Fluorescein and Phenolphthalein—Correlation of Fluorescence and Photoelectric Properties[1]

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Two phthalein dyes in aqueous polyvinyl alcohol solutions of pH 8.5 have been investigated: fluorescein and phenolphthalein. Competitive pathways of deactivation of their energetically excited states have been followed by the use of spectroscopic and photoelectric techniques. Radiative, nonradiative, and charge separation processes followed by electron transfer have been measured by absorption, fluorescence, and photoacoustic spectroscopy accompanied by photovoltage/photocurrent generation in a photoelectrochemical cell. It is shown that, despite their being only a slight difference in molecular structure between the two dyes, their spectral as well as photoelectric behavior is significantly different.

KEY WORDS: Fluorescein; phenolphthalein; photoacoustic spectra; photovoltaic effect; thermal deactivation.

INTRODUCTION

Fluorescein and phenolphthalein are well-known organic dyes of wide application. Fluorescein is used in the laser technique and as a fluorescent marker for determination of fluorescence quantum yields of other dyes [1], whereas phenolphthalein is known as a pH indicator.

Fluorescein and phenolphthalein differ only slightly in molecular structure as shown in Fig. 1. The difference is the O-bridge[4] which links two benzene rings in fluorescein [1,9]. A relatively simple structural modification can substantially alter the photophysical behavior of dyes.

Although some molecules are capable of absorption and fluorescence, charge separation processes upon illumination are not observed in a large number of compounds. Such behavior is closely connected with the molecular structure of the main molecular frame and the substituents attached to the molecular core. Since radiative, nonradiative, and charge separation processes compete with each other, it was deemed interesting to follow the deactivation pathways of the excited states of two phthalein dyes which differ insignificantly in their molecular structures.

Absorption and fluorescence features of some phthalein dyes have been the subject of some papers [9,26]. To date, however, the literature data have not included the study of thermal deactivation processes in both fluorescein and phenolphthalein. As far as we know, the charge separation process in phthalein dyes has never been studied. In the light of our previous spectroscopic and photoelectric studies of porphine dyes, we indicated some correlation between the capability for fluorescence and for charge separation, on one hand, and the molecular structure of dyes, on the other hand [2–8].

The aim of this paper is to follow the absorption, thermal, and fluorescence properties of fluorescein and

[4] *Abbreviations used*: A, absorbance; Φ_{CH}, charge separation yield; Φ_F, fluorescence quantum yield; O-bridge, oxygen bridge; PAS, photoacoustic signal/spectra; PVA, polyvinyl alcohol; TD, thermal deactivation; Φ_{TD}, thermal deactivation yield; Φ_T, triplet quantum yield.

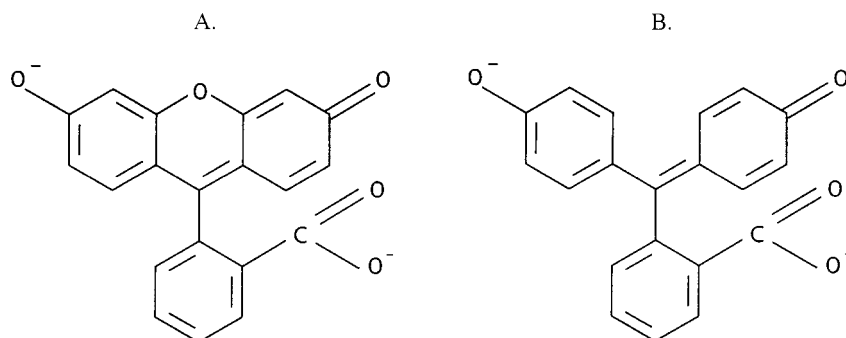A.   B.

Fig. 1. Molecular structure of fluorescein (A) and colored phenolphthalein (B).

phenolphthalein as well as their abilities for charge separation. For that reason the absorption and steady-state fluorescence measurements are accompanied by photoacoustic and photovoltaic experiments. Photoacoustic and absorption spectra provide information on thermal deactivation occurring in nonradiative processes. The charge separation process was monitored as the photoelectric response in a sandwich-like photoelectrochemical cell.

For dyes in the photoelectrochemical cell the photovoltage spectra (the dependence of the photovoltage signal on the excitation wavelength), photocurrent kinetics on the second time scale as well as current–voltage characteristics were measured. Due to the structural differences between fluorescein and phenolphthalein molecules, varying spectroscopic and photoelectric properties are expected.

