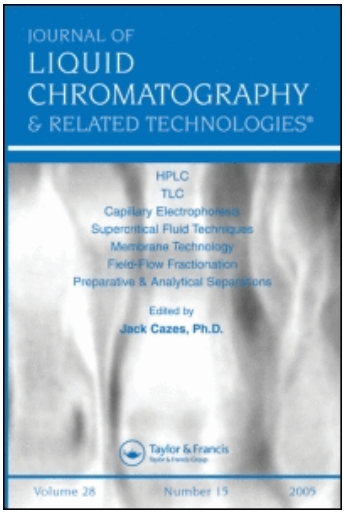

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Photoactivation: A Novel Means to Mediate Electrophoretic Separations

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PHOTOACTIVATION: A NOVEL MEANS TO MEDIATE ELECTROPHORETIC SEPARATIONS

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ABSTRACT

By introducing electromagnetic radiation of the appropriate wavelength, polarization, and power, the electronic charge within a solute molecule can be redistributed in ways that affect its electrophoretic mobility. Consequently, photoactivation may be utilized to control and to enhance electrophoretic separations in a selective and predictable manner. The general concept and theory of the photoactivation method are elucidated, and the feasibility is demonstrated for representative photoionization and photodissociation reactions.

INTRODUCTION

In order to enhance resolution in electrophoretic separations, many parameters have been used to manipulate the solute mobility (1-8, and references therein). For example, the properties of the solution may be varied by means of the buffer type, pH, and ionic strength (4,8-15), as well as the type and concentration of an organic solvent (16,17), complexing agent (18-20), or other modifier (21-23). In addition, a secondary phase

with sieving properties such as a gel (24,25) or entangled polymer (26,27) matrix may be added. While these traditional parameters are effective in altering the solute mobility, the strength and selectivity cannot be easily or rapidly changed during the electrophoretic separation. This limitation is especially important for high-speed applications and for two-dimensional electrophoresis–electrophoresis and chromatography–electrophoresis applications (28-30).

In this work, photoactivation is explored as an alternative approach to mediate electrophoretic separations. In this approach, the solute molecules or ions are exposed to radiant energy (photons) rather than a chemical potential or other source of energy. The molecules that absorb this radiation will selectively undergo a transition to the excited state (Figure 1A). If this transition induces a change in either the mass or the charge of the solute molecule, its electrophoretic mobility will be concomitantly altered. There are two simple mechanisms by which a change in mass and/or charge may occur: photoionization and photodissociation. In the former case (Figure 1B), an electron will be ejected from the molecule if the energy of the absorbed photon exceeds the ionization energy. The resulting positively charged ion will have a higher mobility than the unperturbed molecule. In the latter case (Figure 1C), the equilibrium constant for an acid–base, complexation, or other reversible reaction may differ significantly between the ground and excited states. As the molecules associate or dissociate in order to reachieve equilibrium, their charge and mass will be altered, thereby influencing their mobility.

Unlike other parameters that are used to control electrophoretic separations, the photoactivation method has the advantage that it is

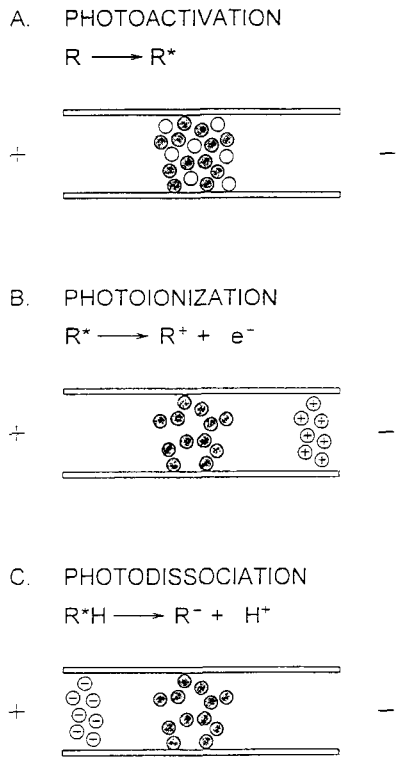


FIGURE 1: Schematic illustration of solute photoactivation (A) with subsequent photoionization (B) and photodissociation (C) reactions.

mediated through an externally applied electromagnetic field that can be rapidly modified in both strength and selectivity. In this paper, the conceptual and theoretical basis of this technique will be described together with preliminary experimental studies that support its feasibility.

