the main process (reaction 8):



(8)

This reaction is supported by the following facts: 1. Assuming the disappearance of R$^\cdot$-species to proceed like SQ (reactions 6 and 7), a total rate constant of about $3 \times 10^{11}\,\mathrm{dm^3\,mol^{-1}\,s^{-1}}$ was calculated, which is not realistic. – 2. The k-value was found to be independent on the dose/pulse at given PFH$^+$-concentration. – 3. A dependency of the k-value from the PFH$^+$-concentration at a constant dose was also observed. These facts are a strong indication for a pseudo-first order reaction. Finally, it can be stated that under acceptance of reaction (8) to be the main decay process of R$^\cdot$-species, the best agreement between the measured and computed results is achieved.

Concerning the H-adducts it might be generally mentioned that transients of this kind can be principally formed on various places on the PFH$^+$-molecule, but preferentially on o- and p-positions of substituted groups on the ring. The applied procedure, however, does not allow a differentiation between them. Based on NMR studies on various acridine dyes in ground state it was established that positions C-4 and C-5 should be the most favourable for an electrophilic H-attack [27]. Principally could be expected that the decay of the H-adducts should follow a second order reaction. A sensitivity analysis [9], however, leads to the conclusion that the influence of these species in respect to the total absorption is very small and therefore the measured data do not contain sufficient information for a satisfactory elucidation of the decay mechanism of the H-adducts. The given pseudo-first order k-value (Table 2) satisfies best the requirements of the applied computation procedure. Similar observations for the H-adducts have been also made in the case of thionine [12, 13], methylene blue [11 – 12] and acridine orange [14].

Nenadovic et al. [8] obtained a transient spectrum resulting from the reaction of proflavin with e_{aq}^- and H-atoms (pH 6.5), which is similar to this shown in Figure 1. The transients were assigned as radical anions ($\dot{\mathrm{P}}\mathrm{F}^-$). This is, however, in contradiction with the structures of the previously studied semireduced forms of proflavin [23] and acridine orange species [14, 28, 29]. The observed decay rate constant, $k = 6.5 \times 10^4\,\mathrm{s^{-1}}$ is in contrast to the expected second order k-values for such type of transients. The photochemical behaviour of aqueous proflavine was also investigated by means of laser flash photolysis technique in the presence of appropriate electron donors [5, 6]. In both cases an absorption spectrum of the nonprotonated form of SQ ($\lambda_{\max} = 520\,\mathrm{nm}$) was registered. These authors did not observe any significant absorption of the protonated SQ-form in the range above 500 nm. By means of our combined method, however, we have been able to measure in acid aqueous solutions (pH = 2.5 – 2.8) for SQ of proflavin two absorption maxima in the visible range: at $\lambda = 440\,\mathrm{nm}$ ($\varepsilon_{440} = 2250\,\mathrm{m^2\,mol^{-1}}$) and 530 nm ($\varepsilon_{530} = 200\,\mathrm{m^2\,mol^{-1}}$).

It might be further of interest to mention that the various protolytic forms of proflavin-semiquinone are often quoted incorrectly [3, 5, 6, 8] and are in discrepancy with the original literature [23]. Therefore, the acid-base equilibria and the pK-values of proflavin in ground state and the SQ-form are presented in set I and II correspondently. The redetermined $\mathrm{pK_1} = 1.1 \pm 0.1$ is in good agreement with the previously reported values of 1.0 and 1.4 [23].

5. Conclusion

The present study provides information about the spectroscopic and kinetic behaviour of proflavin-semiquinone (C-radical) as well as of the $R^{\cdot}$-species (N-radical) and H-adducts in aqueous acid medium. As stated, semiquinone disappeares preferable by dimerisation and only to a certain extent by dismutation. This findings point out, that whenever proflavin-semiquinone is formed (by means of laser- and flash-photolysis, etc.) or H-atoms are available in a proflavin dye solution (e. g. in electrochemical cells), a partial decomposition of the dye takes place. This fact is very important for the application of proflavin as sensitizer in photochemical and photoelectrochemical devices for solar energy utilization. The obtained results may be also of biological and pharmacological interest.

Acknowledgements

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