MATERIALS AND METHODS

Fluorescein and phenolphthalein were purchased from Sigma and used without further purification. The synthesis procedure for these dyes has been described elsewhere [9]. PVA powder was purchased from Aldrich, and the aqueous PVA solution (15%, v/v) was prepared according to the procedure described in Ref. 3.

Particular attention was paid to the pH of dye solvents. Fluorescein (Fig. 1A) used in the study does not show pH sensitivity in the absorbance response. To obtain colored phenolphthalein, commercial colorless phenolphthalein was treated with an aqueous KOH solution. The details of this method are described in Ref. 9. At pH 8.3–10.0 colorless phenolphthalein turns to violet dye. There are several reasons for the use of aqueous PVA in the study. First, the dye is very well dissolved in water and thus its pH can be easily controlled. Second, a PVA solution can serve as a good medium for the investigation of photoelectric properties of dyes immersed in a photoe-

lectrochemical cell as we have shown previously [4]. At pH 8.5 dyes are found to be very stable over time (for days). For these reasons a PVA solution of pH 8.5 was chosen for the experiment. Dyes of $1 \cdot 10^{-3}\,M$ concentration were dissolved in PVA aqueous solutions and used for spectroscopic and photoelectric measurements.

Absorption spectra were carried out with a Specord M40 spectrophotometer (Carl Zeiss, Jena) in the range of 430–800 nm. Fluorescence spectra excited at $\lambda_{ex} = 485$ nm (fluorescein) and at $\lambda_{ex} = 550$ nm (phenolphthalein) were monitored by a homemade device in the range of 490–800 and 560–800 nm, respectively. For the absorption and fluorescence spectra a dye/PVA solution was immersed between two glass plates separated by a spacer (60 μm thick). The 60-μm-pathlength sample holder was used because of the high dye concentration used in the experiment. A similar but uncolored holder was used as a reference sample.

Photoacoustic measurements (in the range of 350–800 nm) were made with a one-beam spectrometer described elsewhere [10] at three light modulation frequencies—8, 15, and 30 Hz—at a constant phase shift ($\phi = -80°$). The dye in the PVA aqueous solution was immersed in the photoacoustic cylindrical cell. Light energy absorbed by a molecule is partially changed into heat and thermal energy reaches the thin layer of gas in a photoacoustic cell. This thin gas layers acts as a pistol on the remaining gas and a photoacoustic signal is created and detected with a very sensitive microphone. A blackbody was used as a reference sample to correct the photoacoustic spectra (PAS) for the spectral distribution of the light source. Thermal deactivation (TD) as the ratio of the photoacoustic signal to the absorption amplitude can be determined [12].

For photovoltaic and photocurrent measurements, dyes in aqueous PVA were immersed in the sandwich-like photoelectrochemical cell constructed with semiconducting (In_2O_3) and gold (Au) electrodes distanced with

the 60-μm Teflon spacer. The details of the arrangement for photovoltaic measurements are described in Ref. 11. The following measurements were made with an automatic potentiostat–galvanostat (Compex; Poland) device: photovoltaic action spectra (the dependence of the photovoltaic signal on the excitation wavelength), the kinetics of the photocurrent in the short circuit, and current–voltage characteristics [$I(V)$]. The photoelectrochemical cell was illuminated from the semiconducting electrode side. For photovoltaic action spectra dyes in the photoelectrochemical cell were excited with a xenon lamp (Oriel) as the light source, and the wavelength was selected with a monochromator. Photovoltaic action spectra were corrected for a number of incident photons. The same photoelectrochemical cell with dyes was used for monitoring of photocurrent kinetics. Photocurrent increases and decreases were measured on the second time scale (0–100 s). Current–voltage characteristics, $I(V)$, were monitored with a scan time of 10 mV/s in the range -100 ± 100 V.

RESULTS AND DISCUSSION

Absorption spectra for fluorescein and phenolphthalein in aqueous PVA are shown in Fig. 2. Each dye shows

Table I. Maximum of the Absorption Band and Extinction Coefficient for Fluorescein and Phenolphthalein-in Aqueous PVA (15%, v/v) of pH 8.5

Dye	λ_{max} (nm), wavelength	$\varepsilon \cdot 10^3 \left(\dfrac{1}{M \cdot cm}\right)$, extinction coefficient
Fluorescein	496	4.6
Phenolphthalein	558	2.1

a broad absorption band in different spectral regions. The absorption bands are located at about 496 nm for fluorescein and 558 nm for phenolphthalein (Fig. 2). This relative shift in the absorption band position is due to the difference in the molecular structure of the dyes. Absorption parameters are listed in Table I, which indicates that the extinction coefficient for phenolphthalein is about two times lower than that for fluorescein.