THEORETICAL CONCEPTS

Upon interaction with electromagnetic radiation in the UV–visible region, molecules will undergo a transition to an electronically and/or

vibrationally excited state (31). The extent of such transitions can be broadly described by the absorption factor (α_o) at a specified frequency (ν), which is related to the power of the incident (ϕ_o) and transmitted (ϕ) radiation passing through a cell of pathlength (b) containing solute molecules of molar absorptivity ($\varepsilon(\nu)$) and molar concentration (C):

$$\alpha_o(\nu) = (\phi_o - \phi) / \phi_o = 1 - 10^{-\varepsilon(\nu)\, b\, C} \qquad [1]$$

This expression can be extended to include optically active molecules (32,33) if linearly polarized light is separated into the individual components, left and right circularly polarized light. Molecules that possess a chiral center exhibit different degrees of absorption for these two components, whereas optically inactive molecules show no preference. For an optically active molecule, the difference in molar absorptivity is represented by

$$\Delta\, \varepsilon(\nu) = \varepsilon(\nu)_L - \varepsilon(\nu)_R \qquad [2]$$

which is known as the circular dichroism. Because each solute has a unique absorption spectrum, it is possible to control selectivity by varying the frequency and polarization. In addition, the strength can be controlled by varying the power of the electromagnetic radiation.

In the simplest case, these transitions lead to the formation of an excited state with a finite lifetime (τ) that decays to the initial ground state *via* radiative or nonradiative relaxation. Alternatively, the molecule may undergo a reversible or irreversible chemical reaction such as ionization, dissociation, isomerization, etc. before relaxation to another, distinctly different ground state. In the former case, the electronic charge distribution within the molecule is temporarily affected during the lifetime of

the excited state, whereas in the latter case, the change is more long-lived and may, in fact, be permanent. These changes in charge distribution may potentially be exploited to alter the solute velocity in electrophoretic separations.

The solute velocity will be altered if the effective electrophoretic mobility differs between the ground and excited states. For a solute that consists of several (n) species in dynamic equilibrium through an acid–base, complexation, or other reversible reaction, the effective mobility (μ_{eff}) is calculated from the following summation (1,8):

$$\mu_{eff} = \sum_{i=1}^{n} \alpha_i \mu_i \qquad [3]$$

where α_i is the fraction and μ_i is the electrophoretic mobility of each species. The fractions α_i are dependent upon the equilibrium constants of the solute, as well as the concentration of the counter ion (e.g., pH or pM) in the buffer medium (34). Because the equilibrium constants are influenced by the electronic charge distribution within the solute molecule, they may differ substantially between the ground (g) and excited (e) states. Therefore, the fraction of each species and, hence, the effective mobility of the solute may be altered by photoactivation:

$$\mu_{eff} = (1 - F) \sum_{i=1}^{n(g)} \alpha_{gi} \mu_{gi} + (F) \sum_{i=1}^{n(e)} \alpha_{ei} \mu_{ei} \qquad [4]$$

In order to establish the potential magnitude of this effect, it is necessary to determine the fraction (F) of solute molecules that will undergo a transition to the excited state. If the transition arises from a single-photon absorption that obeys the Beer–Lambert law (31), then the fraction of molecules in the excited state is given by

$$F = [\phi_o \alpha_o(\nu) w] / [h \nu N b C] \quad [5]$$

where ϕ_o and w are the irradiance and pulse width of the laser source at frequency ν, $\alpha_o(\nu)$ is the absorption factor from Equation [1], h is Planck's constant, and N is Avogadro's number. Because of the assumptions inherent in the Beer–Lambert law, this equation is not applicable at high irradiance where saturation may occur. Under these conditions, the absorption factor in Equation [5] must be replaced by

$$\alpha(\nu) = [\alpha_o(\nu) \phi_{sat}] / [\phi_{sat} + \phi_o] \quad [6]$$

where ϕ_{sat} is the irradiance at which the absorption factor $\alpha(\nu)$ is decreased to one-half of its initial value $\alpha_o(\nu)$ (35). Although it may be advantageous to operate under saturation conditions, the dependence of the fraction of excited-state molecules on experimental variables will be considered only within the linear region of Equation [5] for the purposes of this discussion.