Figure 3 presents the fluorescence spectra of fluorescein ($\lambda_{ex} = 485$ nm) and phenolphthalein ($\lambda_{ex} = 550$ nm) and shows the differences in fluorescence behavior of the investigated dyes. Very intensive fluorescein fluorescence is observed, whereas almost no emission is monitored for phenolphthalein. From literature data it is well known that fluorescein shows a very high fluorescence quantum
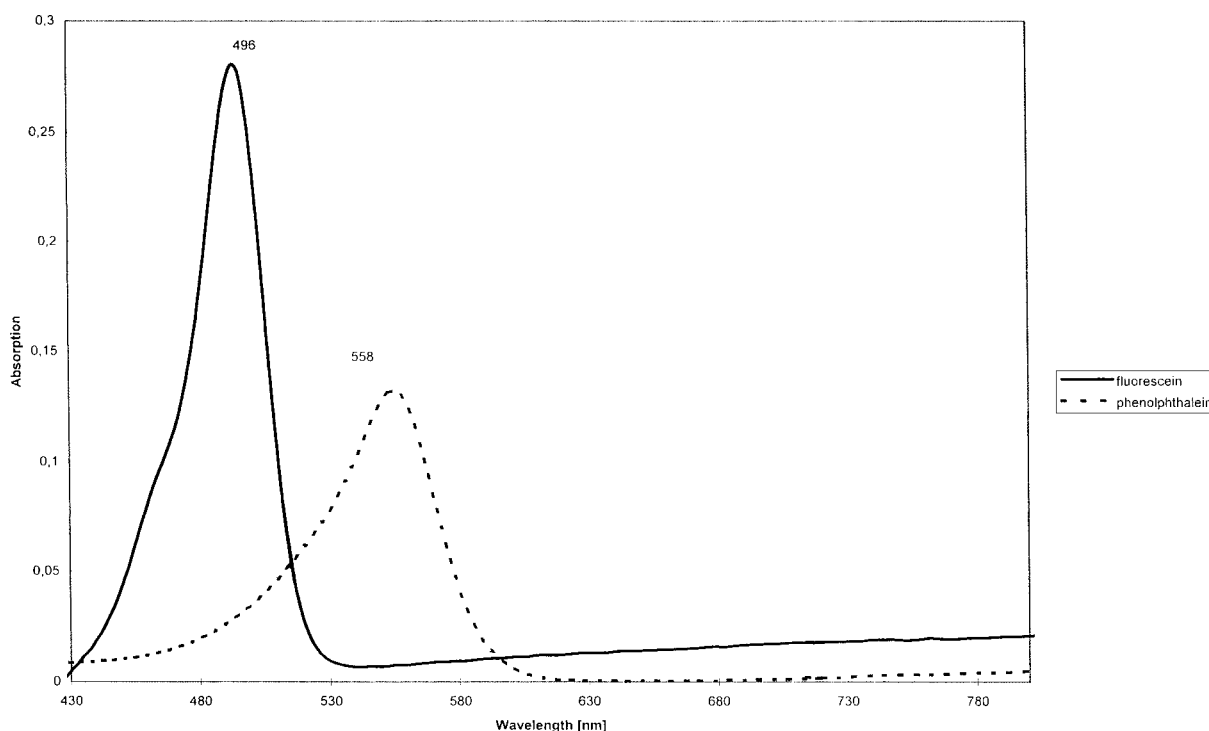
Fig. 2. Absorption spectra of fluorescein and phenolphthalein in an aqueous PVA solution of pH 8.5.
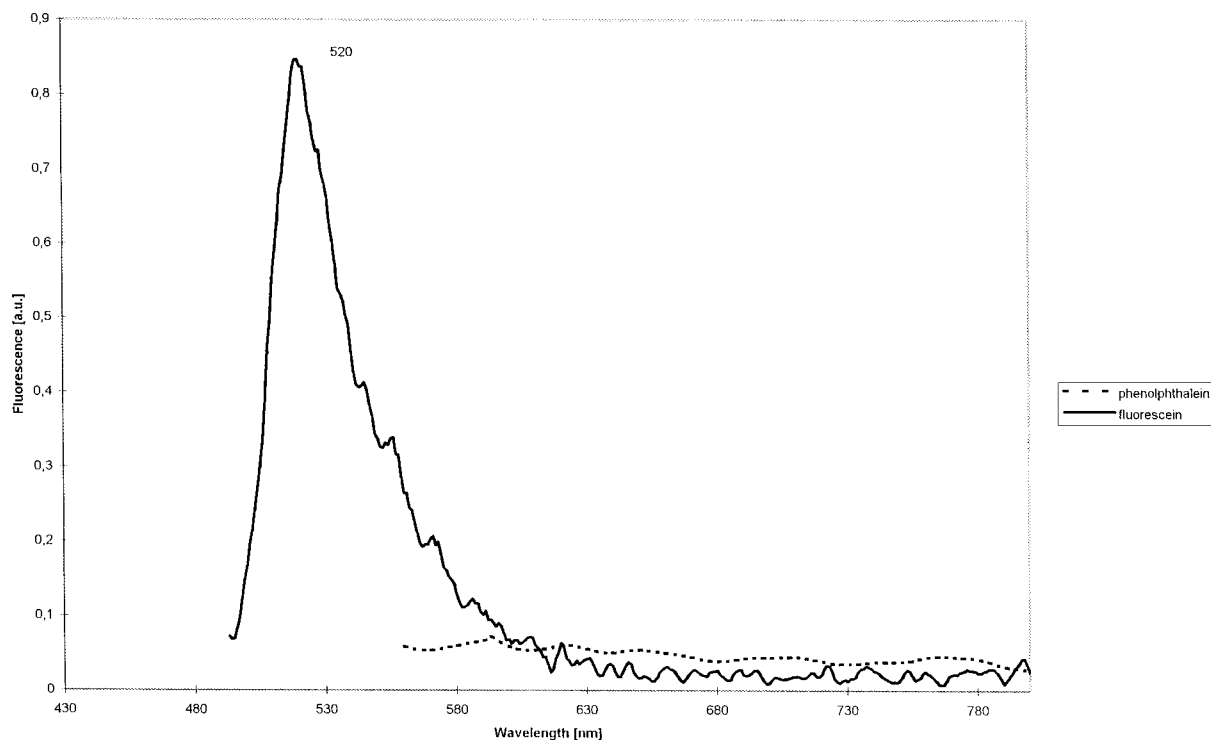
Fig. 3. Fluorescence spectra of fluorescein and phenolphthalein in an aqueous PVA solution of pH 8.5 ($\lambda_{ex} = 485$ nm and $\lambda_{ex} = 550$ nm for fluorescein and phenolphthalein, respectively).

yield, reaching 92% [1]. This obviously indicates that fluorescence emission is a dominating process in deactivation of the fluorescein excited state. The investigated molecules differ from each other by the O-bridge which links the benzene rings in fluorescein. The O-bridge leads to the rigidity of the fluorescein molecular structure and makes this molecule highly fluorescent [1].

The quantum yields of radiative processes determine the quantum yields of other deactivative processes, e.g., as a result of the nonradiative transients. Results of photoacoustic spectroscopy are shown in Fig. 4. Identical experimental conditions allow us to compare the PAS for fluorescein and phenolphthalein directly. The first observation is a two times higher photoacoustic signal in the case of phenolphthalein compared with that for fluorescein.

Taking into account the intensity of absorbance (Fig. 2) and PAS (Fig. 4) results, it is obvious that thermal deactivation in phenolphthalein is several times more effective than that in fluorescein. The photoacoustic results resemble those of the fluorescence study for the two dyes and indicate that in phenolphthalein a large portion of the energy is changed into heat. The kinetics of thermal dissipation appear to be different for fluorescein and phenolphthalein, as different decreases in PAS

signals with light modulation frequency are observed. At the highest modulation frequency, PAS almost vanishes in phenolphthalein. Moreover, only for fluorescein is the decline of PAS in accordance with the Rosencwaig theory [12]. Analysis of the photoacoustic signal as a function of light modulation frequency (using linear regression) gives a correlation factor equal to 1.00, but only in the case of fluorescein. This observation together with the change in the PAS shape of phenolphthalein leads to the conclusion that triplet states can also be involved in thermal deactivation processes. Relatively high concentrations of dyes would give some aggregated forms but there is some evidence of the existence of monomers: fluorescence also occurs at lower concentrations and shows a linear relation of its intensity with concentration (not shown), in the absorption spectra no extra band occurs, aggregated forms are very much involved in thermal deactivation, and we do not observe particular changes in PAS shape also at different frequencies [6,8].