The dependence of the fraction F on the characteristics of the radiant source is shown in Figure 2. Within the typical operating range of laser systems, the fraction is linearly dependent upon the irradiance (10^4 – 10^8 W cm^{-2}) and pulse width (10^{-3} – 10^{-9} s). The dependence of the fraction F on the characteristics of the solute molecule is shown in Figure 3. At low concentration, the fraction remains relatively constant because the concentration-dependent terms in the numerator and denominator of Equation [5] increase proportionately. Under these conditions, the fraction of molecules in the excited state is solely a function of the molar absorptivity. As the concentration increases, however, the absorption factor approaches a limiting value of unity whereas the denominator

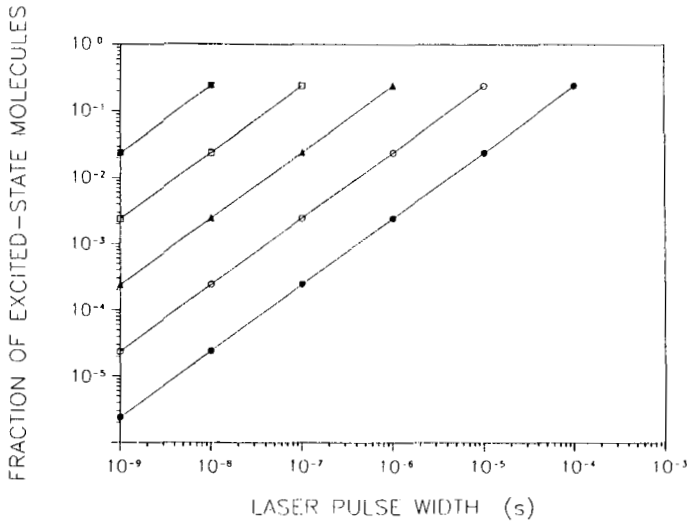


FIGURE 2: Fraction of excited-state molecules (F) *versus* laser pulse width (w) as a function of laser irradiance (ϕ_o) calculated according to Equation [5]. $\phi_o = 10^8$ W cm^{-2} (■), 10^7 W cm^{-2} (□), 10^6 W cm^{-2} (▲), 10^5 W cm^{-2} (○), 10^4 W cm^{-2} (●); $\nu = 1.21 \times 10^{15}$ s^{-1}; $b \equiv [\pi(0.0075/2)^2]^{1/2}$ cm; $C = 10^{-7}$ mole L^{-1}; $\varepsilon(\nu) = 10^4$ L mole^{-1} cm^{-1}.

increases unbound. It is not advisable to operate within the nonlinear region of Figure 3, because the fraction of molecules in the excited state and, hence, the resulting effective mobility of the solute will be concentration dependent. It is noteworthy that the concentration range over which the fraction F remains constant decreases by one order of magnitude for each ten-fold increase in molar absorptivity.

To gain an appreciation for the magnitude of the photoactivation effect, it is instructive to calculate the fraction of molecules in the excited state under conditions that are representative of our experimental system ($\phi_o = 2.17 \times 10^6$ W cm^{-2}, $w = 2.3 \times 10^{-8}$ s, $\nu = 1.21 \times 10^{15}$ s^{-1}). For a very

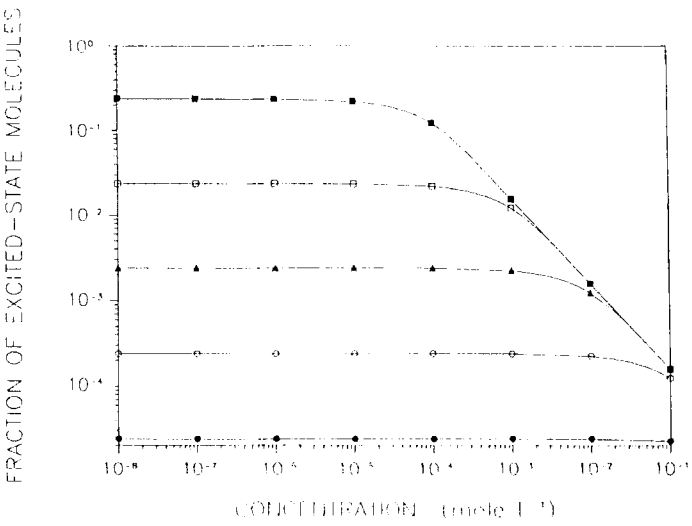


FIGURE 3: Fraction of excited-state molecules (F) *versus* molar concentration (C) as a function of molar absorptivity ($\varepsilon(\nu)$) calculated according to Equation [5]. ϕ_o = 2.17 x 10^6 W cm^{-2}; w = 2.3 x 10^{-8} s; ν = 1.21 x 10^{15} s^{-1}; b ≅ $[\pi(0.0075/2)^2]^{1/2}$ cm; $\varepsilon(\nu)$ = 10^6 L $mole^{-1}$ cm^{-1} (■), 10^5 L $mole^{-1}$ cm^{-1} (□), 10^4 L $mole^{-1}$ cm^{-1} (▲), 10^3 L $mole^{-1}$ cm^{-1} (○), 10^2 L $mole^{-1}$ cm^{-1} (●).

favorable solute ($\varepsilon(\nu)$ = 10^6 L $mole^{-1}$ cm^{-1}, C = 10^{-6} mole L^{-1}), a large fraction of molecules will be in the excited state (F = 24 %). For more typical solutes ($\varepsilon(\nu)$ = 10^3 L $mole^{-1}$ cm^{-1}, C = 10^{-3} mole L^{-1}), the fraction is more modest but still effective (F = 0.024%). Consequently, if the equilibrium constant differs between the ground and excited states, the effective mobility of the solute will be significantly altered by photoactivation.