The photovoltaic spectra (Fig. 5A) show a good correspondence with the absorption spectra (Fig. 2). Photovoltaic bands appear in exactly the same wavelength position as absorption bands. This confirms the responsibility of dyes for the photovoltage generated as was observed for porphyrins [2,3], phthalocyanines [4], and
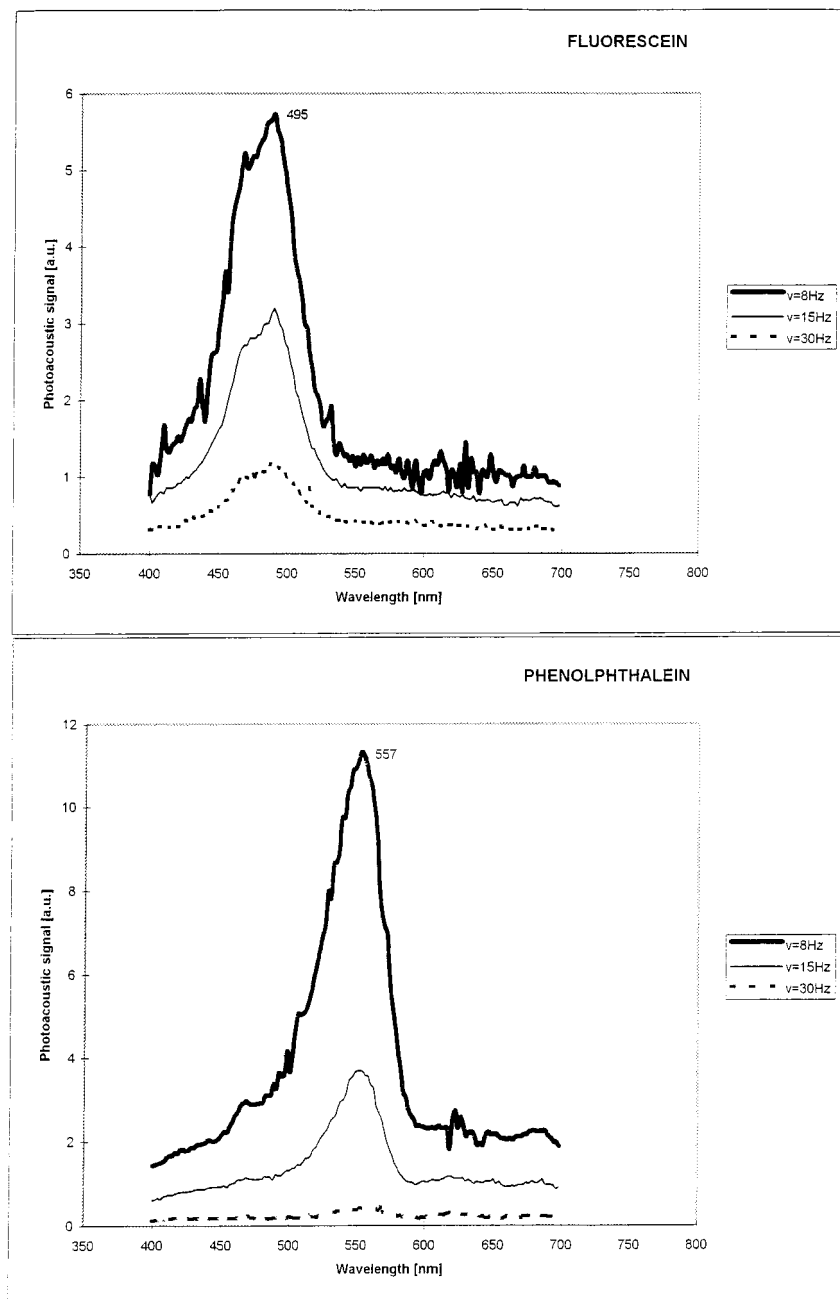
Fig. 4. Photoacoustic spectra and phenolphthalein in aqueous PVA of pH 8.5 at three light modulation frequencies.