This change in the effective mobility will directly influence the resolution in an electrophoretic separation. The electrophoretic resolution (R_s) in the elution mode is calculated as follows:

$$R_s = [N^{1/2} / 2] [(\mu_1 - \mu_2) / (2 \mu_{osm} + \mu_1 + \mu_2)] \quad [7]$$

where N is the number of theoretical plates and μ_{osm} is the electroosmotic mobility. Depending upon the specific circumstances, either the solute with larger effective mobility (μ_1) or with smaller effective mobility (μ_2) can be selectively photoactivated in order to enhance the electrophoretic resolution.

EXPERIMENTAL METHODS

Reagents

Reagent-grade N,N,N',N'-tetramethyl-1,4-phenylenediamine and phenol (Aldrich, Milwaukee, WI, USA) are purified by vacuum sublimation. Stock solutions of these solutes at 10^{-1} – 10^{-4} M concentration are prepared freshly as needed in the appropriate buffer solution. The sodium salts of phosphoric acid (J.T. Baker, Phillipsburg, NJ, USA) are used to prepare buffer solutions in the range of pH 5.0 – 9.0 with ionic strength of 0.01 M. Water is deionized and doubly distilled in glass (Model MP-3A, Corning Glass Works, Corning, NY, USA).

Capillary Electrophoresis System

The experimental system for these studies is illustrated schematically in Figure 4. A regulated high-voltage DC power supply (Model EH50R0.19XM6, Glassman High Voltage Inc., Whitehouse Station, NJ, USA) may be operated under constant-voltage (0 – 50 kV) or constant-current (0 – 190 μA) conditions. The power supply is connected to platinum rod electrodes in two small reservoirs that contain the

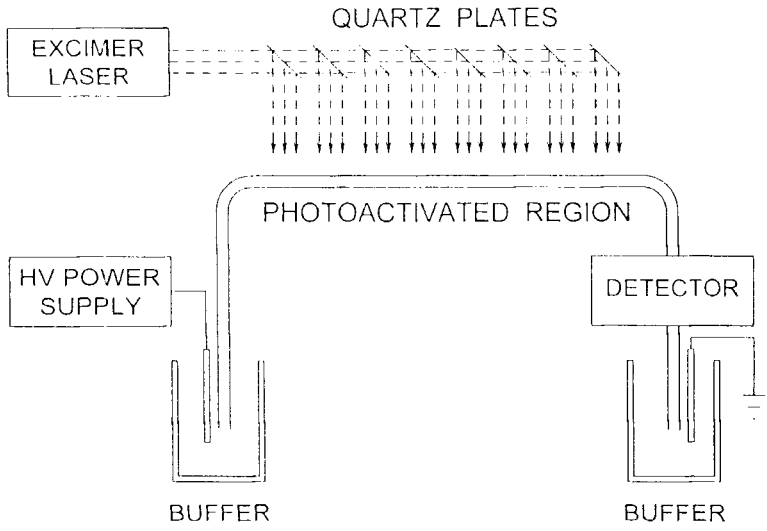


FIGURE 4: Experimental system for capillary electrophoresis with solute photoactivation and detection. Photoactivation is achieved by reflection and transmittance of excimer laser radiation using fused-silica plates. Detection is performed by UV–visible absorbance or fluorescence immediately after the photoactivated region.

phosphate buffer solutions. A straight length of fused-silica capillary tubing (75-μm i.d., 375-μm o.d., 75-cm length, Polymicro Technologies, Phoenix, AZ, USA), which serves as the migration channel, is immersed at each end in the buffer solutions. This tubing is optically transparent, to allow uniform irradiation for the photoactivation studies, and of sufficiently small diameter to dissipate both radiant and Joule heat efficiently.

Photoactivation System

The photoactivation system utilizes an excimer laser (Model EMG 101 MSC, Lambda Physik, Goettingen, Germany) that has beam

dimensions of 1.5 x 3.0 cm at the exit of the laser cavity. Although several wavelengths between 193 and 351 nm are available using different gases, krypton fluoride has been used in the initial studies to provide irradiation at 248 nm with typical pulse energy of 70 – 250 mJ, pulse width of 2.3 x 10^{-8} s, and repetition rate of 10 s^{-1}. A series of fused-silica plates are situated to reflect and transmit this light in order to provide uniform irradiance along a 10- to 25-cm length of the capillary tube.

Detection Systems

Solute detection is accomplished by using either UV–visible absorbance or fluorescence. A variable-wavelength absorbance detector (Model Uvidec 100-V, Japan Spectroscopic Co., Tokyo, Japan), modified to allow the capillary tube to be used as the flowcell, is capable of detecting solutes at the picomole (10^{-12} mole) level with a linear dynamic range of approximately 10^3. In contrast, the laser-induced fluorescence system developed in our laboratory can achieve detection limits at the attomole (10^{-18} mole) level with a linear range of 10^8 (36,37). A helium-cadmium laser (Model 3074-40M, Omnichrome, Chino, CA, USA), with approximately 30 mW of continuous-wave power at 325 nm, is used as the excitation source. The laser radiation is focused directly upon the optically transparent fused-silica capillary and solute fluorescence is collected perpendicular and coplanar to the excitation beam. The emission is then isolated by appropriate interference filters (Corion, Holliston, MA, USA) and is focused onto a photomultiplier tube (Model Centronic Q4249B, Bailey Instruments, Saddle Brook, NJ, USA). The resulting photocurrent is amplified by a picoammeter (Model 480, Keithley Instruments, Cleveland,

OH, USA) and is displayed on a chart recorder (Model 585, Linear Instruments, Reno, NV, USA).

RESULTS AND DISCUSSION

Preliminary Studies of Photoactivation

In preliminary studies, it is necessary to establish that any apparent changes in the electrophoretic separation are due to selective photoactivation of the solute, rather than other non-selective processes that may arise from laser irradiation.

The first potential problem to be addressed is that of photoinduced thermal effects. Because the vibration–translation relaxation time for the aqueous buffer medium is much less than the duration of the laser pulse (38), radiant heat is not expected to accumulate within the capillary tube. For the case where the absorption transition is not saturated, the average change in temperature can be calculated as follows (38):

$$\Delta T = \phi_o \alpha_o(\nu) w \rho / C_p b \quad [8]$$

where ϕ_o and w are the irradiance and pulse width of the laser source, $\alpha_o(\nu)$ is the absorption factor from Equation [1], b is the optical pathlength, and ρ and C_p are the density and heat capacity of the buffer medium. Based on this equation, the estimated temperature change (ΔT) under the representative experimental conditions described previously is 0.03 °C. In practice, the temperature change is likely to be less than this calculated value because of the low repetition rate of the laser as well as the efficient heat dissipation of the capillary with a high surface-area-to-volume ratio.

Although the temperature change is calculated to be quite small, it is known that temperature has a significant effect on both electroosmotic and electrophoretic velocities (39-42). The effect of increasing temperature on electroosmotic velocity is manifested as a decrease in the viscosity and, hence, an increase in the conductivity of the buffer solution within the irradiated region of the capillary. This effect was examined experimentally by monitoring the current generated under constant-voltage conditions or, conversely, the voltage generated under constant-current conditions as a function of the irradiance. From these measurements, the resistance and the equivalent conductivity were calculated by using the classical equations (43). As shown in Figure 5, the equivalent conductivity of the buffer solution does not vary significantly with irradiance in the range from 0 – 1.4 x 10^5 W cm^{-2}. Therefore, photothermal heating may be considered negligible with respect to Joule heating under these experimental conditions.

Another potential problem is that the fused-silica capillary may absorb the laser radiation to a small extent. Because the dissociation constant (pK_a) of the weakly acidic silanol groups may differ between the ground and excited states, the fraction of such groups in the ionic form on the surface may be altered by irradiation. This change in surface charge will influence the zeta potential and, hence, the electroosmotic velocity (44,45). This effect was examined by measuring the velocity as a function of pH under constant-voltage and constant-current conditions. As shown in Figure 6, the electroosmotic velocity increases in the normal and customary manner within the range from pH 5.0 – 9.0 (11,12,46). There is no statistically significant difference in the electroosmotic velocity