other dyes [6,14]. However, the photovoltage signal generated by phenolphthalein is twice as high as that for fluorescein. Considering the relation between the absorbance amplitudes and the photovoltaic responses, it is obvious that phenolphthalein's ability for charge separation predominates over that of fluorescein. It can be suggested that the process of charge separation followed by electron transport on the boundary of the semiconductor electrode and the dye layer is more effective in phenolphthalein than in fluorescein. Photoelectric properties of the two dyes are also confirmed by the photocurrent kinetics presented in Fig. 5B. For phenolphthalein the photocurrent value is two times higher than for fluorescein. It reaches 10 nA and is characterized by
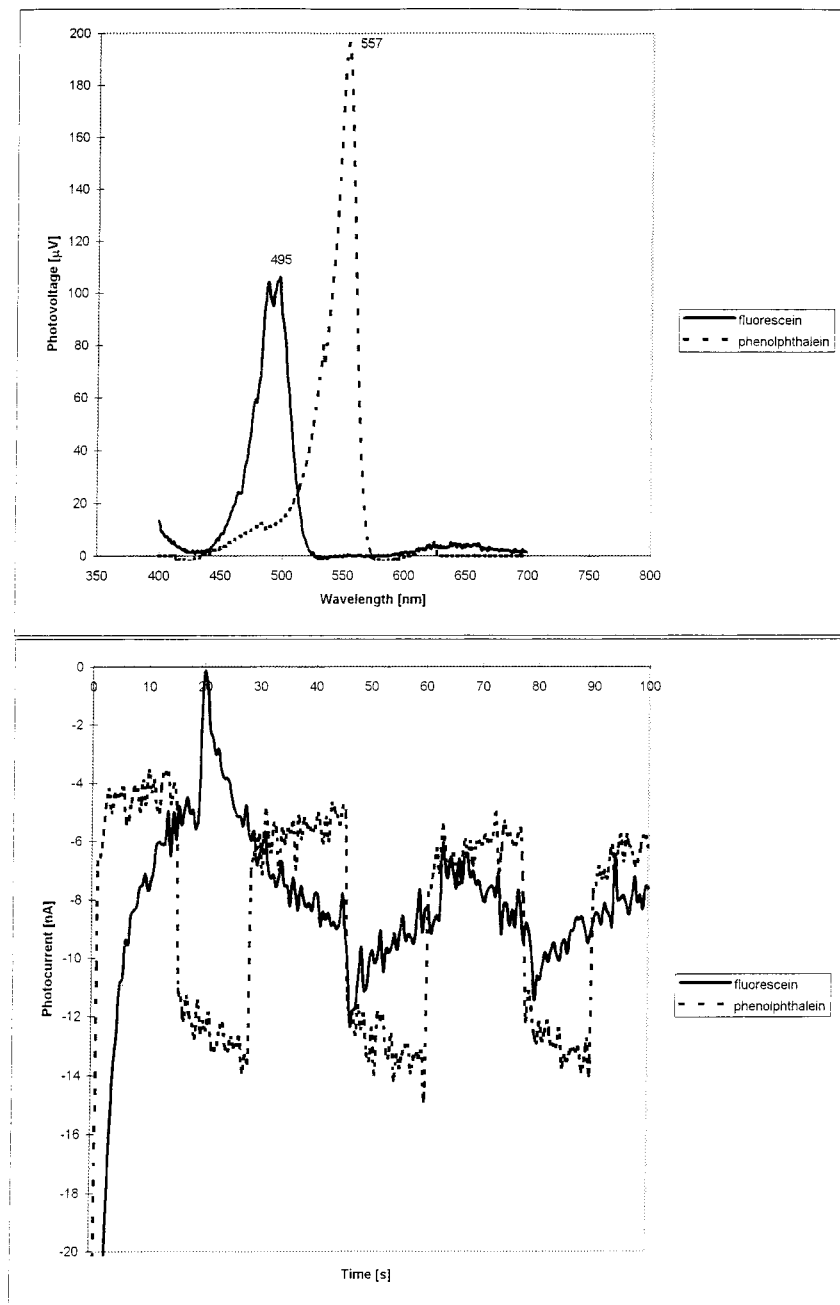
Fig. 5. Photovoltaic action spectra (A) and photocurrent kinetics (B) for fluorescein and phenol-
phthalein in aqueous PVA in the photoelectrochemical cell.

a regular course. A similar character of phenolphthalein kinetics has been observed for porphyrins and phthalocya-nines in our previous papers [2–4]. More complex fluo-rescein kinetics with marked signal increases ("light stress") are usually observed for other dyes and their mixtures [15] and natural photosynthesizing systems such as bacteria [14]. In the light of the results presented in

other papers [15,16] the effect of light stress can be assigned to ion recombination and it can be also connected with the superposition of at least two effects: a fast process occurring immediately after light absorption and a slower motion of the ions across the photoelectrochemical cell.