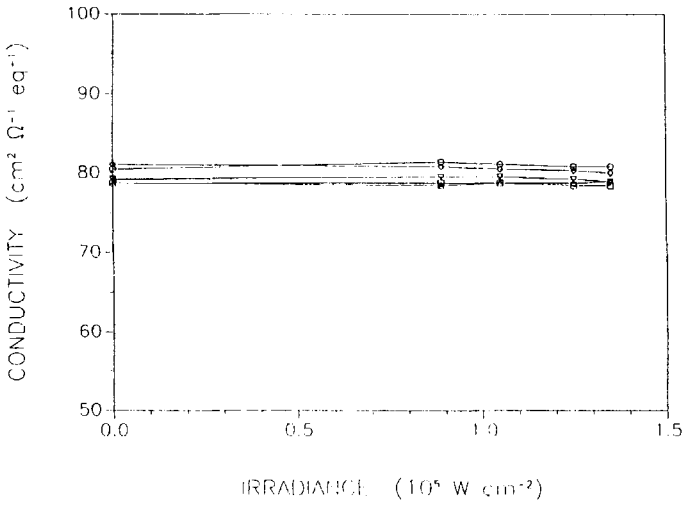


FIGURE 5: Equivalent conductivity *versus* irradiance as a function of the pH of the phosphate buffer solution. pH 5.0 (○), pH 6.0 (□), pH 7.0 (△), pH 8.0 (▽), pH 9.0 (◇).

measured without and with irradiation. Moreover, there is no significant change in the slope of these graphs as the pH approaches the pK_a of the silanol groups (5.2 – 7.7) (45-49). These observations suggest that the charge density of the silica surface is not significantly altered by irradiation.

In order to demonstrate the feasibility of the photoactivation method, it is desirable to employ chemical reactions that are irreversible. Although such reactions do not have analytical utility, they conclusively verify the ability to generate ions in good yield by photoactivation and to separate those ions from the parent neutral solutes. Two validation studies using irreversible photoionization and photodissociation reactions are described in the following sections.

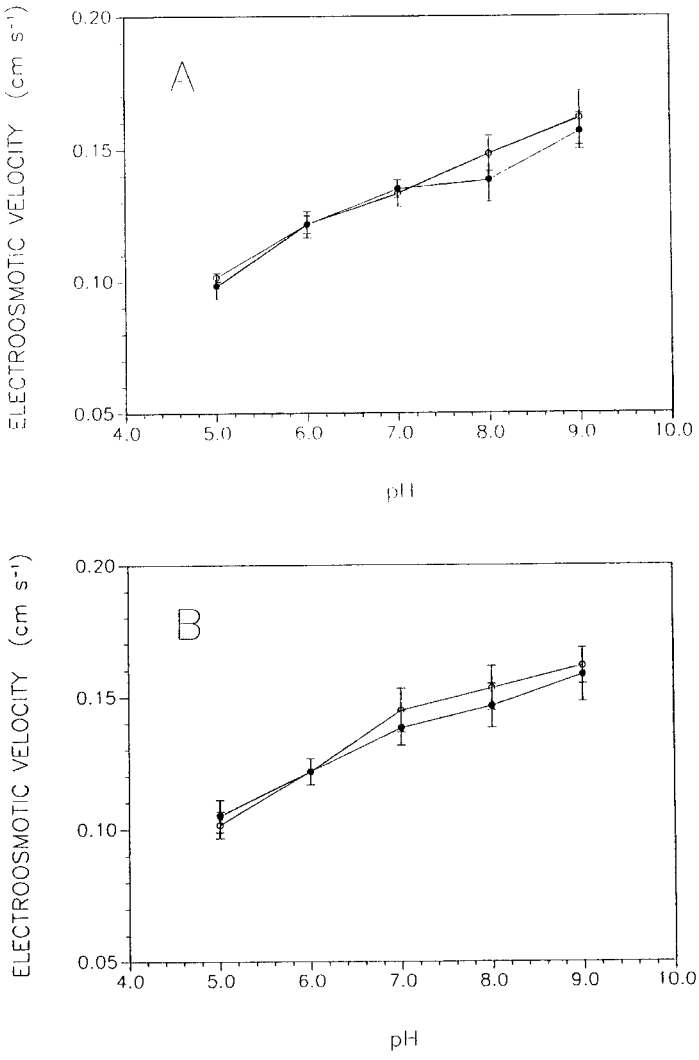
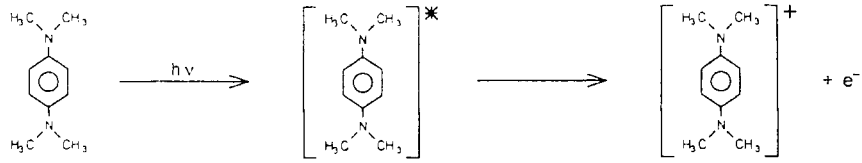


FIGURE 6: Electroosmotic velocity *versus* pH of the phosphate buffer solution (○) without and (●) with photoactivation under (A) constant-voltage conditions (27.5 kV) and (B) constant-current conditions (8.5 μA).

Validation Study with Solute Photoionization

N,N,N',N'-tetramethyl-1,4-phenylenediamine (TMPD) is chosen as the solute for this validation study because it provides visual verification of the photoionization process; the neutral molecule is colorless, whereas the cation has an intense and characteristic blue color. Although ionization can occur by two-photon excitation *via* simultaneous (50) and sequential (51) mechanisms, only one-photon excitation is expected under the experimental conditions employed in this study (52,53).



In the gas phase, the one-photon ionization threshold of TMPD has been reported as 6.2 eV (54). In the liquid phase, the ionization threshold varies from a maximum of 5.0 eV in nonpolar solvents such as hexane (55) to 3.5 eV in polar solvents such as methanol and water (52,56). At the 248 nm (5.0 eV) wavelength of the excimer laser, TMPD has a molar absorptivity of approximately 2800 L mol^{-1} cm^{-1} and, thus, should be readily photoionized in aqueous buffer media. Once the geminate cation–electron pair has become solvated to form free ions, the probability of recombination is low and the reaction is essentially irreversible (57).

The electropherograms of TMPD in phosphate buffer solution were recorded using the experimental system shown in Figure 4. In the absence of irradiation (Figure 7A), the single peak observed in the electropherogram is attributable to neutral TMPD, which migrates at the

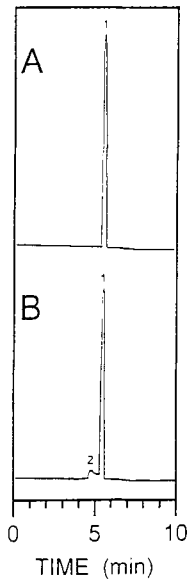


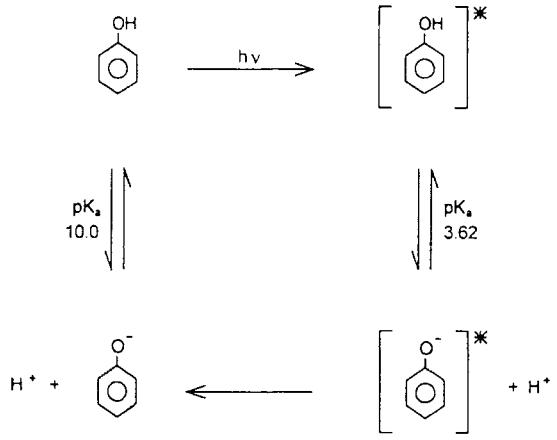
FIGURE 7: Electropherograms of 10^{-3} M N,N,N',N'-tetramethyl-1,4-phenylenediamine (A) without and (B) with photoactivation. Capillary: 75 μm i.d. × 75 cm fused-silica capillary. Buffer: 0.010 M phosphate buffer at pH 6.7. Electrophoresis: 8.5 μA constant-current conditions. Photoactivation: 115 mJ at 248 nm. Detector: UV–visible absorbance detector, 230 nm, 0.005 AUFS. Solutes: (1) TMPD, (2) TMPD cation.

electroosmotic velocity. Upon irradiation of a 25-cm length of the capillary with 115 mJ at 248 nm (Figure 7B), an additional peak appears with a higher electrophoretic mobility and, hence, a more positive charge. The unusual shape of this peak arises because the photoactivation product is not formed instantaneously but, rather, continuously to some extent along the irradiated length of the capillary. In order to confirm the identity of this product, a solution of TMPD in a cuvette was thoroughly irradiated to form the characteristic blue color of the cation. Upon electrophoretic separation of this independently irradiated solution, two peaks are observed with the

same mobilities as those shown in Figure 7B. Thus, this study demonstrates that photoionization reactions can be used to generate cations *in situ*, which can then be separated by electrophoresis.

Validation Study with Solute Photodissociation

Phenol is chosen as the solute for this validation study because the acid–base equilibrium constants are known to differ substantially between the ground and excited states (58-60):



The molar absorptivity of phenol is approximately 440 L mol^{-1} cm^{-1} at the 248 nm wavelength of the excimer laser. If the phosphate buffer solution is maintained at pH 7.0, phenol is neutral in the ground state (pK_a = 10.0) and anionic in the excited state (pK_a = 3.62). When the excited state anion relaxes to the ground state, recombination by diffusion alone is inefficient because of the low concentration of hydrogen ions and the competition with stronger bases (e.g., PO_4^{3-} and OH^-, with pK_a of 12.4 and 14.0, respectively (34,61)). Thus, the photodissociation of phenol is not reversible within the time scale of the electrophoretic experiment.