This suggestion is consistent with the results on the current–voltage characteristics shown in Fig. 6. Using
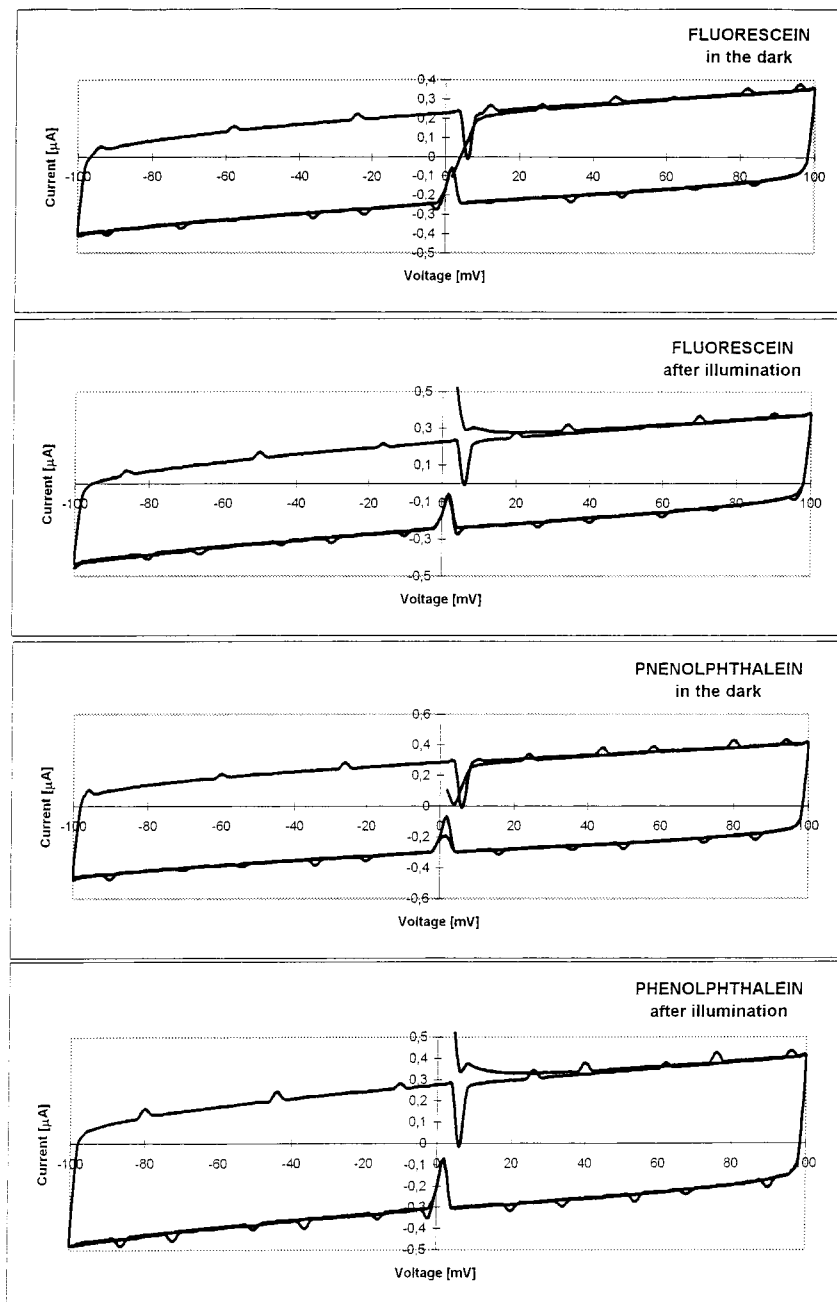
Fig. 6. Current–voltage characteristics for fluorescein and phenolphthalein in the dark and after illumination.

the RC-circuit model proposed in Ref. 7, the electric parameters of the photoelectrochemical cell (conductivity and capacitance) were estimated and are listed in Table II. The capacitance of the photoelectrochemical cell increases under illumination in the case of both dyes, which indicates changes in the charge distribution and an increase in the free charged carriers. Decreasing con-

ductivity of the photoelectrochemical cell with fluorescein after illumination confirms our previous suggestion about charge recombination.