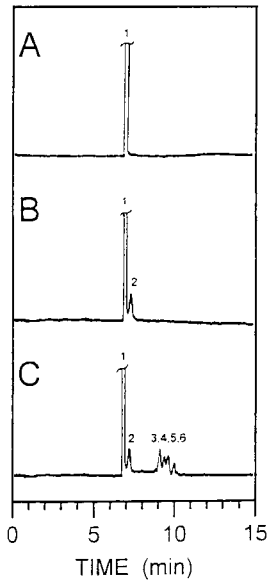


FIGURE 8: Electropherograms of 10^{-2} M phenol (A) without and (B,C) with photoactivation. Photoactivation: (B) 80 mJ at 248 nm, (C) 115 mJ at 248 nm. Solutes: (1) phenol, (2) phenol anion, (3) – (6) photochemical byproducts of phenol or phenol anion. All other experimental conditions as given in Figure 7.

In the absence of irradiation (Figure 8A), the single peak observed in the electropherogram is attributable to neutral phenol, which migrates at the electroosmotic velocity. Upon irradiation of a 25-cm length of the capillary at 80 mJ (Figure 8B), one additional peak appears with a lower electrophoretic mobility and, hence, a more negative charge. This product can be unambiguously identified by comparison of the electrophoretic mobility with that of the phenol anion generated in the absence of irradiation at pH 11.0. Upon irradiation at 115 mJ (Figure 8C), however, several peaks are observed in addition to phenol and the phenol anion.

The number and intensity of the additional peaks appears to be dependent upon the phenol concentration, the pH, and the irradiance. Phenol is known to undergo a wide variety of photochemical reactions in aqueous solution, including oxidation by oxygen, ozone, and peroxide, as well as addition of hydroxyl and phenoxyl radicals (60,62-65). A variety of stable photochemical byproducts have been isolated, such as the positional isomers of dihydroxy- and trihydroxybenzene as well as the isomers of phenoxyphenol (60,64,65). It is noteworthy that the additional peaks shown in Figure 8C are also formed when phenol is irradiated separately in a cuvette, followed by electrophoretic separation.

This study clearly demonstrates that photodissociation reactions can be used to generate ions *in situ*, which can then be separated by electrophoresis. However, the electropherogram shown in Figure 8C illustrates one of the potential problems of this method: the photophysics and photochemistry of the solute must be reasonably well understood in order to provide reliable analytical application in complex mixtures. At the same time, this feature can be used to advantage in order to study the photophysics and photochemistry of solutes in their pure form. For example, benzo[a]pyrene and its monohydroxyl and dihydroxyl isomers are known to undergo photochemical reactions similar to those of phenol (60). The toxicity and carcinogenicity of these isomers varies considerably with the position of hydroxyl substitution (66,67). Although these isomers are difficult to separate chromatographically and electrophoretically (68-71), their acid–base equilibrium constants differ substantially between the ground and excited state (72,73), as summarized in Table 1. Thus, the photoactivation method may provide a unique method to study the

TABLE 1

Acid–Base Dissociation Constants for the Ground (pK_a) and Excited (pK_a^*) States of the Monohydroxyl Isomers of Benzo[a]pyrene (72)

Benzo[a]pyrene Isomer	pK_a	pK_a^*	ΔpK_a
10-hydroxy	11.1	0.0	11.1
5-hydroxy	10.8	1.0	9.8
11-hydroxy	10.2	2.3	7.9
8-hydroxy	10.2	1.6	8.6
9-hydroxy	9.5	5.8	3.7
4-hydroxy	9.4	1.6	7.8
2-hydroxy	9.3	3.2	6.1
6-hydroxy	9.2	1.0	8.2
7-hydroxy	9.2	−0.4	9.6
1-hydroxy	9.0	2.0	7.0
12-hydroxy	9.0	1.6	7.4
3-hydroxy	8.6	4.3	4.3

photochemistry of these environmentally and biochemically important solutes.

CONCLUSIONS

The enhancement of electrophoretic separations by photoactivation of the solutes appears to be a novel and promising approach. Unlike other means to control electrophoretic mobility, this method has the advantage that it is mediated through an externally applied electromagnetic field that can be rapidly modified in both strength and selectivity. The strength of photoactivation may be controlled by the laser irradiance and pulse width, whereas the selectivity may be controlled by the wavelength and polarization. Moreover, stepwise and linear gradients in irradiance and wavelength may be implemented using standard spectroscopic

instrumentation. Although this investigation has been limited to the solute as the photoactive species, it is apparent that this general principle can be extended to other species in the separation system. Thus, this approach can potentially be applied to membrane and chromatographic separation systems where a component of the mobile or stationary phases is photoactivated.

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