The results obtained with the photoelectrochemical cell filled with the dyes used in our experiments resemble those of the spectrophotometric investigations. According to Birks [1] and to other papers [17–21], the sum of the

Table II. Conductivity and Capacitance of the Photoelectrochemical Cell with Fluorescein and Phenolphthalein Dissolved in Aqueous PVA (15%, v/v) of pH 8.5 in the Dark and After Illumination

Dye	$\sigma \cdot 10^{-6}$ (1/Ω)		C (nF)	
	Conductivity in the dark	Conductivity after illumination	Capacitance in the dark	Capacitance after illumination
Fluorescein	1.67	0.99	46.8	47.0
Phenolphthalein	1.10	1.53	58.4	62.3

fluorescence yield (Φ_F) and triplet quantum yield (Φ_T) of fluorescein in aqueous solvent is very close to unity [17]. Due to the internal conversion, thermal deactivation is observed with photoacoustic measurements [12,22]. Evidence presented in this paper also suggests that $\Phi_F + \Phi_T \neq 1$ as shown by our PAS and photovoltaic experiments. An excited molecule gives off heat upon photoexcitation and this process gives rise to the observed Stokes shift (24 nm for fluorescein) due to the vibrational relaxation occurring when the molecule is still in S_1 and in S_0 after photon emission. According to Bowers and Porter [17], $\Phi_F + \Phi_T = 0.97$. This indicates the quantum yields values of the remaining processes: thermal deactivation and charge separation can be estimated at 0.03.

Fluorescence is the predominant decay pathway for fluorescein but not for phenolphthalein, for which electronically excited states are deactivated predominantly in thermal deactivation and in charge separation processes. Taking into consideration the law of energy conservation ($\Phi_F + \Phi_T + \Phi_{TD} + \Phi_{CH} = 1$, where Φ_{TD} is the thermal deactivation yield and Φ_{CH} the charge separation yield) and the competition among radiative (fluorescence), nonradiative (thermal deactivation), and charge separation processes as shown by spectrophotometric and photoelectric results presented in this paper as well as in the literature [1], we suggest that participation of the phenolphthalein triplet state in photothermal processes cannot be excluded. In the light of our spectroscopic and photovoltaic results for phenolphthalein ($\Phi_F = 0$; Φ_{TD} is about 10 times higher than in fluorescein, and Φ_{CH} is at least 2 times higher than in the case of fluorescein), we can roughly estimate the value of Φ_T for phenolphthalein to be about 0.70 in aqueous PVA.

Electron photodetachment from fluorescein dyes has been the subject of only a few studies. The process of electron photoejection in fluorescein was described extensively in Ref. 23. Intensive delayed luminescence and thermoluminescence of dye in boric acid glass have been observed [24,25] and they were related to the photoionization of dyes. In this paper we have shown the capability of fluorescein and phenolphthalein for charge separation

followed by electron transport in a photoelectrochemical cell upon illumination. On the basis of our previous results from optical and photoelectric investigations of porphyrins and phthalocyanines [2–4] as well as on electron spin resonance (data not published), we have indicated the participation of π-electrons in the process of charge separation. Thus we can suggest that in fluorescein and phenolphthalein also, π-electrons could be responsible for the photovoltaic effects observed in our experiment. However, fluorescein shows the minority in the charge separation process, whereas the photocurrent generated in the photoelectrochemical cell based on phenolphthalein is higher.

CONCLUSIONS

In the light of our spectral and photoelectric/electric studies, we can summarize our findings as follows.

- The slight difference in the molecular structures of fluorescein and phenolphthalein leads to a marked difference in the spectral and photoelectric behavior of the dyes.
- Fluorescein shows weak deactivation of excited states by thermal processes but the lack of an O-bridge in phenolphthalein causes an increase in PAS for this dye compared to fluorescein due to the more free oscillation. Participation of the triplet dye state cannot be excluded,
- Phenolphthalein is able to create a photovoltage/photocurrent (when embedded in a photoelectrochemical cell) which is generated more effectively than in the case of fluorescein (charge recombination in fluorescein cannot be excluded).

The results presented though preliminary in nature, indicate that phenolphthalein can be used in the construction of optoelectronic photosensors which do not require large photocurrents.

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